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BRAIN MACRO- AND MICROSCOPIC DAMAGE IN PATIENTS WITH PEDIATRIC MS

Martina Absinta^{1,2}, MD, Maria A. Rocca^{1,2}, MD, Lucia Moiola², MD, Angelo Ghezzi³, MD, Nicoletta Milani⁴, MD, Pierangelo Veggiotti⁵, MD, Giancarlo Comi², MD, Massimo Filippi^{1,2}, MD.

¹Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, and

²Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Milan Italy;

³Multiple Sclerosis Center, Ospedale di Gallarate, Gallarate, Italy; ⁴Child Neurology Department, Neurological Institute 'Carlo Besta', Milan, Italy; ⁵Fondazione "Istituto Neurologico Casimiro Mondino", Pavia, Italy.

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Correspondence to: Dr. Massimo Filippi, Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, Scientific Institute and University Ospedale San Raffaele, Via Olgettina 60, 20132 Milan, Italy. Telephone number: #39-02-26433033; Fax number: #39-02-26435972; E-mail: massimo.filippi@hsr.it

Abstract

Objective. To characterize, using conventional and diffusion tensor (DT) magnetic resonance imaging (MRI), the nature and distribution of lesions and the extent of damage in the brain normal appearing white matter (NAWM) and gray matter (GM) from a relatively large population of pediatric multiple sclerosis (MS) patients.

Methods. Brain conventional and DT MRI scans were acquired from 48 patients with pediatric MS (10 clinically isolated syndromes [CIS], 38 relapsing remitting [RR] MS), 30 adult CIS, 28 adult RRMS, 15 pediatric healthy controls (HC) and 18 adult HC. T2-lesion probability maps and DT MRI of lesions, NAWM and GM were compared among controls and MS groups.

Results. T2-visible lesion volumes did not differ among patient groups, but T2 lesions were more frequently located in the posterior periventricular regions in adult RRMS patients than in adult CIS and pediatric RRMS patients. Adult RRMS patients had significantly higher lesion average mean diffusivity than pediatric RRMS patients. No DT MRI changes in the NA tissues were found in pediatric and adult CIS patients. DT MRI abnormalities were limited to the NAWM in pediatric RRMS patients, while they involved the NAWM and GM in adult RRMS patients. The extent of NAWM involvement was similar between adult and pediatric RRMS patients and was significantly correlated with T2-visible lesion burden.

Conclusions. A less severe intrinsic lesion damage, a less frequent lesion occurrence in the posterior periventricular WM and the sparing of GM may contribute to explain the favourable short/medium term disease outcome of pediatric MS.

Introduction

Although the clinical onset of multiple sclerosis (MS) typically occurs between 20 and 40 years of age, in about 3-12% of cases it can occur before 18 years.[1, 2] Following the first clinical episode of central nervous system involvement (CNS), pediatric MS patients typically have a relapsing-remitting (RR) course, with accumulation of fixed disability 15 years or more after the clinical onset.[2] The median time to reach EDSS 3.0 is shorter in patients with adult-onset MS, being 8-12 years.[3] The factors related to such a better clinical evolution, at least at short-medium term, of pediatric MS patients in comparison to those with an adult-onset of the disease have not been defined yet.

So far, only a few magnetic resonance imaging (MRI) studies have attempted to define the nature and extent of CNS damage in patients with pediatric MS. Two studies, which recruited exclusively pediatric patients with clinically isolated syndromes (CIS) suggestive of MS,[4, 5] found a higher frequency of T2-visible lesions in the brainstem of these patients when compared with adult CIS patients. Magnetization transfer (MT) MRI studies found no abnormalities in the brain normal-appearing white matter (NAWM) and gray matter (GM), as well as in the cervical cord[6, 7] of pediatric patients with relapsing remitting (RR) MS in comparison with matched healthy volunteers. Brain DT MRI studies showed that, contrary to what happens in adult-onset MS,[8] pediatric RRMS patients, compared to matched healthy controls, do not have GM abnormalities and harbor only mild structural abnormalities in the NAWM.[7]

In order to better characterize the disease-related damage in the brain of pediatric MS patients, we obtained conventional and DT MRI scans from a relatively large population of these patients with either clinically isolated syndromes (CIS) suggestive of MS or RRMS and contrasted their findings with those obtained in a group of age-matched healthy subjects. In addition, to test the hypothesis that CNS involvement in pediatric MS patients is less pronounced than that seen in patients with an adult-onset of the disease, we compared measures obtained in pediatric patients with those of adult patients at similar disease stages.

