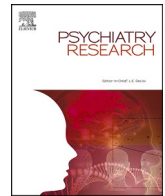




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Two-year follow-up of brain structural changes in patients who recovered from COVID-19: A prospective study

Yanyao Du^{a,1}, Wei Zhao^{a,b,c,1}, Sihong Huang^a, Yijie Huang^a, Yanjing Chen^a, Huiting Zhang^d, Hu Guo^e, Jun Liu^{a,b,c,*}

^a Department of Radiology, Second Xiangya Hospital of Central South University, Changsha, Hunan Province 410011, China

^b Clinical Research Center for Medical Imaging in Hunan Province, Changsha, Hunan 410011, China

^c Department of Radiology Quality Control Center, Changsha, Hunan Province 410011, China

^d MR Scientific Marketing, Siemens Healthineers Ltd., Wuhan 430000, China

^e MR Application, Siemens Healthineers Ltd., Changsha 410011, China

ARTICLE INFO

Keywords:

Gray matter volume changes
COVID-19 clinical sequelae
Voxel-based morphometry
Cross-sectional study
longitudinal follow-up

ABSTRACT

The long-term effects of COVID-19 on brain structure remain unclear. A prospective study was conducted to explore the changes in brain structure in COVID-19 survivors at one and two years after discharge (COVID-19_{one}, COVID-19_{two}). The difference in gray matter volume (GMV) was analyzed using the voxel-based morphometry method, and correlation analyses were conducted. The dynamic changes in clinical sequelae varied. The GMVs in the cerebellum and vermis were reduced in COVID-19_{one} and COVID-19_{two}, positively correlated with lymphocyte count, and negatively correlated with neutrophil count, neutrophil/lymphocyte ratio (COVID-19_{one}), and systemic immune-inflammation index (COVID-19_{two}). The decreased GMVs in the left middle frontal gyrus, inferior frontal gyrus of the operculum, right middle temporal gyrus, and inferior temporal gyrus returned to normal in COVID-19_{two}. The decreased GMV in the left frontal lobe was negatively correlated with the Athens Insomnia Scale (AIS). The GMV in the left temporal lobe was aggravated in COVID-19_{two} and positively correlated with C-reactive protein. In conclusion, GMV recovery coexisted with injury, which was associated with AIS and inflammatory factors. This may shed some light on the dynamic changes in brain structure and the possible predictors that may be related to GMV changes in COVID-19_{two}.

1. Introduction

The global outbreak of COVID-19, caused by SARS-CoV-2, has posed unprecedented challenges to public health. These challenges are manifested not only in providing medical needs after acute infection with COVID-19 pneumonia but also in dealing with the long-term sequelae of various systems. The acute and subacute symptoms and complications of the nervous, respiratory, digestive, and other systems after SARS-CoV-2 infection have been well investigated (Cagnazzo et al., 2021; Caruso et al., 2021; Zhang et al., 2022; Al-Aly et al., 2021), but little is known about the long-term effects on various organs, especially the brain.

Patients with SARS-CoV-2 infections often have neurological symptoms. Acute neurological complications, including dizziness, headache, acute ischemic stroke, encephalitis, and acute necrotizing encephalopathy, are common (Mao et al., 2020; Varatharaj et al., 2020). However,

the mechanism by which SARS-CoV-2 infects the nervous system is still unclear. It may be related to viral neurotropism (e.g., invading olfactory cells or vascular endothelial cells) and the SARS-CoV-2-induced inflammatory storm that damages the blood-brain barrier (Yang et al., 2022; Bourgonje et al., 2020; Speranza et al., 2021). A growing body of research suggests that the neurologic changes associated with COVID-19 exist and can persist for up to one or two years, but the mechanism of neurologic invasion remains unclear. The latest research shows that although the proportion of patients suffering from sequelae of COVID-19 infection two years after discharge has decreased, anxiety or depression and fatigue or muscle weakness were the most common (Huang et al., 2022a). Most current studies are statistical descriptions of patients' clinical subjective symptoms. There is a lack of research support on whether there are changes in brain microstructure and function, whether the changes are long-term, and how they evolve.

