Cognitive Impairment Is Related to Glymphatic System Dysfunction in Pediatric Multiple Sclerosis

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Objective: The aim of this study was to investigate whether, compared to pediatric healthy controls (HCs), the glymphatic system is impaired in pediatric multiple sclerosis (MS) patients according to their cognitive status, and to assess its association with clinical disability and MRI measures of brain structural damage.
Methods: Sixty-five pediatric MS patients (females = 62%; median age = 15.5 [interquartile range, IQR = 14.5;17.0] years) and 23 age- and sex-matched HCs (females = 44%; median age = 14.1 [IQR = 11.8;16.2] years) underwent neurological, neuropsychological and 3.0 Tesla MRI assessment, including conventional and diffusion tensor imaging (DTI). We calculated the diffusion along the perivascular space (DTI-ALPS) index, a proxy of glymphatic function. Cognitive impairment (Co-I) was defined as impairment in at least 2 cognitive domains.
Results: No significant differences in DTI-ALPS index were found between HCs and cognitively preserved (Co-P) pediatric MS patients (estimated mean difference [EMD] = −0.002 [95% confidence interval = −0.069; 0.065], FDR-p = 0.956). Compared to HCs and Co-P patients, Co-I pediatric MS patients (n = 20) showed significantly lower DTI-ALPS index (EMD = −0.136 [95% confidence interval = −0.214; −0.058], FDR-p ≤ 0.004). In HCs, no associations were observed between DTI-ALPS index and normalized brain, cortical and thalamic volumes, and normal-appearing white matter (NAWM) fractional anisotropy (FA) and mean diffusivity (MD) (FDR-p ≥ 0.348). In pediatric MS patients, higher brain WM lesion volume (LV), higher NAWM MD, lower normalized thalamic volume, and lower NAWM FA were associated with lower DTI-ALPS index (FDR-p ≤ 0.016). Random Forest selected lower DTI-ALPS index (relative importance [RI] = 100%), higher brain WM LV (RI = 59.5%) NAWM MD (RI = 57.1%) and intelligence quotient (RI = 51.3%) as informative predictors of cognitive impairment (out-of-bag area under the curve = 0.762).
Interpretation: Glymphatic system dysfunction occurs in pediatric MS, is associated with brain focal lesions, irreversible tissue loss accumulation and cognitive impairment.

ANN NEUROL 2024;00:1–13

Pediatric-onset multiple sclerosis (MS) accounts for about 2–10% of the total MS cases.[1] In comparison to adult-onset, pediatric-onset MS shows higher inflammatory disease activity early in the disease course, but it is typically characterized by better clinical recovery.[2] It has been suggested that the younger age of these patients may provide protection through more efficient brain repair abilities, compensatory mechanisms, and plasticity, thus limiting disability progression over time.[2,3] Conversely, a faster reduction in cognitive performance efficiency was

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.26911

Received Oct 30, 2023, and in revised form Mar 4, 2024. Accepted for publication Mar 4, 2024.

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observed in pediatric-onset compared to adult-onset MS.[3] This, possibly exacerbated by an impaired physiological brain maturation caused by MS, determines the development of cognitive impairment in approximately one-third of patients at a significant younger age.[3]

The study of pediatric patients, in whom the clinical onset is closer to the biological onset of the disease, may improve the understanding of how different pathological processes contribute to the heterogeneous clinical manifestations of MS, including cognitive impairment. Several MRI studies have consistently shown that substantial inflammation, demyelination, and neurodegenerative processes occur from the beginning of the disease.[2,4–9] However, the pathophysiological processes contributing to the accumulation of focal white matter (WM) lesions and irreversible tissue loss still need to be fully recognized.

Recent evidence showed the existence of a physiological central nervous system (CNS) "waste clearance" system, defined as glymphatic system.[10] The glymphatic system denotes a brain-wide perivascular fluid transport system, in which cerebrospinal fluid (CSF) penetrates brain parenchyma through perivascular space around arteries, and then clears interstitial fluid waste products from the brain.[11] The impairment of the glymphatic system has been described in adult subjects with aging[12,13] and in patients with different inflammatory, demyelinating and neurodegenerative diseases of the CNS,[14–17] including MS.[16,18] Interestingly, studies on experimental models of MS showed an association between alterations of parenchymal CSF circulation and occurrences of blood–brain disruption, endothelial activation and leukocyte infiltration.[19] This may be related either to the infiltration of immune cells in the perivascular space altering physiologic glymphatic functioning or vice versa (impairment of CSF circulation may promote immune infiltration).[19] With the aim of evaluating glymphatic function, the index of diffusion along the perivascular space (DTI-ALPS) has recently been applied to measure water diffusion along perivascular spaces identified by their anatomic relationship to surrounding WM tracts.[20] With this approach, a significantly lower DTI-ALPS index was detected in adult MS patients compared to matched healthy controls (HCs), being more severe in progressive than in relapsing–remitting (RR) MS patients.[18] Interestingly enough, a progressive reduction of DTI-ALPS index was found to occur during the first 4 years from disease onset, without further worsening thereafter,[18] suggesting that glymphatic function may be involved early in MS disease course. To address this point, the evaluation of glymphatic function in pediatric patients with MS represents a unique opportunity.

Against this background, by evaluating the DTI-ALPS index in a large cohort of pediatric MS patients we aimed to investigate the potential role of the glymphatic system in the pathophysiology of the disease. Specifically, given the recently described association between glymphatic dysfunction and cognitive decline in adult aging[3,14] and several neurodegenerative conditions,[21] we explored its relationship with cognitive impairment. In addition, we also correlated this measure to clinical disability (Expanded Disability Status Scale [EDSS] score) and MRI measures of brain structural damage (T2 hyperintense lesions, atrophy and microstructural abnormalities).

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Approval was received from the local ethical standards committee on human experimentation (Protocol ID: 25/2007), and written informed consent was obtained from all participants and their parents prior to study enrollment.

Participants

In this cross-sectional, retrospective, observational study, we included 65 consecutive pediatric patients with RRMS,[22] which are part of an ongoing prospective cohort of pediatric MS patients recruited at San Raffaele Hospital, Milan, Italy. Patients with acute disseminated encephalomyelitis or acute disseminated encephalomyelitis-like presentation were excluded according to published criteria.[23] Patients had to be relapse- and steroid-free for at least 1 month prior to clinical and MRI assessment. Whenever needed, appropriate testing was performed to exclude leukodystrophies and myelin oligodendrocyte glycoprotein antibody-associated disease. Exclusion criteria were concomitant therapy with antidepressants, psychoactive drugs, or a history of other primary neurological or psychiatric disorders in addition to MS. Twenty-three HCs with no previous history of neurological dysfunction and a normal neurological examination served as the control group.