Patients and methods

a) Patients. We studied **consecutively** 48 pediatric patients with CIS suggestive of MS (10 subjects)[9] or RRMS (38 subjects)[9, 10] and 15 age-matched healthy individuals with no previous history of neurological dysfunction and a normal neurological exam. **Patients with acute disseminated encephalomyelitis were excluded according to recently published operational criteria.[11, 12]** To serve as additional control groups, we also recruited 30 adult CIS patients,[9] and 27 adult RRMS patients.[9, 10] Adult patients were selected among the population of MS patients enrolled for research studies during the last two years at our Unit in order to match as close as possible the clinical characteristics of the pediatric MS patients. In addition, to rule out an age-effect on our results, another group of 18 healthy controls was selected in order to match as close as possible the demographic characteristics of adult MS patients. All CIS patients had paraclinical evidence of disease dissemination in space.[9] Alternative neurological diseases were excluded by appropriate investigations.[9] The main demographic and clinical characteristics of the six groups of subjects studied are shown in Table 1. **Clinical symptoms of CIS were optic neuritis in 2 (20%) pediatric and 8 (27%) adult patients, hemispheric syndrome in 4 (40%) pediatric and 9 (30%) adult patients, brainstem syndrome in 3 (30%) pediatric and 9 (30%) adult patients, and spinal cord syndrome in 1 (10%) pediatric and 4 (10%) adult patients.**

Table 1. Main demographic and clinical characteristics of the six groups of subjects studied.

	Pediatric healthy volunteers	Adult healthy volunteers	Pediatric CIS patients	Pediatric RRMS patients	Adult CIS patients	Adult RRMS patients
Number of subjects	15	18	10	38	30	27
Women/Men	10/5	11/7	6/4	24/14	21/9	18/9

Mean age (range) [years]	14.7 (9-18)	37.4 (26-60)	15.1 (12-18)	14.7 (7-18)	34.0 (24-56)	41.3 (28-60)
Median EDSS score (range)	-	-	1.0 (1.0-1.5)	1.0 (0.0-2.5)	1.0 (0.0-2.5)	1.5 (0.0-3.0)
Mean disease duration (range) [years]	-	-	1.4 (0.3-4.9)	4.6 (1.2-8.0)	0.2 (0.1-0.25)	5.9 (2.1-9.8)
Therapy: IFN β 1a/glatiramer acetate/mitoxantrone)	-	-	5/0/0	23/3/1	7/2/0	14/1/1

Abbreviations: CIS=clinical isolated syndrome; EDSS= Expanded Disability Status Scale,

IFN β 1a=interferon beta 1a.

At the time MRI was performed, all patients had been relapse- and steroid-free for at least one month. Local Ethical Committee approval and written informed consent from each subject were obtained prior to study initiation.

b) MRI acquisition. In all subjects, brain MRI was obtained using a 1.5 T scanner (Vision, Siemens, Erlangen, Germany). The following brain sequences were collected during a single session: a) dual-echo (DE) turbo spin-echo (TSE) (TR/TE=3300/16-8, matrix size=256x256, FOV=250x250 mm², 24 axial 5 mm-thick slices), and b) pulsed gradient spin echo (PGSE) echo planar (interecho spacing=0.8, TE=123, matrix size=128x128, FOV=250x250 mm², 10 axial 5 mm-thick slices), with diffusion gradients applied in eight non-collinear directions. For DE scans, the slices were positioned to run parallel to a line that joins the most inferoanterior and inferoposterior parts of the corpus callosum. For the PGSE scans, the same orientation as DE scans was used, with the second-last caudal slice positioned to match exactly the central slices of the other image sets. This brain portion was chosen as these central slices are less affected by the distortions due to B₀

