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T1w/FLAIR ratio standardization as a myelin marker in MS patients

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ABSTRACT

Introduction: Calculation of a T1w/T2w ratio was introduced as a proxy for myelin integrity in the brain of multiple sclerosis (MS) patients. Since nowadays 3D FLAIR is commonly used for lesion detection instead of T2w images, we introduce a T1w/FLAIR ratio as an alternative for the T1w/T2w ratio.

Objectives: Bias and intensity variation are widely present between different scanners, between subjects and within subjects over time in T1w, T2w and FLAIR images. We present a standardized method for calculating a histogram calibrated T1w/FLAIR ratio to reduce bias and intensity variation in MR sequences from different scanners and at different time-points.

Material and methods: 207 Relapsing Remitting MS patients were scanned on 4 different 3 T scanners with a protocol including 3D T1w, 2D T2w and 3D FLAIR images. After bias correction, T1w/FLAIR ratio maps and T1w/T2w ratio maps were calculated in 4 different ways: without calibration, with linear histogram calibration as described by Ganzetti et al. (2014), and by using 2 methods of non-linear histogram calibration. The first nonlinear calibration uses a template of extra-cerebral tissue and cerebrospinal fluid (CSF) brought from Montreal Neurological Institute (MNI) space to subject space; for the second nonlinear method we used an extra-cerebral tissue and CSF template of our own subjects. Additionally, we segmented several brain structures such as Normal Appearing White Matter (NAWM), Normal Appearing Grey Matter (NAGM), corpus callosum, thalami and MS lesions using Freesurfer and Samseg.

Results: The coefficient of variation of T1w/FLAIR ratio in NAWM for the no calibrated, linear, and 2 nonlinear calibration methods were respectively 24, 19.1, 9.5, 13.8. The nonlinear methods of calibration showed the best results for calculating the T1w/FLAIR ratio with a smaller dispersion of the data and a smaller overlap of T1w/FLAIR ratio in the different segmented brain structures. T1w/T2w and T1w/FLAIR ratios showed a wider range of values compared to MTR values.

Conclusions: Calibration of T1w/T2w and T1w/FLAIR ratio maps is imperative to account for the sources of variation described above. The nonlinear calibration methods showed the best reduction of between-subject and within-subject variability. The T1w/T2w and T1w/FLAIR ratio seem to be more sensitive to smaller changes in tissue integrity than MTR. Future work is needed to determine the exact substrate of T1w/FLAIR ratio and to obtain correlations with clinical outcome.

1. Introduction

MS is a demyelinating disease with an incidence of 2.3 million people worldwide. The disease is characterized by diffuse myelin destruction and activation of microglia leading to atrophy of grey and white matter (Frischer et al., 2009). MRI is considered one of the most important tools for diagnosis and follow-up in MS for assessing demyelinating lesions. The MRI signal intensity on T1- and T2 weighted images of the white and grey matter of the brain is determined by lipids, free and myelin-bound water and iron Glasser and Van Essen (2011).

In 2011, Glasser et al. proposed the calculation of a T1w/T2w ratio map that is more sensitive to the presence or absence of myelin.

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Calculation of a T1w/T2w ratio increases myelin contrast and is therefore used as a marker of tissue integrity. Since 2011, the T1w/T2w ratio has served as a research topic for many papers. Soun et al. observed a 1.6 higher contrast difference between the posterior part of the internal capsule (highly myelinated) and the optic radiations (little myelinated) on a T1w/T2w ratio image compared to a normal T1w image in 10 newborns (Soun et al., 2016).

It is still unknown whether the T1w/T2w ratio is only reflecting myelin content in the brain. Post-mortem correlations showed in some studies that cortical T1w/T2w ratio correlated with myelin density, but other studies showed an additional contribution of dendrite density to the signal in cortical T1w/T2w ratios (Righart et al., 2017; Nakamura et al., 2017; Preziosa et al., 2021). A possible explanation for these different results could be the stage of the disease of the subject studied. When the variations in myelin content are small, the T1w/T2w ratio might not be sensitive enough to detect it (Mainero and Louapre, 2017). The T1w/T2w ratio has also been extensively studied in MS patients. Li et al. used standardized T1w/T2w ratio images to create myelin maps to investigate people with facial pain due to classic trigeminal neuralgia or due to an MS plaque Li et al. (2021). Beer et al. calculated the T1w/T2w ratio in normal appearing white matter (NAWM) in MS patients with early disease. Compared with healthy controls, they found a significantly lower T1w/T2w ratio in the patient group. Moreover, they found correlations between T1w/T2w ratios and clinical scores (Beer et al., 2016). Pareto et al. demonstrated significant correlations between T1w/T2w ratios and EDSS in relapsing remitting MS (RRMS) patients (Pareto, 2020). Important insights were gained in a recent multicenter study, specially on how T1w/T2w ratio behaves with ageing and how clinical MS phenotypes, severity of disability and structural brain damage affects the ratio. In a healthy control and MS patients cohort, it showed that T1w/T2w ratio is not only reflecting myelin density, but it is also influenced by inflammation, neurodegeneration and iron accumulation. Results showed an increase of T1w/T2w ratio in healthy ageing, significant lower T1w/T2w ratio in WM lesions in all MS phenotypes and in NAWM in RRMS and secondary progressive MS patients already from mild disability levels (Margoni et al., 2022).

