RESEARCH ARTICLE

Association of Choroid Plexus Inflammation on MRI With Clinical Disability Progression Over 5 Years in Patients With Multiple Sclerosis

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Neurology® 2023;100:e911-e920. doi:10.1212/WNL.0000000000201608

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Abstract

Background and Objectives

Inflammation of the choroid plexus (CP) has been reported in multiple sclerosis (MS). The AU1 association between CP inflammation and clinical disability progression is still under debate. The objective of the current study was to assess the relationship between measures of CP inflammation and investigate their associations with clinical disability progression in MS.

Methods

In this retrospective analysis of a longitudinal study, 174 patients with MS (118 with relapsing-remitting MS and 56 with progressive MS [PMS]) and 56 healthy controls (HCs), group matched for age and sex, were imaged on a 3T MRI scanner at baseline and after an average of 5.5 years of follow-up. T2 lesion volume (T2-LV) was assessed. Regional tissue volumes were calculated. CP volume was measured, and pseudo-T2 (pT2) mapping was performed to asses CP inflammation. Group comparisons and correlations were adjusted for age and sex.

Results

Patients with MS presented with significantly larger CP volume ($p = 0.01$) and increased CP pT2 (<0.001) at baseline, when compared with HCs. CP volume and CP pT2 did not significantly increase over the follow-up in the MS sample. However, baseline CP pT2 was associated with clinical disability progression at follow-up ($p = 0.001$), even after controlling for all other factors significantly associated with disability progression ($p = 0.030$), including T2-LV, normalized brain volume, normalized gray matter volume, and normalized thalamic volumes. Changes in CP volume and CP pT2 were not related to changes in clinical parameters such as relapse rate over the course of the follow-up.

Discussion

CP inflammation, as evidenced by MRI, is clinically relevant in MS. CP inflammation may have a relevant role in driving disease progression.

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Glossary

ANCOVA = analysis of covariance; **CP** = choroid plexus; **EDSS** = Expanded Disability Status Scale; **ETL** = echo train length; **GM** = gray matter; **HC** = healthy control; **LPM** = lesion probability mapping; **MS** = multiple sclerosis; **NBV** = normalized brain volume; **NCPV** = normalized choroid plexus volume; **NGMV** = normalized gray matter volume; **NWMV** = normalized white matter volume; **NVV** = normalized ventricular volume; **PMS** = progressive MS; **PPMS** = primary progressive multiple sclerosis; **RRMS** = relapsing-remitting MS; **SPMS** = secondary progressive MS; **TE** = echo time; **TI** = inversion time; **TR** = repetition time; **VBM** = voxel-based morphometry; **WM** = white matter.

Multiple sclerosis (MS) is an immune-mediated disease of the CNS, leading to widespread damage of both the white matter (WM) and the gray matter (GM). It is well-established that immune cell trafficking into the CNS plays a key role in this regard. Leukocyte entry is facilitated by periodic disruptions of the blood-brain barrier[1] that in part characterize the disease. Recent evidence though has highlighted the role that the choroid plexus (CP) may play as well.[2,3] The CP is located within the lateral ventricles of the brain and is the primary producer of CSF. The CP also serves as a critical interface between the CSF and the blood, regulating the ingress of immune cells into the CNS.[4] In the healthy brain, this regulation is part of routine immune surveillance.[5] However, a number of recent studies have highlighted abnormal involvement of the CP in MS pathology.[2,3,6-8] For example, Ricigliano et al.[8] showed an association between active inflammation, as reflected by the presence of contrast-enhancing lesions in the brain tissue, and an enlarged CP, as reflected by greater volume as measured on MRI. Moreover, greater CP volume was also found in patients with MS compared with both healthy controls (HCs) and patients with neuromyelitis optica spectrum disorder, highlighting that CP enlargement may be specifically related to inflammatory processes in MS.[7]

As of now, relatively little is known about the clinical correlates of CP inflammation. In a cross-sectional study, it was shown that CP volume is increased in patients with MS with a history of active disease, as reflected by at least 1 relapse in the previous 2 years.[8] Increased CP volume was hypothesized to reflect CP inflammation, as corroborated by greater CP[18] F-DPA-714 uptake in the CP of patients with MS compared with HCs.[8] The same study did not find an association between CP volume and clinical disability, as measured by the Expanded Disability Status Scale (EDSS), while a study by Fleischer et al.[4] found that cross-sectional CP volume was associated with EDSS scores both cross-sectionally and with EDSS change over time.

With this background, we aimed to assess the relationship between measures of CP inflammation and investigate their associations with clinical disability progression in MS. We hypothesized that CP volume would be greater in patients with MS compared with controls, reflecting inflammation of the CP. We also investigated CP microstructure in vivo using T2 mapping, under the assumption that longer T2 times would reflect greater inflammation;[9] in an inflammatory milieu, increased water content results in T2 prolongation.[10] Furthermore, we aimed to assess the relationship between CP inflammatory markers and other commonly investigated neuroimaging measures in MS. Exploratory analyses were also conducted by separately investigating different MS phenotypes because it is known that neuroinflammatory properties change as the disease progresses, with later stages characterized by inflammation that occurs behind a relatively intact blood-brain barrier.[11] Moreover, we sought to establish how CP volume and CP T2 measures evolve over mid-term follow-up because longitudinal imaging studies are currently lacking in the literature. We hypothesized that the CP would be characterized by a greater increase in inflammatory degree in patients with MS over the follow-up, as reflected by a sharper rate of increase in volume and T2 times. Finally, we hypothesized that quantitative measures of the CP at baseline and over the follow-up would be associated with clinical disability progression.