Clinical Assessment

On the day of MRI acquisition, pediatric MS patients underwent a complete neurologic evaluation, with definition of the clinical phenotype and rating of the EDSS score and recording of ongoing disease-modifying treatments (DMTs).[24] The clinical assessment was performed by an experienced neurologist, unaware of the MRI results.

Neuropsychological Assessment

Within 48 hours from the MRI acquisition, experienced neuropsychologists administered a standardized Neuropsychological Battery for Children with MS[25] which includes Selective Reminding Test to assess verbal memory; 10/36

Spatial Recall Test to assess visuospatial memory; the Trail Making Test and the Symbol Digit Modalities Test to assess attention and information processing speed; the Oral Denomination test from the Aachener Aphasie Test and the Semantic and Phonemic verbal fluency test to assess expressive language; and the Token test and Phrase Comprehension test from the Battery for the Analysis of Aphasic Deficits to assess receptive language. For each cognitive test, z-scores were determined according to normative data.[25]

A z-score was then calculated for each cognitive domain by averaging the z-scores of the corresponding tests.[26] A result below the 5th percentile of the normative sample was defined as test failure.[27] Impairment in a given cognitive domain was defined as failure in at least one test assessing that domain. Cognitive impairment was defined as impairment in at least 2 cognitive domains.[27] Intelligence quotient (IQ) was measured using the Wechsler Intelligence Scale for children[28] (for patients between the ages of 6 and 15 years) and the Wechsler Adult Intelligence Scale[29] (for patients with age ≥ 16 years).

MRI acquisition

Using a 3.0 T Philips Intera MR scanner with 8-channel head coil (Philips Medical System), the following sequences of the brain were acquired from all subjects during a single session: (1) dual-echo turbo spin echo (repetition time [TR] = 2,599; echo time [TE] = 16–80 ms; flip angle = 90°; matrix = 256 × 256; field of view = 240 mm^2; echo train length [ETL] = 6; 44 contiguous axial slices, 3 mm thick); and (2) 3D T1-weighted fast field echo (TR = 25; TE = 4.6 ms; flip angle = 30°; matrix = 256 × 256; field of view [FOV] = 230 mm^2; 220 contiguous axial slices, 0.8 mm thick); (3) pulsed-gradient spin echo echo-planar imaging (repetition time/echo time = 8775/58 ms, matrix size = 112 × 88, field of view = 240 × 231 mm^2, 55 contiguous, 2.3 mm thick axial slices) with SENSE (acceleration factor = 2) and diffusion gradients applied in 35 non-collinear directions. Two optimized b factors were used for acquiring diffusion weighted images (b1 = 0, b2 = 900 s/mm^2).[30]

Conventional MRI Analysis

In pediatric MS patients, T2-hyperintense WM lesion volume (LV) was measured on the dual-echo sequence using a local thresholding segmentation technique (Jim 8, Xinapse Systems). Normalized brain (NBV) and normalized cortical (NCV) volumes were measured on the 3D T1-weighted sequence using the SIENAx2 software, after T1-hypointense lesion refilling.[31] Thalamic segmentation and volume were derived using the FIRST tool and their volume were normalized for head size using the SIENAx scaling factor.[32]

Pre-Processing of Diffusion-Weighted Imaging

Pre-processing of diffusion-weighted images included correction for off-resonance and eddy current-induced distortions and movements using the Eddy tool in the FSL library.[33] A b0 image with reversed polarity of gradients was not available; therefore we simulated an undistorted b0 image from the 3D T1-weighted sequence to be used for distortion correction.[34] The diffusion tensor was estimated by linear regression on diffusion-weighted imaging data at b = 900 s/mm^2.[30] Then, maps of fractional anisotropy (FA) and mean diffusivity (MD) were derived from the diffusion tensor.[35] Dual-echo and 3D T1-weighted sequences were separately co-registered to the distortion-free b = 0 image, and the calculated transformations were applied to the binary masks of T2-hyperintense WM lesions and of WM previously obtained from SIENAx2. To evaluate microstructural tissue damage, FA and MD values within the normal-appearing (NA) WM were derived after removing T2-hyperintense WM lesions from the WM mask.

DTI-ALPS Index Quantification

Using Tract-Based Spatial Statistics, FA images of subjects were registered onto the customized FA atlas previously obtained from a recent published study[14] together with region-of-interest (ROI) probability maps of automatic (a) ROIs from projecting fibers (ROIproj) and associative fibers (ROIassoc) needed for the calculation of the DTI-ALPS index (Fig 1).[20] Then, aROIproj and aROIassoc were transformed back to single-subject space by applying the inverse FA to standard space transformation. Before calculation of the DTI-ALPS index, we visually checked that ROIs did not overlap with WM lesions. In case of overlap (in 3 out of 65 MS patients), we placed a 3 × 3 × 3 mm cubic ROI at least 1 pixel apart from T2 lesion edge. Diffusivity values along the x- and y-axes (ie, frame of reference built with diffusion tensor eigenvectors and the associated eigenvalues, Fig 1) were then extracted from each aROI to calculate the DTI-ALPS index with the following formula:

$$\text{DTI}-\text{ALPS index} = \frac{\text{mean}(\text{Dx proj}, \text{Dx assoc})}{\text{mean}(\text{Dy proj}, \text{Dz assoc})}$$

Statistical Analysis

Demographic and clinical features were compared between HCs and pediatric patients with MS according to their cognitive status using Fisher's exact and Mann–Whitney *U* tests. Age- and sex-adjusted linear models were performed to compare brain lesional and volumetric measures, NAWM FA and MD values and DTI-ALPS index. We retested the Co-I versus Co-P contrast, including age at onset, as additional covariate in a sensitivity analysis.