The technical aspect of calculating the T1w/T2w ratio is not without pitfalls. In 2016, Ganzetti et al. rightly modified the method of (Glasser and Van Essen, 2011; Ganzetti et al., 2014). They published a calibration algorithm using image intensities outside the brain, compared to the internal method of calibration of Glasser et al. An internal calibration using an image intensity histogram of the brain in a population with brain pathology leads to attenuation of differences caused by the disease. Ganzetti et al. used bias corrected T1w and T2w images for calibration to correct for transmission field (B1+) intensity inhomogeneity. They created a mask of the eyeball and temporal muscle in the Montreal Neurological Institute (MNI) atlas. (http://www.bic.mni.mcgill.ca/ServicesAtlases/ICBM152NLin2009). These masks, which both have opposite signal intensities on T1w and T2w images, were used for external linear histogram calibration. This normalization and calibration method of the images improved the reproducibility of the T1w/T2w values in the different datasets, allowing it to be used in multi-center cohorts acquired with different scanners, with different scanning protocols, and for longitudinal data.

New guidelines suggest the replacement of T2w images by 3D FLAIR images in a standard MRI follow-up protocol, because lesion detection is nowadays generally evaluated on FLAIR images (Wattjes et al., 2021). Therefore, T1w/FLAIR ratios could be of more value in future large cohorts and in clinical routine. FLAIR images differ from T2w images in several ways. FLAIR is an inversion recovery sequence typically used in brain imaging to suppress the signal coming from the cerebrospinal fluid. In MS, this sequence makes the detection of bright periventricular lesions in connection with the ventricle wall much easier. FLAIR images make the calculation of a ratio with T1w images particularly challenging. The advantage of the method by Ganzetti et al. of using 2 extracerebral structures with opposite intensities on T1w and T2w images is not applicable on FLAIR images Ganzetti et al. (2014). On fat saturated FLAIR images, the extra-cerebral tissue of the face and neck is more uniform in intensity compared to T2w images. Consequently, there is a current need for another calibration method that is more appropriate for FLAIR images.

The purpose of this article was twofold: first, we want to assess the feasibility of calculating a T1w/FLAIR ratio, which is a new approach, and propose an intensity standardization method for FLAIR images. Second, we want to correlate the T1w/FLAIR ratio with Magnetization Transfer Ratio (MTR), which is known to be a proxy for myelin density.

2. Material and methods

2.1. Patient population

A total of 229 Relapsing Remitting MS patients were scanned on 4 different 3T scanners (Intera, Ingenia or Achieva; Philips, Best, the Netherlands) equipped with an 8-, 15, or 32-channel head coil with the same protocol including 3D T1w, 2D T2w, 3D FLAIR images and Magnetization Transfer Imaging (MTI). All imaging and clinical data were obtained as part of the routine clinical follow-up of these patients. This cross-sectional study population has been described in a previous work (Smets et al., 2021). Due to motion artifacts and errors in image data storage, 207 patients remained for the cross-sectional analysis. Descriptive data of the study population are shown in Table 1 and details of MRI scan protocol are shown in Table 2. Written informed consent was obtained, the study was approved by the ethics committee of our hospital (s60222) and was conducted in accordance with the Declaration of Helsinki.

2.2. Image processing

MRI images were downloaded from the Picture Archiving and Communication System (PACS) and converted to Neuroimaging Informatics Technology Initiative (NIfTI) files with dcm2niix (https://github.com/rordenlab/dcm2niix, v1.0.20220720). During this step, attention was paid to the correct application of the Philips scaling factors (Rescale slope, rescale intercept and Scale slope). Then, images were organized according to the BIDS structure Gorgolewski et al. (2016) Fig. 1 gives a schematic representation of the full processing pipeline.

2.2.1. Bias correction.

The 2D T2w, 3D T1w and 3D FLAIR images were resliced to an isotropic spatial resolution of 1 mm using mrgrid (Tournier et al., 2019). Bias correction is then performed on the T1w, T2w and FLAIR images using N4BiasFieldCorrection from ANTs Avants et al. (2009).

Table 1
Descriptive data of the study population.

Variable	Statistic	All
Sex		
Female	n/N (%)	147/207 (71.01 %)
Male	n/N (%)	60/207 (28.99 %)
Age onset (years)	N	204
	Mean	30.78
	Std	10.458
	Median	29.15
Age scan (years)	N	207
	Mean	40.65
	Std	11.413
	Range	(19.13; 71.23)
Disease duration scan (years)	N	204
	Mean	9.73
	Std	7.360
	Median	8.14

Abbreviations: Std, standard deviation.

Table 2
MRI scan protocol.

Protocol	Number of scans	Scanner	Sequence	FA (°)	TE (ms)	TSE TF	TI (ms)	TR (ms)	Pixel Spacing (mm × mm)	Slice thickness (mm)
A	27 (13 %)	Achieva	MTI	15	7.99 or 8.0			68.62	1.00 × 1.00	3
			3D-TFE	8	4.6	171–175	1000	9.55–9.63	0.87 × 0.87 or 0.98 × 0.98	1.2
			3D-FLAIR FS	90	339.29–406.27	178	1650	4800	1.04 × 1.04	1.12
			T2WI	90	80	15		3000	0.45 × 0.45	5
B	11 (5 %)	Achieva dstream	MTI	10	4.59			67.81	1.00 × 1.00	3
			3D-TFE	8	4.6	171–175	1000	9.53–9.61	0.98 × 0.98	1.2
			3D-FLAIR FS	90	331.86–366.83	178	1650	4800	1.04 × 1.04	1.12
			T2WI	90	80	15		3000	0.45 × 0.45	5
C	19 (9 %)	Ingenia	MTI	10	4.59 or 4.6			70.66	1.14 × 1.14	3
			3D-TFE	8	4.6 or 4.61	171–175	1000	9.59–9.77	0.98 × 0.98	1.2
			3D-FLAIR FS	90	343.86–404.31	178	1650	4800	1.04 × 1.04	1.12 or 1.2
			T2WI	90	80	15		3000	0.45 × 0.45	5
D	124 (60 %)	Ingenia	MTI	10	4.59 or 4.6			67.75	1.00 × 1.00	3
			3D-TFE	8	4.6 or 4.61	171–175	1000	9.57–9.8	0.87 × 0.87 or 0.98 × 0.98 or 0.78 × 0.78	1.2
			3D-FLAIR FS	90	323.75–407.83	178	1650	4800	1.04 × 1.04	1.12
			T2WI	90	80	15		3000	0.45 × 0.45	5
F	26 (13 %)	Intera	MTI	10	4.59			82.58	1.00 × 1.00	3
			3D-TFE	8	4.6	171–175	1000	9.58–9.65	0.98 × 0.98	1.2
			3D-FLAIR FS	90	344.47–392.6	178	1650	4800	1.04 × 1.04	1.2
			T2WI	90	80	15		3000	0.45 × 0.45	5

