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Early perfusion changes in multiple sclerosis patients as assessed by MRI using arterial spin labeling

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Abstract

Background: Gadolinium-perfusion magnetic resonance (MR) identifies gray matter abnormalities in early multiple sclerosis (MS), even in the absence of structural differences. These perfusion changes could be related to the cognitive disability of these patients, especially in the working memory. Arterial spin labeling (ASL) is a relatively recent perfusion technique that does not require intravenous contrast, making the technique especially attractive for clinical research.
Purpose: To verify the perfusion alterations in early MS, even in the absence of cerebral volume changes. To introduce the ASL sequence as a suitable non-invasive method in the monitoring of these patients.
Material and Methods: Nineteen healthy controls and 28 patients were included. The neuropsychological test EDSS and SDMT were evaluated. Cerebral blood flow and bolus arrival time were collected from the ASL study. Cerebral volume and cortical thickness were obtained from the volumetric T1 sequence. Spearman's correlation analyzed the correlation between EDSS and SDMT tests and perfusion data. Differences were considered significant at a level of $P < 0.05$.
Results: Reduction of the cerebral blood flow and an increase in the bolus arrival time were found in patients compared to controls. A negative correlation between EDSS and thalamus transit time, and between EDSS and cerebral blood flow in the frontal cortex, was found.
Conclusion: ASL perfusion might detect changes in MS patients even in absent structural volumetric changes. More longitudinal studies are needed, but perfusion parameters could be biomarkers for monitoring these patients.

Keywords

Perfusion magnetic resonance imaging, arterial spin labeling, multiple sclerosis perfusion, gray matter-multiple sclerosis, cognitive disability, bolus arrival time

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Introduction

There have been great advances in the diagnosis and treatment of multiple sclerosis (MS) in recent years. Magnetic resonance (MR) continues to be the cornerstone in the monitoring of the therapeutic response and clinical evolution of these patients. Nevertheless, the correlation between lesion volume in conventional MR sequences and clinical features, especially cognitive disability, is low (1–6). Cognitive decline affects 40%–65% of patients with MS and is one of the main causes of disability (7). Volumetric MR studies

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are the most commonly used in the assessment of brain atrophy. Many studies have shown an association between cognitive decline in patients with MS and brain atrophy, especially in the cortical gray matter, spinal gray matter, and thalamus (7–13). However, these volumetric changes occur late and only partially demonstrate the damage in gray and white cerebral matter, and their correlation with clinical evolution is not complete either (14,15). Other non-structural MR sequences such as spectroscopy, diffusion imaging, or functional MR have been used to detect metabolic, biochemical, or functional changes in these patients (16–19), but again they usually appear after the structural damage.

Recent studies have demonstrated the potential of perfusion MR imaging (MRI) in identifying cortical abnormalities, even in the absence of structural differences. Cerebral perfusion can assess the oxygenation and the metabolism of different parts of the brain and could, indirectly, analyze the neurodegeneration occurring in this disease (17–20). Dynamic susceptibility contrast MRI (DSC-MRI) evaluates the flow of a paramagnetic contrast agent along cerebral tissue and remains the most widely used method (21). But perfusion can also be assessed using the perfusion sequence called arterial spin labeling (ASL), without needing contrast agents (22–26), making this sequence suitable for research studies. Only a few reports have already shown a correlation between ASL perfusion changes in gray matter and cognitive and physical disability in MS (27–32).

In our study, an ASL sequence was used to quantify absolute perfusion changes, cerebral blood flow (CBF), and bolus arrival time (BAT) within areas implicated in the working memory and executive functions (frontal gray matter and deep basal ganglia) in patients with relapsing-remitting multiple sclerosis (RRMS) and secondary-progressive multiple sclerosis (SPMS). The data showed that patients with SPMS and RRMS had lower CBF and increased BAT compared with controls in superior frontal gray matter and thalamus, even in the absence of structural changes. We hypothesized that frontal lobe and thalamus perfusion changes could be a clinically relevant marker in cognitive dysfunction and disability.

