



# Degree of microstructural changes within T1-SE versus T1-GE hypointense lesions in multiple sclerosis: relevance for the definition of “black holes”

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## Abstract

**Objectives** To retrospectively evaluate the different performances of T1-SE and T1-GE sequences in detecting hypointense lesions in multiple sclerosis (MS), to quantify the degree of microstructural damage within lesions and to correlate them with patient clinical status.

**Methods** Sixty clinically isolated syndrome (CIS) and MS patients underwent brain magnetic resonance imaging (MRI) on 1.5-T and 3-T scanners. We identified T2 fluid-attenuated inversion recovery hyperintense lesions with no hypointense signal on T1-SE/T1-GE (a), hypointense lesions only on T1-GE (b), and hypointense lesions on both T1-SE and T1-GE sequences (c). We compared mean lesion number (LN) and volume (LV) identified on T1-SE and T1-GE sequences, correlating them with Expanded Disability Status Scale (EDSS); fractional anisotropy (FA) and mean diffusivity (MD) values inside each lesion type were extracted and normal-appearing white matter (NAWM).

**Results** Thirty-five patients were female. Mean age was 39.2 ( $\pm 7.8$ ); median EDSS was 3 ( $\pm 2$ ). There were 23 CIS, 21 relapsing–remitting (RR), and 16 progressive MS. T1-GE and T1-SE LN and LV were significantly different ( $p < 0.001$ ), both correlating with EDSS. Both FA and MD metrics resulted significantly different among the three lesion groups and NAWM ( $p < 0.001$ ). FA and MD values extracted from (b) and (c) showed statistically significant differences ( $p < 0.001$ ), while for (a) and (b), the differences were not significant ( $p = 0.31$  for FA and  $p = 0.62$  for MD).

**Conclusion** T1-SE hypointense lesions demonstrated a more pronounced degree of microstructural damage. T1-weighted sequence type must be more carefully evaluated in clinical and research settings.

## Key Points

- T1-weighted spin-echo (T1-SE) images detect chronic hypointense lesions (so called black holes) associated with more severe microstructural changes.
- In the last years, three-dimensional (3D) T1-weighted gradient-echo (T1-GE) sequences are often utilized in lieu of T1-SE acquisition, more so at 3 T or higher fields.
- T1-weighted sequence type must be more carefully evaluated in clinical and research settings in the definition of “black holes” in MS, in order to avoid the overestimation of the effective severe tissue damage.

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**Keywords** Multiple sclerosis · Magnetic resonance imaging · T1-weighted images · Hypointense lesions · Microstructural damage

### Abbreviations

BPF	Brain parenchymal fraction
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIS	Dissemination in space
DIT	Dissemination in time
DTI	Diffusion tensor imaging
EDSS	Expanded Disability Status Scale
FA	Fractional anisotropy
FLAIR	Fluid-attenuated inversion recovery
FSPGR	T1-fast spoiled gradient echo
GM	Gray matter
MD	Mean diffusivity
MPRAGE	Magnetization-prepared rapid gradient echo
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NAWM	Normal-appearing white matter
NODDI	Neurite orientation and dispersion density imaging
RR	Relapsing–remitting
SD	Standard deviation
SNR	Signal-to-noise ratio
T	Tesla
T1-GE	T1-weighted gradient echo
T1-SE	T1-weighted spin echo
TE	Echo time
TR	Repetition time
WM	White matter

### Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), characterized by focal lesions (“plaques”) and parenchymal volume loss (“atrophy”) of the brain and spinal cord. Plaques are characterized by pathological heterogeneity with different inflammatory pathways, variable degrees of demyelination or/and remyelination, and different amounts of axonal loss. Magnetic resonance T2-weighted imaging has a high sensitivity in the detection of plaques but lacks specificity for the underlying pathology and for the degree of tissue destruction. T1-weighted (T1-w) spin-echo (T1-SE) images, conversely, may detect chronic hypointense lesions (so called black holes), which have been associated with more severe pathological changes including more profound demyelination and axonal loss [1–6]. In the last years, three-dimensional (3D) T1-weighted gradient-echo (T1-GE) sequences are often utilized in lieu of T1-SE acquisition, more so at 3 T or higher fields. T1-GE-based sequences are able to detect a significant greater number and volume of hypointense

lesions in MS patients with respect to T1-SE images [7]. The degree of microstructural damage of these hypointensities has not been deeply investigated, and it is unclear if these lesions should be considered to be markers of more severe pathological damage as T1 black holes detected on T1-SE.

