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IN-VIVO MAPPING OF THALAMIC PATHOLOGICAL MECHANISMS IN PEDIATRIC PATIENTS WITH MS

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INTRODUCTION and PURPOSE

During the last decade, neurodegeneration has proven to represent a cardinal feature of multiple sclerosis (MS) pathology, starting from the earliest phases of the disease, and the thalamus has been identified as the first gray matter (GM) compartment involved.[1] In pediatric MS patients, thalamic damage and its role in clinical and neuropsychological impairment[2] have been extensively described. However, despite widely recognized, the mechanism leading to thalamic damage still remains unclear, leaving different hypotheses open. Due to its central location and its double interface, on one side with white matter (WM) and on the other side with CSF, the thalamus is likely to be susceptible to both retrograde Wallerian degeneration from WM lesions and CSF immune cytotoxic factor-mediated damage, in addition to intrinsic thalamic pathology.

In this framework, advanced neuropathological and neuroimaging methods are providing us with an increasingly better view of the full extent of pathological processes underlying thalamic damage in patients with MS. A tract-specific pattern of cortico-thalamic neurodegeneration has been described accounting for WM macroscopic and microstructural damage in the thalamus by adopting DTI.[3] Moreover, recent studies have emphasized the existence of an outside-in CSF-mediated pathophysiological mechanism underlying a neurodegenerative processes in MS. This last hypothesis has been recently proved by *in vivo* studies using magnetization transfer imaging[4] in periventricular WM, and in deep GM in MS patients.[5] Nevertheless, to date, only one study in adult MS patients[6] focused on the pathological processes behind thalamic damage. By adopting ultra-high field quantitative imaging, thalamic focal lesions were attributed to CSF-mediated factors and an association was found between thalamic degeneration and WM lesions outside the thalamus. Recently, a novel MRI method based on the ratio of T_1− and T_2−weighted image signal intensities has been introduced, in order to provide an indirect *in vivo* evidence of myelin content.[7] This method is particularly attractive for MS research and for pediatric subjects and it is characterized by high feasibility, adopting two conventional sequences, which are part of many clinical MRI protocols.

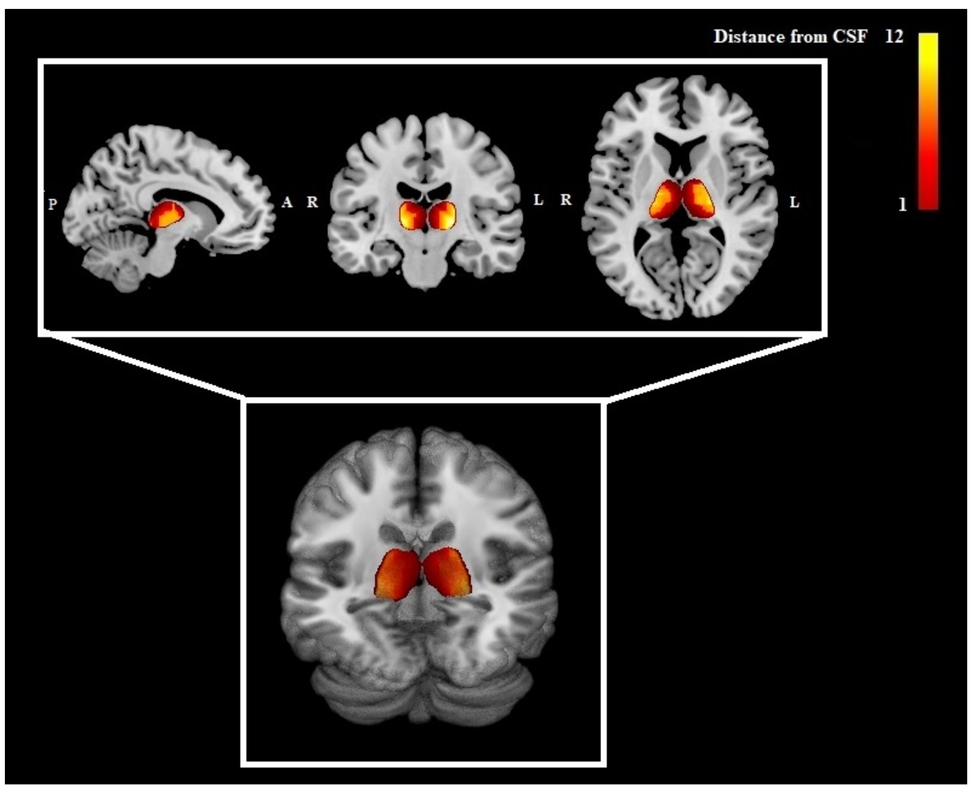
Against this background, we combined different MRI technique to investigate *in-vivo* the pathological processes behind thalamic damage in pediatric MS patients. In details the **aims** of this study are:

