Multimodal neuroimaging in post-COVID syndrome and correlation with cognition

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Abstract

Brain changes have been reported in the first weeks after SARS-CoV-2 infection. However, limited literature exists about brain alterations in post-COVID syndrome, a condition increasingly associated with cognitive impairment. The present study aimed to evaluate brain functional and structural alterations in patients with post-COVID syndrome, and assess whether these brain alterations were related to cognitive dysfunction.

Eighty-six patients with post-COVID syndrome and 36 healthy controls were recruited and underwent neuroimaging acquisition and a comprehensive neuropsychological assessment. Cognitive and neuroimaging examinations were performed 11 months after the first symptoms of SARS-CoV-2. Whole-brain functional connectivity analysis was performed. Voxel-based morphometry was performed to evaluate grey matter volume, and diffusion tensor imaging was carried out to analyse white matter alterations. Correlations between cognition and brain changes were conducted and Bonferroni corrected.

Post-COVID syndrome patients presented with functional connectivity changes, characterized by hypoconnectivity between left and right parahippocampal areas, and between bilateral orbitofrontal and cerebellar areas compared to controls. These alterations were accompanied by reduced grey matter volume in cortical, limbic and cerebellar areas, and alterations in white matter axial and mean diffusivity. Grey matter volume loss showed significant associations with cognitive dysfunction. These cognitive and brain alterations were more pronounced in hospitalized patients compared to non-hospitalized patients. No associations with vaccination status were found.

The present study shows persistent structural and functional brain abnormalities 11 months after the acute infection. These changes are associated with cognitive dysfunction and contribute to a better understanding of the pathophysiology of the post-COVID syndrome.

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Abbreviations: FC = Functional Connectivity; GM = Grey Matter; PCS = Post-COVID syndrome; WM = White Matter

Introduction

Post-COVID syndrome (PCS) has been described in patients with history of SARS-CoV-2 infection, with symptoms developed during or after the infection that persist over 12 weeks.[1] Symptomatology related with PCS is diverse, ranging from chronic fatigue, anosmia, dyspnea, pain, and cognitive symptoms also called "brain fog".[2] The pathophysiology of these symptoms is still under evaluation. Studies have suggested that COVID-19 may damage neurological, vascular, respiratory, and renal structures.[3]

Cognitive dysfunction has been highlighted as one of the most frequent symptoms in PCS.[4] Restricted evidence suggests that SARS-CoV-2 virus may enter the central nervous system through the olfactory fibers or the nasal passages, or by hematogenous spread, causing among others, cerebrovascular disease,[5] but COVID-19 sequelae have also been related to the presence of hypoxia, and inflammatory dysfunction.[6]

Few studies have performed a comprehensive neuropsychological assessment in COVID-19 patients, and showed cognitive dysfunction in a wide range of cognitive domains, including attention, processing speed, memory, executive functions, language and visuospatial ability, being attention, memory and executive functions the most affected capacities in these patients.[7–9] Longitudinal studies revealed that cognitive deficits persist after 1-year follow-up ranging from 12%,[10] 18-19%[11] to 34% of patients.[12]

Limited literature exists about brain alterations in PCS patients. Studies in the post-acute phase found alterations in grey matter (GM) volume, including the hippocampus,[13–16] white matter (WM) changes[13,15] and presence of WM hyperintensities.[17] However, absence of significant changes were also found in other studies regarding GM volume.[18,19] One study evaluated functional connectivity (FC) after three weeks of infection, and revealed alterations in the anterior piriform cortex related to olfactory impairment.[20] One longitudinal study that followed up 401 COVID-19 patients before infection and after four months from the acute phase, evidenced reduced brain volume in orbitofrontal, and parahippocampal gyrus related to the primary olfactory and gustatory systems.[21] However, very few studies have evaluated the brain alterations after longer periods of time following SARS-CoV-2 infection. One study assessed patients 1-year after SARS-CoV-2 infection, and revealed structural WM abnormalities,

specifically, reduced fractional anisotropy and volume fraction of intracellular water compared to controls.[22] In this regard, the role of brain changes in the pathophysiology of cognitive symptoms in the PCS is unknown.

Although some of these studies reported cognitive decline parallel to brain damage, few studies have performed associations between brain alterations and cognitive deficits. Significant associations have been reported between GM reductions and cognitive deficits,[21] while another study showed absence of relationship between WM alterations and cognition.[22]

Multimodal magnetic resonance imaging (MRI) may be useful to disentangle pathophysiological mechanisms of brain disorders. However, there is a lack of multimodal imaging studies in PCS patients, and scarce studies have evaluated associations between brain alterations and cognitive impairment with a comprehensive neuropsychological battery. Therefore, we aimed to investigate patients with PCS and cognitive complaints using a multimodal brain imaging protocol that included T1-weighted, diffusion-weighted and functional MRI sequences, as well as a neuropsychological assessment. We aimed to detect structural and functional brain changes in comparison with a control group, and evaluate whether these brain alterations were related to cognitive dysfunction in PCS.

The first objective of the present study was to evaluate the brain FC alterations in PCS patients. The second objective was to evaluate whether FC alterations were accompanied with GM and WM structural alterations. Finally, we aimed to investigate whether functional or structural alterations were related to clinical or cognitive symptoms in PCS patients 1-year after SARS-CoV-2 Infection.

Materials and methods

Participants

We performed a cross-sectional evaluation of 86 participants with subjective cognitive complaints after SARS-CoV-2 infection (with mean evolution since first symptoms of 11.08 ± 4.47 months). Patients were consecutively recruited through the department of Neurology at Hospital Clínico San Carlos between November 2020 and December 2021. Thirty-six healthy

controls were also recruited. Serological analysis was conducted in controls to ensure they were not exposed to the SARS-CoV-2.