* Corresponding author at: Department of Radiology, Second Xiangya Hospital of Central South University, Changsha, Hunan Province 410011, China.

E-mail address: junliu123@csu.edu.cn (J. Liu).

¹ Yanyao Du and Wei Zhao contributed equally to this manuscript.

Previous studies with follow-up within three to ten months found that rehabilitated COVID-19 patients still had neuropsychiatric symptoms, cognitive impairment, microstructure, and cerebral blood flow changes after discharge (Huang et al., 2021; Tian et al., 2022; Qin et al., 2021; Du et al., 2022; Huang et al., 2022b). One-year follow-up studies found that the brain function index (amplitude of low-frequency fluctuations) increased (Du et al., 2022), and white matter axon density decreased in patients who recovered from COVID-19 (Huang et al., 2022b). A study with follow-up for six months was performed using CT-reconstructed gray matter volume (GMV) data and reported lower GMV in superior/medial/middle frontal gyri (Duan et al., 2021). However, there have been no long-term MRI studies exploring changes in the gray matter. In MRI methods, voxel-based morphometry (VBM) is a traditional noninvasive method for analyzing brain GMV changes and is widely used in the study of various neurological diseases. VBM can obtain more accurate GMV results than CT reconstruction. In addition, longer and multiple longitudinal follow-ups will allow us to further explore the underlying mechanisms of long-term neurological sequelae, but there is a lack of studies to observe dynamic changes in neurological symptoms and brain structure.

Therefore, we aimed to explore the cross-sectional and longitudinal GMV changes by data-driven analysis in patients with COVID-19 at one and two years after discharge (COVID-19_{one}, COVID-19_{two}). Meanwhile, we investigated the possible causes of GMV changes in COVID-19_{two} by correlation analysis.

2. Materials and methods

2.1. Study design and participants

The experimental design is shown in Fig. 1. Of the 237 COVID-19 recovered patients contacted, 22 completed follow-ups at one year after discharge and 18 completed follow-ups at two years after discharge. In the longitudinal analysis, the average gray matter images of two patients could not be segmented; finally, 16 patients were included in the longitudinal analysis. During the same period of the first follow-up of recovered COVID-19 patients, 27 HCs matched by age, sex, and education were recruited. The following comparisons were performed: (a) changes in clinical symptoms of 16 patients during hospitalization and one- and two-year follow-up; (b) comparison of brain structural changes between 22 COVID-19_{one} and HCs; (c) brain effects of COVID-19 on 18 COVID-19_{two} compared to HCs; and (d) GMV differences between COVID-19_{one} and COVID-19_{two}.

In the longitudinal analysis, the average gray matter images of two people could not be segmented, so 16 patients were finally included. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2

infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. HCs, healthy controls. GMV: gray matter volume.

This prospective study was approved by the Ethics Committee of our hospital (approval number: 2020S004), and informed written consent was signed by all participants. Patients with COVID-19 admitted to the First Hospital of Changsha were recruited. Follow-up visits were scheduled at one year and two years post-recovery from the time of discharge. The inclusion criteria were as follows: (a) patients with a diagnosis of COVID-19 according to the guidelines of the National Health Commission; (b) patients who volunteered to participate in the study and completed follow-ups with questionnaires and brain MRI. The exclusion criteria were as follows: (a) patients with contraindications to MRI and (b) patients with a history of structural brain abnormalities. In addition, age-, sex- and education-matched healthy controls (HCs) were recruited through social media. HCs were negative for both nucleic acid and antigen testing. Demographics and laboratory findings were collected during hospitalization. Inflammatory markers were obtained upon admission of COVID-19 patients, including neutrophil count, lymphocyte count, neutrophil/lymphocyte ratio (NLR), systemic immune-inflammation index (SII=platelets*neutrophils/lymphocytes), and C-reactive protein (CRP). On the first and second follow-up MR examinations, the patients were asked to fill out several COVID-19 sequelae questionnaires, including the Athens Insomnia Scale (AIS), Hospital Anxiety and Depression Scale (HADS), Wechsler intelligence scale, and clinical symptoms.