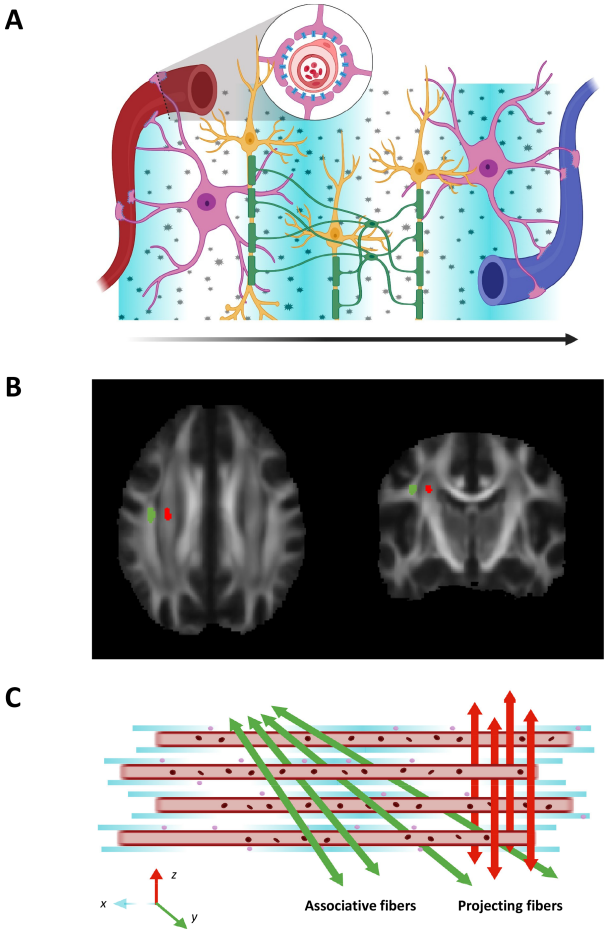

FIGURE 1: Glymphatic schematic picture and MRI processing method for automatic DTI-ALPS index calculation. (A) Overview of glymphatic function. In brief, cerebrospinal fluid originates from arteries and traverses the perivascular space to enter the brain interstitium, subsequently moving toward the perivenous space. During this process, the fluid gathers toxic molecules and cells, effectively cleaning the brain parenchyma. Upon reaching the perivenous space, the fluid progresses toward the brain convexity, where it is gathered in the lymphatic meningeal vessels. Images were created using biorender.com. (B) ROI probability maps from a previously obtained atlas superimposed on the mean fractional anisotropy map. The automatic (a)ROI from projecting fibers (ROIproj) and associative fibers (ROIassoc) were moved back to the single-subject diffusion imaging space. (C) Diffusivity values along the x- and y-axes (ie, frame of reference built with diffusion tensor eigenvectors and the associated eigenvalues) were then extracted from each aROI. DTI-ALPS index was calculated as the ratio of diffusivities perpendicular to fibre bundles and parallel to veins (Dx proj and Dx assoc) over diffusivities perpendicular to fibre bundles and perpendicular to veins (Dy proj and Dz assoc). See text for further details. Assoc = associative; DTI-ALPS = diffusion along perivascular spaces; proj = projecting; ROI = region of interest.

We ran multiple linear regression models to investigate the association of DTI-ALPS index with each studied MRI metrics in both HCs and patients with pediatric MS. Additionally, in patients, the association with IQ, disease duration, EDSS score, and T2-hyperintense WM LV was assessed. Age and sex were included as confounding factors in each model.

In each analysis, false discovery rate (FDR) (Benjamini–Hochberg procedure) correction was carried out to consider the overall number of pairwise comparisons or associations tested.

Random Forest probability models were grown to rank demographic (age, sex, and IQ), clinical (age at onset, disease duration, EDSS score, and DMT) and MRI variables (brain lesional and volumetric measures, NAWM FA and MD values, and DTI-ALPS index) according to their importance in explaining the presence of cognitive impairment in pediatric MS patients. Specifically, we adopted the Boruta algorithm (with 10,000 trees and 2,000 iterations) in order to select a subset of relevant features.[36] The algorithm selects features that outperform (on the basis of a binomial test with multiple comparison adjustment) pure randomness, by iteratively comparing variable importance metrics with those of shadow attributes, created by shuffling the original ones. Features with significantly worse importance than shadow attributes are progressively discarded. The discriminative ability of each selected predictor considered individually and of a model, trained by using the selected predictors, was expressed by the out-of-bag area under the curve (OOB-AUC), computed on the left-out observations.

SAS release 9.4 (SAS Institute, Cary, NC) and Software R (version 4.2.2) were used for computations. *p*-values <0.05 were deemed statistically significant.

Results

Demographic, Clinical and Conventional MRI Findings

Table 1 summarizes the main demographic, clinical and MRI features in HCs and pediatric MS patients according to their cognitive status.

Twenty (30.8%) pediatric MS patients were classified as cognitively impaired (Co-I). The most frequently affected cognitive domains included receptive language (70% of Co-I pediatric MS patients), visuospatial memory (60% of Co-I pediatric MS patients) and verbal memory (55% of Co-I pediatric MS patients) (Table 2). Compared to Co-I pediatric MS patients (median IQ [interquartile range, IQR] = 94.5 [88.0;98.0]), cognitively preserved (Co-P) patients (median IQ [IQR] = 102.0 [94.0;117.0]), had higher IQ (FDR-p = 0.036).

Compared to pediatric HCs, Co-P pediatric MS patients had significantly higher brain T2-hyperintense WM LV (FDR-p < 0.001). No significant differences in terms of age, sex, NBV, and normalized thalamic volume were observed (FDR-p ranging from 0.115 to 0.268).

TABLE 1. Main Demographic, Clinical, and MRI Characteristics of Healthy Controls and Pediatric Patients with Multiple Sclerosis