Abbreviations: MTI, Magnetization Transfer Imaging; FS, Fat Saturated; FA, Flip Angle; TE, Echo Time; TSE TF, Turbo Spin Echo Turbo Factor, TI, Inversion Time; TR, Repetition Time.

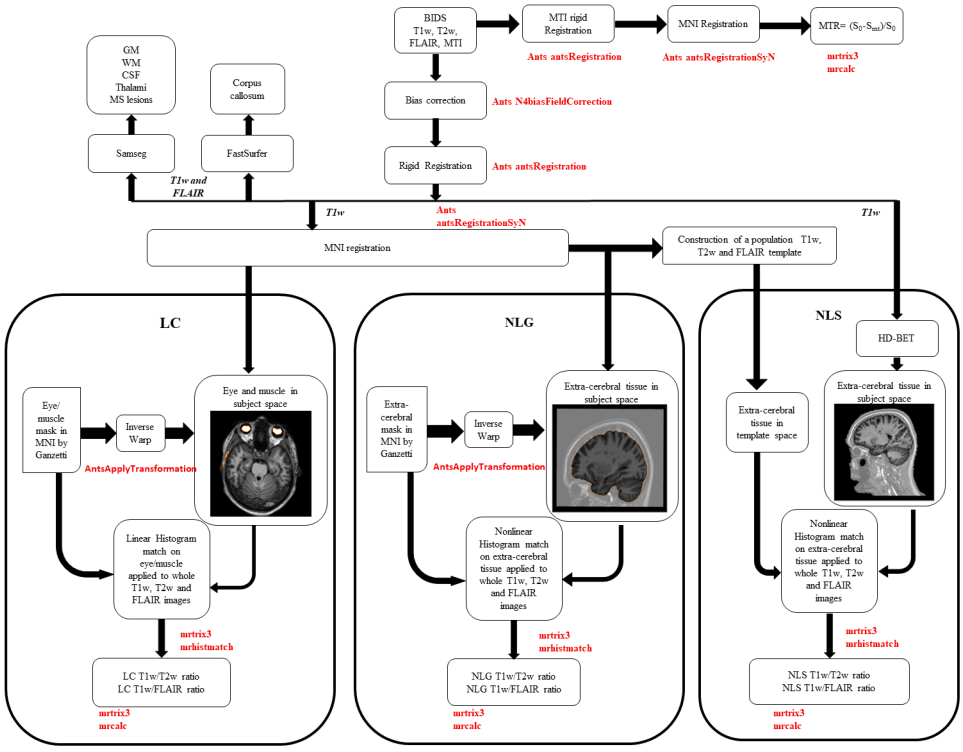

Fig. 1. Image processing pipeline for the different calibration methods. Raw T1w, T2w and FLAIR images undergo a bias correction and rigid registration process. The unbiased and registered T1w and FLAIR images are used for structure segmentation and lesion segmentation. The pipeline describes the warping of the eye/muscle and extra-cerebral masks from MNI space to subject space and the creation of an own population extra-cerebral mask. Finally, T1w/T2w ratio and T1w/FLAIR ratio maps are calculated after each calibration method. The script can be downloaded from GitHub website: https://github.com/treanus/KUL_NIS. *Abbreviations: BIDS, brain imaging data structure; GM, grey matter; WM, white matter; CSF, cerebrospinal fluid; LC, linear calibration; NLG, nonlinear Ganzetti calibration; NLS, nonlinear subject template calibration; MTI: magnetization transfer imaging; MNI, Montreal Neurological Institute; MTR, magnetization transfer ratio.*

2.2.2. Image registration

The bias-corrected T2w and FLAIR images are rigidly registered to the T1w image using ANTS registration (Avants et al., 2009).

2.2.3. Mask creation

HD-BET was used to perform an automated brain extraction on the T1w image for NLS calibration (see method 4) (Isensee et al., 2019). For the LC and NLG calibration method (see method 2 and 3), images were normalized to MNI space using AntsregistrationSyn. A nonlinear warping was determined for the T1w image to the MNI atlas, specifically to the one provided by Ganzetti et al. using Ants registration (Ganzetti et al., 2014; Avants et al., 2009). The inverse transformation was used to

warp the eye and muscle tissue, as well as the extra-cerebral tissue provided by Ganzetti et al. to the individual subject space (Ganzetti et al., 2014).

2.2.4. Own T1w/T2w/FLAIR template creation

An average was computed of the uncalibrated T1w, T2w and FLAIR images registered in MNI space of all 207 subjects.

2.2.5. Segmentation

We segmented white matter (WM), grey matter (GM), cerebrospinal fluid (CSF), left and right thalamus and MS lesions using Samseg (Puonti et al., 2016; Cerri et al., 2021). The corpus callosum was segmented with FastSurfer, a deep-learning based neuro-imaging pipeline (Henschel et al., 2020). Normal appearing white matter (NAWM) was calculated as WM minus MS lesions.