2.2. MRI acquisition parameters

MRI data were obtained on a 3T MRI scanner (MAGNETOM Skyra, Siemens Healthcare, Erlangen, Germany) with a 32-channel head coil. Participants were kept in a supine position with their entire body still while wearing earplugs, and a foam pad was placed between the head and the coil to minimize motion artifacts. The MRI scanning sequences included two-dimensional T1-weighted imaging, T2-weighted imaging, fluid-attenuated inversion recovery imaging, and three-dimensional magnetization-prepared rapid acquisition gradient echo (3D-MPRAGE). The 3D-MPRAGE scanning parameters were as follows: 176 sagittal slices, repetition time = 2000 ms, echo time = 2.26 ms, flip angle = 8°, voxel size = 1 mm × 1 mm × 1 mm, slice thickness = 1 mm, and field of view = 256 mm × 256 mm.

2.3. Cross-sectional analysis

3D-MPRAGE imaging data were analyzed using FSL-VBM with an optimized VBM protocol in the FSL tool (Smith et al., 2004). First, structural data were segmented into gray matter, white matter, and

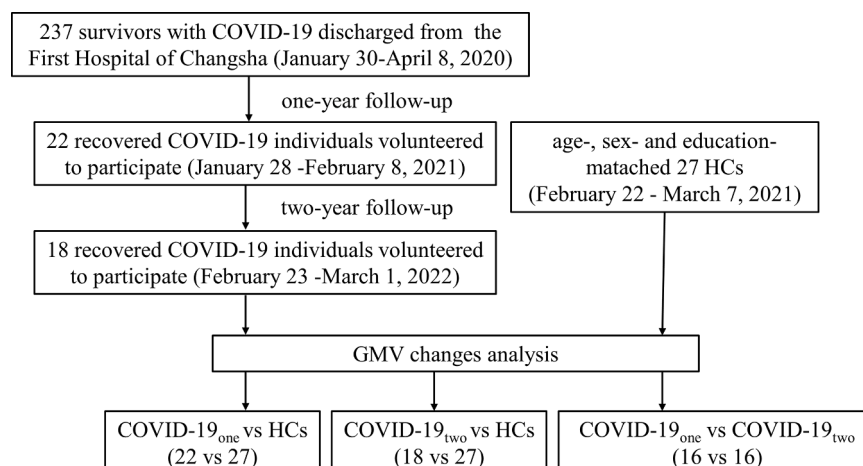


Fig. 1. Flowchart of the study.

cerebrospinal fluid. The segmented gray matter images were registered to the ICBM-152 gray matter template using nonlinear registration, and all the registered images were averaged and flipped along the x-axis. Then, gray matter images of all subjects were nonlinearly registered to the study-specified template, and local expansion or contraction of these images was also corrected by "modulation". Finally, all modulated gray matter images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.

2.4. Longitudinal analysis

Paired structural data were analyzed using the optimized VBM protocol, but the specific protocol for gray matter segmentation was somewhat different from the cross-sectional analysis (Douaud et al., 2009). First, two "halfway" affine matrices between the participants' first and second structural data were generated. The transformation matrices were applied to the corresponding segmented gray matter images and averaged. Then, the averaged gray matter images were nonlinearly registered to a study-specific template constructed after gray matter segmentation of native images. The above nonlinear transformation results were mapped to the "halfway" first and second gray matter images, which were then corrected by "modulation". Smoothing was processed using an isotropic Gaussian kernel with a sigma of 3 mm.