Material and Methods

Patients

We designed a cross-sectional study where we included healthy controls and patients with RRMS—according to the McDonald 2010 criteria (33) and SPSM according to the Lublin 2013 criteria (34)—attending the demyelinating diseases unit at our center, who gave their consent to participate in the study. We excluded patients with primary-progressive multiple sclerosis, patients with a relapse, or corticosteroid treatment during the last month. The study included a total of 19 healthy controls and 28 patients (20 patients with RRMS and eight with SPMS), from whom we collected the following variables: gender; age; age at onset; age at diagnosis; and Expanded Disability Status Scale (EDSS). Cognitive function was evaluated with the Symbol Digit Modalities test (SDMT) (35). Written consent was obtained from all participants.

Image acquisition

MRI was performed on a 1.5-T MR450w MR scanner (General Electric Healthcare, Milwaukee, WI, USA) with an eight-channel phased-array head coil. The acquisitions included our routine structural protocol: isovolumetric sagittal T1 (3D-SPGR); TR = 8.5 ms; TE = 3.2 ms; TI = 700 ms; flip angle (FA) = 12; bandwidth = 31.25 kHz; acquisition matrix = 258 × 258; full brain coverage; reconstructed matrix = 1 × 1 × 1 mm; isovolumetric-FLAIR (CUBE); coronal T2-weighted and a post-gadolinium isovolumetric T1 sequence. Before the contrast gadolinium-enhanced images, we included a prototype sequence to measure perfusion called enhanced ASL. This three-dimensional (3D) stack of spirals sequence (32) has been designed to improve signal-to-noise ratio, reduce artefacts, and acquire multiple post-label delay times that allows us to obtain transit delay time maps, as well as transit time corrected perfusion maps. This sequence was used with the following parameters: TE = 11.5 ms; TR = 5981 ms; post-labeling delay = 1000 ms; perfusion labeling time = 3500 ms; number of delays = 7 (1000 ms, 1220 ms, 1480 ms, 1780 ms, 2150 ms, 2630 ms, and 3320 ms); bandwidth = 62.5 kHz; FA = 155, field of view = 22 cm; spiral acquisition = 640 points × 4 arms; reconstructed image = 128 × 128; and slice thickness = 4.5 mm.

Image processing

To evaluate disability and cognitive dysfunction in working memory and processing speed, our areas of interest were the superior, middle, and inferior gyrus, the caudate nucleus, and the thalamus, areas where a major degree of atrophy is seen in patients with great disability (36–39). From the T1-weighted (T1W) sequences, we computed cortical thickness (CTh) and cerebral volume (CV). Data obtained from the perfusion maps were the corrected CBF and BAT, also called arterial transit delay time, defined as the time from labeling of blood in feeding arteries to its first arrival in the capillary network of the voxel of interest.

To evaluate perfusion and BAT in our areas of interest, the structural T1W volumes were previously segmented using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/). The result is a label map of isotropic voxel size containing a plethora of brain regions, along with meaningful anatomical information—volume, area, and cortical thickness—for each of these regions. Among all the brain regions present in the output label map, 18 regions of interest (ROI) considered significant to our study were selected. In addition, white matter lesions were automatically segmented from each FLAIR volume using the Lesion Segmentation Toolbox (LST) from SPM12 (www.fil.ion.ucl. ac.uk/spm/software/spm12/). The result is a lesion probability map, in which the value of each voxel corresponds to its probability of being a lesion. Each voxel was finally labeled as lesion if its probability ≥ 0.65. The raw ASL volume and FLAIR volume of each patient were rigidly co-registered and resliced to the T1W volumes using SPM12, selecting normalized mutual information as the cost function and b-spline interpolation of order 4. The label map obtained from FreeSurfer was rigidly registered to the T1 volume to recover the original resolution. In this case, a nearest neighbor interpolation was chosen to preserve the label values in the voxels. The transformation resulting from the co-registration of the ASL volume to the T1 volume was applied to the perfusion and BAT maps. Similarly, the transformation obtained for the FLAIR volume was applied to the lesion segmentation map.

After this step, the BAT, CBF, and lesion segmentation maps are all co-registered to the anatomical segmentation map, which allows to evaluate these features locally per region. Quantification of perfusion (mL/100g/min) and bolus arrival time (ms) were assessed by calculating the mean and SD for each of the ROIs. Quantification of lesion load was assessed by estimating the lesion volume for each of the ROIs. All calculations required in the quantification step were conducted by a dedicated software, which was developed for this purpose.

