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Mid-term MRI evaluation reveals microstructural white matter alterations in COVID-19 fully recovered subjects with anosmia presentation

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Abstract

Background: Little is still known about the mid/long-term effects of coronavirus disease 2019 (COVID-19) on the brain, especially in subjects who have never been hospitalized due to the infection. In this neuroimaging exploratory study, we analyzed the medium-term effect of COVID-19 on the brain of people who recovered from COVID-19, experienced anosmia during the acute phase of the disease, and have never been hospitalized due to SARS-Co-V-2 infection.

Methods: Forty-three individuals who had (COV+, $n = 22$) or had not (COV–, $n = 21$) been infected with SARS-Co-V-2 were included in the study; the two groups were age- and sex-matched and were investigated using 3T magnetic resonance imaging (MRI). Gray matter (GM) volume, white matter (WM) hyperintensity volume, WM microstrutural integrity (i.e. fractional anisotropy [FA], mean diffusivity [MD], axial diffusivity [AD], radial diffusivity [RD]) and cerebral blood flow (CBF) differences between the two groups were tested with either analysis of covariance or voxel-wise analyses. Results were family wise error (FWE) corrected.

Results: No significant differences between COV+ and COV– groups were observed in terms of GM volume, WM hyperintensity volume, and CBF. Conversely, local WM microstructural alterations were detected in COV+ when compared with COV– with tract-based spatial statistics. Specifically, COV+ showed lower FA (pFWE-peak = 0.035) and higher RD (pFWE-peak = 0.038) than COV– in several WM regions.

Conclusion: COVID-19 may produce mid/long-term microstructural effect on the brain, even in case of mild-to-moderate disease not requiring hospitalization. Further investigation and additional follow-ups are warranted to assess if the alterations reported in this study totally recover over time. As brain alterations could increase the risk of cognitive decline, greater knowledge of their trajectories is crucial to aid neurorehabilitation treatments.

Keywords: COVID-19, mid-term effects, neuroimaging, non-hospitalized subjects

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Introduction

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV2), a virus responsible for the global pandemic that is still ongoing (January 2022).[1] COVID-19 is mainly characterized by respiratory symptoms, but it is now well established that COVID-19 is a systemic disease.[2] Multiple organs other than lungs are affected, such as the heart, the liver, kidneys, and the brain.[2] The involvement of the central nervous system in SARS-COV2 infection was proved both by clinical neurological manifestations reported by the affected population,[3] and by

imaging evidence of brain alterations during the acute phase of the disease.[4,5]

A very common and specific neurological symptom for COVID-19 is hyposmia/anosmia. A large multicenter European study reported olfactory dysfunctions in 85.6% of the enrolled 417 patients with laboratory-confirmed mild-to-moderate SARS-COV2 infection.[6] In addition to this common symptom, however, other neurological manifestations have also been observed, such as disorientation, confusion, headache, hypogeusia/cacogeusia, asthenia, vertigo, delirium, ataxia, myalgia, allodynia, and acroparesthesia.[5,7–9]

All these symptoms may be associated with the neurotropic and neuroinvasive potential of SARS-COV2.[10] Although the pathophysiologic mechanism underpinning COVID-19 is not clear yet,[11] several magnetic resonance imaging (MRI) studies showed brain abnormalities including cerebrovascular lesions, perfusion abnormalities, white matter (WM) enhancing lesions, basal ganglia alterations, leptomeningeal enhancement, and acute to subacute cerebral infarction during the acute phase of severe SARS-COV2 infection.[4,5,12] Despite the substantial heterogeneity of the reported neuroimaging findings, the considerable incidence of brain abnormalities suggests that COVID-19 highly impacts the central nervous system in severe cases.[12] Furthermore, the lung disease severity was reported to be potentially predictive of acute abnormalities detected in neuroimaging data.[13]