Inclusion criteria for the PCS group were: 1) Diagnosis of COVID-19 confirmed by RT-PCR at least three months before the inclusion in the study; 2) Cognitive complaints temporally related to the SARS-CoV-2 infection. Patients were excluded if they presented with other neurological, or psychiatric disorders that could affect the study outcomes. Specifically, exclusion criteria included: 1) Any cognitive complaint before COVID-19; 2) History of stroke, traumatic brain injury, or any neurological disorder potentially associated with cognitive impairment; 3) Active psychiatric disorder or previous psychiatric disease with a potential cognitive effect (e.g. schizophrenia); 4) History of abuse of alcohol or other toxics; 5) Drugs or uncontrolled medical conditions associated with cognitive impairment at the moment of the assessment 6) Sensory disorder potentially biasing cognitive assessments; 7) Deep WM cerebral small vessel disease (Fazekas grade 2 or higher). Inclusion and exclusion criteria for controls are detailed in Supplementary Materials. The main clinical and demographic characteristics of COVID-19 patients are shown in Table 1. Vaccination status from patients was also retrieved. PCS patients underwent a clinical and neuropsychological assessment. All neuroimaging analyses were performed on 86 patients with PCS and 36 controls, except for WM analyses in which two controls were excluded due to differences in DWI parameter acquisition.

Clinical and Neuropsychological assessment

Clinical evaluation included the Modified Fatigue Impact Scale (MFIS) for fatigue assessment.[23] In addition, State-Trait Anxiety Inventory (STAI),[24] Beck Depression Inventory-II,[25] Brief Smell Identification Test (BSIT)[26] and the Pittsburgh Sleep Quality Index (PSQI)[27] were also administered to PCS patients. The following cut-offs were used according to the previous literature: BSIT ≤ 8 was categorized as having abnormal olfaction; STAI-S ≥ 40 was considered clinically significant anxiety; BDI-II ≥ 19 was regarded as moderate or severe depression;[28] PSQI >5 defined poor sleep quality and MFIS ≥ 38 was considered as having fatigue.[29]

PCS patients underwent a comprehensive neuropsychological evaluation. A trained neuropsychologist administered the cognitive protocol including attention, working memory, processing speed, executive functions, memory, language, and visuoperceptive and visuospatial abilities. Specifically, the tests included 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 Color-Word Interference Test, Boston Naming Test (BNT), Judgment Line Orientation (JLO), and the Visual Object and Space Perception Battery (VOSP). These tests were validated and normative data are available in our country, adjusted by age and education level.[30,31] 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 at the scaled-score of seven or less, which is equivalent to a percentile of ≤ 16 or z-score ≤ 1.

Neuroimaging acquisition and analysis

Patients and controls were scanned using a 3.0T Magnet (GE Signa Architect) and a 48-channel head coil. Resting-state fMRI, T1-weighted images, T2 FLAIR, and diffusion-weighted images were acquired in a single session. Acquisition parameters are shown in Supplementary Materials.

Resting-state fMRI

Functional connectivity analysis was performed using Conn Functional Connectivity Toolbox 18.b.[32] After removing the first 5 scans, each subject' 200 functional images were realigned and unwraped, non-linear coregistered with structural data, slice timing corrected (interleaved bottom-up), spatially normalized into the standard MNI space (Montreal Neurological Institute), then, outliers were detected (ART-based scrubbing) and finally, images were smoothed using a Gaussian kernel of 8 mm FWMH. As recommended, band-pass filtering was performed with a frequency window of 0.008 to 0.09 Hz.[33]

Because SARS-CoV-2 infection is a novel disease, we have no prior hypothesis on brain functional alterations. Therefore, we performed a whole-brain Region of interest (ROI) ROI-to-ROI approach analysis according to Conn toolbox options to test differences between PCS patients and controls. Atlases used were the Automated Anatomical Labeling atlas (AAL) atlas parcellation included in CONN toolbox, and supplementary materials show results with the Brodmann's area atlas.[34] BrainNet Viewer software was used for FC visualization purposes.[35]

T1-weighted images

T1-weighted images were preprocessed and analysed with the DARTEL tool (Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra) in SPM12.[36] After orientation and segmentation, the mean template was created, then performed spatial normalization into the Montreal Neurological Institute (MNI) template space. Then, images were modulated, and smoothing with isotropic Gaussian kernel of 8 mm full-width at half maximum (FWHM) was applied. Two sample t-test were performed with age as nuisance covariate and Total Intracranial Volume introduced for proportional scaling in analysis. The AAL atlas parcellation was used for GM results localization.

White Matter Lesion Segmentation

3D FLAIR images were used for WM lesion segmentation using SPM12. WM hyperintensities (WMH) were automatically extracted using the Lesion Prediction Algorithm from the LST toolbox.[37,38] Initial threshold mask was set at 0.3, and the number of iterations was set at 50. WMH volume and the number of lesions were calculated from lesion maps using a threshold of 0.5.[37,38] WM lesion maps were calculated in MRIcron, creating overlap image for PCS and control group.

DWI images

Diffusion data were preprocessed and analyzed in FMRIB Software Library (FSL) (v.6.0.5). First, each subject's images were concatenated and radiologically oriented. Then, topup was applied to estimate and correct susceptibility-induced distortions (fieldmap estimation).[39] Then, BET brain extraction was applied.[40] Eddy command was used to correct for distortion (eddy currents, susceptibility-induced distortions, and subject's motion)[41] with a fieldmap estimated by topup. After, dtifit command was applied to fit diffusion tensors into the eddy-corrected data. For voxelwise statistical analyses, data were processed applying the standard FSL pipeline for Tract-Based Spatial Statistics (TBSS).[42] Finally, fractional anisotropy, mean diffusivity, radial diffusivity and axial diffusivity whole-brain maps were calculated. Randomise was performed with 5000 permutations. Mean values of whole-brain fractional anisotropy, mean diffusivity, radial diffusivity and axial diffusivity were calculated and extracted for analyses in SPSS. Additionally, WM indexes were also calculated for the main WM tracts that showed significant differences among groups. WM tract masks were extracted from the JHU White-Matter Tractography Atlas from FSL.