- To assess **thalamic atrophy** in pediatric MS patients;
- To explore **lesion distribution within the thalamus;**
- To investigate **microstructural pathology (as measured by DTI metrics and T1/T2 ratio) in the thalamus and thalamic WM** (in order to explore the additional variance in MRI microstructural metrics due to this compartment) as whole and as a function of distance from the ventricles;
- To explore the **relationship between** the previous abnormalities and **WM lesions and cortical pathology** as well as their contribution to **clinical measures.**

MATERIALS and METHODS

- **Subjects:** 70 pediatric MS patients and 26 age and sex-matched healthy controls (HC) were enrolled. Patients with acute disseminated encephalomyelitis were excluded according to published operational criteria [8]. None of the patients had a diagnosis of clinically isolated syndrome at the time of study inclusion and all of them had at least two clinical attacks and the formation of new central nervous system lesions on serial MRI.
- **Neurological examination:**
  - Clinical evaluation;
  - EDSS score rating.
- **MRI Acquisition (3.0 T scanner):**
  - Dual-echo TSE;
  - 3D T1-weighted fast field-echo scan;
  - Pulsed-gradient SE EPI 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 (b=0 and b=900 s mm-2).
- **Conventional MRI analysis:**
  - Measurements of T2 hyperintense and T1 hypointense lesion volumes (LV);
  - Quantification of normalized brain (NBV), WM (NWMV) and GM (NGMV) volumes (SIENAx).
- **Quantitative MRI analysis:**
  - T1/T2-weighted ratio maps were estimated using an in-house implemented method;
  - Thalamic segmentation (FIRST software);
  - Thalamic segmentation into concentric bands according to geodesic distance from the thalamus/CSF interface;
  - Tract-based spatial statistic to isolate thalamic WM;
  - Assessment of DTI metrics - fractional anisotropy (FA) and mean diffusivity (MD) – and T1/T2 ratio values in whole thalamus, thalamic WM and their concentric bands;
  - Cortical surface reconstruction and mean cortical thickness measurements (FreeSurfer 6.0.0).

Figure 1. Example of thalamic segmentation obtained in a healthy subject is provided. A red-yellow scale shows the different thalamic bands according to the geodesic distance from the CSF.



Abbreviations: A=anterior; P=posterior; R=right; L=left.

- **Statistical analysis**
  - Demographics and clinical characteristics were compared between pediatric MS patients and HC using Mann–Whitney U test or χ^2 test for sex;
  - Multivariate models were used to compare global MRI characteristics between groups;
  - Quantitative MRI metrics in bands within thalamus defined as geodesic distance from CSF were compared between groups by linear regression adjusted for age, sex and thalamic volume;
  - Multiple linear regression models were used to test the correlation between thalamic, cortical, and WM metrics, and the contribution of MRI metrics to clinical disability;
  - Bonferroni method was applied to correct for multiple comparisons.