2.2.6. T1w/T2w ratio and T1w/FLAIR ratio

T1w/T2w ratio and T1w/FLAIR ratio maps were calculated in 4 different ways: without calibration, with linear histogram calibration as described by Ganzetti et al. and by using 2 methods of nonlinear histogram calibration (Margoni et al., 2022). To see the difference in intensity standardization we applied all methods for each ratio to see what the effect of calibrating would be on the dispersion of the data. Each method is described in a different section below. MRcalc was used to calculate the ratios (Tournier et al., 2019).

2.2.6.1. Method 1; no calibration (NC). In the first method T1w/T2w ratio and T1w/FLAIR ratios were calculated without any calibration.

2.2.6.2. Method 2; linear histogram calibration (LC). This is the method described by Ganzetti et al. to calculate T1w/T2w ratio using eye and temporal muscle tissue for linear histogram matching (Ganzetti et al., 2014). The workflow of image processing is described in detail in the article mentioned. An intensity histogram was obtained of eye and temporal muscle on the T1w images and on the T1w in MNI space using the eye and temporal muscle masks defined by Ganzetti et al. (2014) Next, a linear histogram match was calculated using mrhistmatch (Tournier et al., 2019). Afterwards, we applied the linear histogram transformation to the T1w images of our subject to obtain a calibrated T1w image. For T2w images we use the same strategy except with T2w images in MNI space and subject space. For the calibrated FLAIR images, we used the linear histogram transformation obtained from the T2w histogram matching.

2.2.6.3. Method 3; nonlinear histogram calibration conforms to Ganzetti et al (NLG calibration). In this method we use the extra-cerebral mask obtained by inversely warping this mask provided by Ganzetti et al. from MNI space to subject space to perform a nonlinear histogram matching (Ganzetti et al., 2014). First, an intensity histogram of extra-cerebral tissue was obtained from the T1w images of the subjects and from the T1w images in MNI space. Second, a nonlinear histogram match was calculated using mrhistmatch. Tournier et al. (2019). Finally, we applied the nonlinear histogram transformation to the T1w images of our subject to obtain a calibrated T1w image. The same strategy was used for the T2w images in MNI and subject space. To obtain the calibrated FLAIR images, we used the nonlinear histogram transformation obtained from the T2w histogram matching.

2.2.6.4. Method 4; nonlinear histogram calibration with subject template (NLS calibration). In this method we used the extra-cerebral mask obtained from our own population created T1w template. An intensity histogram from extra-cerebral tissue was obtained from the T1w image of our template and the T1w images of our subjects to perform a histogram matching with mrhistmatch (Tournier et al., 2019). The obtained nonlinear histogram transformation was applied on the T1w images of our subjects to get a calibrated T1w image. The same strategy was used for both the T2w and the FLAIR images. For each subject and template T2w and FLAIR image, we obtained an own nonlinear histogram transformation that we could apply on each subject T2w and each FLAIR image respectively to obtain calibrated T2w and FLAIR images.

2.2.7. MTR calculation

MTI images were rigidly registered to the T1w image using ANTS registration and were normalized to the MNI space using AntsregistrationSyN. MTR was calculated as follows: $(S_0\text{-}S_{mt})/S_0$.

2.3. Statistical analysis

Descriptive information was provided as a mean with standard deviation for continuous variables, or frequencies with percentages for categorical variables (see Table 1). The coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean, for the different calibration methods in the NAWM, NAGM, MS lesions, corpus callosum and thalamus to determine the dispersion in the data. CV's determined by different calibration methods were compared statistically, in the entire population and per scan protocol (Kalkur and Rao, 2015) Scatter plots were used to graphically present the associations between MTR and T1w/T2w or T1w/FLAIR ratios. All tests were performed at a two-sided 5 % significance level. Analyses have been performed using SAS software (version 9.4 of the SAS System for Windows).

3. Results

3.1. Image processing

Fig. 2 is a visual illustration of the intensity histograms, the input images and the obtained T1w/T2w and T1w/FLAIR ratios for 2 different subjects scanned with a different MR device. In box A, histograms and input T1w images without calibration, with linear calibration, with NLG calibration and with NLS calibration are visualized. The same is true for box B and box C, where respectively the T2w images and FLAIR images with different calibration methods are given. For the input images, the same windowing scale is used for the 2 different subjects. Without calibration, the intensity histograms derived from the T1w, T2w and FLAIR images do not show a good overlay due to receiver gain adjustments during scan preparation and the fact that different MR devices are used. This can be the case within one subject scanned repeatedly, but moreover is the case between several subjects. For the T1w images, the NLG and NLS calibration methods give a better fit of intensity histograms in these patients. For the T2w images, the LC gives the best overlay of intensity histograms and for the FLAIR images it is the NLS calibration method. Boxes D and E show T1w/T2w ratios and T1w/FLAIR ratios, respectively, with higher contrast between grey and white matter and MS lesionscompared to raw T1w images.

3.2. Coefficients of variations

The dispersion for the T1w/T2w ratio and T1w/FLAIR ratio in the NAWM under different calibration methods (no calibration, linear calibration, NLG calibration and NLS calibration) can be appreciated in Fig. 3. In Fig. 3A we see a small decrease in dispersion of the T1w/T2w ratio and a small decrease in the CV with the NLS calibration method compared to the other methods. As reported in Table 3, for the T1w/T2w ratio there is no significant difference in CV between no calibration and any calibration method in NAWM. Only for the MS lesions, there is a statistically significant difference in CV for uncalibrated and NLG calibrated images. In Fig. 3B, we see the largest decrease in data dispersion for T1w/FLAIR ratio in NAWM for the NLG calibration method compared to the no calibration method. A statistically significant difference between no calibration and the 2 nonlinear calibration methods (NLG and NLS) in different structures (NAWM, NAGM, corpus callosum