A portion of subjects who have been hospitalized due to COVID-19 and recovered from moderate to severe infection was reported to still display brain abnormalities at 2/3-month MRI follow-up.[14] This suggests that SARS-COV2-induced alterations may be not limited to the acute illness. The actual impact of long-term sequelae persisting after recovering from COVID-19, referred to as long COVID, is still currently unknown and it represents a crucial issue that needs to be addressed. Although some information is currently available about COVID-19 effects post-hospital discharge,[14] monitoring brain alterations over time in subjects who suffered from COVID-19 with neurological manifestations but without having ever been hospitalized due to the acute infection may be more challenging. However, increasing the understanding of post-acute COVID-19 effects is essential to drive guideline updates for rehabilitation services, in order to provide personalized and evidence-based care for all the subjects who experienced the infection.[15]

We assessed potential brain alterations in a group of subjects who recovered from COVID-19, presented with neurological symptoms during the acute phase of the disease but who have never been hospitalized due to the infection. The individuals who were enrolled in the study had recovered from COVID-19 2 to 12 months prior to undergoing MRI analyses. Because we are following all these individuals and we are planning to check the presence of possible brain alterations in the next 5 to 7 years, we use the definition of 'mid-term follow-up' for the analyses presented herein. A multi-modal MRI study was performed to explore brain SARS-COV2-induced alterations from multiple points of view: (1) gray matter (GM) volume, (2) WM hyperintensities, (3) WM microstructural damage, and (4) brain perfusion. We expected some brain alterations could be present in these subjects, even if totally recovered from acute SARS-COV2 infection.

Methods

Participants

A group of subjects who recovered from SARS-COV2 infection (COV+) and a group of subjects who was never SARS-CoV-2 infected (COV−) were enrolled in the study at IRCCS Fondazione don Gnocchi. The inclusion criteria were defined as follows: (1) being older than 18 years; (2) having no history of brain tumors and/or neurologic diseases and/or psychiatric diseases. For COV+ group only, these additional inclusion criteria were defined: (3) having been diagnosed with COVID-19 (positive realt time polymerase chain reaction (RT-PCR) test) but not having required hospitalization during the infection; (4) having recovered from COVID-19 infection at the time of the study, from at least 2 months; (5) presenting with either hyposmia or anosmia during the acute stage. The latter inclusion criterion for COV+ group was introduced as hyposmia/anosmia is a very common and specific neurological symptom of SARS-COV2 infection, and we aimed to obtain a COV+ group as clinically homogeneous as possible. A questionnaire was used to record COVID-19 neurological symptoms (both at the time of the acute infection and at the MRI time) for all subjects belonging to the COV+ group. Self-reported

symptoms had to be graded using a rating scale (none, mild, moderate, or severe).

MRI acquisition

All the participants were scanned with the same PRISMA Siemens 3 T scanner, equipped with a 64-channel coil. A 3D sagittal magnetization-prepared rapid acquisition with gradient echo (MPRAGE) sequence was acquired to quantify gray matter volume and as anatomical reference, with the following parameters: repetition time (TR) = 2300 ms, echo time (TE) = 3.1 ms, in-plane resolution = 0.8 × 0.8 mm^2, acquisition matrix = 300 × 320, slice thickness = 0.8 mm^3, 224 slices. A sagittal fluid attenuated inversion recovery (FLAIR) sequence was also acquired (TR = 5000 ms, TE = 394 ms, in-plane resolution = 0.8 × 0.8 mm^2, acquisition matrix = 288 × 320, slice thickness = 1 mm, 176 slices) to assess macrostructural white matter (WM) damage. Furthermore, the acquisition protocol included an axial diffusion-weighted imaging (DWI) sequence (TR = 3600 ms, TE = 92 ms, in-plane resolution = 2 × 2 mm^2, acquisition matrix = 104 × 104, slice thickness = 2 mm, 72 slices) to assess WM microstructural integrity. The DWI sequence consisted of 5 b0 images, 50 diffusion-encoding directions with b = 1000 s/mm^2 and 50 diffusion directions with b = 2000s/mm^2, and it was acquired twice with reversed phase encoding direction (i.e. anterior-posterior and posterior-anterior).[16] Finally, an axial multi-delay pseudo-continuous arterial spin labeling (pCASL) sequence [TR = 4100 ms, TE = 30.56 ms, in-plane resolution = 3.5 × 3.5mm^2, acquisition matrix = 64 × 64, slice thickness = 3.5 mm, 32 slices, labeling duration = 1500 ms, 5 post-labeling delays (PLD) = [500, 1000, 1500, 2000, 2500] ms, phase-encoding direction = anterior-posterior] was acquired to assess brain perfusion.[17] M0 image for cerebral blood flow (CBF) calibration was acquired with the same parameters, with reversed phase-encode blips (i.e. anterior-posterior and posterior-anterior).