field inhomogeneity, which can affect image co-registration. Additional information about the MR acquisition protocol is given elsewhere.[6, 7]

c) MRI analysis. T2 lesion volume (LV) of the whole brain as well as of the supra- and infra-tentorial compartments were measured using a local thresholding segmentation technique (Jim 4.0, Xinapse System, Leicester, UK). T2 lesion probability maps (LPMs) were created for each group of individuals. First, a binarized lesion mask from the segmented lesions visible on the T2-weighted images was produced. Then, using statistical parametric mapping (SPM2), the non-linear transformation between the T2-weighted images and the Montreal Neurological Institute (MNI) space was calculated and applied to lesion masks. LPMs were produced by averaging normalized T2 lesion masks (Figure 1). Finally, lesion masks were smoothed with an 8 mm³ FWHM Gaussian kernel, before their use as input for the statistical analysis.

PGSE images were first corrected for distortion induced by eddy currents using an algorithm which maximizes mutual information between the diffusion un-weighted and weighted images.[13] Then, the DT was calculated, and mean diffusivity (MD) and fractional anisotropy (FA) derived for every pixel, as previously described.[14] The diffusion images were interpolated to the same image matrix size as the T2-weighted images, and then the b=0 step of the PGSE scans were co-registered with the T2-weighted images. Using SPM2 and maximum image in-homogeneity correction,[15] brain GM, WM, and cerebrospinal fluid (CSF) were automatically segmented from T2-weighted images. Each pixel was classified as GM, WM or CSF, dependent on which mask had the greatest probability (maximum likelihood) at that location. This generated mutually exclusive masks for each tissue. **In each subjects, intracranial volume (ICV) was calculated as the sum of GM+WM+CSF maps.** The resulting masks were superimposed onto the MD and FA maps (on which hyperintense lesions were masked out previously), and the corresponding MD and FA histograms of the NAWM and GM were produced. FA histograms were derived only for the NAWM, since no preferential direction of water molecular motion is expected to occur in the GM,

due to the absence of a microstructural anisotropic organization of this tissue compartment. For each histogram, the average MD and FA were measured.

d) Statistical analysis. Voxel-wise between-group comparisons of LPMs were performed using SPM2 and an ANCOVA model, including age as a nuisance covariate ($p < 0.05$, family-wise error [FWE] corrected). Using SPSS software, an ANOVA was used to compare structural MRI quantities between: 1) pediatric and adult healthy controls; 2) pediatric healthy controls and pediatric CIS and RRMS patients; and 3) adult healthy controls and adult CIS and RRMS patients. An analysis of covariance (ANCOVA) adjusted for age and disease duration was used to compare structural MRI quantities between pediatric and adult MS patients. False Discovery Rate (FDR)[16] was applied to correct for multiple comparisons. The continuous dependent variables with a skewed distribution were log-transformed and tested for normality before ANCOVA was performed. Normality of the data was confirmed by Shapiro-Wilk test ($p > 0.05$)[17] and visual inspection after plotting the histograms. Univariate correlations between clinical findings and structural MR measures were assessed using the Spearman Rank Correlation Coefficient.

Results

All controls had normal brain MRI scans. **ICV**, whole brain, supratentorial and infratentorial LVs did not differ among patients (Table 2). To take into account head size, an analysis was also run where T2 LV was expressed as a percentage of ICV. Also this analysis did not show between-group differences of T2 LV (Table 2).

Table 2. Conventional and diffusion tensor MRI findings in the six groups of subjects studied.