2.5. Statistical analysis

Clinical and imaging data were analyzed by IBM SPSS Statistics 24.0 and permutation-based nonparametric testing, respectively. Quantitative data are described as the mean \pm standard deviation or median with interquartile range according to data distributions. Categorical variables are shown as numbers and percentages. Two-sample *t*-test, Wilcoxon-Mann-Whitney *U* test or Chi-square test was used for the comparison of two groups as appropriate, and Cochran's *Q* test was used for the differences in clinical symptoms among the three groups. Cross-sectional and longitudinal VBM results were corrected for multiple comparisons by 5000 permutation tests with threshold-free cluster enhancement, and age, sex, education and BMI were used as covariates. Then, a post hoc region-of-interest (ROI) analysis was performed by extracting the GMV values of each participant's different brain regions. Pearson's or Spearman's correlation was performed according to the data distributions. $P < 0.05$ was considered indicative of a statistically significant difference.

Table 1

Neuropsychiatric and neurocognitive test scales of the COVID-19_{one}, COVID-19_{two} and HC groups.

	COVID-19 _{one} (N=22)	COVID-19 _{two} (N=18)	HCs (N=27)	$t_1 / \chi^2_1 / Z_1$	P_1	$t_2 / \chi^2_2 / Z_2$	P_2
Age (years) ^a	54.14 \pm 9.76	53.50 \pm 10.03	50.81 \pm 11.48	1.076	0.287	0.807	0.424
Gender (male/female) ^b	11/11	9/9	7/20	3.023	0.082	2.732	0.098
Education (years) ^a	13.45 \pm 3.57	13.56 \pm 3.87	12.56 \pm 3.99	0.822	0.415	0.839	0.407
HADS-A ^c	1.00(0.00, 3.00)	2.00(0.00, 3.50)	3.00(1.00, 5.00)	-1.578	0.115	-1.289	0.197
HADS-D ^c	1.50(0.00, 4.50)	1.00(0.00, 2.00)	2.00(0.00, 4.00)	-0.072	0.942	-1.190	0.234
AIS ^a	7.82 \pm 4.63	6.06 \pm 4.81	5.15 \pm 2.94	2.454	0.018*	0.786	0.436
Neurocognitive tests							
LM-A ^c	7.00(5.00, 9.00)	7.00(5.75, 9.00)	6.00(3.00, 9.00)	-1.033	0.301	-1.274	0.203
LM-B ^c	5.00(3.75, 8.25)	4.00(3.00, 10.25)	6.00(4.00, 8.00)	-0.233	0.816	-0.373	0.709
DSST ^c	72.00(56.00, 89.50)	76.50(49.75, 92.00)	56.00(36.00, 91.00)	-1.297	0.195	-0.696	0.487
Knowledge subscale of Wechsler Intelligence scale ^a	18.27 \pm 4.41	17.83 \pm 5.27	17.85 \pm 4.64	0.323	0.748	-0.012	0.990
FDS ^a	11.36 \pm 1.92	11.17 \pm 2.23	10.96 \pm 2.44	0.628	0.533	0.284	0.778
BDS ^c	6.50(5.00, 7.50)	6.50(4.00, 8.25)	6.00(5.00, 8.00)	-0.580	0.562	-0.633	0.527
WFT ^a	20.00 \pm 5.49	19.33 \pm 5.85	18.07 \pm 6.51	1.104	0.275	0.661	0.512

^a Two-sample *t*-test.

^b Chi-square test.

^c Wilcoxon-Mann-Whitney *U* test. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. N, number of subjects. HCs, healthy controls. AIS, Athens Insomnia Scale. HADS, Hospital Anxiety and Depression Scale. LM, logical memory task; DSST, digital symbol substitution test; DS, digit span task; FDS, forward digit span; BDS, backward digit span; WFT, Word fluency test.

* *P* values less than 0.05 indicate statistical significance.

3. Results

3.1. Participant characteristics and clinical symptoms

The neuropsychiatric and neurocognitive test scales of COVID-19_{one} and COVID-19_{two} compared with HCs are shown in Table 1. There was no statistically significant difference in age, sex, education, HADS scores, or neurocognitive tests. The COVID-19_{one} demonstrated significantly increased AIS scores compared to the HCs.