MRI processing

MRI processing was performed with FMRIB's Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) unless otherwise specified. The FLAIR and MPRAGE images were bias-corrected for magnetic resonance field inhomogeneity. Then, FLAIR images were coregistered to the respective MPRAGE image. WM hyperintensities were semi-automatically segmented by an experienced operator with Jim software package (http://www.xinapse.com). The volume of the segmented hyperintensities was computed for all the recruited subjects. The masks of hyperintense regions identified on FLAIR data were used to correct MPRAGE data for concurrent WM T1-hypointensities.[18] Then, non-brain tissue was removed from T1-weighted images.

DWI data were simultaneously corrected for eddy currents, subject movement, and susceptibility-induced geometric distortions.[19–21] Then, diffusion tensor was estimated for each voxel. Only DWI data acquired with b = 0 and b = 1000 were used in this study for diffusion tensor imaging (DTI) analysis because lower b-values fit better with the Gaussian diffusion model assumed in DTI. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps were computed and coregistered to MNI standard space (resolution = 1 × 1 × 1 mm^3).[22] Registration parameters were estimated by non-linearly registering a b0 image corrected for susceptibility-induced geometric distortions to MNI standard space, *via* MPRAGE.[23,24]

pCASL raw data were corrected for movement. Distortions were also corrected, using phase-encode-reversed calibration image.[25] Brain extraction was performed and brain perfusion maps were computed (tissue T1 = 1.3 s, T1 of blood = 1.65 s, tagging efficiency = 0.85).[26] Then, calibration was performed using a voxel-wise approach, to derive quantitative CBF maps.[26] Finally, CBF maps were non-linearly registered to MNI standard space (resolution = 2 × 2 × 2 mm^3), *via* respective 3D-T1-weighted images with Advanced Normalization Tools (ANTs, http://stnava.github.io/ANTs)

Statistical analysis

Demographic characteristics were compared between COV+ and COV− groups with SPSS (Version 24; IBM, Armonk, New York). Parametric or non-parametric statistics was used in case of normally and non-normally distributed data, respectively. Then, explorative analyses were performed to test the differences between COV+ and COV− groups in terms of (1) local GM volume; (2) WM hyperintensity volume; (3) local WM microstructural integrity; (4) local brain perfusion.

Specifically, voxel-based morphometry (VBM) was performed to assess GM volume differences.[27] Brain-extracted T1-weighted images were segmented into GM, WM, and cerebrospinal fluid (CSF), and a study-specific GM template was created. All GM images were non-linearly coregistered to the study-specific template and smoothed (Gaussian kernel sigma = 3 mm).[24] Modulation for the contraction/enlargement due to the non-linear component of the transformation was included in the processing. Finally, voxel-wise comparison between the two groups was performed with randomize tool (5000 permutations, cluster detection with threshold-free cluster enhancement), including age and gender as covariates.[28] The GM mask used for this voxel-wise statistics was defined by merging Harvard-Oxford cortical structural atlas (threshold = 0.25) to subcortical GM regions defined in Harvard-Oxford subcortical structural atlas (threshold = 0.25),[29] to test local GM volume differences both in cortical and subcortical GM areas.

WM hyperintensity volumes derived from FLAIR data were compared between COV+ and COV− groups with analysis of covariance (ANCOVA), correcting for age with SPSS (Version 24; IBM, Armonk, New York).

