Supplementary Materials

Choroid plexus volume is enlarged in long COVID and associated with cognitive and brain changes

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Neuroimaging acquisition parameters

Patients were scanned using a 3.0T Magnet (GE Signa Architect) and a 48-channel head coil. 3D T1-weighted images were acquired in a Sagittal MPRAGE sequence with the following parameters: number of slices = 200, slice thickness = 1 mm, field of view 256 mm, matrix = 256x256, flip angle = 8, preparation time = 974 ms, recovery time = 700 ms, TR = 2450 ms , TE = 3.1 ms, NEX= 1, acquisition time = 9:27 min.

Diffusion-weighted images were acquired using an axial multishell diffusion one shot echo-planar sequence, with 3 b values (500, 1000, 2000), 14 b= 0 s/mm^2, and 125 diffusion directions, and the following parameters: number of slices = 64, slice thickness = 2.2 mm, no gap, field of view 256 mm, matrix = 116x166, TR = 6843 ms, TE = 88.6 ms , NEX = 1, acquisition time = 14:35 min. An additional sequence on opposing direction was acquired, to correct for geometrical distortions with 2 b= 0 s/mm^2.

Resting-state fMRI data were obtained in an axial orientation using a sequence sensitive to blood oxygen level-dependent (BOLD) multi-slice gradient echo EPI sequence (TR = 3000 ms, TE = 30 ms, matrix size = 64 x 64, flip angle = 90°, field of view 220 mm, slice thickness = 3.4 mm, no gap, 205 volumes, 48 slices, voxel size = 3.4 x 3.4 x 3.4 mm, acquisition time = 10:15 min).

Neuropsychological assessment

Specifically, the cognitive tests administered in the protocol were: Forward and Backward Digit Span, Corsi Block-Tapping Test (forward and backward), Symbol Digit Modalities Test (SDMT), Free and Cued Selective Reminding Test (FCSRT), Rey-Osterrieth Complex Figure (ROCF) (copy and recall at 3, 30 min, and recognition), verbal fluency (animals and words beginning with "p", "r", and "m" in one minute each one), Stroop Word-Color Interference Test, Boston Naming Test (BNT), Judgment Line Orientation (JLO), and the Visual Object and Space Perception Battery (VOSP). These tests have been validated and normative data are available in our country, adjusted by age and education level.[1,2] Cognitive impairment was set at two cut-off scores: first, at the scaled-score of five or less, which is equivalent to a percentile of ≤ 5 or z-score ≤ 1.65; and second, at the scaled-score of seven or less, which is equivalent to a percentile of ≤ 16 or z-score ≤ 1.

Clinical assessment

Clinical assessment included fatigue with the Modified Fatigue Impact Scale (MFIS) questionnaire,[3] anxiety with the State-Trait Anxiety Inventory (STAI),[4] depression with the Beck Depression Inventory-II (BDI-II),[5] olfactory symptoms with the Brief Smell Identification Test (BSIT) [6] and sleep quality with the Pittsburgh Sleep Quality Index (PSQI) [7]. According to the previous literature, the following cut-offs were used: MFIS ≥ 38 was considered as having fatigue [8]; STAI-S ≥ 40 was considered clinically significant anxiety; BDI-II ≥ 19 was regarded as moderate or severe depression [9]; PSQI >5 defined poor sleep quality and BSIT ≤ 8 was categorized as having abnormal olfaction.

Supplementary Table S1: Neuropsychological results in PCC

		Raw Score	Max – Min score	Impairment (%)*	
				≤ 5 (1.65 SD)	≤ 7 (1 SD)
Neuropsychological results					
Digit Span	Forward	5.89 (1.52)	2 -9	12.4%	24.8%
	Backward	4.14 (1.23)	2 -8	7.8%	26.4%
Corsi test	Forward	5.49 (1.17)	2 -8	9.3%	11.6%
	Backward	4.69 (1.14)	2 -7	7.8%	24%
SDMT		41.7 (12.84)	8 -67	20.2%	37.2%
FCSRT	Free Recall Trial 1	7.67 (2.22)	2 -15	3.9%	13.2%
	Total Free Recall	27.85 (7.23)	7 -43	18.6%	34.1%
	Total Recall	40.47 (7.77)	4 -48	25.6%	41.9%
	Delayed Free Recall	10.05 (3.41)	0 -16	18.6%	36.4%
	Delayed Total Recall	14.07 (2.7)	3 -16	16.3%	33.3%
ROCF	Copy (score)	33.03 (3.54)	19 -36	3.1%	11.6%
	Copy (Time)	132.36 (63.15)	10 -424	0.8%	7%
	Recall (3 mins)	19.44 (6.2)	4,5 -34	3.1%	12.4%
	Recall (30 mins)	19.18 (6.32)	4,5 -35	3.1%	13.2%
	Recognition	19.11 (2.57)	12 -27	15.5%	31.8%
Verbal Fluency	Phonetic P	15.8 (5.19)	4 -32	10.9%	23.3%
	Phonetic M	13.55 (4.21)	2 -25	5.6%	12.0%
	Phonetic R	12.84 (4.33)	2 -23	4.8%	14.5%
	Semantic	21.05 (5.52)	10 -36	12.4%	28.7%
Stroop	Words	89.84 (23.41)	37 -131	31%	50.4%
	Color	61.59 (15.67)	23 -102	34.1%	45.7%
	W-C	36.14 (12.41)	12 -82	25.8%	45.3%
BNT		52.46 (5.34)	34 -60	6.2%	16.3%
JLO		21.6 (6.1)	0 -30	17.8%	32.6%
VOSP	Object Decision	16.64 (2.39)	8 -20	5.4%	26.4%
	Pr. Silhouettes	9.04 (3.03)	4 -20	7.8%	24.8%
	Position Discrimination	18.66 (2.83)	2 -20	12.4%	29.5%
	Number Localization	9.21 (2.21)	3 -20	17.1%	38%