	Pediatric healthy volunteers	Adult healthy volunteers	Pediatric CIS patients	Pediatric RRMS patients	Adult CIS patients	Adult RRMS patients
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T2 LV (SD) (ml)	0	0	12.6 (16.3)	9.5 (10.9)	4.8 (0.54)	11.1 (12.6)
Infratentorial LV (SD) (ml)	0	0	0.6 (1.1)	0.7 (1.4)	0.3 (0.7)	0.5 (0.8)
ICV (SD) (ml)	1361 (149)	1402 (90)	1435 (148)	1380 (92)	1412 (109)	1398 (106)
T2 LV/ICV % (SD)	-	-	1.0 (1.0)	0.6 (0.9)	0.3 (0.6)	0.7 (1.0)
Average lesion FA (SD)	-	-	0.26 (0.03)	0.26 (0.03)	0.28 (0.04)	0.25 (0.03)
Average lesion MD (SD)	-	-	1.06 (0.01)	1.03 (0.08)	1.07 (0.01)	1.18 (0.01)
NAWM average FA (SD)	0.30 (0.01)	0.30 (0.01)	0.27 (0.02)	0.26 (0.02)	0.28 (0.02)	0.27 (0.03)
NAWM average MD (SD)	0.83 (0.02)	0.81 (0.03)	0.84 (0.02)	0.86 (0.04)	0.82 (0.03)	0.86 (0.07)
GM average MD (SD)	0.98 (0.02)	0.98 (0.04)	0.98 (0.04)	1.00 (0.05)	0.99 (0.03)	1.06 (0.08)

Abbreviations: CIS=clinical isolated syndrome; LV=lesion volume; NAWM=normal appearing white matter; FA=fractional anisotropy; SD=standard deviation; MD=mean diffusivity; GM=gray matter; ICV=intracranial volume.

Note: average MD is expressed in units of $\text{mm}^2\text{s}^{-1} \times 10^{-3}$, FA is a dimensionless index. For statistical analysis: see the text.

Figure 1 shows T2 lesion distribution in the four groups of patients, separately. In all groups, T2-visible lesions were mostly located in the corpus callosum, periventricular, and juxtacortical areas. The between-group comparisons of lesion frequency, showed that in adult RRMS patients lesions were more frequently located in the posterior periventricular WM than in pediatric RRMS patients and in adult CIS patients ($p < 0.05$, FWE corrected) (Figure 2). No difference was found between pediatric CIS patients and the remaining groups. **No interaction was found between the factor pediatric/adult and the factor CIS/RRMS.**

In Table 2, DT MRI quantities from the six groups of subjects are reported. NAWM and GM DT MRI measures did not differ between pediatric and adult healthy controls (Table 2).

The comparison of DT MRI quantities between pediatric healthy controls and pediatric MS patients showed a significant between-group difference of NAWM average FA ($p = 0.004$). At post-hoc analysis, no difference was detected between pediatric healthy controls and pediatric CIS patients, while pediatric RRMS patients had a significantly reduced NAWM average FA ($p = 0.003$) compared to pediatric healthy controls.

The comparison of DT MRI quantities between adult healthy controls and adult MS patients showed a significant between-group difference of NAWM average FA ($p = 0.003$), NAWM average MD ($p = 0.003$), and GM average MD ($p < 0.0001$). At post-hoc analysis, no difference was detected between adult healthy controls and adult CIS patients (this difference was significant only when the correction for multiple comparisons was not applied; data not shown). Compared to adult healthy controls, adult RRMS patients had significantly reduced NAWM average FA ($p = 0.002$), and increased NAWM ($p = 0.01$) and GM ($p < 0.0001$) average MD. Compared to adult CIS patients, adult RRMS patients had significantly decreased average lesion FA ($p < 0.0001$), and increased average lesion MD ($p = 0.006$), NAWM average MD ($p = 0.008$), and GM average MD ($p < 0.0001$).

The comparison of DT MRI findings between the four groups of patients, corrected for age and disease duration, showed significant between-group differences of average lesion FA

($p=0.001$), average lesion MD ($p<0.0001$), NAWM average MD (0.02) and GM average MD ($p<0.0001$). At post-hoc analysis, no difference was detected between pediatric and adult CIS patients, whereas adult RRMS patients had significantly higher lesion average MD ($p<0.0001$) and GM average MD ($p=0.009$) in comparison with pediatric RRMS ones.