RESULTS

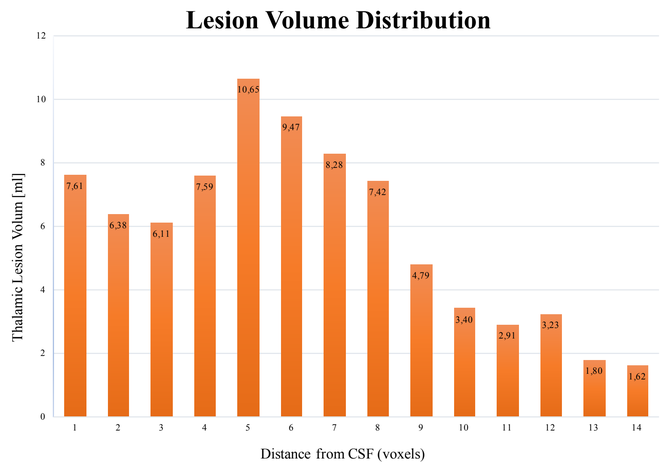
Table 1 Shows the main demographic and clinical characteristics of the study subjects.

	Pediatric HC	Pediatric MS patients	*p* values§
Number of subjects	26	70	-
Female/male	15/11	42/28	0.78*
Mean age (SD) [years]	15.2 (8.5-18.0)	15.5 (7.6-18.0)	0.92
Median disease duration (range) [years]	-	1.8 (0.1-8.1)	-
Median EDSS [range]	-	1.5 (0.0-4.0)	-
Mean T2 LV (SD) [ml]	-	6.2 (7.6)	-
Mean T1 LV (SD) [ml]	-	3.5 (4.6)	-
Mean NBV [ml] (SD)	1715 (91)	1660 (84)	**0.01**
Mean NGMV [ml] (SD)	862 (72)	831 (55)	**0.03**
Mean NWMV [ml] (SD)	852 (51)	829 (51)	**0.04**
Mean Left thalamus volume [ml] (SD)	11.0 (12.4)	10.5 (8.6)	0.06
Mean Right thalamus volume [ml] (SD)	11.2 (11.8)	10.8 (9.3)	0.06
Mean Cortical Thickness [mm] (SD)	2.4 (0.1)	2.3 (0.1)	**0.04**

*Chi square test.

Abbreviations: HC=Healthy Controls; MS=Multiple Sclerosis; SD=standard deviation; EDSS=Expanded Disability Status Scale; LV=lesion volume; NBV=normalized brain volume, NGMV=normalized gray matter volume; WMV=normalized white matter volume.

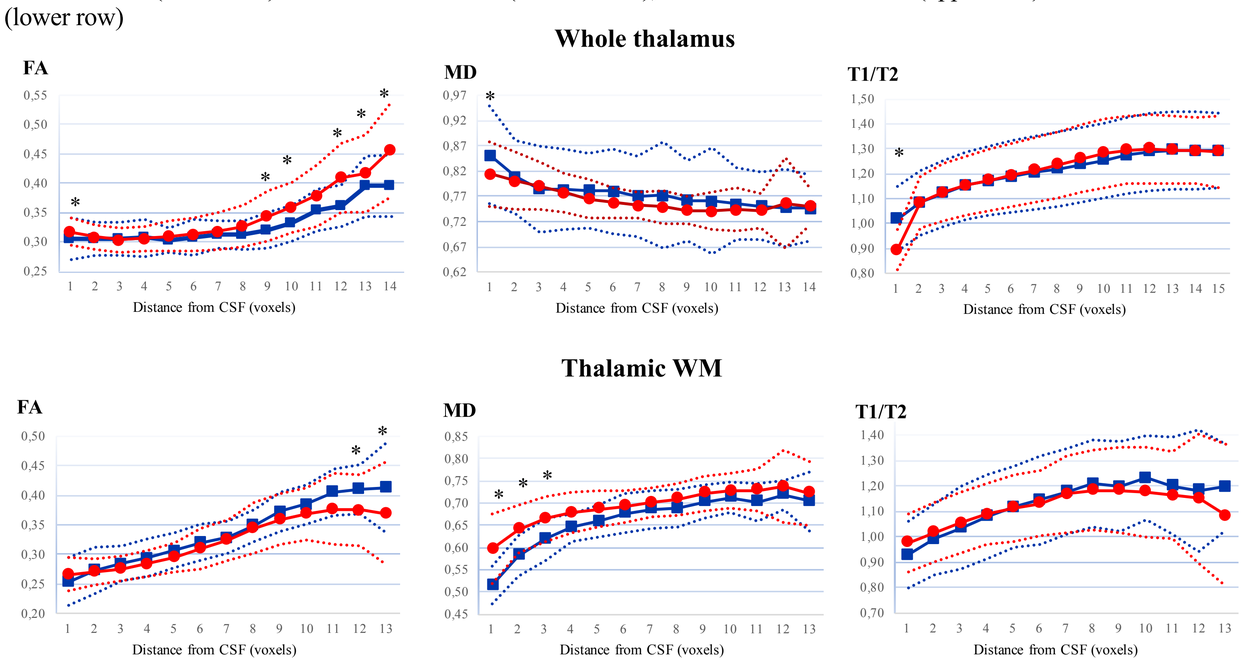
Considering the thalamus as a whole, we found significant differences in thalamic FA (p=0.03) as well in WM FA (p=0.006) and MD (p=0.009).