As shown in Table 2, the number of patients with mild type and severe type were the same, and body mass index (BMI) was not significantly different in the longitudinal follow-up study. They were hospitalized for 21.00 \pm 10.54 days. The follow-up time was 342.81 \pm 15.13 days in one year and 731.75 \pm 13.21 days in two years. At the one-year follow-up after SARS-CoV-2 infection, except for a significant reduction in insomnia symptoms, there were no statistically significant differences in HADS scores and neurocognitive tests. The mean or median values of neutrophil count, lymphocyte count, NLR, SII, and CRP were 2.82 \times 10⁹/L (range 1.51–6.12), 1.14 \times 10⁹/L (range 0.54–2.55), 2.46 (range 1.00–7.46), 326.48 (range 167.61–2492.78), and 25.17 mg/L (range 7.68–91.35), respectively.

During hospitalization, the main symptoms appeared in the nervous system and respiratory system and less frequently in the digestive system. Myalgia, cough, and dyspnea were persistent in COVID-19_{one}. The frequency of fever, fatigue, olfactory loss, and taste loss decreased, but the frequency of headache, expectoration, and chest tightness increased. Compared with COVID-19_{one}, headache, fatigue, dyspnea, and chest pain were the most common symptoms of COVID-19_{two}, while cough continued to improve (Table 2, Fig. 2).

The dynamic changes in clinical sequelae varied, and some symptoms resolved (fever, taste loss, etc.), whereas some symptoms persisted (fatigue and dyspnea) in COVID-19_{one} and COVID-19_{two}. Some patients newly developed headache and chest pain in COVID-19_{two}. Orange on the brain and lungs indicates the presence of clinical symptoms in COVID-19_{one}, and dark red indicates the persistence of clinical symptoms in COVID-19_{two}. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection.

3.2. Cross-sectional VBM analysis

Compared with the HCs, the COVID-19_{one} showed significantly reduced GMV in the bilateral cerebellum and vermis, left middle frontal

Table 2Clinical information in the acute stage, COVID-19_{one}, and COVID-19_{two}.