Statistical analyses

Statistical analyses were performed in SPSS v26 program. Normality of data was tested with Kolmogorov-Smirnov. Sociodemographic, clinical and cognitive characteristics of the sample were calculated using U-Mann Whitney or Chi-squared tests for quantitative or categorical data, respectively. For FC analyses, two-sided T-test of CONN toolbox was used with FDR-corrected $p < 0.05$ threshold. Regarding GM volume differences, results are reported at $p < 0.05$ FWE-corrected and $p < 0.001$-uncorrected ($k \geq 200$) for exploratory purposes. WM alterations cluster significance was set at $p < 0.05$ FWE-corrected, Threshold (T value) > 2 and k >200 voxels. Covariates were demeaned before including in neuroimaging analyses. All neuroimaging analyses included age as nuisance covariate. Moreover, GM volume analyses were also controlled for Total Intracranial Volume. Finally, we aimed to investigate whether brain alterations were related with cognitive dysfunction. Therefore, variables were extracted and introduced in SPSS for correlation analysis. Correlation analyses were performed with Pearson's correlation coefficient, and set at $p < .01$. Additionally, Bonferroni correction was also indicated, corrected by the number of cognitive tests, at $p < 0.0017$. Post-hoc analyses were performed to test differences between hospitalized patients and non-hospitalized patients. Moreover, the influence of vaccination status in cognitive and neuroimaging results was also evaluated. Finally, supplementary analysis was performed to explore the effect of arterial hypertension in neuroimaging results; it was introduced as covariate in neuroimaging analyses, but results remained significant (Supplementary Materials).

Ethics Committee

The present study was approved by the ethics committee from Hospital Clínico San Carlos (reference: 21/062-E) and participants provided written informed consent prior to research participation.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request and once the project is finalized.

Results

Clinical and neuropsychological characteristics

The mean age of PCS patients was 50.71 ± 11.20 years old, and 67.44% were women. During the acute phase of COVID-19, 29 (33.72%) were hospitalized. Main clinical and demographic characteristics are depicted in Table 1. Patients showed no significant differences in age, sex or education with controls.

Regarding clinical profile, PCS patients reported fatigue in 82.4% (*mean (m)* = 53.76 ± 15.19), depression in 27.1% (*m* =14.42 ± 8.94), sleep quality dysfunction in 82.1% (*m* = 9.71 ± 4.73), 32.9% presented with olfaction problems (*m* = 9.12 ± 2.36) and 9.3% showed anxiety symptoms (*m* = 21.36 ± 11.90).

Cognitive impairment was present in PCS patients. Most cognitive alterations were detected in attention and working memory (up to 44.2%), but deficits were also found in memory (up to 40.7%) and executive functions (up to 39.5%), followed by visuospatial ability (up to 36%), and language (up to 18.6%) (Figure 1, Supplementary Table 1).

Brain alterations

Functional connectivity

PCS patients showed FC alterations compared to controls (Figure 2). Specifically, PCS patients presented with reduced FC between left and right parahippocampal gyrus (t = 3.63; p = 0.048-FDR) compared to controls. In addition, PCS patients showed reduced connectivity from the left cerebellar III (vermis) to the left frontal superior orbital cortex and (t = 3.54; p = 0.047-FDR) and right frontal superior orbital cortex (t = 3.43; p = 0.047-FDR).

Grey matter volume

We found no statistically significant differences in GM volume between PCS patients and controls at $p < .05$ FWE corrected. Exploratory analyses showed lower GM volumes in PCS patients compared to controls ($p < .001$-uncorr; $k > 200$). GM volume reductions were found in the parahippocampal gyrus, frontal gyrus, anterior cerebellar, occipital lobe, and bilateral superior temporal lobe (Figure 3.A, Table 2).

White matter hyperintensities

Results showed no significant differences in WMH total lesion volume between PCS patients ($m = 1.60 \pm 2.92$ ml) and controls ($m = 1.66 \pm 2.68$ ml) ($F = 0.010$; $p = .922$). Similarly, the number of lesions did not show significant differences in PCS patients ($m = 9.18 \pm 8.86$); compared to controls ($m = 9.75 \pm 7.91$) ($F = 0.109$; $p = .742$). However, after controlling for age, statistically significant differences emerged, showing the control group increased WMH total lesion volume and increased number of lesions compared to PCS patients ($p < .001$). Supplementary Figure S1 shows WM lesion maps, revealing very low percentage of overlap of WM lesions within each group, and similar WM lesion location and distribution in PCS and control groups.

White matter alterations

WM alterations were present in PCS compared to controls. PCS patients revealed reduced mean diffusivity and axial diffusivity in Corpus Callosum, Forceps Minor, Middle Longitudinal Fasciculus, Uncinate tract, and Fronto-Occipital fasciculus compared to controls ($p < .05$-FWE corrected). Mean diffusivity alterations were found mostly in the right hemisphere, while axial diffusivity alterations were found bilaterally in frontal (near the orbital area), temporal (next to the angular gyrus and parahippocampal area), parietal (next to the precuneus), occipital, and subcortical areas (proximal to the lentiform nucleus). Fractional anisotropy and radial diffusivity showed no significant differences between groups in specific tracts. WM alterations are depicted in Figure 3.B and peak coordinates are described in Supplementary Table 2.