Furthermore, to check for local microstructural WM alterations, tract-based spatial statistics (TBSS) was performed.[30] The FA image of each subject was non-linearly registered to MNI standard space (resolution = $1 \times 1 \times 1\,mm^3$) and averaged. The mean FA image was skeletonised and the FA images of all subjects were projected on the mean FA skeleton, resulting in skeletonised FA maps for all the subjects. The nonlinear warps and skeleton projection were also applied to MD, AD, and RD maps of all the participants. Finally, voxel-wise statistics was performed to test group differences in terms of FA, MD, AD, and RD (5000 permutations, cluster detection with threshold-free cluster enhancement, age and gender as covariates).[28] The mean skeleton was used as mask for these voxel-wise tests.

Finally, CBF differences were locally tested for the whole brain in MNI standard space (resolution = $2 \times 2 \times 2\,mm^3$) with FSL randomize tool (5000 permutations, cluster detection with threshold-free cluster enhancement, age and gender as covariates).[28]

All voxel-based analyses results were family wise error (FWE) corrected to account for multiple comparisons. Significance level was set to 0.05 for all the statistics of this study.

If statistically significant results were obtained at voxel-wise analyses, the position of the clusters of voxels, where significant difference was observed, was mapped according to Harvard-Oxford atlas[29] and XTRACT atlas[31] for GM and WM respectively. Furthermore, for any MRI-derived index showing any difference between COV+ and COV−, the correlation with elapsed time between COVID-19 acute phase and the MRI scan was assessed in the regions occupied by the significant clusters of voxels.

Results

Sample

Forty-three subjects were included in the study: 22 subjects constituted COV+ group (9 males, median age [25th percentile–75th percentile] = 45.7 [34.8–53.4] years old), while 21 subjects were included as COV− group (6 males, median age [25th percentile–75th percentile] = 37.6 [28.4–56.6] years old). The two groups were age-matched (Independent samples Mann–Whitney U test, $p = 0.827$) and sex-matched (Fisher's exact test, $p = 0.526$). The two groups were also matched for the following factors: hypertension (Fisher's exact test, $p = 0.233$), hyperlipidemia (Fisher's exact test, $p = 0.488$). None of the recruited subjects suffered from diabetes. The mean elapsed time [standard deviation] between COVID-19 onset (i.e. positive RT-PCR) and the MRI scan (Δt) for COV + group was 7.3 [3.2] months (interquartile range = [5.3–10.3] months). All the recruited COV+ subjects experienced hyposmia during the acute phase of the infection, as required by the inclusion criteria. The additional neurological symptoms self-reported by the subjects are reported in Table 1.

GM volume

No significant GM volume differences were locally observed between COV+ and COV− groups with VBM.

Table 1. Prevalence of self-reported mild, moderate, and severe neurological manifestations in COV+ group, both in the acute phase and at the mid-term follow-up (when the MRI scan was performed).

COV+ group ($n = 22$) symptoms	Acute phase				Mid-term follow-up			
	None	Mild	Moderate	Severe	None	Mild	Moderate	Severe
Hyposmia (%)	0.0	0.0	18.2	81.8	54.5	31.8	4.5	9.1
Headache (%)	13.6	13.6	40.9	31.8	50.0	27.3	13.6	9.1
Vertigo (%)	45.5	22.7	22.7	9.1	72.7	27.3	0.0	0.0
Confusion (%)	81.8	18.2	0.0	0.0	95.5	4.5	0.0	0.0
Hypogeusia (%)	9.1	4.5	9.1	77.3	59.1	18.2	9.1	13.6
Myalgia (%)	18.2	9.1	18.2	54.5	63.6	9.1	22.7	4.5
Asthenia (%)	0.0	9.1	4.5	86.4	31.8	40.9	13.6	13.6
Allodynia (%)	72.7	4.5	18.2	4.5	86.4	13.6	0.0	0.0
Acroparesthesia (%)	40.9	13.6	27.3	18.2	63.6	22.7	9.1	4.5

MRI, magnetic resonance imaging.