Variable		HC (n = 23)	Co-P pedMS (n = 45)	Co-I pedMS (n = 20)	Co-P pedMS versus HC		Co-I pedMS versus HC		Co-I versus Co-P pedMS	
					EMD (95% CI)	p (FDR-p)	EMD (95% CI)	p (FDR-p)	EMD (95% CI)	p (FDR-p)
Sex	Male (%)	13 (56)	18 (40)	7 (35)	-	0.211	-	0.223	-	0.787
	Female (%)	10 (44)	27 (60)	13 (65)		(0.268)		(0.268)		(0.814)
Median age (IQR), (range) (yr)		14.1	15.5	15.2	-	0.077	-	0.269	-	0.772
		(11.8;16.2)	(14.5;17.0)	(14.2;17.0)		(0.136)		(0.310)		(0.814)
		(8.5–17.9)	(12.4–17.9)	(7.6–17.9)						
Median IQ (IQR)		-	102.0 (94.0;117.0)	94.5 (88.0;98.0)	-	-	-	-	-	0.018 (0.036)
Median age at onset (IQR)		-	13.1 (10.1;14.7)	13.9 (12.3;15.1)	-	-	-	-	-	0.158 (0.215)
Median DD (IQR) (years)		-	1.0 (0.4;2.5)	2.0 (1.0;4.3)	-	-	-	-	-	0.088 (0.146)
Median EDSS (IQR)		-	1.0 (1.0;1.5)	1.5 (1.2;2.2)	-	-	-	-	-	0.110 (0.174)
Patients receiving DMTs (%)		-	37 (82)	17 (90)	-	-	-	-	-	0.711 (0.790)
Median brain T2-hyperintense WM LV[a] (IQR) (ml)		0.0 (0.0;0.1)	2.6 (1.3;5.5)	7.5 (4.6;15.2)	-	**<0.001** (**<0.001**)	-	**<0.001** (**<0.001**)	-	**0.003** (**0.010**)
EM (SE)	NBV (ml)	1714 (11)	1,693 (8)	1,649 (12)	−21 (−49;6)	0.128 (0.184)	−65 (−98;−33)	**<0.001** (**0.001**)	−44 (−72;−16)	**0.002** (**0.009**)
	NCV (ml)	727 (9)	707 (6)	693 (9)	−20 (−42;1)	0.061 (0.115)	−34 (−59;−9)	**0.008** (**0.021**)	−14 (−35;8)	0.215 (0.268)
	NThalV (ml)	23.0 (0.3)	22.4 (0.2)	21.3 (0.3)	−0.6 (−1.5;0.2)	0.121 (0.181)	−1.7 (−2.6; −0.7)	**<0.001** (**0.004**)	−1.0 (−1.9;−0.2)	**0.015** (**0.034**)
	DTI-ALPS index	1.645 (0.027)	1.643 (0.019)	1.509 (0.028)	−0.002 (−0.069;0.065)	0.956 (0.956)	−0.136 (−0.214;−0.058)	**<0.001** (**0.004**)	−0.134 (−0.202;−0.067)	**<0.001** (**0.001**)
	NAWM FA	0.460 (0.005)	0.442 (0.003)	0.4250 (0.005)	−0.018 (−0.029;−0.006)	**0.003** (**0.010**)	−0.035 (−0.049;−0.022)	**<0.001** (**<0.001**)	−0.018 (−0.030;−0.006)	**0.003** (**0.010**)
	NAWM MD	0.777 (0.006)	0.793 (0.004)	0.818 (0.006)	0.017 (0.003;0.030)	**0.017** (**0.036**)	0.042 (0.025;0.058)	**<0.001** (**<0.001**)	0.025 (0.011;0.039)	**<0.001** (**0.004**)

Note: Comparisons performed by Fisher's exact test (sex, DMT status) and Mann–Whitney test (age, disease duration, IQ, age at onset and EDSS). Age- and sex-adjusted linear models were performed for MRI variables. Bold values denote statistical significance ($p < 0.05$). MD is expressed in units of $mm^2/s \times 10^{-3}$. FA and DTI-ALPS are dimensionless indexes.

Abbreviations: CI = confidence interval; Co-P = cognitive preserved; Co-I = cognitive impaired; DD = disease duration; DMT = disease-modifying therapy; DTI-ALPS = diffusion tensor imaging along perivascular spaces; EDSS = Expanded Disability Status Scale; EM = estimated mean; EMD = estimated mean difference; FA = fractional anisotropy; FDR-p = false discovery rate p value; HC = healthy controls; IQ = intelligence quotient; IQR = interquartile range; MD = mean diffusivity; ml = milliliter; MS = multiple sclerosis; NBV = normalized brain volume; NAWM = normal appearing; NCV = normalized cortical volume; ped = pediatric; NThalV = normalized thalamic volume; SE = standard error; WM = white matter.

[a]T2-hyperintense white matter lesion volume was log transformed.

Compared to HCs, Co-I pediatric MS patients had significantly higher brain T2-hyperintense WM LV, lower NBV, lower NCV and lower normalized thalamic volume (FDR-p ranging from <0.001 to 0.021).

Compared to Co-P pediatric MS patients, Co-I patients had significantly higher brain T2-hyperintense WM LV, lower NBV and lower normalized thalamic volume (FDR-p = 0.010; FDR-p = 0.009 and FDR-p = 0.034).

NAWM Damage

Compared to HCs, both Co-P and Co-I pediatric MS patients showed significantly lower FA (Co-P pediatric MS:

TABLE 2. Mean z-Scores and Prevalence of Impairment at the Neuropsychological Tests and at Each Cognitive Domain Explored by the Neuropsychological Battery for Children With Multiple Sclerosis According to Cognitive Status

	Z-score[a]		No. of impaired[b] (%)			Z-score[a]		No. of Impaired[c] (%)	
Cognitive Tests	Co-P pedMS (n = 45)	Co-I pedMS (n = 20)	Co-P pedMS (n = 45)	Co-I pedMS (n = 20)	Cognitive Domains	Co-P pedMS (n = 45)	Co-I pedMS (n = 20)	Co-P pedMS (n = 45)	Co-I pedMS (n = 20)
SRT lts	0.2 (1.1)	−1.1 (1.6)	1 (2)	6 (30)	Verbal memory	0.1 (0.9)	−1.2 (1.7)	3 (7)	11 (55)
SRT crtl	0.0 (1.0)	−1.1 (1.6)	1 (2)	6 (30)					
SRT recall	0.1 (1.1)	−1.3 (2.0)	3 (7)	8 (40)					
SPART	0.0 (1.6)	−1.7 (2.0)	6 (13)	11 (55)	Visuospatial memory	0.0 (1.5)	−1.7 (2.0)	7 (15)	12 (60)
SPART recall	0.0 (1.4)	−1.7 (2.1)	7 (15)	9 (45)					
SDMT	0.1 (0.9)	−0.7 (0.8)	0 (0)	2 (10)	Attention and information processing speed	0.1 (0.8)	−1.0 (1.2)	1 (2)	10 (50)
TMT-A	0.1 (0.8)	−0.9 (1.4)	1 (2)	5 (25)					
TMT-B	0.0 (0.9)	−1.3 (2.0)	0 (0)	7 (35)					
ODT	−0.2 (1.0)	−0.9 (1.8)	3 (7)	5 (25)	Expressive language	−0.1 (0.7)	−0.7 (1.1)	4 (9)	6 (30)
Semantic verbal fluency	−0.1 (0.8)	−0.3 (0.8)	0 (0)	0 (0)					
Phonemic verbal fluency	0.0 (1.0)	−0.5 (0.9)	1 (2)	2 (10)					
Token test	−0.3 (1.4)	−2.3 (2.8)	7 (15)	11 (55)	Receptive language	−0.3 (1.1)	−2.1 (2.6)	12 (27)	14 (70)
PCT	−0.4 (1.5)	−2.0 (4.0)	6 (13)	5 (25)					