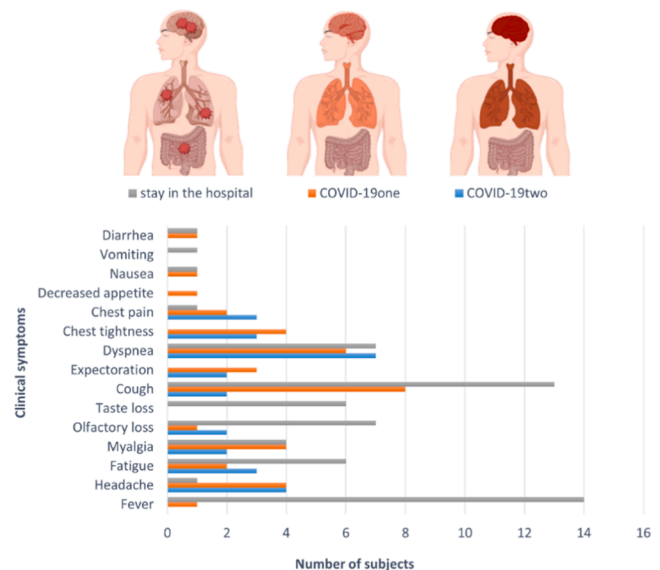
	stay in the hospital (N=16)	COVID-19 _{one} (N=16)	COVID-19 _{two} (N=16)	t/Q/Z	P
Clinical type (mild/severe)	9/9	-	-	-	-
Hospital day (days)	21.00 ±10.54	-	-	-	-
Follow-up time (days) ^a	-	342.81 ±15.13	731.75 ±13.21	-77.443	<0.001*
BMI	-	25.39 ±2.31	25.39 ±2.19	0.006	0.995
HADs-A ^b	-	1.00 (0.00, 3.00)	2.00 (0.00, 4.50)	0.088	0.931
HADs-D ^b	-	1.50 (0.00, 4.00)	1.00 (0.00, 2.00)	0.584	0.568
AIS ^a	-	8.00 ±3.88	5.75 ±4.75	2.697	0.017*
Neurocognitive tests					
LM-A ^a	-	7.13 ±3.03	7.31 ±2.27	-0.269	0.791
LM-B ^a	-	5.38 ±2.85	5.56 ±4.27	-0.176	0.863
DSST ^b	-	75.50 (50.00, 92.50)	67.50 (49.25, 91.00)	0.959	0.353
Knowledge subscale of Wechsler Intelligence scale ^a	-	17.75 ±4.68	17.44 ±5.37	0.359	0.724
FDS ^a	-	11.38 ±2.09	11.06 ±2.32	0.863	0.401
BDS ^b	-	6.50 (4.25, 7.00)	6.50 (4.00, 8.00)	-0.642	0.521
WFT ^a	-	20.25 ±6.05	19.81 ±5.98	0.378	0.711
Inflammatory markers					
Neutrophil count (×10 ⁹ /L)	2.82 ±1.10	-	-	-	-
Lymphocyte count (×10 ⁹ /L)	1.14 ±0.48	-	-	-	-
NLR	2.46 (1.76, 3.11)	-	-	-	-
SII	326.48 (243.35, 443.02)	-	-	-	-
CRP(mg/L)	25.17 (14.05, 42.73)	-	-	-	-
Symptoms, n (%)^c					
Fever	14 (87.50%)	1 (6.25%)	0 (0.00%)	26.143	<0.001*
Headache	1 (6.25%)	4 (25.00%)	4 (25.00%)	3.000	0.223
Fatigue	6 (37.50%)	2 (12.5%)	3 (18.75%)	2.889	0.236
Myalgia	4 (25.00%)	4 (25.00%)	2 (12.5%)	1.000	0.607
Olfactory loss	7 (43.75%)	1 (6.25%)	2 (12.5%)	8.857	0.012*
Taste loss	6 (37.50%)	0 (0.00%)	0 (0.00%)	12.000	0.002*
Cough	13 (81.25%)	8 (50.00%)	2 (12.5%)	12.133	0.002*
Expectoration	0 (0.00%)	3 (18.75%)	2 (12.5%)	3.500	0.174
Dyspnea	7 (43.75%)	6 (37.50%)	7 (43.75%)	0.200	0.905

Table 2 (continued)

	stay in the hospital (N=16)	COVID-19 _{one} (N=16)	COVID-19 _{two} (N=16)	t/Q/Z	P
Chest tightness	0(0.00%)	4 (25.00%)	3 (18.75%)	4.333	0.115
Chest pain	1(6.25%)	2(12.5%)	3 (18.75%)	1.000	0.607
Decreased appetite	0(0.00%)	1(6.25%)	0(0.00%)	2.000	0.368
Nausea	1(6.25%)	1(6.25%)	0(0.00%)	1.000	0.607
Vomiting	1(6.25%)	0(0.00%)	0(0.00%)	1.000	0.607
Diarrhea	1(6.25%)	1(6.25%)	0(0.00%)	1.000	0.607

^a Paired-sample *t*-test.^b Wilcoxon-Mann-Whitney *U* test.

^c Cochran's Q Test. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. N, number of subjects. BMI, body mass index. AIS, Athens Insomnia Scale. HADS, Hospital Anxiety and Depression Scale. LM, logical memory task; DSST, digital symbol substitution test; DS, digit span task; FDS, forward digit span; BDS, backward digit span; WFT, Word fluency test. NLR, neutrophil/lymphocyte ratio. SII, systemic immune-inflammation index. CRP, C-reactive protein.