Moreover, PCS patients compared to controls showed reduced whole-brain mean values in fractional anisotropy, mean diffusivity, radial diffusivity and axial diffusivity (Supplementary Table 3).

Cognitive and Brain Correlations in PCS patients

We aimed to investigate whether brain alterations in PCS patients were related to cognitive dysfunction.

GM atrophy showed significant relationships with cognitive dysfunction, mostly with attention, working memory and processing speed, showing greater GM volume loss associations with poorer cognitive performance (Figure 4). Stronger correlations were found between the attention and processing speed and the left parahippocampal area, the left superior temporal gyrus, and the anterior cerebellar area. Additionally, GM volume also showed positive correlations with memory and visuospatial test performance (Figure 4).

Moreover, FC alterations between the bilateral frontal superior orbital cortex and cerebellar area III (vermis) correlated with memory (learning and recall), showing that reduced FC was associated with poorer learning and recall performance (Figure 4).

On the contrary, reduced WM alterations showed scarce associations and barely significant with cognitive dysfunction (Supplementary Figure S2).

Additionally, relationships between brain alterations and the days of evolution of PCS was explored (interval from first symptoms of SARS-CoV-2 to the enrollment). Results showed no significant associations between GM, WM or FC alterations and the days of evolution.

Hospitalized versus non-hospitalized patients

Post-hoc analyses were performed to test whether these cognitive and brain alterations differed between hospitalized and non-hospitalized patients. Sociodemographic and clinical differences between groups are shown in Supplementary Table 4. Age was included as covariate. Among the 29 hospitalized patients (days of hospitalization: 22.00 ± 19.10), eight were admitted in the intensive care unit.

Hospitalized patients revealed greater cognitive deterioration compared to non-hospitalized patients, in attention and working memory, processing speed, memory and visuospatial ability and language (Supplementary Figure S3).

Hospitalized patients showed brain differences compared to non-hospitalized patients (Supplementary Figure S4). Specially, hospitalized patients showed reduced FC between the left and right parahippocampal areas compared to non-hospitalized patients. In addition, reduced GM volume was also found in hospitalized patients in most of the brain areas showing alterations in PCS patients compared to controls, including the superior temporal gyrus, frontal and cerebellar areas. In addition, hospitalized patients showed increased WM mean diffusivity compared to non-hospitalized patients (Supplementary Figure S4).

Association with vaccination status

None of the patients was vaccinated before COVID-19 infection. We performed statistical analyses to test differences between vaccinated and non-vaccinated patients at time of enrollment. Fifty (58.13%) patients were not vaccinated before enrollment on the study and 36 (41.86%) had vaccination before enrollment (17 patients received 1 dose before enrollment, and 19 patients received 2 doses before enrollment). Results showed no statistically significant differences in cognition, GM, WM, WMH, or FC among groups, except for one cognitive subtest, VOSP discrimination (p = .003), showing vaccinated patients better performance compared to non-vaccinated patients.

Discussion

The present study evaluated patients with post-COVID syndrome after 11.08 ± 4.47 months since first symptoms of SARS-CoV-2, with multimodal functional and structural neuroimaging analyses and a comprehensive neuropsychological battery. Findings showed presence of hypoconnectivity changes that were accompanied by WM alterations and slight GM volume reduction. These alterations were related to cognitive performance, mostly attention and working memory deficits, which are the most common cognitive deficits involved in these patients. We found cognitive dysfunction mainly in attention and working memory (up to 44.2%) followed by memory (up to 40.7%), executive functions (up to 39.5%), visuospatial abilities (up to 36%) and language (up to 18.6%). These results are in line with previous cognitive studies in post-COVID patients, showing cognitive dysfunction after several months from the acute infection.[8,12,43]

These confirmed cognitive symptoms in PCS patients were accompanied by brain functional and structural GM and WM alterations.

First, PCS patients presented hypoconnectivity between bilateral orbitofrontal areas and cerebellar area III (vermis) and between left and right parahippocampal areas compared to controls. These FC alterations were related with learning memory and recall deficits in PCS. Extensive literature has related learning and memory deficits with alterations in parahippocampal and orbitofrontal areas,[44–46] but also with the cerebellum.[47] Previous COVID-19 studies also showed reduced FC in COVID patients compared to controls, including the hippocampal and cerebellar areas.[48] Supplementary FC analyses with Brodmann's area atlas were also performed to test the consistency across brain atlas parcellations.[34] Results showed some consistency, reporting both atlases FC alterations in frontal and parahippocampal regions in PCS patients compared to controls. These results go in line with a recent longitudinal study in post-COVID patients that reported brain alterations in orbitofrontal and parahippocampal areas.[21] The reduced FC in these areas is also consistent with previous studies that have found reduced perfusion in orbitofrontal and temporal areas in COVID-19 patients.[49,50] In fact, these regions are located in areas adjacent to the olfactory regions, and previous studies proposed the olfactory system as the entry route of SARS-CoV-2 infection to the central nervous system.[51]

These FC alterations were accompanied by structural WM diffusivity abnormalities. PCS patients presented mainly widespread reduced axial diffusivity and reduced mean diffusivity mostly lateralized in the right hemisphere. Following previous literature in neurodegenerative diseases, these results are unexpected. Patients with neurodegenerative disorders usually show increased diffusivity and reduced anisotropy values compared to controls.[52–54] However, in the case of PCS patients from this study, reduced axial and mean diffusivity was found, and mostly located in the following WM tracts: the callosal body, forceps minor, superior longitudinal fasciculus, inferior fronto-occipital fasciculus and the uncinate tract. Peak values were located in WM areas adjacent to the hippocampal area, frontal orbital lobe, basal ganglia, cuneus, precuneus and supramarginal gyrus, among others. WM alterations from the present study showed no relevant association with cognitive dysfunctions, similar to a previous study in PCS.[22] Interestingly, similar results were found in previous studies in COVID-19 patients, which reported reduced mean and axial diffusivity, showing significant differences compared to controls[13] or slight differences but not statistically significant.[43]