Methods

Participants

This study was a retrospective analysis of a larger prospective study investigating cerebrovascular, environmental, and genetic associations in the MS study.[12] The inclusion criteria were (1) age between 18 and 75 years and (2) diagnosed as MS, based on the 2010 McDonald criteria. At the time of MRI acquisition, patients with MS were relapse-free and steroid-free within the last 30 days. Clinical disability in patients was quantified through EDSS. In addition, a group of HCs, group matched for age and sex, were included and had to have a normal neurologic examination without a history of neurologic or psychiatric disorders. Participants were included in this subanalysis if their imaging data were free from artifacts that would have otherwise interfered with the postprocessing analysis (e.g., subject movement). The study flowchart is shown in eFigure 1 (links.lww.com/WNL/C528).

Patients and HCs returned for follow-up after an average of 5.5 ± 0.6 years for both imaging and neurologic assessments. At follow-up, disease progression was defined as having an EDSS change of ≥1.5 if baseline EDSS was <1.0, an EDSS change of ≥1.0 if baseline EDSS was 1.0–5.5, and an EDSS change of ≥0.5 if baseline EDSS ≥5.5, as previously reported.[13,14] Stable/improved was defined as those who did not meet the above criteria.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the local Institutional Review Board at the University at Buffalo, and written informed consent was obtained from all participants.

MRI Acquisition

All scans at both baseline and follow-up were acquired on the same 3T GE Signa Excite HD 12.0 MRI scanner (GE, Milwaukee, WI) with an 8-channel head and neck coil. The imaging system did not undergo any major hardware or software upgrades during the study. The imaging protocol included that a 2D T2-weighted fluid-attenuated inversion recovery (FLAIR) was acquired with echo time (TE)/inversion time (TI)/repetition time (TR) = 120/2,100/8,500 ms, flip angle = 90°, echo train length (ETL) = 24; 2D fast spin echo dual-echo proton density-weighted/T2-weighted acquisition was also acquired before gadolinium injection with TE1/TE2/TR = 10/90/3000 ms, flip angle = 90°, ETL = 14, and a pair of 2D T1-weighted spin echo sequences was acquired before and after administration of gadolinium with TE/TR = 9/600 ms, flip angle = 90°. The aforementioned sequences were all acquired with a 256 × 192 matrix (frequency × phase) and FOV = 256 × 192 mm^2 and 48 3 mm thick slices without gap, for a nominal resolution of 1 × 1 × 3 mm^3. In addition, a magnetization-prepared 3D T1-weighted fast spoiled gradient echo sequence was acquired without gadolinium contrast with TE/TI/TR = 2.8/900/5.9 ms, flip angle = 10°, with a 256 × 256 × 180 matrix and 1-mm isotropic resolution.

Image Processing

T2 lesion volume and contrast-enhancing lesion volume were calculated using a semiautomated contouring/thresholding technique, as previously described.[15] The 3D T1-weighted images were then lesion filled (using the FMRIB's Software Library [FSL] tool lesion_filling) to reduce the effect of T1 hypointensities on subsequent tissue segmentation.[16] Measures of normalized brain volume (NBV), normalized GM volume (NGMV), normalized WM volume (NWMV), and normalized ventricular volume (NVV), all normalized for head size, were then calculated using SIENAX.[17] The same lesion filled image was then processed with the FIRST tool[18] to obtain absolute volumes of the thalamus and the putamen, which were multiplied by the SIENAX-derived scaling factor to obtain normalized measures (NThalV and NPutV, respectively). The image was then processed with the recon-all stream from the FreeSurfer software package to obtain an initial segmentation of the CP.[19] The CP segmentation was subsequently refined using a Gaussian Mixture Model method, as previously described, which is much more accurate than that obtained directly from FreeSurfer.[20] CP segmentations were reviewed and manually corrected, as needed while remaining blinded to all clinical data. Nearly all segmentations required the removal of small clusters of incorrectly classified voxels. CP volume was then multiplied by the SIENAX-derived scaling factor to obtain normalized CP volume (NCPV).

As our imaging protocol did not include a dedicated acquisition for T2 mapping for quantifying inflammation (e.g., multiecho spin echo with several echoes), we calculated pseudo-T2 (pT2) maps of the whole brain as previously described.[21,22] Briefly, the following equation was used:

$$(TE2 - TE1)/\ln(S1/S2)$$

where S1 and S2 are the measured image intensities at each echo time, TE1 and TE2. The PD-weighted image was registered to the 3D T1-weighted image using FMRIB's Linear Image Registration Tool with a rigid body model (i.e., 6 degrees of freedom), and the resulting registration matrix was used to bring along the pT2 map. Finally, the mean pT2 value of the CP was measured.

Longitudinal changes in NBV and NVV were calculated using SIENA[17] and the VIENA extension,[23] respectively, while changes in NGMV and NWMV were obtained using the SIENAX multitimepoint method.[24] Changes in other measures were derived from cross-sectional measures obtained at baseline and follow-up.