WM focal lesions

The ANCOVA of WM hyperintensity volume did not show any significant difference between COV+ and COV− groups ($p = 0.479$).

WM microstructural integrity

Significant differences were observed in terms of WM microstructural integrity (Figure 1). Specifically, significantly lower FA was observed for COV+ group with respect to COV− group in the right arcuate fasciculus (cluster of 102 mm^3, pFWE-peak = 0.037), right middle longitudinal fasciculus (26 mm^3, pFWE-peak = 0.047), right superior longitudinal fasciculus II (363 mm^3, pFWE-peak = 0.035), right superior longitudinal fasciculus III (162 mm^3, pFWE-peak = 0.035). Furthermore, COV+ showed significantly higher RD when compared with COV− group in the right arcuate fasciculus (168 mm^3, pFWE-peak = 0.039), acoustic radiation (28 mm^3, pFWE-peak = 0.049), dorsal cingulum (15 mm^3, pFWE-peak = 0.047), corticospinal tract (33 mm^3, pFWE-peak = 0.048), frontal aslant tract (38 mm^3, pFWE-peak = 0.050), inferior fronto-occipital fasciculus (5 mm^3, pFWE-peak = 0.046), middle longitudinal fasciculus (153 mm^3, pFWE-peak = 0.042), optic radiation (4 mm^3, pFWE-peak = 0.047), superior longitudinal fasciculus I (37 mm^3, pFWE-peak = 0.044), superior longitudinal fasciculus II (448 mm^3, pFWE-peak = 0.038), superior longitudinal fasciculus III (237 mm^3, pFWE-peak = 0.038), and superior thalamic radiation (23 mm^3, pFWE-peak = 0.050). No significant correlation was observed in the COV+ group between Δt and either FA or RD within the regions for which either FA or RD alterations were detected.

Brain perfusion

The explorative voxel-wise analysis across the whole brain detected no significant CBF differences between COV+ and COV−.

Discussion

In this exploratory study, COVID-19-related brain alterations were assessed in a group of non-hospitalized subjects who recovered from SARS-COV2 infection and presented with neurological symptoms during the acute phase of the disease. No significant SARS-COV2-induced abnormalities were found in terms of GM volume, WM focal lesions and brain perfusion, while WM microstructural alterations were detected. These results suggest that in these patients (1) COVID-19 may have no mid-term effect on GM and WM at the structural and metabolic/vascular macroscopic level, and (2) WM may be persistently damaged at the microstructural level due to SARS-COV2 infection.

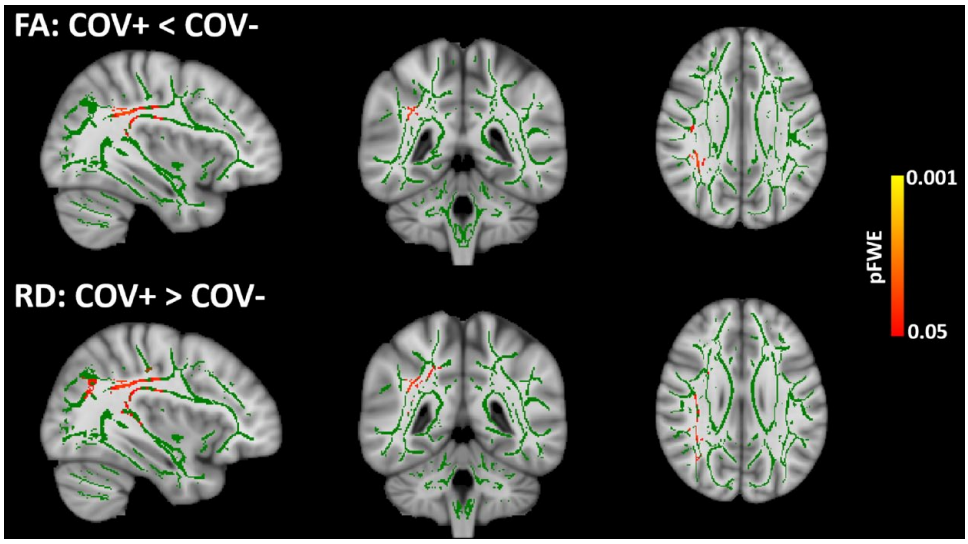

Figure 1. Significant local microstructural alterations observed in COV+ group compared with COV– group. The upper panel shows voxels where FA is significantly lower in COV+, while the lower panel shows voxels where higher RD was observed in COV+. Significance level is represented with red-yellow scale, and the respective colorbar is reported. The results are represented in MNI standard space, superimposed to the mean skeleton mask (in green).