Statistical analysis

Due to the exploratory nature of the study and the low sample size, statistical analyses were carried out using non-parametric techniques and without multivariate analysis. Quantitative variables were summarized as median and interquartile range (IQR) and categorical variables as number and percentage. Mann–Whitney U test was used for independent group comparison. Spearman's correlation was used to analyze the correlation between the results of the EDSS and SDMT tests and CBF, BAT, and CV. Differences were considered significant at a level of $P < 0.05$. We used the IBM SPSS statistical software program, version 21.0 (IBM, Armonk, NY, USA).

Results

A total of 19 controls and 28 patients were included in the study. The median age of patients with MS was 45 years (age range = 39–52 years) and the median age of controls was 44 years (age range = 28–55 years) ($P = 0.46$). Of the 28 patients and 19 controls, 64.3% and 63.2%, respectively, were women ($P = 0.94$). The median time since diagnosis of MS in patients with MS was eight years (range = 5–14 years). With regard to the results of tests, the median EDSS was 1 (range = 0.3–3.6) and the median SMDT was 51 (range = 42–62).

We found statistically significant differences in the CBF (Fig. 1) between patients with MS and controls in the superior frontal gyrus (median = 44.2 vs 63.36, $P < 0.001$), middle frontal gyrus (49.9 vs. 71.8, $P < 0.001$), inferior frontal gyrus (53 vs. 70.6, $P < 0.001$), caudate (31 vs. 40.5, $P < 0.001$), and thalamus (37.1 vs. 50, $P < 0.001$) (Fig. 1). There were no significant differences in brain volume values and BAT between patients with MS and controls in any of the areas studied (Table 1).

The differences between patients with RRMS and patients with SPMS are shown in Table 2. As expected, patients with RRMS were younger ($P = 0.002$), with shorter diseases ($P = 0.004$), lower disability in the EDSS ($P < 0.001$), and better cognitive functioning ($P < 0.001$). We found statistically significant differences in the CBF between RRMS and SPMS in the superior frontal gyrus (median = 49.4 vs, 37.8, $P = 0.033$), middle frontal gyrus (59.5 vs. 39.7, $P = 0.01$), inferior frontal gyrus (56.1 vs. 41, $P = 0.025$), and thalamus (39.2 vs. 31.5, $P = 0.028$). There were no significant differences in the BAT data, although the values were higher in patients with SPMS in all areas studied. Brain volume values were lower in all areas in patients with SPMS, but we found no statistically significant differences (Table 2).

We found a negative correlation between EDSS and CBF in the superior frontal gyrus (Spearman's rho = –0.52, $P = 0.005$), middle frontal gyrus (rho = –0.53, $P = 0.003$), inferior frontal gyrus (rho = –0.49, $P = 0.007$), thalamus (rho = –0.47, $P = 0.01$), and caudate (rho = –0.40, $P = 0.04$). There was no correlation between CBF and the SDMT. A negative correlation was found between BAT in the thalamus and EDDS (rho = –0.43, $P = 0.02$) but not with the SDMT, although there was a tendency towards a statistically significant correlation (rho = –0.35, $P = 0.07$). A moderate correlation between SDMT and CV in the superior frontal gyrus (rho = 0.4, $P = 0.048$) was observed (Table 3).