[a]Mean (SD) of z-scores according to the normative data of an Italian representative sample.[25]
[b]Number of patients (frequency) who scored below the 5th percentile of the normative sample on at least 2 tests examining different cognitive domains were classified as cognitively impaired.
[c]Number of patients (frequency) with ≥1 abnormal neuropsychological tests of Neuropsychological Battery for Children with MS for each cognitive domain.
Abbreviations: Co-I = cognitively impaired; Co-P = cognitively preserved; MS = multiple sclerosis; ODT = Oral Denomination test; ped = pediatric; PCT = Phrase Comprehension test; ped = pediatric; SDMT = Symbol Digit Modalities Test; SPART = 10/36 Spatial Recall Test; SRT = Selective Reminding Test; TMT = Trail Making Test.

estimated mean difference [EMD] = −0.018 [95% confidence interval, [CI] = −0.029; −0.006], FDR-p = 0.010; Co-I pediatric MS: EMD = −0.035 [95% CI = −0.049; −0.022], FDR-p < 0.001) and higher MD values (EMD = 0.017 [95% CI = 0.003;0.030], FDR-p = 0.036; EMD = 0.042 [95% CI = 0.025;0.058], FDR-p < 0.001).

Co-I pediatric MS patients presented significantly lower FA (EMD = −0.018 [95% CI = −0.030; −0.006], FDR-p = 0.010) and higher MD values (EMD = 0.025 [95% CI = 0.011;0.039], FDR-p = 0.004) compared to Co-P patients (Table 1).

DTI-ALPS Index

No significant differences in DTI-ALPS index were observed between Co-P pediatric MS patients and HCs (EMD = −0.002 [95% CI = −0.069; 0.065], FDR-p = 0.956) (Table 1; Fig 2).

Compared to HCs and Co-P patients, Co-I pediatric MS patients showed significantly lower DTI-ALPS index (EMD = −0.136 [95% CI = −0.214; −0.058], FDR-p = 0.004; EMD = −0.134 [95% CI = −0.202; −0.067], FDR-p = 0.001) (Table 1; Fig 2).

The results for the Co-I versus Co-P contrast were not affected by the inclusion of age at onset as additional confounding factor.

Analysis of Associations

In pediatric HCs, no significant associations were observed between DTI-ALPS index and NBV, NCV, normalized

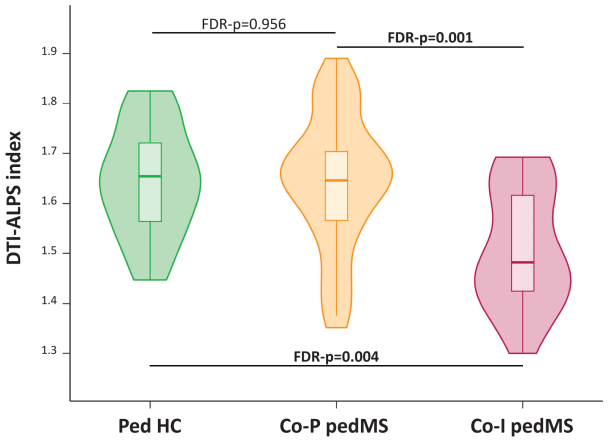

FIGURE 2: DTI-ALPS index distribution in pediatric healthy controls and multiple sclerosis patients according to their cognitive status. Violin plots show the distribution of DTI-ALPS index in pediatric HCs and pediatric MS patients stratified according to their cognitive status. *p*-values of statistically significant between-group comparisons are reported in bold. See main text and Table 1 for further details. Co-I = cognitively impaired; Co-P = cognitively preserved; HC = healthy controls; MS = multiple sclerosis; Ped = pediatric.

thalamic volume, FA, and MD NAWM (FDR-p ≥ 0.348) (Table 3).

In pediatric MS patients, a lower DTI-ALPS index was significantly associated with a higher brain T2-hyperintense WM LV and NAWM MD (standardized beta [β] [standard error, SE] = −0.345 [0.095], 95% CI = [−0.534; −0.156], FDR-p = 0.003; β [SE] = −0.381 [0.106], 95% CI = -[0.593; −0.169], FDR-p = 0.003), and a lower normalized thalamic volume and NAWM FA (β [SE] = 0.290 [0.099], 95% CI = [0.093;0.488], FDR-p = 0.016; and β [SE] = 0.402 [0.098], 95% CI = [0.207;0.596], FDR-p = 0.001) (Table 3; Fig 3). No significant associations were observed with IQ, disease duration, EDSS score, NBV, and NCV (FDR-p ranging from 0.174 to 0.902) (Table 3).

Predictors of Cognitive Impairment in Pediatric MS

Random Forest selected lower DTI-ALPS index (relative importance [RI] = 100%), higher brain WM LV (RI = 59.5%), higher NAWM MD (RI = 57.1%), and lower IQ (RI = 51.3%) as informative predictors of cognitive impairment (OOB-AUC = 0.762) (Table 4, Fig 4). When considering each selected predictor individually, DTI-ALPS index showed the highest discriminative ability (OOB-AUC = 0.731).

Discussion

By using a recently proposed non-invasive diffusion-derived MRI index of diffusivity along the perivascular space

TABLE 3. Associations of Clinical and MRI Features with DTI-ALPS Index in Pediatric Healthy Controls and Multiple Sclerosis Patients

	Pediatric HCs		Pediatric MS Patients	
Variable	**β (SE) (95% CI)**	**p (FDR-p)**	**β (SE) (95% CI)**	**p (FDR-p)**
IQ	-	-	−0.029 (0.141) (−0.312;0.254)	0.837 (0.902)
Disease duration	-	-	−0.119 (0.134) (−0.387;0.149)	0.379 (0.535)
EDSS score	-	-	−0.130 (0.124) (−0.378;0.119)	0.301 (0.526)
Brain T2-hyperintense WM LV[a]	-	-	−0.345 (0.095) (−0.534; −0.156)	**<0.001 (0.003)**
NBV	0.429 (0.312) (−0.193;1.050)	0.174 (0.348)	0.261 (0.138) (−0.013;0.536)	0.062 (0.174)
NCV	0.064 (0.261) (−0.455;0.583)	0.807 (0.902)	0.259 (0.150) (−0.040;0.558)	0.089 (0.208)
NThalV	0.139 (0.262) (−0.383;0.660)	0.598 (0.762)	0.290 (0.099) (0.093;0.488)	**0.005 (0.016)**
NAWM FA	0.154 (0.175) (−0.194;0.502)	0.382 (0.535)	0.402 (0.098) (0.207;0.596)	**<0.001 (0.001)**
NAWM MD	−0.037 (0.323) (−0.681;0.606)	0.909 (0.909)	−0.381 (0.106) (−0.593; −0.169)	**<0.001 (0.003)**

Note: Standardized beta coefficients (β), related SE, 95% CI, and *p*-values from age- and sex-adjusted multiple linear regression models are reported. Bold values denote statistical significance ($p < 0.05$). MD is expressed in units of $mm^2/s \times 10^{-3}$. FA and DTI-ALPS are dimensionless indexes.