* *P* values less than 0.05 indicate statistical significance.**Fig. 2.** Clinical symptoms in the acute stage, COVID-19_{one}, and COVID-19_{two}.

gyrus (MFG) and inferior frontal gyrus of the operculum (IFGoperc), and right middle temporal gyrus (MTG) and inferior temporal gyrus (ITG); and the COVID-19_{two} showed significantly reduced GMV in the bilateral cerebellum and vermis, left MTG and fusiform. Details and post hoc ROI analysis are shown in Table 3 and Fig. 3. The GMV in the bilateral cerebellum and vermis was significantly positively correlated with the lymphocyte count (Fig. 4A, $r = 0.44$, $P = 0.040$) in COVID-19_{one}, and the GMV in the left MFG and IFGoperc was significantly negatively correlated with AIS (Fig. 4B, $r = -0.34$, $P = 0.017$). However, these two correlations were not significantly different after false discovery rate (FDR) correction for multiple comparisons. The GMVs in the bilateral cerebellum and vermis were significantly negatively correlated with the neutrophil count (Fig. 4C, $r = -0.60$, $P = 0.008$, $P_{FDR} = 0.040$), NLR (Fig. 4D, $r = -0.63$, $P = 0.005$, $P_{FDR} = 0.025$), and SII (Fig. 4E, $r = -0.62$, $P = 0.007$, $P_{FDR} = 0.035$) in COVID-19_{two}.

Table 3Significant GMV differences between COVID-19_{one} and HCs, COVID-19_{two} and HCs, and COVID-19_{one} and COVID-19_{two}.

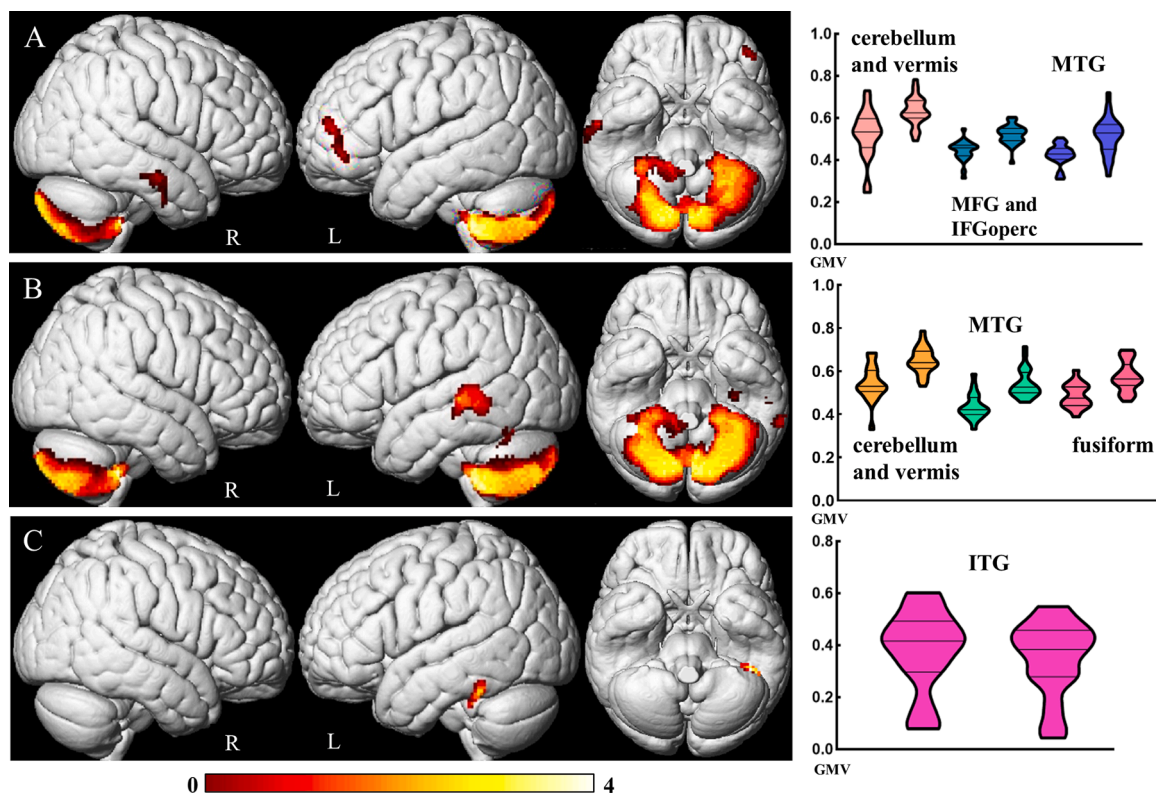
Brain regions (AAL)	Side (R/L)	Size (Voxels)	MNI coordinate			GMV		P
			X	Y	Z			
COVID-19_{one} vs HCs (22 vs 27)								
Cerebellum, vermis	R, L	5167	56	44	9	0.52±0.12 ^a	0.63±0.07 ^b	0.001
MFG, IFGoperc	L	154	65	89	43	0.44±0.05	0.52±0.05	0.021
MTG, ITG	R	141	15	57	26	0.42±0.05	0.51±0.09	0.038
COVID-19_{two} vs HCs (18 vs 27)								
Cerebellum, vermis	R, L	8102	50	20	13	0.54±0.08 ^c	0.65±0.06 ^b	<0.001
MTG	L	570	73	44	36	0.44±0.06	0.54±0.07	0.006
Fusiform	L	41	62	51	22	0.49±0.06	0.57±0.07	0.042
COVID-19_{one} vs COVID-19_{two} (16 vs 16)								
ITG	L	90	69	41	25	0.38±0.16 ^a	0.35±0.15 ^c	0.042