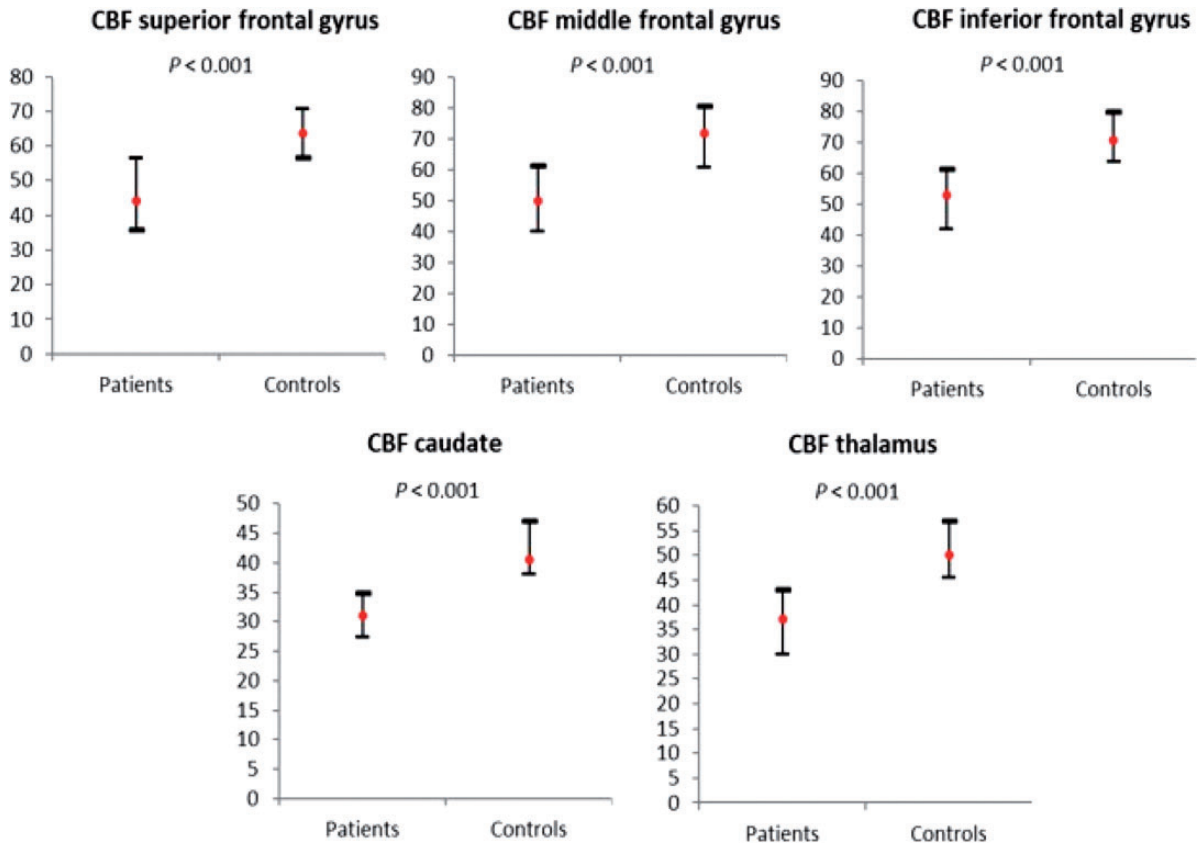

Fig. 1. CBF in mL/100g/min: differences between patients with MS and controls. Median (red point) and IQR. CBF, cerebral blood flow, MS, multiple sclerosis.

Table 1. BAT and CV: differences between patients with MS and controls.

	Patients with MS (n = 28)	Controls (n = 19)	*P*
BAT superior frontal gyrus	1290.8 (245.3–1403.2)	1321.9 (1283–1424.7)	0.43
BAT middle frontal gyrus	1401.6 (1301.6–1503.2)	1387.3 (1327.2–1448)	0.74
BAT inferior frontal gyrus	1210.6 (1166.8–1291.1)	1206.5 (1176.5–1304.4)	0.66
BAT caudate	1130.8 (1087.7–1201.3)	1155.3 (1113.9–1249.6)	0.15
BAT thalamus	1240.4 (1194.8–1311.9)	1264.5 (1203.9–1355.9)	0.26
CV superior frontal gyrus	22,993 (20,968.8–24,993)	21,983 (19,426–26,163)	0.91
CV middle frontal gyrus	22,378.5 (20,274.8–23,827)	23,014 (19,786–25,419)	0.39
CV inferior frontal gyrus	10,745 (10,072.2–11,732)	10,704 (9758–12,394)	0.7
CV caudate	3526.5 (3118.3–3922)	3307.4 (3060.7–3779.9)	0.32
CV thalamus	7601.5 (6813.5–8754.5)	8344.2 (7725.9–8854.1)	0.07

Values are given as median (IQR).
BAT, bolus arrival time (in ms); CV, cerebral volume (in mm^3); EDSS: Expanded Disability Status Scale; IQR, interquartile range; MS, multiple sclerosis; SDMT: Symbol Digit Modalities Test.