In the whole group of MS patients, significant correlations were found between:

- EDSS vs. age at onset of the disease ($r=0.31$, $p=0.006$), disease duration ($r=0.29$, $p=0.002$), and T2 LV ($r=0.31$, $p=0.001$);

- T2 LV vs. average lesion FA ($r=-0.40$, $p<0.0001$), average lesion MD ($r=0.30$, $p=0.003$), NAWM average FA ($r=-0.44$, $p<0.0001$), NAWM average MD ($r=0.52$, $p<0.0001$), and GM average MD ($r=0.44$, $p<0.0001$),

- average lesion MD and GM average MD ($r=0.34$, $p<0.0001$).

When the analysis of correlations was re-run in the two groups of adult and pediatric patients, separately, all the correlations with T2 LV (including EDSS) remained significant in the adult cohort, while the correlation with GM average MD was not found in the pediatric cohort (data not shown). Similarly, the correlation between average lesion MD and GM average MD remained significant in adult patients only ($r=0.40$, $p=0.002$).

Discussion

In this study, we wished to characterize the nature and topographical distribution of T2 lesions, as well as the extent of NAWM and GM damage in patients with pediatric MS in comparison with adult patients at similar disease stages.

Previous studies suggested that the extent and distribution of T2-visible lesions differ between pediatric and adult MS patients. In particular, pediatric CIS patients less frequently than adult CIS patients meet the MRI criteria for disease dissemination in space,[18] and show a higher involvement of the posterior fossa,[4, 5] especially the pons.[4] These studies recruited exclusively patients with an initial demyelinating event, within a few months from the onset of the disease, who

were studied with conventional MRI only. As a consequence, they were unable to rate the severity of intrinsic lesion damage and to provide a picture of lesion distribution in patients with established MS, likely more representative of the general pediatric MS population.

In our study, the T2 lesion burdens of the whole brain and the supra- and infratentorial compartments, separately, were not significantly different between the two cohorts of pediatric MS patients (CIS and RRMS) as well as between them and the corresponding adult groups. However, the analysis of the regional patterns of lesion frequency revealed a higher occurrence of lesions in the posterior periventricular regions in adult patients with RRMS in comparison with adult CIS and pediatric RRMS patients. Remarkably, we did not find any difference between pediatric patients with CIS and those with RRMS, or between pediatric and adult CIS patients. Several factors might contribute to explain the discrepancy between our findings and those of previous studies.[4, 5] First, our pediatric patients were “older” than those of the previous two studies. This might have had an impact on our and previous data since timing of myelin maturation has been considered as a possible explanation of the selective infratentorial involvement in pediatric CIS patients. Second, the time elapsed between the onset of the clinical event in pediatric CIS patients and the acquisition of the MRI scan was more than three months in our study *vs.* less than 3 months in the study of Waubant et al.[5] Since a significant resolution of the initial T2 lesions has been shown to occur in pediatric CIS patients, it is likely that some lesions possibly present at onset were not anymore detectable in our cohort. Finally, all our patients were studied using the same scanner and acquisition protocol, while those of the previous two studies were scanned at different centers and with different sequences.[4, 5, 19] Variability of scanners and sequences might have influenced lesion identification and quantification and their distribution on LPMs.[4, 5, 19] Clearly, considering the relatively small number of pediatric CIS patients we recruited, we can not completely rule out that our study was underpowered for detecting any significant difference between pediatric CIS patients and the remaining groups. Nevertheless, the demonstration that adult RRMS patients have a higher occurrence of lesions in the posterior periventricular WM, where

several “clinically eloquent” pathways run, and the demonstration that infratentorial structures are similarly involved in all patients (Figure 1) fit with the notion of a favourable disease outcome in this latter group.