Lesions were mainly located in thalamic bands nearest to CSF.

Figure 2. Mean lesion volume values [ml] are reported for each thalamic concentric band originating from the CSF/thalamus-interface.
Abbreviation: CSF=cerebrospinal fluid.

Figure 3 shows MRI quantitative metrics in thalamic concentric bands obtained as function of their geodesic distance from CSF as mean (solid lines) ± standard deviation (dashed lines), for the whole thalamus (upper row) and thalamic WM (lower row)



- **Correlation analysis:**
  - No correlations were found between thalamic quantitative MRI metrics and EDSS;
  - Thalamic WM MD was significantly correlated with disease duration (r=0.4; p=0.009), while thalamic WM FA and T1/T2 ratio values in the band nearest to CSF/thalamus-interface significantly correlated to a younger age of disease onset (r=0.3; p=0.003-0.03);
  - Thalamic FA abnormalities in the thalamic bands nearest to thalamus/WM interface significantly correlated with T2 and T1 LV (r=0.3-0.4; p=0.009-0.01);
  - Thalamic DTI metrics in the bands nearest to CSF/thalamus interface significantly correlated with mean cortical thickness (FA: r=-0.3; MD: r=0.4; p=0.003-0.03).

DISCUSSION

- By assessing diffuse microstructural damage, this study confirmed the presence of thalamic damage from the earliest stages of MS.
- In accordance with the hypothesis of an immune cytotoxic factor in the CSF mediating thalamic damage, a prominent involvement of the bands nearest to CSF/thalamus interface was found as well as the preferential location of thalamic lesions in these regions. To support this hypothesis, a correlation between thalamic damage in the regions nearest to CSF and cortical thickness was found, thus suggesting a common inflammation-mediated pathogenetic mechanism.
- The involvement of thalamic regions nearest to thalamus/WM interface appeared to be related to Wallerian neurodegenerative phenomena, as suggested by the correlation with T2 lesion volume.
- In conclusion, the thalamus may represent a critical barometer of diffuse neuronal pathology in MS mirroring the different pathogenetic mechanisms of brain damage.

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DISCLOSURES

E. De Meo, and L. Storelli report no conflict of interest. L.Moiola has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with Sanofi-Genzyme, Novartis, Teva, Merck-Serono, Biogen, Roche, Excemed. M.P. Amato has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with Biogen, Byer, Merck, Roche, Sanofi, and Teva, she has received research support from Biogen, Byer, Italian MS Society, Novartis, Sanofi, and Teva. A. Ghezzi has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with Biogen Idec, Merk-Serono, Novartis, Genzyme, Teva Neuroscience, Serono Symposia Int Found., and Almirall. P. Veggiotti has nothing to disclose. R. Capra has received personal compensation for consulting, serving on a scientific advisory board, speaking, or other activities with Novartis, Biogen, Teva, Genzyme and Sanofi-Aventi. M.A. Rocca received speakers honoraria from Biogen Idec, Novartis, Genzyme, Sanofi-Aventis, Teva, Merck Serono, and Roche and receives research support from the Italian Ministry of Health and Fondazione Italiana Sclerosi Multipla. M. Filippi is is Editor-in-Chief of the Journal of Neurology; received compensation for consulting services and/or speaking activities from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Novartis, Teva Pharmaceutical Industries, Roche, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA).

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