Statistical Analysis

Differences in demographic characteristics between the groups were assessed using the Student *t*-test and chi-squared, as appropriate. For statistical analyses, a cube root transform was applied to T2 lesion volume to reduce skew. Associations between baseline and follow-up CP measures were assessed using Pearson correlations. Comparisons between baseline imaging measures were made using univariate analysis of covariance (ANCOVA) models, adjusting for age and sex. Similarly, partial correlations, adjusted for age and sex, were used to assess the relationship between CP measures and other tissue measures separately in HC and MS groups as well as between patients with relapsing-remitting MS (RRMS) and progressive multiple sclerosis (PMS). Correlation analyses were corrected for the false discovery rate using the Benjamini-Hochberg method. Baseline CP measures were compared between patients with gadolinium-enhancing lesions and those without as well as those with disability progression vs those who remained stabled or improved, while adjusting for age and sex. In addition, univariate ANCOVA models, controlling for age and sex, were used to evaluate the evolution of CP measures over the course of the follow-up, with either HC vs patient with MS or stable/improved vs progressed as the fixed factor. All analyses were performed using IBM SPSS Statistics for Windows (version 27.0. Armonk, NY: IBM Corp.) with *p*-values ≤0.05 considered significant.

Finally, voxel-wise analyses were performed to investigate the association between CP assessments and lesion location (that is, lesion probability mapping [LPM]) as well as GM volume (that is, voxel-based morphometry [VBM]) at baseline and changes over the course of the follow-up. A longitudinal, study-specific template was first constructed as previously described.[25] Briefly, subject-specific templates were first constructed from the baseline and follow-up lesion-filled 3D T1-weighted images using Advanced Normalization Tools.[26] Next, a study-specific template was created from the individual subject templates. Baseline lesion masks and GM

probability maps as well as their changes over time were then brought into template space. General linear models were constructed, and statistical inference was performed with the randomise tool[27] from FSL using threshold-free cluster enhancement[28] with 5,000 permutations for each test and age, sex, and SIENAX-derived scaling factor (to control for head size) entered as nuisance covariates. Statistical significance was set at a family-wise error corrected p-value ≤0.025 to control the error rate across statistical contrasts.

Data Availability

All data are available on reasonable request by qualified researchers by contacting the corresponding author (NB).

Results

Demographic and Clinical Characteristics

For the current substudy, 174 patients with MS were included, consisting of 118 RRMS, 46 with secondary progressive MS (SPMS), and 10 with primary PMS (PPMS) along with 56 HCs. Table 1 provides an overview of the demographic and clinical characteristics of the enrolled participants. HC and patients with MS were not different at the group level in age or sex. Approximately 68% of the MS sample consisted of patients with RRMS. As there were only 10 patients with PPMS in our cohort, they were merged with SPMS into a single PMS group for subsequent analyses. At follow-up, 20 patients were missing EDSS assessments and could not be classified about disease progression, and were not used in the longitudinal analyses of CP and clinical outcomes. Of the remaining participants with MS, 55 progressed in their disease, while 99 remained either stable or improved in their EDSS scores. Thirteen patients with RRMS converted to SPMS over the course of the follow-up. The percentage of patients progressing in their disease was relatively similar between patients with RRMS (32.7%) and PMS (42.0%) (p = 0.284).

MR Imaging Characteristics at Baseline

Table 2 provides an overview of the MR imaging comparisons between the HCs and the patients with MS as well as between patients with RRMS and PMS. Compared with HCs, the MS group presented with significantly lower NBV, NGMV, NTalV, NPutV, and WMV (all $p \leq 0.001$) as well as significantly increased NVV and T2 LV (both $p < 0.001$) as well as NCPV (p = 0.024). NCPV differences were no longer significant after additionally controlling for NBV (p = 0.524) or NVV (p = 0.248). pT2 was significantly greater in patients with MS at $p < 0.001$, even after correcting for NCPV as well. Compared with patients with RRMS, patients with PMS presented with significantly lower NBV, NWMV, and NThalV (all $p \leq 0.005$). Other measures, including NCPV and pT2, were not significantly different. As expected, patients with RRMS were significantly more likely to present with contrast-enhancing lesions compared with their PPMS

Table 1 Demographics of the Study Participants

	HC (n = 56)	MS (n = 174)	*p* Value	RRMS (n = 118)	PMS (n = 56)	*p* Value
Age at baseline in years, mean (SD)	45.4 (13.7)	47.5 (10.8)	0.293	44.4 (10.9)	54.0 (7.2)	<0.001
Sex, female, N (%)	39 (69.6)	129 (74.1)	0.495	83 (70.3)	46 (82.1)	0.137
Years of education at follow-up in years, mean (SD)	14.5 (2.5)	14.7 (2.9)	0.662	14.6 (2.6)	15.0 (3.5)	0.542
Follow-up period, mean (SD)	5.5 (0.5)	5.5 (0.6)	0.846	5.4 (0.6)	5. 5(0.5)	0.378
Disease duration at baseline—mean in years (SD)	—	15.2 (10.0)	—	12.6 (8.3)	21.0 (10.7)	<0.001
Relapse rate, mean (SD)	—	0.2 (0.4)	—	0.2(0.4)	0.1 (0.2)	0.036
EDSS at baseline—median (interquartile range)	—	2.5 (1.5–5.5)	—	2.0 (1.5–2.6)	6.0 (4.0–6.5)	<0.001
EDSS at follow-up—median (interquartile range)	—	3.5 (2.0–6.0)	—	2.5 (1.5–3.5)	6.5 (4.5–6.6)	<0.001
EDSS absolute change—mean (SD range)	—	0.4 (1.0)	—	0.4(1.0)	0.5 (0.5)	0.941
DMT use at baseline, n (%)						
Interferon β	—	74 (42.5)	—	49 (41.5)	25 (44.6)	0.021
Glatiramer acetate	—	36 (20.7)	—	21 (17.8)	15 (26.8)	
Natalizumab	—	27 (15.5)	—	24 (20.3)	3 (5.4)	
Off-label medications	—	9 (5.2)	—	3 (2.5)	6 (10.7)	
No DMT	—	28 (16.1)	—	18 (15.3)	10 (17.9)	