In this study, we compared 2D T1-SE and 3D T1-GE sequences both at 1.5-T and at 3-T in patients with clinically isolated syndrome (CIS), relapsing–remitting (RR), and progressive MS (PMS), to quantify the degree of microstructural changes within the lesions detected on the different sequences and to correlate them with patient clinical status.

### Methods

#### Subjects

In this retrospective analysis, we studied 60 consecutive patients with CIS, RRMS, and PMS, diagnosed according to the 2017 revisions to McDonald criteria [8]. In particular, patients who developed a monophasic clinical episode reflecting an inflammatory demyelinating event in the CNS, similar to a typical MS (attack and exacerbation) but not contemporary fulfilling dissemination in time (DIT) and dissemination in space (DIS) criteria, were diagnosed as CIS patients. According to these criteria, we defined patients who met the definition of CIS at the time of MRI. The other patients were defined as MS according the 2017 criteria and included in the RRMS and PMS groups according to Lublin and co-workers [9]. All patients were assessed by a neurologist within 1 week of magnetic resonance imaging (MRI) for disability by using the Expanded Disability Status Scale (EDSS).

Out of 60 patients, 35 (68.3%) were female and 25 (41.7%) were male; mean age (SD) was 39.2 (7.8) years. Median (IQR) EDSS was 2.5 (1–5.5). Regarding MS phenotypes, 23 (38.3%) were CIS, 21 (35%) were RRMS, and 16 (26.7%) were PMS patients. The mean (SD) time interval between CIS onset and the first MRI imaging was 4.7 (3.1) months.

In 32 (53.3%) patients, MRI was performed at 1.5 T and in 28 (46.7%) at 3 T. The study was performed at Ospedale Policlinico IRCCS San Martino, Genoa, Italy.

#### MRI acquisition

Thirty-two patients underwent brain MRI on a 1.5-T (Signa HDxT 1.5 T, General Electric) scanner using the same acquisition protocol, including (i) a GE sequence, in particular 3D T1-fast spoiled gradient echo (FSPGR) (repetition time (TR) 4.98 ms, echo time (TE) 1.348 ms, voxel size  $1 \times 1 \times 1$  mm, inversion time (IT) 500 ms, acquisition time 3.50 min); (ii) 3D

T2-weighted fluid-attenuated inversion recovery (3D T2-FLAIR) (TR 6000 ms, TE 128.17 ms, voxel size  $1 \times 1 \times 1$  mm); (iii) diffusion tensor imaging (DTI) (TR 14,000 ms, TE 95.7 ms; voxel size  $2.5 \times 2.5 \times 2.5$  mm, 30 directions with  $b$  factor  $1000 \text{ s/mm}^2$ ); and (iv) 2D T1-SE post-gadolinium administration (TR 480 ms, TE 20 ms, voxel size  $1 \times 1 \times 3$  mm, acquisition time 2.45 min).

Twenty-eight patients underwent brain MRI on a 3 T (Signa HDxT 3 T, General Electric) scanner using the same acquisition protocol, including (i) 3D T1-FSPGR (TR 6.99 ms, TE 2.856 ms, IT 0 ms, voxel size  $1 \times 1 \times 1$  mm, acquisition time 4.25 min); (ii) 3D T2-weighted fluid-attenuated inversion recovery (3D T2-FLAIR) (TR 6000 ms, TE 125.15 ms, voxel size  $1 \times 1 \times 1$  mm); (iii) DTI (TR 14,125 ms, TE 97.3 ms; voxel size  $2.5 \times 2.5 \times 2.5$  mm, 30 directions with  $b$  factor  $1000 \text{ s/mm}^2$ ); and (iv) 2D T1-SE post-gadolinium administration (TR 640 ms, TE 9 ms, voxel size  $1 \times 1 \times 3$  mm, acquisition time 2.45 min).