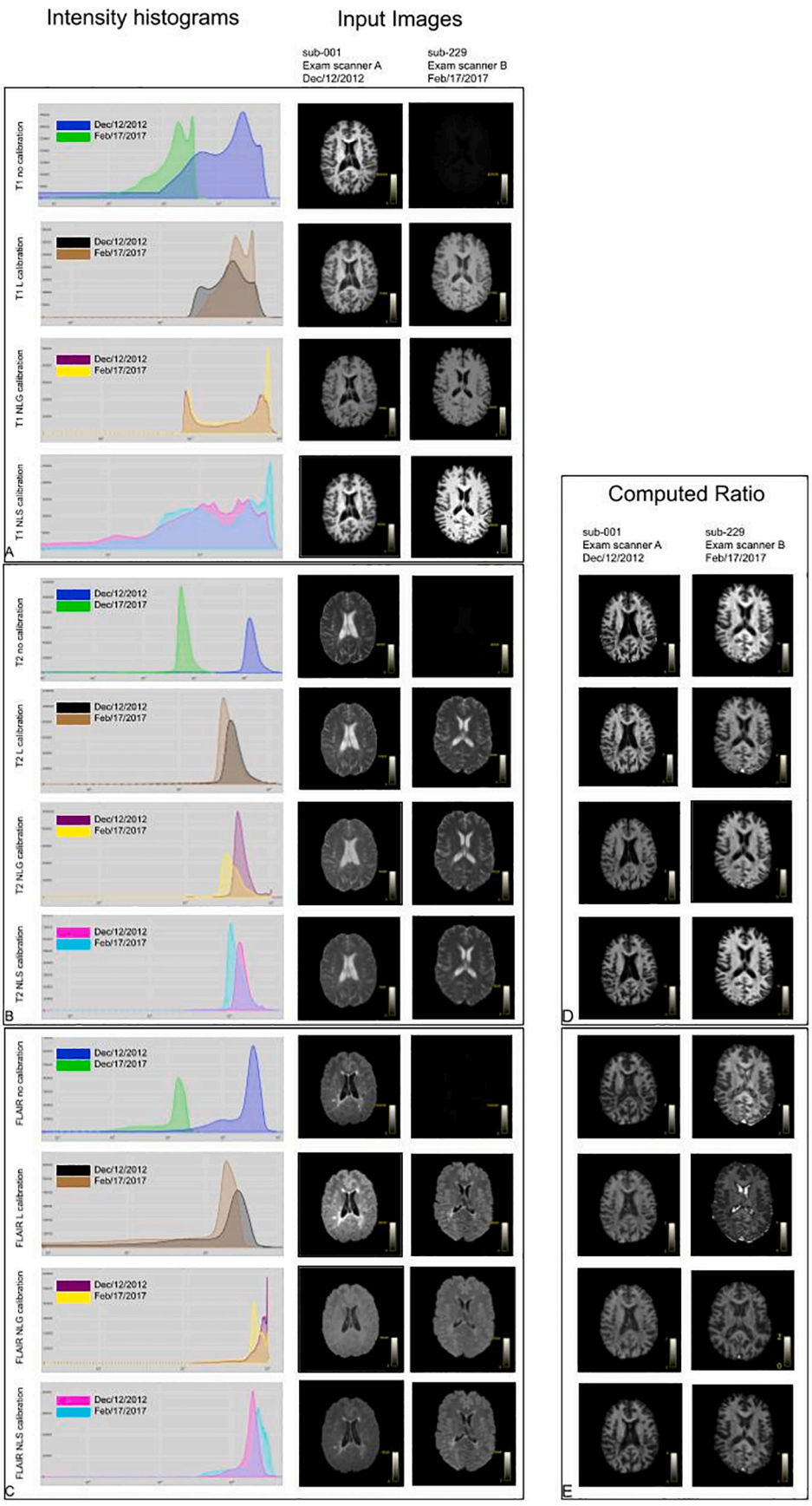

Fig. 2. Intensity histograms, input images and computed T1w/T2w ratio and T1w/FLAIR ratio maps. T1w, T2w and FLAIR intensity histograms and input images in [A], [B] and [C], respectively, from 2 different patients scanned (subject 001 and subject 229) on a different MR device on different dates. Top raw without calibration, 2nd raw with linear calibration, 3rd raw with NLG calibration and bottom raw with NLS calibration. T1w/T2w ratio and T1w/FLAIR ratio maps for the 2 patients [D] and [E]. Top raw without calibration, 2nd raw with linear calibration, 3rd raw with NLG calibration and bottom raw with NLS calibration. *Abbreviations: L calibration, linear calibration; NLG, nonlinear Ganzetti calibration; NLS, nonlinear subject template calibration;*