The interpretation of WM integrity and diffusivity measures is complex and should always be taken with caution.[55] Mean diffusivity represents the average mobility of water molecules, and has been described in some cases as an inverse measure of membrane density, sensitive to edema, and necrosis.[56] The interpretation of axial diffusivity has been more variable. Axial diffusivity reflects diffusivity parallel to axonal fibers, and it has been interpreted as a marker of axonal damage but also has been related with brain maturation.[55,56] The combination of reduced axial and mean diffusivity may reflect axonal injury.[57] Therefore, following previous literature, we hypothesized that WM alterations in PCS patients seem not to resemble a neurodegenerative process, but seem more likely to be a consequence of axonal damage or reduced perfusion, or a consequence of neuroinflammation. Studies combining MRI and histopathological analysis and/or other biomarkers (CSF, specific PET tracers) could be useful to disentangle the pathophysiology of MRI alterations. In one hand, patients from this study suffered from COVID-19 symptoms, including respiratory problems, and 80% of them presented headache. Remarkably, a previous study showed widespread reduced WM mean diffusivity without changes in fractional anisotropy in patients with hypoxia state with subsequent headache, and hypothesized that it might be indicative of intracellular swelling added to extracellular edema.[58] Another study also found reduced mean diffusivity in hypoxia exposure.[59] Additionally, decreased mean diffusivity has been associated with reduced perfusion, suggesting primary ischemia.[60] Moreover, one of the main symptoms of COVID-19 patients is the presence of hyposmia, and a previous study in Parkinson's disease patients with olfactory impairment showed reduced axial diffusivity and mean diffusivity in the left uncinate tract, in WM areas adjacent to olfactory sulcus, in the orbitofrontal cortex and the entorhinal cortex.[61]

Patients from the present study (1-year follow-up from diagnosis) presented with WM alterations similar to patients at the post-acute phase.[13,43] However, another study that also evaluated patients after 1-year follow up did not find similar results, but found reduced intracellular water volume fraction in the corpus callosum and superior longitudinal fasciculus tracts.[22] One possible explanation could be the degree of symptomatology presented by the patients. In the study of Huang et al.,[22] 22.73% of the patients presented with fatigue, and during the acute phase 4.55% of the patients presented headache, and 40.91% hyposmia, while patients from the present study presented with more severe symptoms, showing fatigue in 82.4% of the patients, and during the acute phase presented headache in 80.23% and hyposmia in 53.48%. Therefore, severity of

symptoms at infection may influence WM alterations at 1 year follow-up. Overall, WM results from the present study added to previous findings suggest variability in WM alterations among COVID-19 patients, which may indicate a dynamic process and not a permanent alteration, which could be mediated by the severity of the symptoms.

The present study also found slight GM volume reductions in PCS patients, in the anterior part of the cerebellum, parahippocampal gyrus, frontal, temporal, parietal and occipital areas. A recent longitudinal study in post-COVID patients revealed GM atrophy in specific regions, including parahippocampal and orbitofrontal gyrus, and tissue damage in brain areas that are functionally connected to the olfactory cortex.[21] GM alterations in the present study were related to presence of cognitive deficits, mainly, the reduced volume in the parahippocampal, superior temporal gyrus and anterior cerebellar area showed relationships with attention and working memory and processing speed deficits. Interestingly, Douaud et al. [21] also found associations between cognitive deficits and the cerebellum in PCS. The many regions involved and the correlation between these regions and cognitive function suggest the existence of multiple networks and systems implicated in the generation of cognitive deficits in patients with PCS. On the one hand, this could explain the heterogeneous cognitive deficits found in recent studies in PCS population.[8,9,22] On the other hand, our findings confirm that although the prefrontal cortex is still considered the main region associated with attention and executive function, many other cortical and subcortical regions are participating in this function. Specifically, attention tests were correlated with superior medial frontal and precentral gyri, but also with the superior temporal gyrus, lingual gyrus and cuneus, paracentral lobe, parahippocampal gyrus and cerebellar vermis. Some of these regions have been associated with a network related to attention and processing speed in healthy volunteers[62] and suggest the impairment in top-down and bottom-up attentional processes in the PCS.

Worth to highlight, the parahippocampal region in PCS patients from this study showed FC alterations, accompanied by GM volume reduction and presented adjacent WM abnormalities. Atrophy in the parahippocampal region was also previously found in PCS patients, showing reduced volume compared to controls[21] but also enlarged GM volume.[16] GM volume alterations in the hippocampal area have been reported since the post-acute phase.[13,15] These findings suggest that this region might be a target of COVID-19 infection.

WM lesions have been found to influence neuroimaging results, such as FC alterations.[63] PCS patients from the present study presented reduced WM lesion volume and reduced number of lesions compared to the control group, however, these differences were minor (lesion volume: 1.60 vs 1.66; number of lesions: 9.18 vs 9.75). Moreover, lesion maps showed an unspecific pattern of WM lesions in PCS patients and very reduced overlap within the group. Indeed, WM lesion map from the PCS group revealed similarities with the lesion map in the control group. Therefore, these results suggest that FC, GM, or WM results from the present study were not influenced by WM lesions.