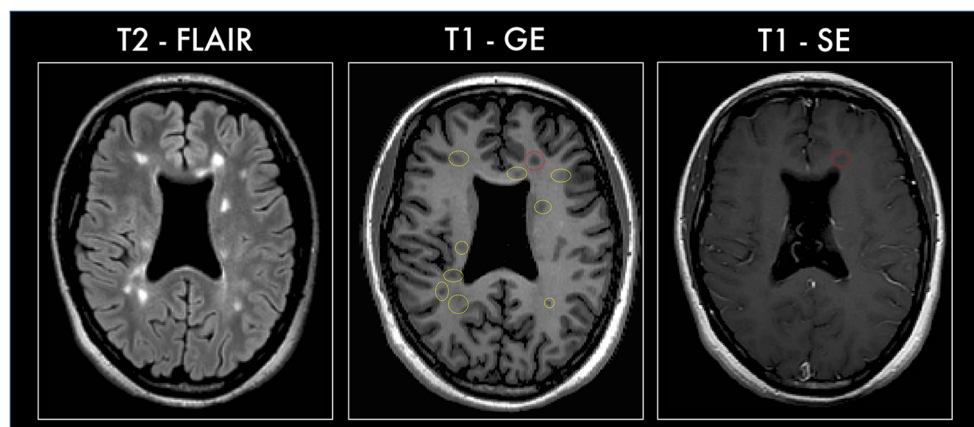
### MRI analysis

All the 3D images were resliced to 3-mm slice thickness to obtain a better comparison among T1-SE, 3D T2-FLAIR, and T1-GE images. Thus, lesions on each of the T2-FLAIR, T1-SE, and T1-GE images (Fig. 1) were marked by consensus by two experienced observers (one neurologist, one neuroradiologist) and thus verified by a third experienced observer and converted in lesion masks (semi-automatic segmentation, Jim version 7.0 Xinapse System; [www.xinapse.com](http://www.xinapse.com)). The differences in tissue contrast between T1-SE and T1-GE made impossible for the raters to be blinded to the type of T1-weighted MRI sequence used to segment lesions. Only T1-SE/T1-GE hypointense lesions which showed at least partial hyperintensity on T2-FLAIR images were considered, in order to avoid the

inclusion of enlarged perivascular spaces. On T1-SE sequences, only hypointense lesions that did not show gadolinium enhancement were segmented. After the detection of the “acute” hypointense lesions on contrast-enhanced T1-SE images, we identified the correspondent hypointensities on T1-GE images and removed them from the analysis. Lesion volumes and numbers for each type of sequence were then extracted. T1-GE, T1-SE, and T2-FLAIR lesion masks were thus linearly registered to T1-GE native space by using FLIRT [10], thus obtaining all lesion types in the same (T1-GE) brain volume. Then, we distinguished:

- Lesion masks of T2-FLAIR hyperintense lesions with no correspondent hypointense signal on T1-SE/T1-GE sequences (from now on called *T2-FLAIR hyper-T1-w isointense lesions*)
- Lesion masks of hypointensities detected only on T1-GE sequences (from now on called *only T1-GE hypointense lesions*)
- Lesion masks of hypointensities detected both on T1-SE sequences and on T1-GE sequences (from now on called *T1-SE-GE hypointense lesions*)

Brain parenchymal fraction (BPF) was obtained through white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF) volume segmentation from T1-GE images by using Statistical Parametric Mapping (SPM12; <http://www.fil.ion.ucl.ac.uk/spm>; MATLAB toolbox). Maps of fractional anisotropy (FA) and mean diffusivity (MD) were obtained for all subjects by fitting of the diffusion tensor images after eddy current correction, by using DTIFit (FMRIB Diffusion Toolbox, part of the FMRIB Software Library, FSL; <http://www.fmrib.ox.ac.uk/fsl>).



**Fig. 1** The different performances of T1-SE and T1-GE on the detection of hypointense lesions in a patient with multiple sclerosis. Example of axial MRI images showing the different sensitivity of T2-FLAIR, T1-GE, and T1-SE in the detection of white matter lesions in a patient with

multiple sclerosis. In particular, note the higher sensitivity of T1-GE sequence in detecting hypointense lesions: yellow dotted circles show the *only T1-GE hypointense lesions*, while red dotted circle shows the *T1-SE-GE hypointense lesion*

Normal-appearing white matter (NAWM) was created by subtracting FLAIR hyperintense lesions from the WM mask obtained by SPM12. To ensure the reliability of NAWM and avoid the inclusion of deep gray matter structures, a visual quality check of the images was performed for each patient.

Finally, FA and MD values inside normal-appearing white matter (NAWM) and *T2-FLAIR hyper-T1-w isointense, only T1-GE hypointense*, and *T1-SE-GE hypointense lesions* were extracted, after performing linear registration with boundary-based constraints (FLIRT) between the abovementioned lesion masks and b0 images.