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; HC = healthy controls; MS = relapsing-remitting multiple sclerosis; n = number.
Student *t*-test, Mann-Whitney *U* test, and Fisher exact test were used as appropriate.

Table 2 MR Imaging Characteristics at Baseline of Healthy Controls and Patients With Multiple Sclerosis

	HC (n = 56)	MS (n = 174)	*p* Value	Partial η^2	RRMS (n = 118)	PMS (n = 56)	*p* Value	Partial η^2
Presence of gadolinium-enhancing lesions, N (%)	—	21 (13.0%)[a]			20[b] (18.0%)	1[c] (2.0%)	0.004	—
T2 lesion volume (mL)	0.3 (0.8)	16.1 (19.5)	<0.001	0.475	12.7 (18.0)	23.2 (20.6)	0.001	0.060
NBV (mL)	1,529.2 (87.8)	1,455.1 (93.5)	<0.001	0.109	1,477.7 (89.1)	1,407.5 (85.0)	0.005	0.046
NGMV (mL)	776.5 (53.9)	734.8 (64.8)	<0.001	0.079	744.9 (67.9)	713.5 (51.9)	0.650	0.001
NThalV (mL)	20.9 (1.8)	18.6 (2.7)	<0.001	0.134	19.3 (2.5)	17.3 (2.4)	0.001	0.065
NPutV (mL)	12.9 (1.1)	12.0 (1.7)	<0.001	0.061	12.2 (1.7)	11.4 (1.5)	0.105	0.015
NWMV (mL)	752.6 (44.7)	720.4 (59.9)	0.001	0.051	732.9 (61.3)	694 (47.4)	<0.001	0.075
NVV (mL)	35.3 (15.0)	52.5 (25.0)	<0.001	0.090	48.0 (22.7)	60.0 (28.2)	0.134	0.013
NCPV (mL)	2.3 (0.9)	2.6 (0.9)	0.024	0.022	2.6 (0.9)	2.8 (0.9)	0.995	<0.001
CP pT2 (milliseconds)	636.3 (498.3)	1,015.5 (454.5)	<0.001	0.108	956 (434.8)	1,128.8 (474.1)	0.369	0.006

Abbreviations: ANCOVA = analysis of covariance; CP tT2 = choroid plexus pseudo-T2; HC = healthy controls; MS = relapsing-remitting multiple sclerosis; n = number; NBV = normalized brain volume; NGMV = normalized gray matter volume; NThalV = normalized thalamic volume; NPutV = normalized putaminal volume; NWMV = normalized white matter volume; NVV = normalized ventricular volume; NCPV = normalized choroid plexus volume; PMS = progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis.
Differences between the groups were assessed using ANCOVA models, adjusting for age and sex. Cells represent mean (standard deviation).
[a] Gadolinium was not administered to 12 patients.
[b] Gadolinium was not administered to 7 patients with RRMS.
[c] Gadolinium was not administered to 5 patients with PMS.

counterparts (20 vs 1, $p = 0.004$). There were no differences in NCPV nor CP pT2 between patients with or without contrast-enhancing lesions ($p = 0.957$ and $p = 0.340$, respectively).

Associations With Choroid Plexus Measures in HCs and Patients With MS at Baseline

In the HC sample, the only association with NCPV was for NVV, while no associations were detected for CP pT2. No voxel-wise associations were detected in the HC sample for either NCPV or CP pT2. In the MS sample; however, widespread correlations were detected for both NCPV and CP pT2, with the strongest correlations for both being with NVV ($r = 0.635$ and $r = 0.341$, respectively). Full details are reported in Table 3. LPM analysis revealed associations primarily with periventricular WM lesions for both NCPV and CP pT2 (Figure 1, eFigure 2 and eFigure 3, links.lww.com/WNL/C528). In GM volume, NCPV was largely linked to midline structural and insular volumes (Figure 2, eFigure 4), while CP pT2 showed widespread associations throughout the entire cortex (Figure 2 and eFigure 5).

Associations With Choroid Plexus Measures in Patients With RRMS and PMS at Baseline

Correlations with NCPV in the RRMS sample were largely consistent with those found in the MS sample as a whole (Table 3). No voxel-wise associations were detected between either CP measure and T2 lesion location in LPM analyses (Figure 1). VBM analyses revealed a more restricted pattern of GM volumes linked to NCPV compared with the cohort as a whole, with the main clusters linked to the anterior cingulate gyrus and insular cortex (Figure 2, eFigure 6, links.lww.com/WNL/C528). Associations with CP pT2 were generally stronger in patients with RRMS than those seen in the combined MS sample, with concordant results seen for the voxel-wise analyses with GM volume (Figure 2 and eFigure 7).