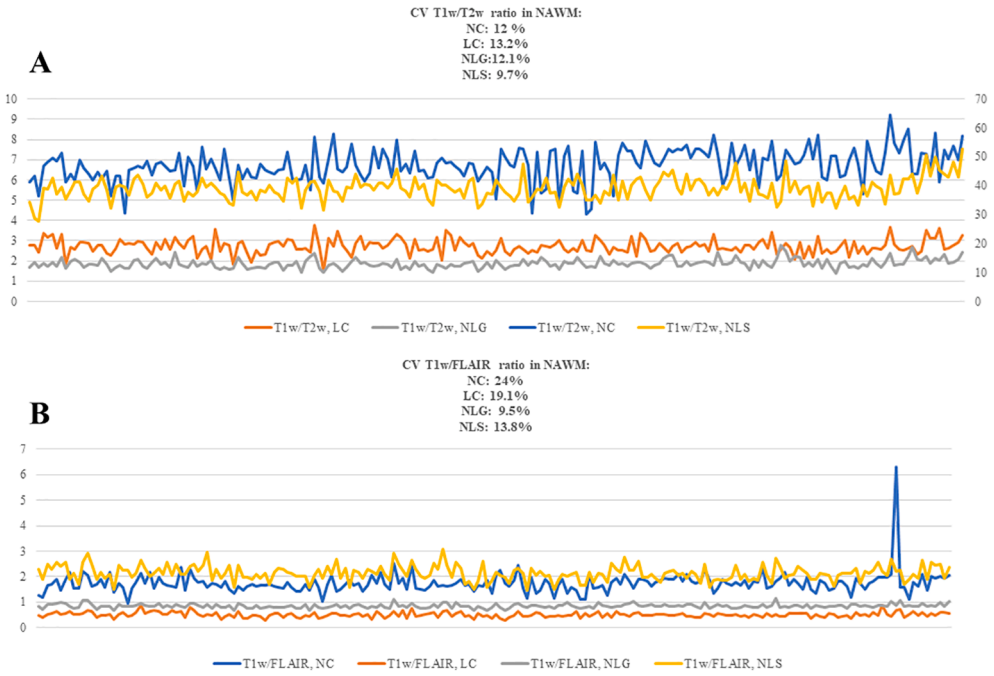

Fig 3. Dispersion in the data due to intensity scale variation. T1w/T2w ratio [A] and T1w/FLAIR ratio [B] in NAWM with 4 different calibration methods: NC, LC, NLS and NLG calibration. In [A], T1w/T2w, LC and T1w/T2w, NLG are located on the primary axis; T1w/T2w, NC and T1w/T2w, NLS are located on the secondary axis. *Abbreviations: NAWM, normal appearing white matter; NC, no calibration; LC, linear calibration; NLG, nonlinear Ganzetti calibration; NLS, nonlinear subject template calibration; CV, coefficient of variation.*

Table 3
Coefficient of variation for T1w/T2w ratio in NAWM, NAGM and MS lesions.

	CV (%)				P-values					
Structure	NC	LC	NLG	NLS	NC-LC	NC-NLG	NC-NLS	LC-NLG	LC-NLS	NLG-NLS
NAWM	12.0	13.2	12.1	9.7	1.0000	1.0000	0.2116	0.4417	0.1279	0.1938
NAGM	15.1	13.0	11.7	13.0	0.2796	0.1593	0.2838	0.3800	1.0000	1.0000
MS Lesions	23.7	20.0	13.5	17.3	0.1749	**0.0119**	0.0560	**0.0479**	0.2311	0.1229
CC	18.5	18.2	13.0	13.2	0.6981	0.0680	0.0689	0.0799	0.0813	0.7628
Thalamus	13.7	13.6	11.0	10.6	0.7590	0.1895	0.1513	0.2045	0.1654	1.0000

CV: coefficient of variation for T1w/T2w ratio.
P-value for comparison of paired coefficients of variation (Kalkur and Rao, 2015). Statistically significant results are indicated in bold.

and thalamus) was found, as reported in Table 4.

3.3. Associations T1w/T2w ratio, T1w/FLAIR ratio and MTR

The correlations between T1w/T2w ratio and MTR (left column) and T1w/FLAIR ratio and MTR (right column) are shown in Fig. 4, for each calibration method in each row. MTR ranges from 0.3 to 0.5, while T1w/T2w ratios have a larger range from 0.5 to 4.0 for linear calibration and NLG calibration to 10–60 for no calibrated and NLS calibrated ratios. The T1w/FLAIR ratios have a more diverse range depending on the type of calibration used. The ratios for MS lesions, NAWM and NAGM are rendered in different colors. For T1w/T2w ratio it is the NLS method and for T1w/FLAIR ratio the NLG method that gives the best spread of the values for the ratios in the different structures and are thus most sensitive to distinguish different structures. (see G and H, Fig. 4).

4. Discussion and limitations

To our knowledge this is the first time the T1w/FLAIR ratio has been proposed as a possible alternative for the T1w/T2w ratio. In 2011, Glasser et al. introduced a T1w/T2w ratio map to evaluate myelin content in the cortical grey matter. Creating a ratio between T1w and T2w images increases the contrast between highly myelinated and mildly myelinated structures (Glasser and Van Essen, 2011). This can be appreciated in the created ratio maps seen in Fig. 2 box E and is relevant in demyelinating diseases such as MS, where myelin is destroyed. With

Table 4
Coefficient of variation for T1w/FLAIR ratio in NAWM, NAGM and MS lesions. *Abbreviations: NC, no calibration; LC, linear calibration; NLG, nonlinear Ganzetti calibration; NLS, nonlinear subject template calibration; CV, coefficient of variation, NAWM, normal appearing white matter; NAGM, normal appearing grey matter.*

	CV (%)				P-values					
Structure	NC	LC	NLG	NLS	NC-LC	NC-NLG	NC-NLS	LC-NLG	LC-NLS	NLG-NLS
NAWM	24.0	19.1	9.5	13.8	0.1334	**0.0022**	**0.0189**	**0.0105**	0.0861	0.0836
NAGM	26.1	17.0	12.0	15.1	**0.0351**	**0.0048**	**0.0182**	0.0856	0.3122	0.1742
MS Lesion	27.5	23.0	9.7	22.0	0.1634	**0.0006**	0.1139	**0.0027**	0.4907	**0.0027**
CC	28.6	24.8	12.4	18.6	0.2066	**0.0018**	**0.0273**	**0.0050**	0.0760	**0.0453**
Thalamus	26.5	23.4	11.7	16.5	0.2483	**0.0027**	**0.0242**	**0.0060**	**0.0570**	0.0748

CV: coefficient of variation for **T1w/FLAIR ratio.**
P-value for comparison of paired coefficients of variation (Kalkur and Rao, 2015). Statistically significant results are indicated in bold.