## Statistical analysis

Data were reported as mean  $\pm$  standard deviation (SD) and median with interquartile range (IQR) where appropriate. Non-parametric Wilcoxon's test for paired data was used to compare lesion number and volume between T1-SE and T1-GE sequences. Repeated measures ANOVA was instead used to compare FA and MD inside lesion groups. *p* values on single pairwise comparisons were adjusted for multiple comparisons using the false discovery rate approach. Stata (v.14; StataCorp) was used for the computation.

## Results

Out of 60 CIS and MS patients, 50 showed chronic hypointense lesions both on T1-GE and on T1-SE images.

None of the patients presented hypointensities detectable only on T1-SE sequences ("only T1-SE hypointense lesions").

T1-SE mean lesion number and volume were 15.9 ( $\pm$  16.9) and 3.2 ( $\pm$  5.2)  $\times 10^3$  mm<sup>3</sup>, respectively; T1-GE mean lesion number and volume were 37.6 ( $\pm$  30.6) and 6.8 ( $\pm$  8.7)  $\times 10^3$  mm<sup>3</sup>, respectively; T2-FLAIR hyperintense mean lesion number and volume were 48.2 ( $\pm$  35.4) and 12.5 ( $\pm$  13.5)  $\times 10^3$  mm<sup>3</sup>, respectively.

In Table 1, the same quantities were reported separately for 1.5 T and 3 T and according to the disease phenotypes.

The differences between the T1-GE hypointense lesions' and T1-SE hypointense lesions' mean lesion number and volume were statistically significant (*p* < 0.001) considering the entire cohort. We found similar results also in patients' subgroup analysis based on field strength and disease phenotype (*p* < 0.001).

Mean FA and MD values inside NAWM and *T2-FLAIR hyper-T1-w isointense, only T1-GE hypointense*, and *T1-SE-GE hypointense lesions* are reported in Table 2. Mean FA and MD values inside the *T1-SE-GE hypointense lesions* were 0.26 ( $\pm$  0.05) and 0.0001 ( $\pm$  0.0002) mm<sup>2</sup>/s, respectively. Both FA and MD metrics resulted significantly different among NAWM

**Table 1** Lesion number and volume in all patients stratified for field strength and MS phenotype. The differences between T1-GE and T1-SE hypointense lesions' mean lesion number and volume are statistically

significant (*p* < 0.001) considering the entire cohort. The results survived also in patients' subgroups based on field strength and disease phenotype (*p* < 0.001)

	All ( <i>n</i> = 60)	1.5 T ( <i>n</i> = 32)	3 T ( <i>n</i> = 28)
Lesion number			
T2-FLAIR hyperintense	48.3 (35.4); 41.5 (17–77)	29.3 (26); 23 (9–39)	69.9 (32.5); 67 (47–88)
T1-GE hypointense	37.6 (30.6); 30.5 (15–52)	23.2 (22.3); 17 (6–30)	54.1 (30.8); 47 (32–69)
T1-SE-GE hypointense	15.9 (16.9); 11 (1–25.5)	5.6 (7.5); 2 (0–9.5)	27.6 (17); 26.5 (17–38)
Lesion volume <sup>1</sup>			
T2-FLAIR hyperintense	12.5 (13.5); 10.4 (1.5–17.6)	10.5 (16.7); 1.8 (0.6–12.7)	14.9 (8.1); 13.2 (10.4–18.4)
T1-GE hypointense	6.8 (8.7); 3.6 (0.8–9.2)	6.8 (11.1); 1.3 (0.4–7.9)	6.9 (4.7); 5 (3.4–10.3)
T1-SE-GE hypointense	3.2 (5.2); 1.1 (0.1–3.8)	2.6 (5.9); 0.2 (0–1.2)	4 (4.2); 2.1 (1.2–4.3)
	CIS ( <i>n</i> = 23)	RR ( <i>n</i> = 21)	Progressive ( <i>n</i> = 16)
Lesion number			
T2-FLAIR hyperintense	16.5 (11); 15 (8–25)	66.1 (29); 67 (46–80)	70.4 (33.8); 68 (47–89.5)
T1-GE hypointense	12.7 (9.9); 10 (5–17)	51 (26); 47 (31–66)	55.9 (33.3); 49 (32–75)
T1-SE-GE hypointense	1.9 (2.9); 1 (0–2)	22.1 (15.5); 19 (11–30)	27.7 (17); 27 (16–36)
Lesion volume <sup>1</sup>			
T2-FLAIR hyperintense	1.6 (1.9); 0.8 (0.3–2.7)	19.9 (12.1); 15.3 (12.1–23.3)	18.5 (14.8); 13.9 (9.7–22.2)
T1-GE hypointense	1 (1.1); 0.6 (0.2–1.7)	9.9 (8.2); 7.9 (3.6–11.5)	11.2 (11); 7.8 (3.9–14.8)
T1-SE-GE hypointense	0.2 (0.3); 0.07 (0–0.3)	4.1 (4.7); 2.3 (1.1–4.3)	6.4 (7.2); 4.1 (1.6–8.9)