We also quantified the extent of microscopic damage in lesions, NAWM and GM by means of DT MRI, which has been shown to be sensitive to disease-related changes both in adult and pediatric MS patients.[6-8] Noteworthy, in a preliminary study,[7] which included part of the pediatric patients of the present analysis, we did not find DT MRI changes in the brain GM of pediatric RRMS patients when contrasted to matched healthy controls. The previous study was limited by the small sample of pediatric patients enrolled[7] and by the fact that pediatric MS findings were not compared with those from adult patients. The present results are, therefore, important, since they provide a compelling evidence for a sparing of brain GM in pediatric-onset MS. Indeed, we found no GM abnormalities in both CIS and RRMS patients in comparison with healthy controls. In addition, GM average MD was significantly higher in adult RRMS patients in comparison with the corresponding pediatric group. Diffuse damage to the brain GM has been shown virtually in all adult MS patients with different disease courses.[14, 20-23] The relative preservation of GM integrity in pediatric MS is in line with the recent demonstration, using voxel-based morphometry, of a selective atrophy of the thalamus in these patients, with sparing of the cortical GM and of the remaining deep GM nuclei.[24] It also fits with the results of a functional MRI study of the motor system in pediatric MS, which showed a maintenance of a selective and strictly lateralized pattern of brain activations, thus supporting the notion of a preserved functional reserve in pediatric MS that might be the result of a structural GM integrity.[25] Recently, the introduction of double inversion recovery (DIR) sequences has allowed to demonstrate macroscopic GM lesions in the majority of the adult MS phenotypes.[26-28] These GM lesions have been related to the accumulation of widespread GM damage, in terms of atrophy, and to the development of irreversible disability.[28, 29] Unfortunately, we did not acquire DIR sequences in our patients, as a consequence we can not exclude that a reduced amount of GM macroscopic lesions might be

among the factors responsible for the different GM DT MRI findings between our pediatric and adult cohorts.

Another intriguing finding of this study derives from the analysis of NAWM involvement, which showed no DT MRI changes in the CIS cohorts (independently of the age at onset), and a similar extent of DT MRI changes (reduction of FA and increase of MD) in the RRMS forms of the disease. The finding of a relatively mild NAWM involvement in CIS patients agrees with the results of previous studies in adult patients, which demonstrated absent or only subtle NAWM abnormalities.[30, 31] The correlation found between NAWM DT MRI abnormalities and the extent of T2-visible lesions suggests that Wallerian degeneration of axons passing through focal, macroscopic lesions is likely to be one of the factors contributing to NAWM damage in this disease. Finally, we also quantified the extent of intrinsic damage to T2-visible lesions using DT MRI. Such an analysis showed a higher lesion average MD in adult than in pediatric RRMS patients, which might be a reflection of a better capability of the “young” brain to react to the inflammatory process through a more efficient tissue repair. Remarkably, in the whole cohort of patients and in the adult cohort separately, average lesion MD was correlated with MD changes in the GM, while such a correlation was not found in pediatric patients. These results suggest that the severity of damage of macroscopic T2-visible lesions might be an additional factor contributing to the observed GM changes in adult MS patients in comparison with the pediatric group.

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Figure legends

Figure 1. Representative slices showing lesion probability maps, superimposed on a T1-weighted scan in the standard Montreal Neurological Institute (MNI), of pediatric patients with clinically isolated syndromes (CIS) suggestive of MS (A), pediatric patients with relapsing remitting (RR) MS (B), adult patients with CIS suggestive of MS (C), and adult patients with RRMS (D). The frequency of occurrence of brain lesion in each group (%) is colour coded scale.

Figure 2. Areas of significant increased lesion occurrence in adult patients with relapsing remitting (RR) MS compared to adult patients with clinically isolated syndromes (CIS) suggestive of MS (A) and to pediatric patients with RRMS (B) superimposed on the glass brain ($p < 0.05$, corrected for multiple comparisons).

Pediatric CIS

Pediatric RRMIS

Adult CIS

Adult RRMIS

Adult RRMS vs. Adult CIS

Glass brain

Adult RRMS vs. Pediatric RRMS

Glass brain