Multi-focal WM lesions, compatible with cerebral small-vessel disease, and perfusion abnormalities were frequently observed in critically ill patients with COVID-19.[5,32,33] Although the pathophysiological mechanisms underlying the COVID-19-induced cerebrovascular disease are still unclear, angiotensin-converting enzyme 2 receptor downregulation, which induces disruption of the renin-angiotensin system, was hypothesized to play a role in cerebrovascular dysregulation, altered cerebral perfusion, and endothelial dysfunction observed in COVID-19.[34] GM volume alterations were less found in the acute phase of the disease, also because most of the published COVID-19 neuroimaging studies so far have been based on visual MRI assessment by the radiologist. A recent computed tomography study reported no significant difference in total GM volume between COVID-19 patients and healthy controls. However, the same study showed that lower GM volume in frontal regions was linked to more severe disability in COVID-19 patients, suggesting that frontal areas could be affected in COVID-19, beyond the presence of focal damage.[35] This mounting evidence suggests that alterations at the macroscopic level are present in case of severe acute COVID-19, supporting that brain integrity is vulnerable to SARS-COV2. Interestingly, at 3-month follow-up, persistent GM hypoperfusion and reduced cortical thickness in the left insula, left hippocampus, and left superior temporal gyrus were reported by Qin *et al.*[36] in patients recovered from severe COVID-19, with no specific neurological manifestation. Nevertheless, the same study also reported that subjects who recovered from a milder form of the disease and with no specific neurological manifestation did not show any GM volume and perfusion alteration.[36] The latter finding is similar to the one that was obtained in our current study, performed in non-hospitalized subjects, who though experience neurological manifestations during COVID-19 acute phase. Therefore, the absence of significant GM volume loss, WM focal lesion increase, and perfusion abnormalities could be related to the fact that non-hospitalized subjects had experienced mild-to-moderate COVID-19. However, a large recent longitudinal UK Biobank study, including 394 participants having tested positive for SARS-CoV2 infection between the two scans, investigated the effect of COVID-19 on structural and functional brain imaging, and identified significant effects of COVID-19 in the brain with GM loss in the left parahippocampal gyrus, the left lateral orbitofrontal cortex, the left insula, anterior cingulate cortex, supramarginal gyrus and temporal pole, even for COVID-19 patients presenting with neurological manifestation who had never been hospitalized.[37] The discrepancy

between our GM volume findings and UK Biobank's ones might be ascribed both to the inclusion of younger participants (age range = 20.5–67.7 *versus* 51.3–81.4 years old, respectively) and to the relatively small sample size of our current study. Further investigations, comparing clinically well-characterized, non-hospitalized, recovered patients who experienced neurological symptoms and patients who did never experience neurological symptoms may help to clarify the relationship between clinical severity, neurological manifestations, and COVID-19 effect on GM volume.