[a]T2-hyperintense WM LV was log transformed.

Abbreviations: CI = confidence interval; DTI-ALPS = diffusion tensor imaging along perivascular spaces; EDSS = Expanded Disability Status Scale; FA = fractional anisotropy; FDR-p = false discovery rate p value; HC = healthy controls; IQ = intelligence quotient; LV = lesion volume; MD = mean diffusivity; MS = multiple sclerosis; NA = normal-appearing; NBV = normalized brain volume; NCV = normalized cortical volume; NThalV = normalized thalamic volume; SE = standard error; WM = white matter.

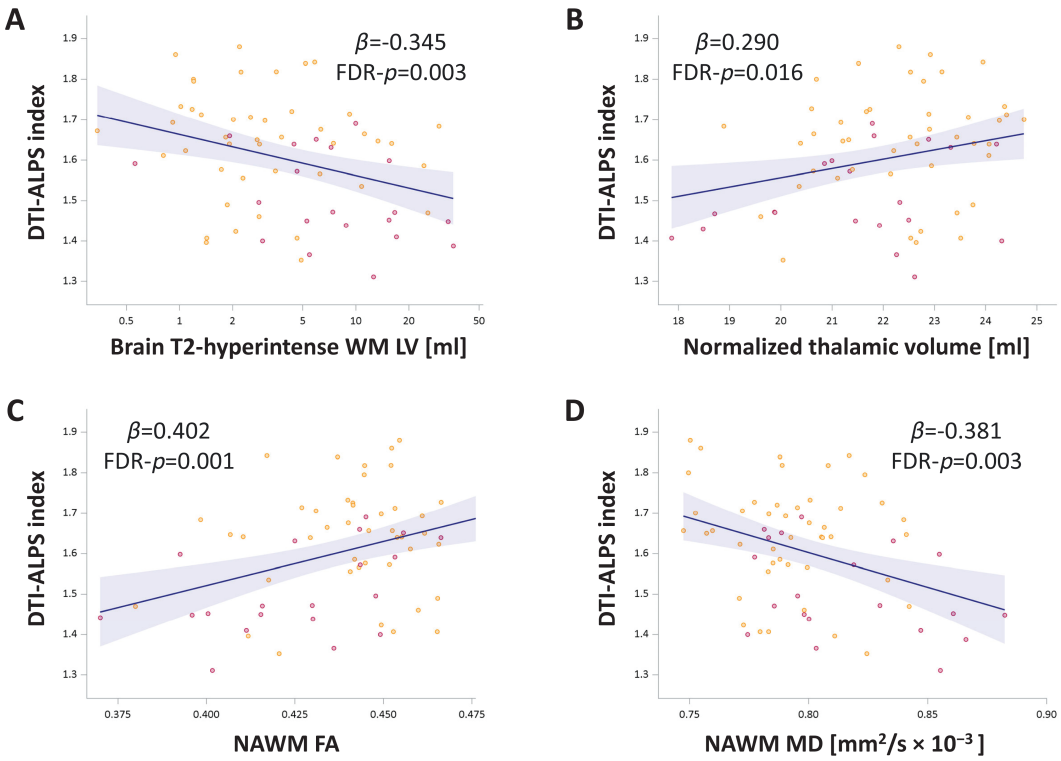

FIGURE 3: Significant associations of DTI-ALPS index with conventional MRI measures in pediatric multiple sclerosis patients. Scatter plots showing significant age- and sex-adjusted associations of DTI-ALPS index with (A) T2-hyperintense WM LV (on log scale), (B) normalized thalamic volume, (C) NAWM FA, and (D) NAWM MD in pediatric multiple sclerosis patients. Dots are color-coded according to cognitive status (Co-P in orange, Co-I in red). See text for further details. DTI-ALPS = diffusion along perivascular spaces; FA = fractional anisotropy; LV = lesion volume; MD = mean diffusivity; NA = normal-appearing; WM = white matter.

TABLE 4. Random Forest Informative Predictors of Cognitive Impairment in Pediatric Multiple Sclerosis Patients

Outcome	Predictor	Median Importance (IQR)	Relative Importance	OOB-AUC	
Cognitive impairment	DTI-ALPS index	32.2 (28.2;36.4)	100.0	0.731	0.762
	T2-hyperintense WM LV	19.2 (16.7;21.8)	59.5	0.651	
	NAWM MD	18.4 (17.1;20.9)	57.1	0.636	
	IQ	16.5 (14.2;19.5)	51.3	0.571	

Note: Variables selected by Boruta algorithm as relevant predictors of the presence of cognitive impairment in pediatric MS patients are listed. Median and relative importance of each predictor, achieved across iterations, are reported. The individual discriminative ability of each predictor and the performance of a final random Forest model including the selected variables are provided in last column.

Abbreviations: DTI-ALPS = diffusion along perivascular spaces; IQ = intelligence quotient; LV = lesion volume; MD = mean diffusivity; MS = multiple sclerosis; NA = normal-appearing; OOB-AUC = out-of-bag area under the curve; WM = white matter.

(ie, DTI-ALPS index), we investigated in vivo glymphatic system function in a relatively large cohort of pediatric MS patients stratified according to their cognitive status and its correlation with structural brain damage and clinical disability. While DTI-ALPS index was not significantly different between Co-P pediatric MS patients and pediatric HCs, Co-I pediatric MS patients had significantly lower DTI-ALPS index compared to both HCs and Co-P pediatric MS patients. Moreover, a lower DTI-ALPS index was significantly associated with MRI measures of brain structural damage, including higher focal T2-hyperintense WM LV, lower normalized thalamic volume and more severe NAWM microstructural abnormalities in pediatric MS patients. Finally, lower DTI-ALPS index, higher brain T2-hyperintense WM LV, NAWM MD, and lower IQ values were found to be informative predictors of cognitive impairment in pediatric MS patients.