When considering only the PMS group, NCPV was more strongly correlated with all of the investigated measures compared with as in the MS group as a whole (Table 3). LPM analysis revealed a more widespread pattern of periventricular lesions being associated with NCPV (Figure 1 and eFigure 8, links.lww.com/WNL/C528). However, unlike that which was seen in the RRMS sample, the VBM analysis resulted in a limited number of small clusters of voxels (Figure 2, eFigure 9). CP pT2 measures were not associated with other imaging measures in the PMS group for neither summary correlation analyses (Table 3) nor voxel-wise analyses (Figures 1 and 2).

Longitudinal Evolution of Imaging Measures

NCPV at follow-up was moderately correlated with baseline NCPV ($r = 0.674$, $p < 0.001$), while CP pT2 at follow-up was more weakly associated with the corresponding baseline measure ($r = 0.543$, $p < 0.001$). The correlation between percent changes of the 2 measures was not significant ($r = 0.028$, $p = 0.726$).

The rate of increase in NCPV was similar between HCs and patients with MS (10.3% vs 7.7%, $p = 0.602$). However, CP pT2 showed a significantly greater percent increase over the follow-up in HCs compared with patients with MS (81.1% vs 24.5%, $p < 0.001$). No other regional measures progressed at significantly different rates between the 2 groups. No

Table 3 Partial Correlations, Adjusted for Age and Sex, Showing the Association Between Choroid Plexus Measures and Other Imaging Measures at Baseline

	HC		MS		RRMS		PMS	
	NCPV	CP pT2	NCPV	CP pT2	NCPV	CP pT2	NCPV	CP pT2
T2 lesion volume	0.002 (0.990)	0.062 (0.782)	**0.322 (<0.001)**	**0.255 (0.005)**	0.222 (0.056)	**0.275 (0.015)**	**0.547 (<0.001)**	0.224 (0.199)
NBV	−0.171 (0.351)	−0.032 (0.896)	**−0.333 (<0.001)**	−0.165 (0.082)	**−0.270 (0.017)**	**−0.278 (0.013)**	**−0.454 (0.003)**	0.052 (0.812)
NGMV	−0.106 (0.588)	−0.014 (0.945)	**−0.282 (0.002)**	**−0.231 (0.012)**	−0.204 (0.082)	**−0.418 (<0.001)**	**−0.463 (0.003)**	0.187 (0.301)
NThalV	−0.204 (0.269)	−0.125 (0.541)	**−0.461 (<0.001)**	**−0.224 (0.013)**	**−0.394 (<0.001)**	**−0.327 (0.003)**	**−0.607 (<0.001)**	0.021 (0.936)
NPutV	−0.106 (0.588)	−0.085 (0.682)	**−0.405 (<0.001)**	−0.157 (0.097)	**−0.369 (<0.001)**	**−0.309 (0.005)**	**−0.476 (0.002)**	0.119 (0.541)
NWMV	−0.184 (0.326)	−0.040 (0.869)	**−0.200 (0.030)**	−0.009 (0.945)	−0.155 (0.210)	0.049 (0.744)	**−0.320 (0.049)**	−0.103 (0.588)
NVV	**0.659 (<0.001)**	0.231 (0.332)	**0.635 (<0.001)**	**0.341 (0.001)**	**0.577 (<0.001)**	**0.481 (<0.001)**	**0.735 (<0.001)**	0.168 (0.349)

Abbreviations: CP tT2 = choroid plexus pseudo-T2; HC = healthy controls; MS = relapsing-remitting multiple sclerosis; NCPV = normalized choroid plexus volume; NBV = normalized brain volume; NGMV = normalized gray matter volume; NThalV = normalized thalamic volume; NPutV = normalized putaminal volume; NWMV = normalized white matter volume; NVV = normalized ventricular volume; PMS = progressive multiple sclerosis; RRMS = relapsing-remitting multiple sclerosis.
Cells represent the partial correlation value, corrected for age and sex, with false discovery rate-corrected *p*-values in parentheses and those <0.05 in bold.

significant differences were detected between patients with RRMS and SPMS. Full results are summarized in eTable 1 (links.lww.com/WNL/C528).

No associations were found between changes in NCPV and CP pT2 with changes in other imaging measures. Full results are summarized in eTable 2 (links.lww.com/WNL/C528). No voxel-wise associations were detected.

Associations With Clinical Parameters

At baseline, EDSS was correlated with both NCPV (r = 0.185, p = 0.019) and CP pT2 (r = 0.267, p = 0.002) but were no longer significant after correcting for age and sex. When considering patients who progressed in their disability status over the follow-up compared with those who remained stable or improved, baseline NCPV was not significantly different between the groups (p = 0.365). However, baseline CP pT2 was significantly greater in patients who progressed at p = 0.001 with the largest effect size out of all investigated measures (Table 4). CP pT2 remained significant at p = 0.030 even after controlling for all other measures that were significantly different between the 2 groups (i.e., T2 lesion volume, NBV, NGMV, and NThalV).