Overall, although the olfactory region was emphasized as a potential entry route of the virus in the central nervous system and subsequent damage of adjacent or connected brain regions,[51] findings from the present study seem to partially but not totally sustain this hypothesis. PCS patients from this study showed FC alterations in regions that have been found to be directly or indirectly functionally connected to the olfactory system, including orbitofrontal region and parahippocampal gyrus.[64] Furthermore, no structural alterations in the olfactory bulb or piriform cortex were reported. Conversely, patients presented multifocal changes in both cortical and subcortical regions including GM and WM alterations. These could support the existence of neuroinflammatory mechanisms and/or endothelial damage, brain-blood disruption and passage of neurotoxins that could induce a generalized or multifocal brain damage.[65] Thus, findings suggests the existence of several pathophysiological mechanisms in patients with persistent symptoms after COVID-19, which may be different according to each deficit. The clinical similarities of some manifestations of PCS with neuroinflammatory disorders, such as Multiple Sclerosis (e.g. fatigue, cognitive deficits with predominant attention deficits, mood disorders) could also suggest shared mechanisms with these diseases that should be specifically investigated. [66]

The cognitive and brain alterations found in PCS patients, were more severe in hospitalized patients compared to non-hospitalized patients. Cognitive deficits were more severe in hospitalized patients, mainly in attention and working memory, processing speed, memory and visuospatial ability. Moreover, brain GM volume reductions, WM and FC alterations in PCS patients were more accentuated in hospitalized patients compared to non-hospitalized patients. However, although hospitalized patients present with greater brain and cognitive changes, our findings suggest that these changes are not exclusive to the severe forms of the disease, because

most of our sample included patients that suffered relatively mild forms of COVID-19 during the acute stage. Moreover, brain alterations were not related with the days of evolution from infection to enrolment, as previously found in another study.[21]

Furthermore, the influence of vaccination status at the time of enrollment was also inspected. Results suggested that vaccination status had no influence on cognitive deficits or neuroimaging alterations in PCS patients. A recent study also reported absence of differences in the evolution of clinical symptoms between vaccinated and non-vaccinated post-COVID patients.[67] Despite these results, survey studies with long-COVID patients declared improvements in cognitive and clinical symptoms after receiving vaccination.[68] Therefore, future studies should address this subject.

Some limitations should be considered. First, the present study presents a cross-sectional design, without neuroimaging or cognitive data from these patients before the SARS-CoV-2 infection. Therefore, the present study can only identify brain alterations in post-COVID syndrome compared with a control group. In addition, cognitive or mood was not evaluated in the control group, a limitation also shared with the majority of post-COVID studies.[8,9,12,69] Despite that previous studies already reported greater cognitive decline in post-COVID patients compared to controls,[10,70] the evaluation of the control group would have been of interest. Moreover, longitudinal studies are needed to study whether these changes are permanent or dynamic and to identify if these brain changes are compensatory mechanisms or part of a recovery process. It would be interesting to identify whether these changes are a consequence of the SARS-CoV-2 infection or if there is still an active pathological process.

In conclusion, our study shows persistent brain functional and structural abnormalities 1-year after the acute infection. These changes involved GM and WM, and are associated with FC disturbances including cortical and subcortical structures. These findings provide novel insights to the understanding of the neural underpinnings of cognitive dysfunction of post-COVID syndrome and the pathophysiology of this disorder. Future studies with longitudinal designs should evaluate the dynamics of brain and cognitive changes over time to further understand the pathophysiological events that occur during the post-acute stages of COVID-19 and their neurological consequences.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Percentage of cognitive impairment in PCS patients. Impairment is shown at the adjusted scaled-score of ≤ 5 with solid fill and ≤ 7 with striped fill. Color bars represent cognitive domains evaluated: Green= Attention and Working Memory; Red= Executive Functions; Blue = Learning and Memory; Orange: Visuospatial and visuoconstructive; Purple = Language. 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.

Figure 2 Functional connectivity alterations in PCS patients compared to controls. Blue connections indicate PCS < Controls. Boxplots are shown with the distribution of mean FC values of each group (z-score). L= Left; R= Right; Cerebellar= Cerebellar Area III (Vermis); FS Orbital = Frontal Superior Orbital Cortex; PaH= Parahippocampal gyrus.

Figure 3 Structural brain alterations in PCS patients compared to controls. (A) GM areas showing volume decrease in PCS compared to controls are shown in red-yellow. Coordinates are shown in MNI space (Montreal Neurological Institute) (p < .001-uncorr); **(B)** Significant WM differences between groups are shown in red-yellow; the WM skeleton is shown in green. Coordinates are shown in MNI space (Montreal Neurological Institute). The red-yellow scale represents t-scores of those areas that showed significant differences between PCS patients and HC (p<.05 FWE corrected). R= Right; L= Left.

Figure 4 Heatmap showing correlations between GM and FC alterations with cognitive dysfunction. Significant associations are shown in color heatmap. *Show significant associations (p < .01). **Bonferroni Corrected (p < .0017). Stroop W= Stroop Words; Stroop C = Stroop Color; Stroop WC= Stroop Word-Color; SDMT= Simbol Digit Modality Test; ROCF= Rey-Osterrieth Complex Figure; FCSRT = Free and Cued Selective Reminding Test; VOSP= Visual Object and Space Perception Battery; JLO= Benton Judgment Line Orientation; BNT=

Boston Naming Test. FS Orbital = Frontal Superior Orbital Cortex; CB = Cerebellar Area III (Vermis); Green line = Attention and Working Memory; Red line = Executive Functions; Blue line = Learning and Memory; Orange line = Visospatial and visoconstructive ability; Purple line = Language.