We also analyzed the correlation between the data of the superior frontal gyrus cortical thickness (CTh) and neuropsychological tests. There was no significant correlation either with EDSS test (rho = –0.37, $P = 0.054$) or with the SDMT (rho = 0.24, $P = 0.24$). In the same way, we found no statistically significant correlation between cortical thickness and CBF values in the superior frontal gyrus (rho = 0.21, $P = 0.28$). These results support the hypothesis that changes in perfusion occur before loss of cortical volume.

Table 2. Comparative analysis between clinical forms.

	Clinical forms		
	RRMS (n = 20)	SPMS (n = 8)	*P*
Age (years)	41 (37–47)	51 (48–57)	**0.002**
Time since diagnosis (years)	6 (5–10)	13 (11–18)	**0.004**
EDSS	1 (0–1)	4.5 (4–6.3)	**<0.001**
SDMT	58 (50–64)	36 (24–43)	**<0.001**
CBF superior frontal gyrus	49.4 (39.4–59.2)	37.8 (29.6–42.8)	**0.033**
CBF middle frontal gyrus	59.5 (42.3–67.1)	39.7 (29.8–47.7)	**0.01**
CBF inferior frontal gyrus	56.1 (47.3–64.4)	41 (33.6–53)	**0.025**
CBF caudate	31.8 (28–35.3)	28.1 (26.7–30.5)	0.182
CBF thalamus	39.2 (30.8–46.9)	31.5 (26–37.1)	**0.028**
BAT superior frontal gyrus	1288.2 (1246.7–1380.1)	1348.8 (1251.2–1464.5)	0.328
BAT middle frontal gyrus	1347.8 (1292.9–1469.3)	1458.7 (1382.1–1538.9)	0.182
BAT inferior frontal gyrus	1185.3 (1149.9–1258)	1228.1 (1209.6–1327.9)	0.165
BAT caudate	1115.1 (1080.5–1192)	1145.9 (1126.5–1228.4)	0.136
BAT thalamus	1210.4 (1164.7–1304.7)	1298.4 (1251.60–1327.6)	0.07
CV superior frontal gyrus	23,003 (2207–25,775)	22,105 (16,649–24,761)	0.231
CV middle frontal gyrus	22,531.5 (21,416.5–23,783.5)	20,404.5 (17,965.5–23,401.5)	0.15
CV inferior frontal gyrus	10,839 (10,277–11,894)	9902.5 (9198.5–11,123)	0.079
CV caudate	3597 (3225–3983.5)	3315 (3018–3735)	0.237
CV thalamus	7669.5 (7097.5–9087.5)	6313 (6125.5–8391)	0.079

Values are given as median (IQR).

Values in bold represent statistically significant differences.

BAT, bolus arrival time (in ms); CBF, cerebral blood flow (in mL/100g/min); CV, cerebral volume (in mm^3); EDSS, Expanded Disability Status Scale; IQR, interquartile range; RRMS, relapsing-remitting multiple sclerosis; SDMT, Symbol Digit Modalities Test; SPMS, secondary progressive multiple sclerosis.

Discussion

In recent years, several studies have demonstrated the potential for perfusion MRI in identifying cortical and deep gray matter abnormalities in patients with MS, even in the absence of structural differences (29–32). Almost all studies have used contrast media agents to perform the perfusion sequences. Patients with early RRMS with relatively preserved clinical and cognitive functions have shown multiple regions with reduced cerebral blood volume, but no loss of gray matter volume has been commonly found.

The goal of our study was to confirm these previously published results using a perfusion sequence that does not require any contrast media, the ASL sequence. It uses the water in arterial blood as an endogenous, freely diffusible contrast medium. The main physiological parameters measured with ASL are the CBF and the BAT (33). Its non-invasive and quantitative nature makes the technique especially attractive for longitudinal studies and clinical research. There is currently sufficient evidence to support its clinical application, with clear advantages in terms of improved and earlier diagnosis.

A statistically significant reduction in CBF has been found in the frontal cortices, the thalamus, and the caudate in patients with MS, with no evidence of loss of gray matter volume and no reduction in cortical thickness. The data included in this study reveal that these abnormalities are more evident in SPMS as compared with RRMS. Regarding disability, a negative correlation was discovered between EDSS and CBF in the frontal cortex and the basal ganglia, as well as between EDSS and the BAT in the thalamus.