*Impairment = Percentage of patients showing ≤ 5 or ≤ 7 in the scaled score in cognitive tests. SD = Standard Deviation. Stroop W= Stroop Words; Stroop C = Stroop Color; Stroop WC= Stroop Word-Color; SDMT= Simbol Digit Modality Test; ROCF= Rey-Osterrieth Complex Figure; VOSP= Visual Object and Space Perception Battery; JLO= Benton Judgment Line Orientation; BNT= Boston Naming Test.

Supplementary Table S2: Clinical results in PCC

		Raw Score	Max – Min score	Impairment (%)
Fatigue	MFIS total score	55.96 (15.26)	16 - 84	86.8%
Depression	BDI-II total score	14.60 (8.68)	0 - 43	26.4%
Anxiety	STAI State total score	40.49 (11.70)	20 -75	49.6%
Sleep quality	PSQI total score	10.15 (4.60)	0 - 19	84.5%
Smell quality	BSIT total score	9.41 (2.20)	1 - 12	26.4%

Supplementary Table S3: VBM GM volume reduction in PCC patients compared to HC

GM Brain Area	MNI Coordinates			K (cluster)	T	*p* FWE-corrected
	x	**y**	**z**			
Caudate L Putamen L Insula L Inferior Orbitofrontal L Middle Oribitofrontal L Inferior Frontal Tri. L Inferior Frontal Operc. L Insula L Rolandic Operc. L	-24	15	13.5	5337	5.2125	.009
Thalamus BIL Hippocampus L Pallidum L	-1.5	-15	4.5	5925	4.3676	.005
Caudate R Anterior Cingulum BIL Inferior Orbitorfrontal R Middle Orbitofrontal R Superior Orbitofrontal R Rectus BIL Frontal Middle R Superior Frontal R Superior Frontal Medial BIL Insula R	24	12	16	6400	4.1323	.003

GM = Grey Matter; L = Left; R = Right; BIL = Bilateral; MNI = Montreal Neurologic Institute coordinates.

Supplementary Table S4: WM tracts showing associations with ChP volume

WM tract	R/L	MNI coordinates			K	T	*p* FWE-corrected
		x	y	z			
FA correlations							
Fornix Anterior Thalamic Radiation	L	-18	-28	14	430	5.014	.033
Fornix Callosal Body	R	13	-33	12	55	3.896	.046
Anterior Thalamic Radiation	R	1	-13	1	17	4.107	.050
Anterior Thalamic Radiation	R	3	-20	-4	5	4.850	.050
AD correlations							
Anterior Thalamic Radiation Callosal Body	L	-24	-45	24	3444	3.294	.019
Inferior Fronto-occipital Fasc. Uncinate Tract	R	27	19	5	1321	5.398	.020
Fornix	L	-3	-9	13	363	5.186	.025
Inferior Fronto-occipital Fasc. Middle Longitudinal Fasc.	R	31	-33	11	243	2.681	.046

L= Left; R= Right; MNI = Montreal Neurologic Institute coordinates.

Supplementary Table S5: Sociodemographic and clinical differences between PCC subgroups

	HC (n=36)	**ChP-small (n=61)**	**ChP-large (n=68)**	***p* (ANOVA)***
Age (years)	49.33 (15.99)	46.20 (9.93)	52.18 (9.84)	.014[a]
Sex (Women) (n,%)	22 (61.11%)	53 (86.88%)	42 (61.76%)	.003[a,b]
Education (years)	15.43 (3.28)	14.82 (3.60)	15.10 (3.67)	.781
Evolution (months)	-	15.14 (7.26)	14.48 (7.12)	.603
Hospital Admission (n,%)	-	14 (22.95%)	22 (32.35%)	.235
Hypertension (n,%)	-	9 (14.75%)	24 (35.29%)	.008
Diabetes (n,%)	-	5 (8.19%)	8 (11.76%)	.502
Dyslipemia (n,%)	-	15 (24.59%)	16 (23.52%)	.888

* p (ANOVA): ANOVA 3 group**:** HC; ChP-small; ChP-large; a = ChP-small vs ChP-large ($p<.05$); b= HC vs ChP-small ($p<.05$).

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