Table 1 Sociodemographics and clinical characteristics of the sample

	PCS (n = 86)	Controls (n=36)	U/ χ^2	*p*
Age	50.71 (11.20)	49.33 (15.99)	1524.00	0.893
Sex (women%)	67.44%	61.11%	0.451	0.502
Education (years)	14.20 (2.34)	15.43 (3.28)	741.00	0.178
Premorbid risk factors				
Hypertension	25 (29%)	4 (11.1%)	4.24	0.039
Diabetes	10 (11.62%)	1 (2.77%)	2.31	0.128
Dyslipidemia	23 (26.74%)	4 (11.1%)	3.36	0.067
Neurological symptoms in the acute stage				
Headache	69 (80.23%)	-		
Hyposmia+ageusia	46 (53.48%)	-		

Table 2 GM volume reduction in PCS patients compared to HC

GM Brain Area	Coordinates			K	T*
	x	y	z		
Precuneus, Cuneus L & R	−8	−88	40	780	3.92
Lingual L / Cuneus	−4	−90	−14	685	4.38
Superior Motor Area R / Paracentral R	10	−3	64	670	4.03
Fusiform L / Parahippocampal L	−14	0	−38	539	4.32
Vermis IV-V / Lingual Gyrus R	3	−56	3	394	4.76
Superior Temporal L	−44	−30	2	241	3.95
Precentral, Postcentral gyrus R	36	−22	50	221	3.70
Frontal Sup Medial L	−2	64	6	218	3.71

*$P < 0.001$-uncorrected, $k > 200$.

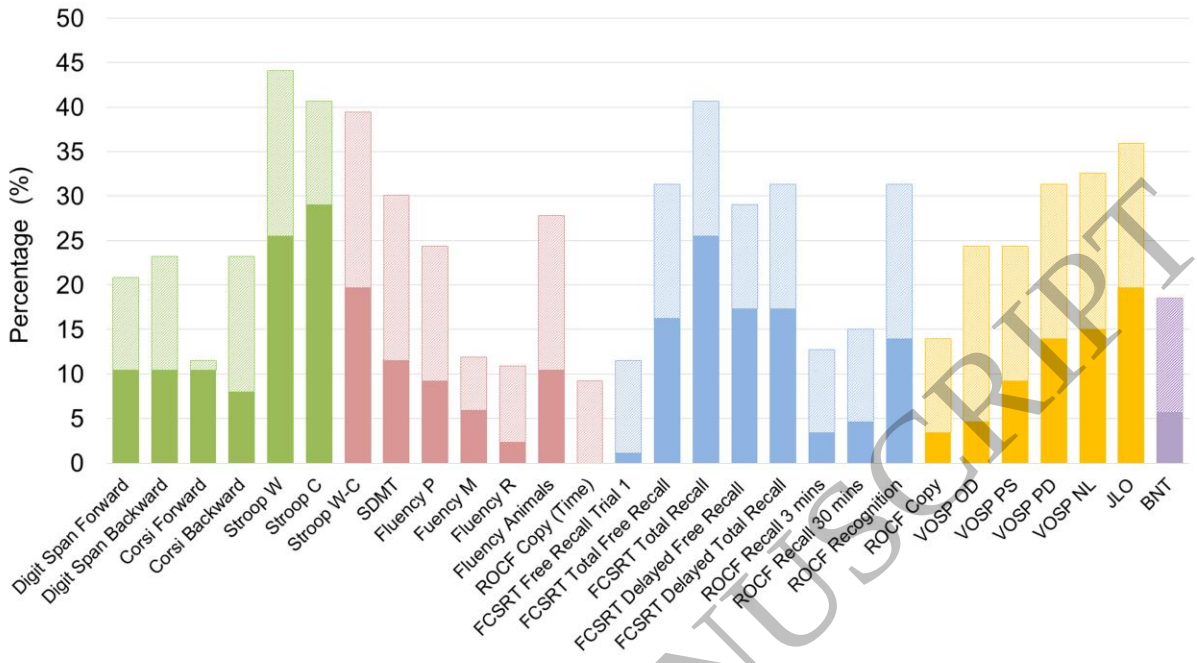

Figure 1
159x88 mm (1.1 x DPI)