Despite the heterogeneity of the cohorts participating in the previous studies investigating SARS-COV2 mid-term effects on WM microstructural integrity and in the current one (i.e. hospitalized/non-hospitalized subjects, with/without neurological symptoms), DWI-based WM abnormalities have already been observed in hospitalized subjects recovered from COVID-19, at 3 months from the acute infection.[14,36] Therefore, our WM DWI findings in non-hospitalized subjects are in line with previous neuroimaging studies that have evaluated the evolution of brain changes over time in hospitalized subjects, after the acute phase of the disease. Specifically, a study by the Oxford group about the medium-term effects of SARS-COV2 reported higher MD in the left posterior thalamic radiation and in the right sagittal stratum of subjects who had been hospitalized with moderate/severe COVID-19.[14] In addition, Qin *et al.*[36] showed that subjects recovered from severe COVID-19 had greater and more widespread brain abnormalities than those who had suffered from moderate COVID-19, but WM alterations were detected even for the latter group. Notably, the alterations of the WM bundles reported in Qin's study have been observed in subjects who have never experienced neurological symptoms due to SARS-COV2, suggesting that microstructural brain damage may be indirectly produced by the inflammation caused by the disease.[36] This previous result, together with the findings reported in our study, suggests that COVID-19 might impact WM microstructural integrity even for mild-to-moderate forms of the infection. Nevertheless, it is still unclear whether brain alterations induced by COVID-19 will last over time, as, to the best of our knowledge, neuroimaging longitudinal studies with follow-ups greater than 6 months are currently not available yet. Clarifying the longitudinal trajectory of brain alterations is crucial for aiding rehabilitation treatments. Indeed, it was recently reported that people who had recovered from COVID-19, including non-hospitalized cases, exhibit cognitive deficits when compared with healthy controls,[38] which mirrors the alterations of the neural substrate.[37] Dealing with an increased risk for cognitive decline may be one of the greatest post-pandemic future challenges. Future neuroimaging and behavioral longitudinal studies, including a large and more homogeneous cohort of subjects and multiple follow-ups, are warranted to clarify whether the microstructural damage detected even in non-hospitalized subjects will be either persistent or fully recovered over time. Furthermore, including susceptibility-weighted imaging (SWI) in the MRI protocol is recommended for future studies, as SWI provides information about the presence of a higher burden of microvascular events, which are an additional relevant aspect to assess in a COVID-19 neuroimaging study.[4,14]

The relatively limited sample size and the cross-sectional design of the study are the main limitations of this exploratory study. Subjects included in COV+ group were scanned with MRI just once, after recovering from COVID-19. No MRI was acquired during the acute phase of the infection. However, this limitation could be overcome only by including hospitalized patients in the study, as isolation and quarantine are mandatory for any confirmed COVID-19 case, but this was out of the scope of the study. Although scanning the participants during the acute infection was not possible, planning future follow-ups is warranted to assess how the brain alterations reported in this study will evolve over time.

In conclusion, this exploratory neuroimaging study showed some COVID-19 mid-term effects on the brain in non-hospitalized subjects who recovered from the infection. Brain alterations were detected at the microstructural level, suggesting that even subjects who have never been hospitalized may present with brain changes due to COVID-19. However, previous studies and the current one have not produced sufficient evidence yet to determine the impact of COVID-19 on the central nervous system over time. It cannot be excluded that WM microstructural alterations that have been observed will be totally recovered over time. Future longitudinal studies are warranted to clarify

the evolution of COVID-19-induced brain changes. As COVID-19 was hypothesized to result in a higher risk factor for developing cognitive decline,[38] knowledge deriving from longitudinal studies may be relevant to guide neurorehabilitation treatments, in terms of duration, intensity, and target regions.[15,39]

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Helsinki Declaration[40] and it was approved by IRCCS Fondazione Don Carlo Gnocchi Ethics Committee (approval number: 06_16042020). All the participants provided their written informed consent to participate in this study, according to the local Institutional Ethic Committee recommendations.

Consent for publication

Not applicable.

Author contributions

Laura Pelizzari: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing.

Marta Cazzoli: Data curation; Investigation; Writing – review & editing.

Susanna Lipari: Investigation; Writing – review & editing.

Maria Marcella Laganà: Conceptualization; Writing – review & editing.

Monia Cabinio: Investigation; Writing – review & editing.

Sara Isernia: Investigation; Writing – review & editing.

Alice Pirastru: Investigation; Writing – review & editing.

Mario Clerici: Conceptualization; Writing – review & editing.

Francesca Baglio: Conceptualization; Project administration; Writing – review & editing.

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Competing Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

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