Results are reported as mean (SD); median (IQR)

CIS, clinically isolated syndrome; RR, relapsing–remitting

<sup>1</sup> Lesion volumes are reported in "value"  $\times 10^3$  mm<sup>3</sup>



**Table 2** DTI metrics according to lesion groups and 1.5 T and 3 T. Both FA and MD metrics resulted significantly different among NAWM and the three lesion groups. FA and MD values extracted from *T1-SE-GE hypointense lesions* and *only T1-GE hypointense lesions* showed statistically significant differences, while for *T2-FLAIR hyper-T1-w isointense lesions* and the *only T1-GE hypointense lesions*, the differences were not significant. Similar results were obtained stratifying for 1.5 T and 3 T

	All (n = 60)		1.5 T		3 T	
	FA	MD	FA	MD	FA	MD
1. NAWM	0.34 (0.05)	0.00082 (0.000006)	0.38 (0.03)	0.00079 (0.000004)	0.30 (0.03)	0.00085 (0.000006)
2. T2-FLAIR hyper-T1-w isointense lesions	0.320 (0.051)	0.0009 (0.0001)	0.316 (0.061)	0.0009 (0.0001)	0.324 (0.038)	0.0009 (0.0001)
3. Only T1-GE hypointense lesions	0.313 (0.045)	0.0009 (0.0001)	0.311 (0.043)	0.0009 (0.0001)	0.316 (0.047)	0.001 (0.0001)
4. T1-SE-GE hypointense lesions	0.26 (0.05)	0.0011 (0.00002)	0.25 (0.06)	0.0011 (0.00002)	0.27 (0.05)	0.0011 (0.00002)
<i>p</i> value	Overall: <i>p</i> < 0.001	Overall: <i>p</i> < 0.001	Overall: <i>p</i> < 0.001	Overall: <i>p</i> < 0.001	Overall: <i>p</i> < 0.001	Overall: <i>p</i> < 0.001
	2 versus 3: <i>p</i> = 0.31	2 versus 3: <i>p</i> = 0.62	2 versus 3: <i>p</i> = 0.64	2 versus 3: <i>p</i> = 0.64	2 versus 3: <i>p</i> = 0.20	1 versus 2: <i>p</i> = 0.22
	2 versus 4: <i>p</i> < 0.001	2 versus 4: <i>p</i> < 0.001	2 versus 4: <i>p</i> < 0.001	2 versus 4: <i>p</i> < 0.001	2 versus 4: <i>p</i> < 0.001	1 versus 3: <i>p</i> < 0.001
	2 versus 1: <i>p</i> = 0.005	2 versus 1: <i>p</i> < 0.001	2 versus 1: <i>p</i> < 0.001	2 versus 1: <i>p</i> < 0.001	2 versus 1: <i>p</i> < 0.001	1 versus 4: <i>p</i> < 0.001
	3 versus 4: <i>p</i> < 0.001	3 versus 4: <i>p</i> < 0.001	3 versus 4: <i>p</i> = 0.002	3 versus 4: <i>p</i> < 0.001	3 versus 4: <i>p</i> < 0.001	2 versus 3: <i>p</i> < 0.001
	3 versus 1: <i>p</i> < 0.001	3 versus 1: <i>p</i> < 0.001	3 versus 1: <i>p</i> < 0.001	3 versus 1: <i>p</i> < 0.001	3 versus 1: <i>p</i> = 0.06	2 versus 4: <i>p</i> < 0.001
	4 versus 1: <i>p</i> < 0.001	4 versus 1: <i>p</i> < 0.001	4 versus 1: <i>p</i> < 0.001	4 versus 1: <i>p</i> < 0.001	4 versus 1: <i>p</i> = 0.032	3 versus 4: <i>p</i> < 0.001

Results are reported as mean (SD). *p* values for paired comparisons were adjusted for multiple comparisons. MD values are reported in mm<sup>2</sup>/s. *p* values < 0.05 are considered significant as indicated by italics. NAWM, normal-appearing white matter; FA, fractional anisotropy; MD, mean diffusivity

and the three lesion groups (*p* < 0.001). FA and MD values extracted from *T1-SE-GE hypointense lesions* and *only T1-GE hypointense lesions* showed statistically significant differences (*p* < 0.001), while for *T2-FLAIR hyper-T1-w isointense lesions* and the *only T1-GE hypointense lesions*, the differences were not significant (*p* = 0.31 for FA and *p* = 0.62 for MD). Similar results were obtained stratifying for 1.5 T and 3 T.