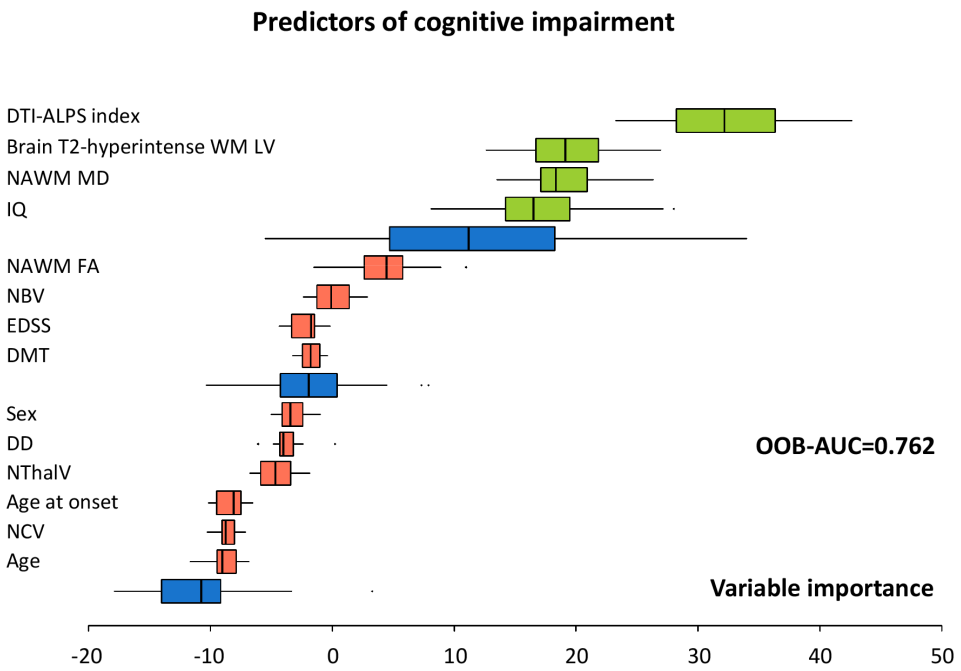

FIGURE 4: Random Forest informative predictors of cognitive impairment in pediatric multiple sclerosis patients. Distribution of variable importance, achieved across iterations of Boruta algorithm, of demographic, clinical, and MRI features to explain cognitive impairment. Boruta compares the importance of the original variables with the highest feature importance of the shadow features, obtained using features permuted copies. Poorly performing variables are progressively discarded. Selected features are shown in green, discarded features in red. Maximum, mean, and minimum importance achieved by shadows attributes are shown in blue. DD = disease duration; DMT = disease modifying treatment; DTI-ALPS = diffusion along perivascular spaces; EDSS = Expanded Disability Status Scale; FA = fractional anisotropy; IQ = intelligence quotient; LV = lesion volume; MD = mean diffusivity; MS = multiple sclerosis; NA = normal-appearing; NBV = normalized brain volume; NCV = normalized cortical volume; NThalV = normalized thalamic volume; OOB-AUC = out-of-bag area under the curve; WM = white matter.

In the past few years, the study of glymphatic function is dramatically risen in adult aging[12,13] and neurodegenerative conditions,[14–17] including MS.[16,18] Classically, glymphatic function has been explored through contrast-based imaging (ie, MRI after intrathecal or intravenous gadolinium-based contrast agent [GBCA] administration). However, more recent works have proposed noninvasive MRI-based approaches to measure glymphatic function in humans.[37] Among them, DTI-ALPS index holds considerable promise, as it quantifies the small diffusion component in the direction of the perivascular spaces, when the influence of diffusivity of major WM fibers has been removed.[20]

Given the consistent evidence of glymphatic dysfunction in various neurological disorders characterized by cognitive deterioration,[15,21,38] we explored whether glymphatic system impairment can be found also in pediatric MS patients. However, we specifically focused on cognition, given also its high frequency of involvement in pediatric MS.[3] Consistently with previous studies,[39–41] a significant proportion of our patients had cognitive impairment, with the most frequently affected cognitive domains including receptive language, visuospatial memory and verbal memory.

Interestingly, Co-P pediatric MS patients showed a significant accumulation of brain T2-hyperintense WM lesions and presence of NAWM abnormalities compared to pediatric HCs, but no significant alterations in DTI-ALPS index. A previous study in adult MS patients suggested that a gradual decrease in the DTI-ALPS index takes place within the initial 4 years following the onset of the disease.[18] Accordingly, the short disease duration of this patients' group (median = 1.0 year) together with the mild disease severity in terms of clinical disability, cognitive dysfunction, and structural brain damage accumulation, may contribute to explain the lack of significant difference compared to pediatric HCs.

On the other hand, in line with previous studies,[2,9,42] Co-I pediatric MS patients showed not only a significant accumulation of brain T2-hyperintense WM lesions, irreversible tissue loss and NAWM abnormalities, but also a significantly lower DTI-ALPS index compared to both pediatric HCs and CoP MS patients.

The relevance of glymphatic system impairment in explaining cognitive dysfunctions is supported by the random Forest analysis showing that lower DTI-ALPS index was the most informative predictor of cognitive impairment in pediatric

MS patients, followed by higher brain T2-hyperintense WM LV, higher NAWM MD, and lower IQ.

Although the developmental trajectories of glymphatic system in pediatric HCs are still not fully explored,[43] our findings imply that an early impairment of glymphatic function may be associated with worse cognitive performance in pediatric MS patients. Our results are also consistent with a growing body of evidence showing an association between glymphatic dysfunction and cognitive decline in adult aging[12,13] and different neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and idiopathic normal pressure hydrocephalus.[15,44] These findings suggest that glymphatic failure may represent a common pathway leading to cognitive impairment in neurodegenerative conditions.[21] Emerging evidence also revealed that pathological proteins and iron deposition in the brain are cleared via the glymphatic pathway.[10,38,45] In this view, the accumulation of pathological proteins may trigger neurotoxicity and inflammation,[21,46] while iron accumulation may compromise synaptic transmission and neuronal function.[47] Additionally, impaired glymphatic function could reduce the clearance of inflammatory cells and exacerbate inflammation.[48] Ultimately, the buildup of metabolic products and inflammation could initiate a cascade of pathological damage, leading to cognitive impairment.

Notably, current evidence had shed light on the potential for treating cognitive impairment by improving glymphatic function. A study performed on experimental models of aging showed that voluntary exercise accelerated glymphatic clearance, attenuated the accumulation of amyloid plaques and ultimately protected mice against a decline in spatial cognition.[49] Another experimental study found that a polyunsaturated fatty acid supplement could improve glymphatic transport and protect cognitive function.[50] Thus, glymphatic pathway might be considered as a potential prevention or treatment target for cognitive decline in the future, also in MS patients.