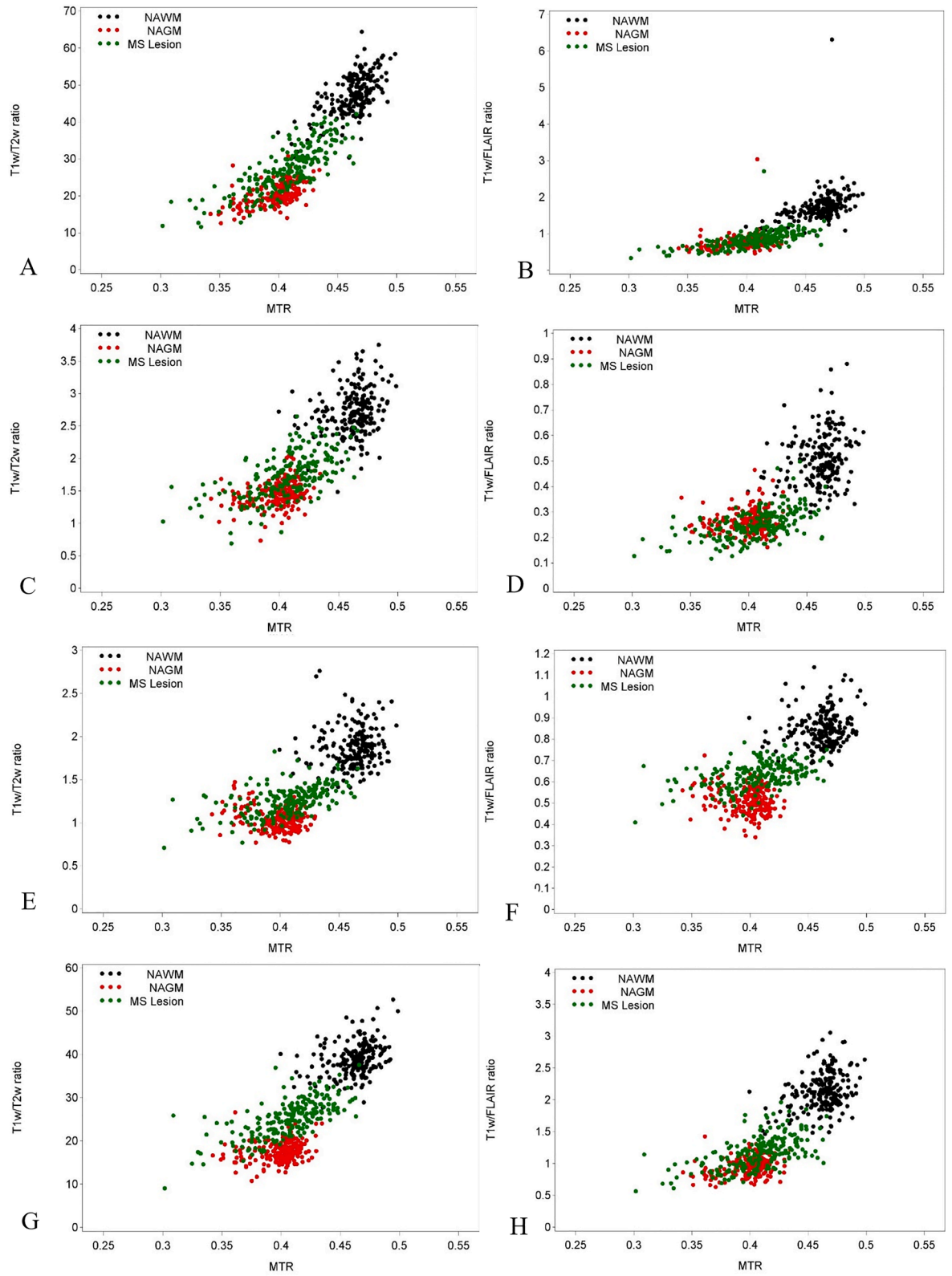

Fig 4. Correlation of T1w/T2w ratio and T1w/FLAIR ratio in NAWM, NAGM and MS lesions to MTR. [A, B] T1w/T2w ratio and T1w/FLAIR ratio without calibration. [C,D] T1w/T2w ratio and T1w/FLAIR ratio with linear calibration by Ganzetti et al (Beer et al., 2016). [E,F] T1w/T2w ratio and T1w/FLAIR ratio with nonlinear calibration conform to the method of Ganzetti et al (NLG). [G,H] T1w/T2w ratio and T1w/FLAIR ratio with nonlinear calibration with own subject template (NLS). *Abbreviations: NAWM, normal appearing white matter; NAGM, normal appearing grey matter; MTR, magnetization transfer ratio.*

this T1w/T2w ratio we can get a proxy of tissue integrity without the need for specialized and time-consuming MRI sequences in the daily clinical routine. The method of Glasser et al. was adapted by Ganzetti et al. in 2014. They emphasized the importance of eliminating the bias in the images and calibrating the images before calculating the T1w/T2w ratio Ganzetti et al. (2014). We know that bias and intensity variation are widely present between different scanners, between subjects and within subjects over time in T1w, T2w and FLAIR images. It is secondary to transmission field (B1 +) intensity inhomogeneity, receiver gain adjustments, scanner updates, different positioning of patients in the scanner, interval between follow-up scans, etc.

Based on Fig. 2, we conclude that the intensity histogram of the T1w and T2w images for 2 particular patients scanned on 2 different MR devices with the same window scale is far from identical. This leads to considerable variation in the calculated T1w/T2w ratios of these 2 patients, which cannot be explained by pathology and intersubject differences alone. Consequently, we obtain incomparable values of T1w/T2w ratios between different scanners. By calibrating the images, we limit this variation caused by previously mentioned sources without attenuating the differences caused by brain pathology.