When considering patients who progressed in their disability status over the follow-up compared with those who remained stable or improved, change in NCPV was not significantly different between the groups (7.1% vs 8.4%, p = 0.887). However, change in CP pT2 was significantly lower in those

Figure 1 Orthogonal Views of the Lesion Probability Mapping (LPM) Analysis Showing the Association Between Lesion Location and Either Choroid Plexus Volume or Choroid Plexus pT2 Measures

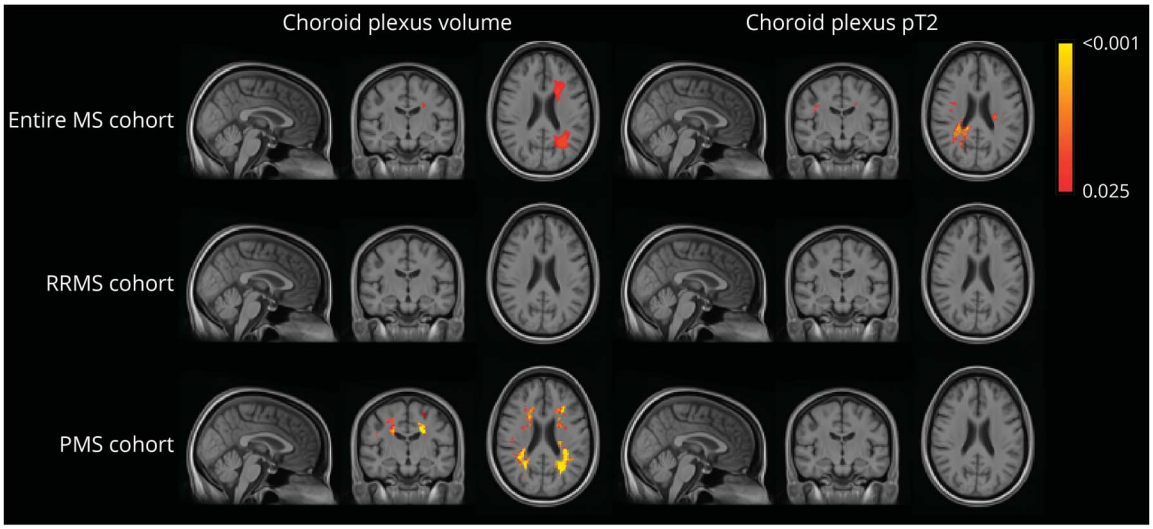
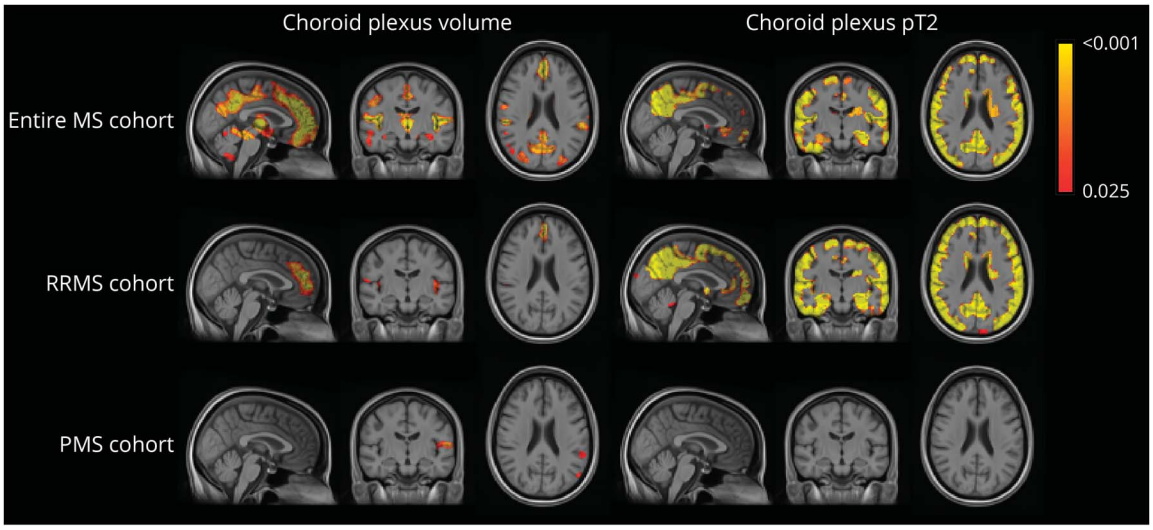


Figure 2 Orthogonal Views of the Voxel-Based Morphometry (VBM) Analysis Showing the Association Between Lesion Location and Either Choroid Plexus Volume or Choroid Plexus pT2 Measures



who progressed compared with those who remained stable or improved (3% vs 39%, $p = 0.011$).

Changes in CP parameters over the course of the follow-up were not associated with the number of relapses during the same time frame ($r = -0.168$, $p = 0.065$ and $r = -0.077$, $p = 0.402$ for percent changes in NCPV and pT2, respectively).