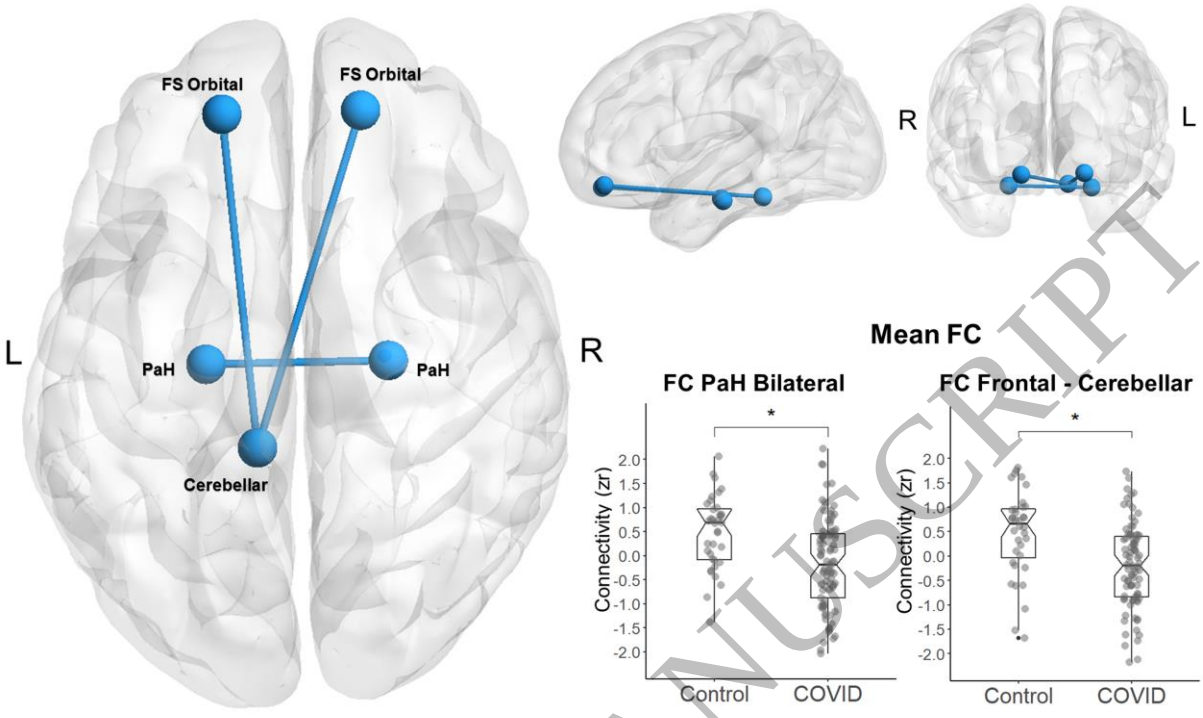

Figure 2
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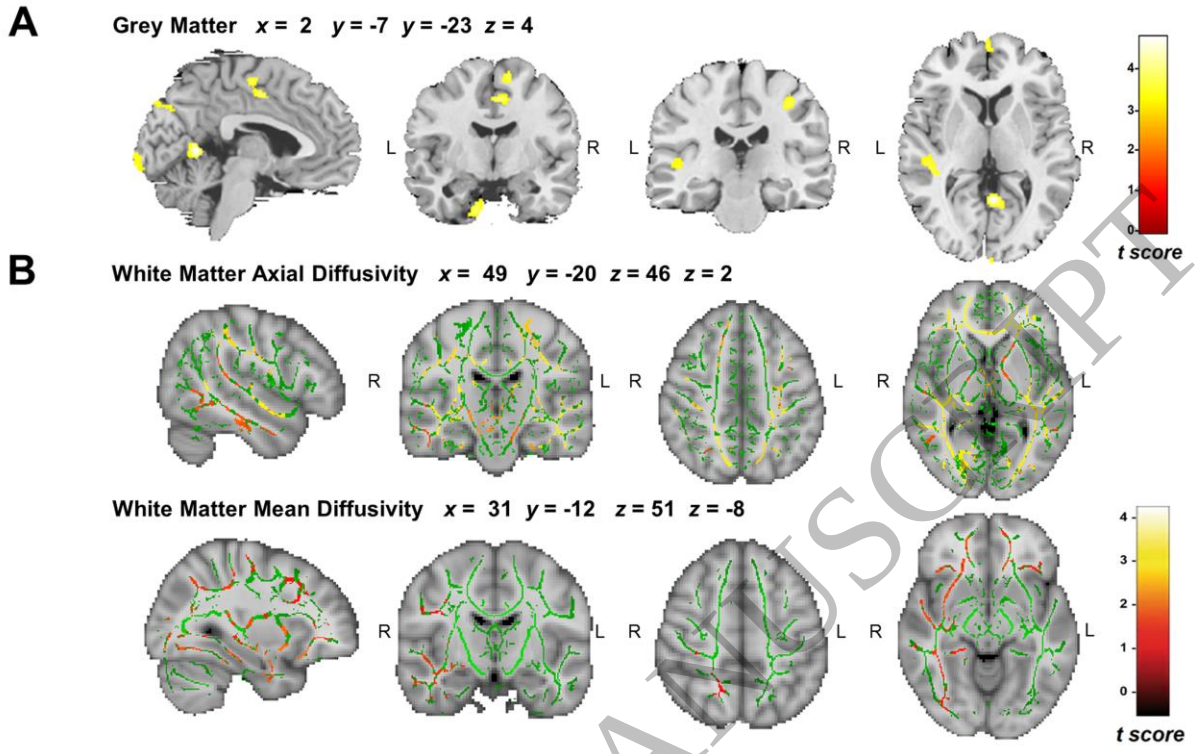

Figure 3
159x100 mm (1.1 x DPI)

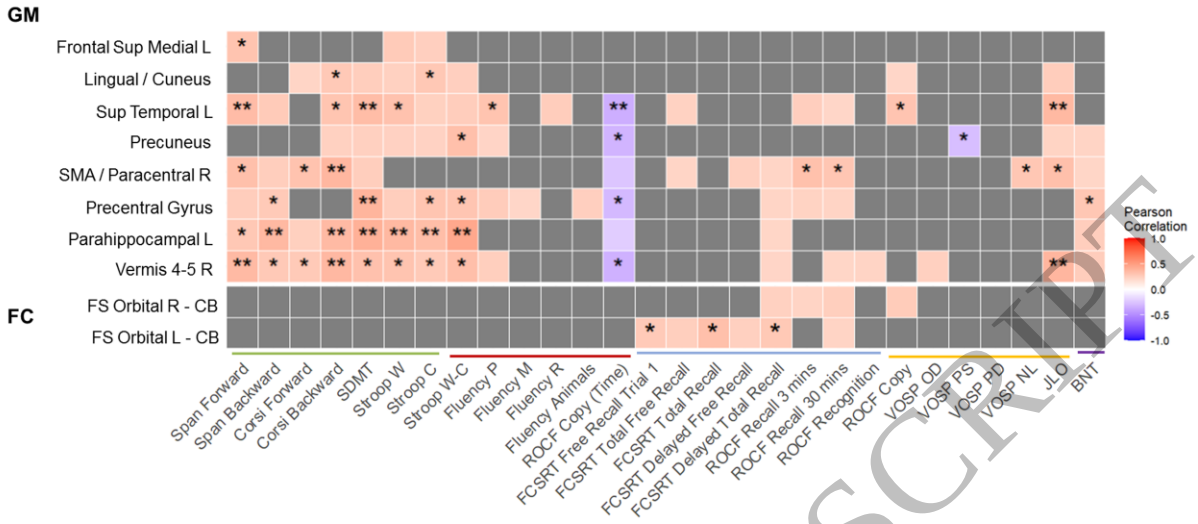

Figure 4
159x70 mm (1.1 x DPI)