Since nowadays 3D FLAIR images are generally used for lesion detection instead of T2w images in the MS population, we wanted to know whether it was feasible to calculate a calibrated T1w/FLAIR ratio as an alternative to the T1w/T2w ratio. Given the characteristics of our fat saturated 3D FLAIR images, we needed to work out a different method of calibration. We proposed a calibration method based on nonlinear intensity histogram matching between an extra-cerebral mask of our subjects and an extra-cerebral template mask obtained from the mean of all our subjects. Using our own study population to create a template instead of using the MNI template has 2 advantages: first, we are working with a template scanned on the same scanners as the study population and second, the availability of a FLAIR template in addition to T1w and T2w templates available in the MNI atlas. Our method, together with the other nonlinear calibration method, showed the best results for calculating the T1w/FLAIR ratio with a smaller spread of data and a smaller overlap of T1w/FLAIR ratio in the different structures (NAWM, NAGM, MS lesions) (see Fig. 4 F,H). Especially for T1w/FLAIR ratios it seems more important to use nonlinear calibration methods compared to the T1w/T2w ratios where the 4 calibration methods do not give a statistically different result (see Tables 3 and 4).

Similar to the results of Pareto et al. we see a wider range of values given by T1w/T2w ratios and T1w/FLAIR ratios compared to MTR values (Pareto, 2020) This could mean that T1w/T2w and T1w/FLAIR ratios are more sensitive to smaller degrees of myelin destruction. However, there is still doubt about the specificity of the T1w/T2w ratio to measure myelin content. Arshad et al. showed low to intermediate significant correlation between myelin water fraction (MWF) and T1w/T2w ratio and assumed T1w/T2w ratio is more associated with axonal diameter in the subcortical white matter Arshad et al. (2017). These results were in line with the study by Uddin et al. who showed no correlation between T1w/T2w ratio and MWF, which is a histologically validated measurement (Uddin et al., 2018; Dula et al., 2010). According to a postmortem study of 9 MS patients, the cortical T1w/T2w ratio seems to reflect dendrite density rather than myelin density (Righart et al., 2017; Nakamura et al., 2017). Shafee et al. showed that in the cortical grey matter of healthy subjects, T1w/T2w ratio represented higher values in the inner cortical layer compared to the outer cortical layer, which could be validated in a postmortem brain (Shafee et al., 2015). A high field magnetic resonance study on 9.4 Tesla showed that in cortical grey matter of MS patients, the T1 signal may be a predictor of neuronal density and the T2 signal of myelin content (Schmierer et al., 2010). Therefore, a ratio of these 2 values would be influenced by both myelin content and neuronal density. We know that T1w/T2w ratios at least are influenced by the myelin content and some studies could even show significant correlations between T1w/T2w ratios in NAWM and clinical scores in MS patients (Beer et al., 2016; Pareto, 2020). Hagiwara et al. studied 20 healthy adults and examined the correlation between T1w/T2w ratio, MTI and synthetic MRI. This last one is a quantitative MRI technique which yields us T1w, T2w and FLAIR images based on acquired quantitative values. In the post-processing phase it is even possible to calculate relaxometry of R1 and R2 relaxation rates based on these quantitative values. R1 correlates well with myelin content in a post-mortem study of MS patients. Their study suggested that MTR and synthetic MRI measurements were more optimal to measure myelin in white matter, whereas in cortical grey matter T1w/T2w ratio was strongly correlated to R1 (Hagiwara et al., 2018; Mottershead et al., 2003).

There are some limitations to this study. First, we do not yet have neuropathological correlations for the T1w/FLAIR ratio. Second, we used “real world clinical” data, as opposed to a study in a healthy population. This likely increases the observed variability in the ratios, which is thus influenced by the presence of disease. Our scans were obtained over a time period of 10 years, on 4 different scanners with 5 different scanning protocols. It would have been very difficult to conduct a volunteer study over this period of time with the same number of healthy subjects and differing variables (scanner, coil, software version).

Third, the nonlinear histogram matching we apply in the NLS method needs further improvement. The main reason why it is not yet optimal, is probably because we standardize with extra-cerebral tissue. An alternative method could be to use NAWM for standardization, although diffusion studies have shown that NAWM is also not ‘normal’ in MS patients (Filippi et al., 2001; Rahmanzadeh et al., 2021). Fourth, calibration of FLAIR images in the linear calibration method and nonlinear NLG calibration method by using the intensity histogram transformation derived from the T2w images is not recommended. Fifth, the values of T1w/T2w ratios and T1w/FLAIR ratios are comparable between different centers only if the same approach to calibration is used. Finally, it is yet unclear what the optimal T1w and T2w (or FLAIR) mean image intensities (or weights) should be for the computation of the ratio maps in order to obtain optimal “myelin” contrast. We have not taken this into account. Future work is needed to determine this optimal ratio, by comparing differently weighted ratios to other white matter lesion measurements.

In conclusion, calibration of T1w/T2w and T1w/FLAIR ratio maps is absolutely necessary to account for the sources of intensity variation described above. The nonlinear calibration methods NLG and NLS showed the best reduction in between-subject and within-subject variability. We were able to create a standardized pipeline to calibrate raw T1w and FLAIR images to create a T1w/FLAIR ratio. Future work including correlations of T1w/FLAIR ratios with MTR and clinical parameters should give us better insight into the neuropathological substrate that drives T1w/FLAIR ratios. Finally, it is of utmost importance that the comparison of T1w/T2w ratios and T1w/FLAIR ratios is only possible when they have been calculated in the same standardized way.

CRediT authorship contribution statement

S. Cappelle: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. **D. Pareto:** Conceptualization, Methodology, Writing – review & editing, Supervision. **S. Sunaert:** Conceptualization, Methodology, Software, Formal analysis, Writing – review & editing. **I. Smets:** Investigation, Resources, Writing – review & editing. **A. Laenen:** Formal analysis. **B. Dubois:** Investigation, Resources, Supervision. **Ph. Demaerel:** Writing – review & editing, Supervision.

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.

Data availability

The authors do not have permission to share data.

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