Interestingly enough, glymphatic dysfunction was not associated with disability (EDSS score) and disease duration in our patients. This is in contrast with a previous study in adult patients with MS showing an association between DTI-ALPS index and the severity of EDSS score.[18] These findings may be related, at least partially, to the limited span of disease duration and the mild disability observed in our pediatric patients. However, these results may also suggest that, in pediatric MS patients, the evaluation of cognitive deficits may be more sensitive to capture the early detrimental impact of MS compared to the assessment of clinical disability with EDSS score.

In addition to glymphatic system dysfunction, several pathological processes may associate with cognitive dysfunction in MS.[42,51]

In particular, the evidence that a higher T2-hyperintense WM LV and higher MD NAWM were also informative predictors of cognitive impairment, support the hypothesis that a "disconnection syndrome" caused by the accumulation of focal lesions and microstructural tissue abnormalities in cognitively related WM tracts may be particularly relevant in explaining cognitive impairment in pediatric MS patients. Although normalized brain and thalamic volumes were significantly lower in Co-I compared to Co-P pediatric MS patients, these volumetric measures were not significant informative predictors of cognitive impairment. Brain and, in particular, regional gray matter volume loss have been shown to be more relevant predictors in adult MS patients and in MS patients with a longer disease course. In addition, while thalamic atrophy has been suggested as relevant predictor of cognitive impairment in pediatric MS,[52] a multiparametric study identified other MRI metrics as significant predictors of cognitive impairment.[42] Discrepancies among studies could be related to the heterogeneity in the cohort of pediatric MS studied as well as different neuropsychological criteria applied to define cognitive impairment and in the MRI analysis performed. Furthermore, it is likely that atrophy of specific brain regions such as the precuneus,[42] which were not explored in this study, may contribute to explain cognitive impairment in pediatric MS patients.

The evidence that lower IQ score represents an informative predictor of cognitive impairment underscores the potential protective role of cognitive reserve against cognitive impairment in pediatric MS population.[53,54] It is likely that the promotion of intellectual enrichment in pediatric MS patients may prevent the onset and/or the evolution of MS-related cognitive dysfunction.

The analysis of associations between DTI-ALPS index and structural MRI measures provided also interesting results that may contribute to better understand the interplay between glymphatic system dysfunction and structural brain damage accumulation.

We observed a significant association between higher brain T2-hyperintense WM LV and lower DTI-ALPS index. Although the pathophysiological mechanisms underlying glymphatic dysfunction in MS have not been fully elucidated yet, our results seem to suggest that the relationship between brain focal WM lesion formation and alterations in the glymphatic system could be bidirectional. The infiltration of immune cells into the perivascular space, the impairment of blood–brain barrier integrity, and the presence of astrogliosis may impair the physiological function of the glymphatic system. On the other hand, an impaired glymphatic system may prolong the detrimental exposure of the CNS to harmful elements, such as pro-inflammatory cytokines and reactive

oxygen species, thus promoting the formation of focal inflammatory demyelinating lesions and neurodegenerative processes.

DTI-ALPS index was also negatively associated with normalized thalamic volume, supporting the notion that thalamic atrophy represents an early pathological process in patients with pediatric MS.[7,55] Indeed, the thalamus, due to its central location between the WM and the CSF, is vulnerable to a range of different pathological processes.[56] These encompass CSF-mediated damage fueled by soluble inflammatory and neurodegenerative factors, which accumulate in the presence of glymphatic dysfunction.

Another interesting result from our work is the association between glymphatic system impairment and microstructural tissue damage in the NAWM, in terms of reduced FA and increased MD. This suggests a significant interaction within the interstitium between these variables. On the one hand, as previously mentioned, the reduced clearance within the interstitium due to the compromised glymphatic system may lead to tissue abnormalities by promoting neuronal loss, inflammation, and gliosis. On the other one, it is also plausible that MS demyelination could impact the astrocytes' end feet, potentially disrupting aquaporin-4-dependent glymphatic fluid circulation. In acute MS lesions, astrocytes undergo a morphological transformation characterized by soma enlargement and reduced feet density, accompanied by a retraction of the glia limitans that delineates the perivascular space, leading to glymphatic dysfunction.[18]

This study has some limitations. First, we evaluated the DTI-ALPS index with a cross-sectional approach. Future longitudinal studies are needed to explore the dynamic abnormalities of glymphatic impairment in pediatric MS and their associations with measures of disease activity and progression (eg, paramagnetic rim lesions). Second, due to the difficulty in the enrolment of pediatric healthy subjects in MRI studies, we only included 23 HCs, potentially limiting the extent of normal variability of DTI-ALPS index. Future studies should explore the glymphatic system maturational processes occurring in a population of pediatric HCs. Third, our pediatric MS patients were characterized by a relatively narrow range of age and disease duration, with a limited number of subjects in each subgroup when stratifying according to cognitive status. These limitations did not enable some analyses of interests between the Co-I and Co-P groups. Fourth, although the method we used to assess glymphatic system was based on diffusion, thus not implying invasive approaches, it does not completely reflect the overall glymphatic function. Indeed, the DTI-ALPS index is based on the orthogonal geometric relationship between projection and association fibers and medullary arteries and veins in the lateral ventricle body.[20] Therefore, the DTI-ALPS index can only be evaluated at the level of the lateral ventricle body. Moreover, although the DTI-ALPS index is based on the diffusivity measure of the perivenous space around the deep medullary vein, it might be also influenced by the surrounding WM microstructure included in the ROI, which could be affected in MS. A critical next step for understanding the validity of this method will be comparing it with the reference standard contrast-based approaches (ie, intrathecally or intravenously administrated GBCA-enhanced MRI) and evaluating its sensitivity in the detection of glymphatic function across different physiologic and pathologic states. However, such approaches may be more invasive, especially for pediatric subjects.

In summary, a decreased DTI-ALPS index was observed in cognitively impaired pediatric MS patients; such alteration associated with MRI measures of brain structural damage in terms of focal WM lesions, thalamic atrophy, and microstructural abnormalities. Our results suggest that glymphatic system dysfunction is an early phenomenon in pediatric MS pathophysiology, and associated with cognitive impairment in this population.

Acknowledgment

Open access funding provided by BIBLIOSAN.

Author Contributions

M.M., M.F., and M.A.R. contributed to the conception and design of the study. M.M., E.P., A.M., P.P, D.M., M.G., L.M., M.F., and M.A.R. contributed to the acquisition and analysis of data. M.M., E.P., A.M., P.P, D.M., M.G., L.M., M.F., and M.A.R. contributed to drafting the text and preparing the figures. M.M., E.P., A.M., P.P, D.M., M.G., L.M., M.F., and M.A.R. approved the final draft of the manuscript.

Potential Conflicts of Interest

Nothing to report.

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