Discussion

In this study, we segmented the CP from 3D T1-weighted MRI images in a fairly large cohort of patients with MS and HCs. We found that CP pT2, a putative marker of inflammation in the CP, is highly associated with future disease progression. Our results extend previous imaging findings of CP involvement in MS[4,8] but also raise further questions. In contrast to our initial hypothesis, we did not find evidence of a greater rate of increase in either CP volume or CP pT2 in patients with MS over 5 years of follow-up compared with the HC group. Moreover, we did not find any longitudinal associations between changes in CP measures and clinical parameters.

NCPV was significantly larger in the MS group as a whole compared with in HCs, as previously reported.[4,8] It should be noted though that group differences in CP volume were no longer significant after controlling also for brain and/or ventricular volumes, unlike previously reported.[8] Although the reason for these differences remains unknown at this time,

Table 4 Baseline Differences Between Patients Split by Disability Status Progression Over the Follow-up, Adjusted for Age and Sex

Measure	Stable/Improved (n = 99)	Disability Progression (n = 55)	*p* Value	Partial η^2
NCPV (mL)	2.5 (0.8)	2.7 (0.8)	0.365	0.005
CP pT2 (ms)	**919.7 (456.6)**	**1,185.4 (418.2)**	**0.001**	**0.078**
T2 lesion volume (mL)	**13.1 (18.1)**	**22.6 (22.1)**	**0.001**	**0.067**
NBV (mL)	**1,472.3 (92.1)**	**1,423.9 (93.2)**	**0.006**	**0.049**
NGMV (mL)	**747.4 (59.2)**	**710.6 (68.5)**	**0.002**	**0.063**
NThalV (mL)	**19.1 (2.4)**	**17.8 (2.9)**	**0.006**	**0.049**
NPutV (mL)	12.1 (1.6)	11.6 (1.8)	0.054	0.024
NWMV (mL)	724.9 (57.4)	713.2 (69.4)	0.337	0.006
NVV (mL)	50.0 (25.6)	59.0 (25.4)	0.069	0.022

Abbreviations: CP tT2 = choroid plexus pseudo-T2; NBV = normalized brain volume; NCPV = normalized choroid plexus volume; NGMV = normalized gray matter volume; NThalV = normalized thalamic volume; NPutV = normalized putaminal volume; NWMV = normalized white matter volume; NVV = normalized ventricular volume.
Cells represent mean (SD) and *p*-values <0.05 in bold.

differences in disease duration between this study (median 13.0 years) vs that in the former study (median 4.8 years) may account for at least some of the discrepancy. It may be the case that more advanced atrophy associated with longer disease duration simply overwhelms any differences in CP volume between the groups. Moreover, we did not find any evidence of NCPV being different between patients with the RRMS phenotype compared with those with a PMS one, suggesting that CP enlargement may be a phenomenon that occurs earlier in MS. In line with this hypothesis is that CP inflammation has been reported to be mild in the progressive stages of the disease,[3] although comparisons with patients with RRMS were not performed in that study.

Our data support a link between CP inflammation, as reflected by greater CP volume and increased CP pT2, and injury throughout the brain as shown by the associations with T2 LV and structural volumes. The LPM analyses revealed a link between CP volume and lesion accumulation occurring mostly in the periventricular WM, at least for patients with PMS. As regards CP pT2, the VBM analyses showed a strong link between CP inflammation and most of the cortical GM in patients with RRMS. In contrast to that which has been previously reported though, we did not replicate previous findings of increased CP volume in patients with contrast-enhancing lesions compared with those without nor with respect to the relapse rate.[8] Similarly, no associations were found with CP pT2. As of now, it is not clear why such a discrepancy exists, although again, differences in disease duration between the cohorts may in part be the reason. A recent study found moderate correlations with inflammatory activity, both about clinical activity (i.e., annualized relapse rate) and imaging characteristics (e.g.,, WM lesion load, ^{18}F-DPA-714 binding [a PET marker of neuroinflammation] in the thalamus and normal-appearing WM).[8] Moreover, LPM analyses in patients with RRMS did not reveal any significant clusters of voxels, whereas widespread periventricular WM lesion location associations were found in the PPMS cohort. In this regard, our results are also discrepant with the aforementioned study[8] where CP volume was associated with T2 LV in patients with RRMS, but not PMS. Unlike in the RRMS sample, we did not find an association between CP pT2 and T2 LV in the PMS group. In fact, CP pT2 was not associated with any other imaging measure in the PMS sample, suggesting that both CP volume and CP pT2 may be complementary measures in assessing underlying CP pathology. Nevertheless, our finding suggests that CP inflammation is likely to be relevant throughout the entire course of the disease. It is tempting to speculate that there might be an association between chronic active lesions, which are more prevalent in the progressive stage of the disease,[29] and an inflamed CP. Future studies by our group will investigate this hypothesis by incorporating data on lesions with paramagnetic rims, which reflect active microglia at the periphery of lesions with ongoing, active inflammation.[30]

We found that CP inflammation, particularly as assessed by pT2, is highly associated with future disability progression. It is important that the association remained significant even after correcting for all other measures that were significantly different between the group that progressed in their disability vs those who remained stable or improved. It is not clear why there were such striking differences between the 2 putative measures of CP inflammation in that CP volume was not associated with disability progression, while CP pT2 was. Our findings suggest that both imaging measures may be useful in characterizing pathology associated with the CP. In fact, Fleischer et al. highlighted the relevance of CP volume in determining future EDSS change.[4] Nevertheless, there are limited studies in the literature that have integrated multiple imaging modalities. One notable exception is the aforementioned study where a PET tracer sensitive to inflammation was used.[8] Future multimodal studies may shed further light on the temporal relationship between changes in CP microstructure and its enlargement.