Mean FA and MD values inside NAWM and T2-FLAIR hyper-T1-w isointense, only T1-GE hypointense, and T1-SE-GE hypointense lesions among CIS, RR, and PMS patients' groups are reported in Table 3. Both FA and MD values extracted from NAWM of CIS patients resulted significantly different with respect to those extracted from MS patients (*p* < 0.001). Inside T2-FLAIR hyper-T1-w isointense lesions, only MD was significantly different between CIS and MS patients (*p* = 0.026 and *p* = 0.014, respectively). Conversely, FA and MD values inside only T1-GE and T1-SE-GE hypointense lesions did not result significantly different among CIS, RR, and PMS patients. Both *only T1-GE* and *T1-SE-GE lesion* number and volume correlated with EDSS (Pearson for T1-GE: *r* = 0.55, *p* < 0.001; and *r* = 0.53, *p* < 0.001, respectively; for T1-SE: *r* = 0.56, *p* < 0.001; and *r* = 0.59, *p* < 0.001, respectively). A similar correlation was found also with BPF (mean 0.83, SD 0.14): for T1-GE: *r* = -0.72, *p* < 0.001; and *r* = -0.67, *p* < 0.001, respectively; for T1-SE: *r* = -0.67, *p* < 0.001; and *r* = -0.57, *p* < 0.001, respectively (Table 4). When both *only T1-GE* and *T1-SE-GE lesion* number and volume were considered in a linear multivariable model, exclusively *T1-SE-GE lesion* volume remained significantly associated with EDSS (*p* < 0.001) and *T1-SE-GE lesion* number with BPF (*p* < 0.001).

## Discussion

In this study, we demonstrated that hypointense lesions detected on T1-SE hypointense lesions had a more pronounced degree of microstructural damage with respect to hypointense lesions detected only on T1-GE, to T2-FLAIR hyper-T1-w isointense lesions, and to the NAWM, regardless of MRI field strength and MS phenotype.

MRI and histopathological correlative studies showed that MS lesions that appear hypointense on T1-w images—the so called black holes—represent the more severely damaged areas of the whole MS-related lesion load in the brain, characterized by irreversible axonal loss, demyelination, and matrix disruption [11]. Chronic hypointense lesions are reported to correlate better with clinical disability and brain atrophy compared with T2-w lesion load [12] and are therefore suggested as additional outcome measures in clinical studies. Furthermore, the detection of chronic hypointense lesions has a critical role in pediatric patients who experience a first acute demyelinating attack, where the presence of one or more

**Table 3** DTI metrics according to lesion groups and phenotype. Both FA and MD values extracted from NAWM of CIS patients resulted significantly different with respect to those extracted from MS patients. Inside T2-FLAIR hyper-T1-w isointense lesions, only MD was significantly different between CIS and MS patients. Conversely, FA and MD values inside only T1-GE and T1-SE-GE hypointense lesions did not result significantly different among CIS, RR, and PMS patients