The reasons for these changes in cerebral perfusion in MS are not fully understood. There are several hypotheses that may address these findings. First, hypoperfusion could be related to a neuroaxonal loss. However, most of the studies have not found a relationship between perfusion and brain atrophy (18,19,25,38), while other articles have only described a partial association with T2 lesion load (23,39). In this study, the reduction of perfusion was not associated with measures of brain atrophy either, so our results reinforce the idea that these changes are caused by other mechanisms. Other possible explanations include a reduction in energy demand, or metabolic consumption (39,40), primary ischemia (41), dysfunction of cerebrovascular reactivity (42–44), mitochondria dysfunction (45–47), or even a previous step in the neurodegeneration process before tissue loss is established. In this case, this parameter could allow for greater

Table 3. Correlation analysis between perfusion results and neurological test (SDMT and EDSS) in patients with MS.

		SDMT n = 27	EDSS n = 28
CBF superior frontal gyrus	rho	–0.07	**–0.52**
	P	0.75	**0.005**
CBF middle frontal gyrus	rho	–0.02	**–0.53**
	P	0.92	**0.003**
CBF inferior frontal gyrus	rho	–0.06	**–0.49**
	P	0.76	**0.007**
CBF caudate	rho	–0.15	**–0.40**
	P	0.45	**0.04**
CBF thalamus	rho	–0.08	**–0.47**
	P	0.68	**0.01**
BAT superior frontal gyrus	rho	–0.15	0.19
	P	0.45	0.33
BAT middle frontal gyrus	rho	–0.17	0.22
	P	0.40	0.26
BAT inferior frontal gyrus	rho	–0.16	0.31
	P	0.44	0.11
BAT caudate	rho	–0.27	0.26
	P	0.17	0.19
BAT thalamus	rho	–0.35	**0.43**
	P	0.07	**0.02**
CV superior frontal gyrus	rho	**0.4**	–0.25
	P	**0.048**	0.21
CV middle frontal gyrus	rho	0.16	–0.23
	P	0.42	0.27
CV inferior frontal gyrus	rho	0.37	**–0.39**
	P	0.06	**0.04**
CV caudate	rho	0.26	–0.25
	P	0.18	0.21
CV thalamus	rho	0.33	–0.32
	P	0.09	0.1

Values in bold represent statistically significant correlations.
BAT, bolus arrival time; CBF, cerebral blood flow; CV, cerebral volume; EDSS, Expanded Disability Status Scale; SDMT, Symbol Digit Modalities Test.

therapeutic opportunities than the detection of a more intense and widespread damage (48).

Moreover, we did not find a positive correlation between SDMT—an indirect measure of cognitive impairment—with the CBF or BAT, as previously reported (31). This fact could be due to methodological issues. On the one hand, we had a low percentage of cognitive dysfunction. Thus, only three patients with RRMS and seven with SPMS showed medium to low scores in SDMT. Even in this situation, we observed a tendency towards a statistically significant correlation between SDMT score, the CBF of the superior frontal gyrus, and the BAT in the thalamus, suggesting that they are interesting parameters for this component of the disease.

Nevertheless, there is growing evidence of the alterations in cerebral perfusion in patients with MS that would be independent from or before the structural volumetric changes. Our results, as well as the data from other studies, support the idea that this technique could be useful for monitoring the neurodegeneration occurring in MS, with a progressive reduction in the CBF and an increase in BAT, as the disease evolves. In this regard, the ASL sequence could be a suitable method, as it is a non-invasive, feasible, and reproducible technique (49,50).

It remains to be decided which measures or which brain areas would offer the most relevant information to be implemented as a routine evaluation. Methods and results are very variable in this regard (21,29,30). In our study, as pointed out previously, these regions could be the frontal cortex and the thalamus, where we obtained a negative correlation between EDSS and thalamus transit time, and between EDSS and CBF in different areas of the frontal cortex.

In order to better understand these perfusion alterations in patients with MS, and to clarify their possible uses in clinical practice, further studies with longitudinal designs and more participants are needed.

In conclusion, early perfusion changes occurring in patients with MS can be assessed with ASL sequence, even in those with absent or minimal structural changes. The perfusion data from the frontal cortex and the thalamus could offer relevant information to evaluate the cognitive disability in these patients.

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