In our MS sample, we did not find evidence that CP volume increased at a greater rate compared with that found in the HC group. This suggests that there may a potential ceiling effect in CP enlargement or that it increases in volume at a slower rate than we could detect over about 5 years of follow-up. We did find, however, that the HC group increased in CP pT2 at a significantly greater rate (although the actual pT2 values remained below those observed in patients with MS). Nevertheless, this was an unexpected finding, but it must be noted that it has previously been shown that CP microstructure changes over time in normal aging.[31] Specifically, Alisch et al. found that advancing age is associated with increased T1 times, T2 times, and mean diffusivity as well as volume of the CP coupled with decreased fractional anisotropy and blood flow.[31] The authors of that study hypothesized that such changes are reflective of decreased structural integrity of the blood-CSF barrier, reflecting the so-called inflammaging,[32] that increases with age. This would potentially be in line with the putative ceiling effect of CP volume and that there simply is not very much room for T2 values to increase further in an already inflamed CP in our patients with MS with a relatively long disease duration of about 15 years. Thus, the factors that contribute to prolonged T2 times in the CP of healthy individuals may possibly be overwhelmed by existing pathology in the patients with MS. Support for this hypothesis is that when splitting patients into those with <10 years of disease duration and those with >10 years, we found that the group with lower disease duration had a significantly greater, 2.5-fold increase in CP pT2 compared with those with longer length of disease (results not shown). Similarly, percent changes in CP pT2 over the follow-up were significantly lower in patients who progressed compared with those who remained stable or improved. This seemingly unexpected finding would also be in line with the notion that patients who are worse off (i.e., those who progress in their disability) are characterized by a CP that has effectively reached its maximum state of inflammation. Nevertheless, additional studies with more frequent serial imaging and patients earlier in their disease would be useful to clarify the questions raised by our findings.

Certain limitations of our study need to be considered. First, we measured the CP within the lateral ventricles but did not consider the infratentorial portions, which contain approximately one-third of the total volume. However, a previous study comparing CP volume between patients with MS and those with neuromyelitis optica spectrum disorder found consistent results when including the infratentorial portion of the CP.[7] Thus, we do not suspect that having excluded the infratentorial portions of the CP has had a substantial effect on our findings. Second, differences in CP volume were no longer significant after controlling for NBV and NVV. However, it should be noted that CP volume was moderately associated with both measures. The association may be driven by various factors, such as inflammation driving simultaneous ventricular and CP volume increases.[4,33] In this context, correcting for NVV or NBV would weaken the NCPV effect. Moreover, we used a relatively crude technique for mapping T2 times and the use of more advanced techniques is warranted to validate our findings and shed further light on the microstructure of the CP. The use of a more advanced T2 mapping technique and/or other methods, such as magnetization transfer ratio or diffusion tensor imaging, may further inform on CP pathology in MS.[31] Future work should also incorporate correction for partial voluming effects, which were not considered in this study when assessing pT2 values. Finally, EDSS was not confirmed after an additional follow-up (e.g., after 6 or 12 months).

In conclusion, our findings suggest that quantitative CP assessment may be an informative, as of yet fully explored, measure of MS-related pathology associated with disease progression. As of now, most MRI studies in MS have focused only on enlarged CP volumes as a measure of inflammation. Future work should continue to incorporate complementary multimodal imaging measures to better disentangle the evolution of CP pathology in MS.

Study Funding

The authors report no targeted funding.

Disclosure

N. Bergsland reports no disclosures. M.G. Dwyer has received personal compensation from Keystone Heart for consultant fees. He received financial support for research activities from Bristol Myers Squibb, Mapi Pharma, Keystone Heart, Protembis, and V-WAVE Medical. D. Jakimovski reports no disclosures. E. Tavazzi reports no disclosures. R.H.B. Benedict has received consultation or speaking fees from Bristol Myer Squibb, Biogen, Merck, EMD Serono, Roche, Verasci, Immune Therapeutics, Novartis, and Sanofi-Genzyme. B. Weinstock-Guttman has participated in speaker's bureaus and/or served as a consultant for Biogen, EMD Serono, Novartis, Genentech, Celgene/Bristol Meyers Squibb, Sanofi &Genzyme, Janssen, Horizon, Bayer, and Labcorp. Dr. Weinstock-Guttman has also received grant/research support from the agencies listed in the previous sentence. She serves in the editorial board for BMJ Neurology, Children, CNS Drugs, MS International, and Frontiers Epidemiology. R. Zivadinov has received personal compensation from Bristol Myers Squibb, EMD Serono, Sanofi, Keystone Heart, Protembis, and Novartis for speaking and consultant fees. He received financial support for research activities from Sanofi, Novartis, Bristol Myers Squibb, Octave, Mapi Pharma, Keystone Heart, Protembis, and V-WAVE Medical. Go to Neurology.org/N for full disclosures.

Publication History

Received by *Neurology* March 29, 2022. Accepted in final form October 11, 2022. Submitted and externally peer reviewed. The handling editor was Deputy Editor Olga Ciccarelli, MD, PhD, FRCP.

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