	CIS ( <i>n</i> = 23)		RR ( <i>n</i> = 21)		Progressive ( <i>n</i> = 16)		<i>p</i> values	
	FA	MD	FA	MD	FA	MD	FA	MD
1. NAWM	0.39 (0.02)	0.00076 (0.00003)	0.33 (0.05)	0.00085 (0.00004)	0.30 (0.03)	0.00086 (0.00007)	RR versus CIS: <i>p</i> < 0.001 Progressive versus CIS: <i>p</i> < 0.001 Progressive versus RR: <i>p</i> = 0.052	RR versus CIS: <i>p</i> < 0.001 Progressive versus CIS: <i>p</i> < 0.001 Progressive versus RR: <i>p</i> = 0.39
2. T2-FLAIR hyper-T1-w isointense lesions	0.319 (0.067)	0.0009 (0.0001)	0.329 (0.042)	0.0010 (0.00007)	0.309 (0.034)	0.0010 (0.0001)	RR versus CIS: <i>p</i> = 0.63 Progressive versus CIS: <i>p</i> = 0.63 Progressive versus RR: <i>p</i> = 0.42	RR versus CIS: <i>p</i> = 0.026 Progressive versus CIS: <i>p</i> = 0.014 Progressive versus RR: <i>p</i> = 0.18
3. Only T1-GE hypointense lesions	0.312 (0.048)	0.001 (0.0002)	0.327 (0.042)	0.0009 (0.0001)	0.298 (0.041)	0.001 (0.00007)	RR versus CIS: <i>p</i> = 0.36 Progressive versus CIS: <i>p</i> = 0.36 Progressive versus RR: <i>p</i> = 0.12	RR versus CIS: <i>p</i> = 0.39 Progressive versus CIS: <i>p</i> = 0.47 Progressive versus RR: <i>p</i> = 0.59
4. T1-SE-GE hypointense lesions	0.25 (0.07)	0.0011 (0.0002)	0.26 (0.05)	0.0011 (0.0001)	0.28 (0.04)	0.0011 (0.0002)	RR versus CIS: <i>p</i> = 0.55 Progressive versus CIS: <i>p</i> = 0.28 Progressive versus RR: <i>p</i> = 0.46	RR versus CIS: <i>p</i> = 0.66 Progressive versus CIS: <i>p</i> = 0.60 Progressive versus RR: <i>p</i> = 0.77

Results are reported as mean (SD). *p* values for paired comparisons were adjusted for multiple comparisons. MD values are reported in mm<sup>2</sup>/s. *p* values < 0.05 are considered significant as indicated by italics CIS, Clinically isolated syndrome; RR, relapsing–remitting; NAWM, normal-appearing white matter; FA, fractional anisotropy; MD, mean diffusivity

**Table 4** Only T1-GE and T1-SE-GE hypointense lesion load and volume correlations with EDSS and BPF. Both only T1-GE and T1-SE-GE lesion number and volume correlated with EDSS and BPF

		EDSS		BPF	
Only T1-GE hypointense	Lesion number	$r = 0.55$	$p < 0.001$	$r = -0.72$	$p < 0.001$
	Lesion volume	$r = 0.53$	$p < 0.001$	$r = -0.67$	$p < 0.001$
T1-SE-GE hypointense	Lesion number	$r = 0.56$	$p < 0.001$	$r = -0.67$	$p < 0.001$
	Lesion volume	$r = 0.59$	$p < 0.001$	$r = -0.57$	$p < 0.001$

EDSS, Expanded Disability Status Scale; BPF, brain parenchymal fraction

T1-w hypointense lesions is highly associated with subsequent confirmation of MS [13].

In recent years, besides the traditional T1-SE sequences, T1-GE images are increasingly utilized in clinical and research settings; particularly at 3 T or higher fields, T1-GE images have demonstrated to be able to detect a greater number and volume of MS chronic hypointense lesions with respect to T1-SE images [7]. This may be related to the higher signal-to-noise ratio (SNR) of 3D T1-GE than SE sequences making hypointense lesions more conspicuous [14, 15].

Nevertheless, microstructural damage of hypointense lesions detected by only T1-GE sequences has not been deeply assessed. It has been hypothesized that they might represent a wide spectrum of less severe structural changes, such as inflammation, edema, gliosis, and partial demyelination [7]. Furthermore, also remyelination processes may partially reverse plaque hypointensity [16, 17].

An attempt to improve clinical–radiological correlations between black hole lesion volume and patients' clinical disability was made by Thaler et al [18], by stratifying hypointense lesions detected on MP2RAGE images according to T1 relaxation time (T1-RT) thresholds. In this paper, the subgroup of black holes exhibiting the highest T1-RT showed the best correlation with patients' disability status; thus, the authors suggested that T1-RT was able to reveal the different degrees of tissue damage inside hypointense lesions.

Thus, the main purpose of our study was the quantification of the degree of microstructural changes within the lesions detected on the different sequences. To this aim, we extracted DTI metrics inside the following 3 types of MS lesions: T2-FLAIR hyperintense-T1-w isointense, only T1-GE, and T1-SE-GE hypointense lesions. We found that FA (and MD) were respectively lower (and higher) inside T1-SE-GE hypointense lesions with respect to only T1-GE hypointense lesions, T2-FLAIR hyper-T1-w isointense lesions, and NAWM. Conversely, no significant differences between FA and MD values inside the T2-FLAIR hyper-T1-w isointense and the only T1-GE hypointense lesions were observed. The same findings were obtained when subgroups of patients studied at 1.5 T and 3 T were separately analyzed. According to these results, T1-SE-GE hypointense lesions have a more pronounced degree of microstructural damage, which is statistically significant with respect to T2-FLAIR hyper-T1-w isointense and only T1-GE hypointense lesions. In particular, a continuum of

microstructural changes among NAWM and T2-FLAIR hyper-T1-w isointense, only T1-GE, and T1-SE-GE hypointense lesions is suggested by our findings. Interestingly, both FA and MD values extracted from NAWM of CIS patients resulted significantly different with respect to those extracted from MS patients, while FA and MD values inside only T1-GE and T1-SE-GE hypointense lesions were not significantly different among CIS, RR, and PMS patients. These findings are in line with those of previous reports in the literature [19], suggesting that the degree of microstructural damage within NAWM significantly increases throughout the course of the disease from CIS to MS. Conversely, we may suppose that within MS plaques, once T1-w hypointense lesions have developed and become detectable on T1-SE and T1-GE images, MS phenotype has no direct impact on the degree of microstructural damage.

In this scenario, more complex DTI analysis, such as Neurite Orientation and Dispersion Density Imaging (NODDI) [20] or, in addition, myelin-weighted sequences, might allow to better identify the different components of tissue damage inside MS lesions.

Due to the lack of histological analysis on brain tissue and MRI–pathology correlations, we are not able to confirm which degree of tissue damage is indeed present within T1 hypointense lesions detected on the different pulse sequences. Nevertheless, our findings suggest that the definition of T1 “black hole” (i.e., lesions associated with severe tissue damage) which is valid for T1 hypointense lesions detected on SE sequences might not be applicable to all the lesions characterized by hypointensity on T1-GE sequences.

However, this surely represents an interesting issue, particularly considering that T1-GE seems to have an even more sensitivity at ultra-high MRI field, where all T2-w lesions have demonstrated to be also detectable on magnetization-prepared rapid gradient-echo (MPRAGE) sequences [21].

In our study, we also confirmed that T1-GE images show a higher sensitivity with respect to T1-SE images [7]; in particular, compared with T1-SE images, T1-GE images were able to reveal a significantly higher number and volume of MS chronic hypointense lesions, also when patients' subgroups based on disease phenotypes were separately analyzed.

Interestingly, when both only T1-GE and T1-SE-GE lesion number and volume were considered in a linear multivariable model, exclusively T1-SE-GE lesion volume remained

significantly associated with EDSS and T1-SE lesion number with BPF. This may suggest the presence of a stronger correlation between *T1-SE-GE hypointense lesions*, clinical disability, and brain atrophy with respect to *only T1-GE hypointense lesions*.

A combined approach, i.e., the analysis of diffusion metrics inside T1-w hypointense lesions stratified according to a T1-threshold [18], may be an interesting approach for future investigations.

The main limitations of our study are represented by the lack of a longitudinal assessment and by the acquisition of only T1-SE images after contrast administration. In fact, we excluded from our analysis focal lesions characterized by contrast enhancement, typically hypointense in T1-weighted images as result of the presence of edema (thus sometimes reported in the literature as “wet black holes” [22]). Nevertheless, by basing the detection of lesion enhancement only on post-contrast T1-SE images, we might have undervalued the number of enhancing lesion given the higher sensitivity to contrast of 3D GRE T1-weighted images [23].

Finally, due to the lack of a direct comparison in patients performing MRI both at 1.5 T and at 3 T, we could not test the influence of the magnetic field in the detection of hypointense lesions on T1-GE and T1-SE images. Further studies are necessary to assess this aspect.

## Conclusions

In this study, we demonstrated that *T1-SE-GE hypointense lesions* show a more severe degree of microstructural damage, with respect to NAWM and *T2-FLAIR hyper-T1-w isointense and only T1-GE hypointense lesions*. Not taking into account the differences in pulse sequences used to classify lesions as T1 hypointense “black holes,” thus considering all T1 hypointensities as lesions with more severe microstructural damage, may lead to an overestimation of the effective irreversible pathological burden. Therefore, the type of T1-weighted sequence must be carefully evaluated in this setting, as already suggested [8]. These issues point to the need of a standardization and definition of MRI sequences used to detect MS hypointense lesions.

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## Methodology

- Retrospective
- Observational
- Performed at one institution

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