AAL, anatomical automatic labeling atlas. R, right. L, left. MNI, Montreal Neurological Institute. GMV, gray matter volume. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. HCs, healthy controls. MFG, middle frontal gyrus. IFGoperc, inferior frontal gyrus of operculum. MTG, middle temporal gyrus. ITG, inferior temporal gyrus.

^a One-year post-recovery.

^b Healthy controls.

^c Two-year post-recovery. In the longitudinal analysis, the average gray matter images of 2 people could not be segmented, so 16 patients were finally included.

**Fig. 3.** Results of VBM analysis and post hoc ROI analysis between COVID-19_{one} and HCs, COVID-19_{two} and HCs, and COVID-19_{one} and COVID-19_{two}.

(A) Compared with the HCs, the COVID-19_{one} showed significantly reduced GMV in the bilateral cerebellum and vermis, left MFG and IFGoperc, and right MTG and ITG. (B) Compared with the HCs, the COVID-19_{two} showed significantly reduced GMV in the bilateral cerebellum and vermis, left MTG and fusiform. (C) Significant difference in the left ITG between COVID-19_{one} and COVID-19_{two}. VBM, voxel-based morphometry. ROI, region of interest. R, right. L, left. GMV, gray matter volume. COVID-19_{one}, patients one year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. HCs, healthy controls. MFG, middle frontal gyrus. IFGoperc, inferior frontal gyrus of operculum. MTG, middle temporal gyrus. ITG, inferior temporal gyrus.

3.3. Longitudinal VBM analysis

COVID-19_{two} had significantly reduced GMV in the left ITG compared with COVID-19_{one}, as shown in Table 3 and Fig. 3. The difference in GMV in the left ITG between the two groups was significantly positively correlated with CRP (Fig. 4F, $r = 0.60$, $P = 0.015$) but not with survival after FDR.

4. Discussion

This study used longitudinal MR imaging data to explore brain structural damage in COVID-19_{two}. The main findings of our two-year follow-up study were as follows: (a) insomnia gradually improved with longer follow-up; (b) clinical sequelae were mainly present in the nervous system and respiratory system, and headache, fatigue, dyspnea, and chest pain were the most common symptoms; (c) GMV reduction in the cerebellum and vermis was persistently observed, and reversible changes in the left frontal and temporal GMV were found; and (d)

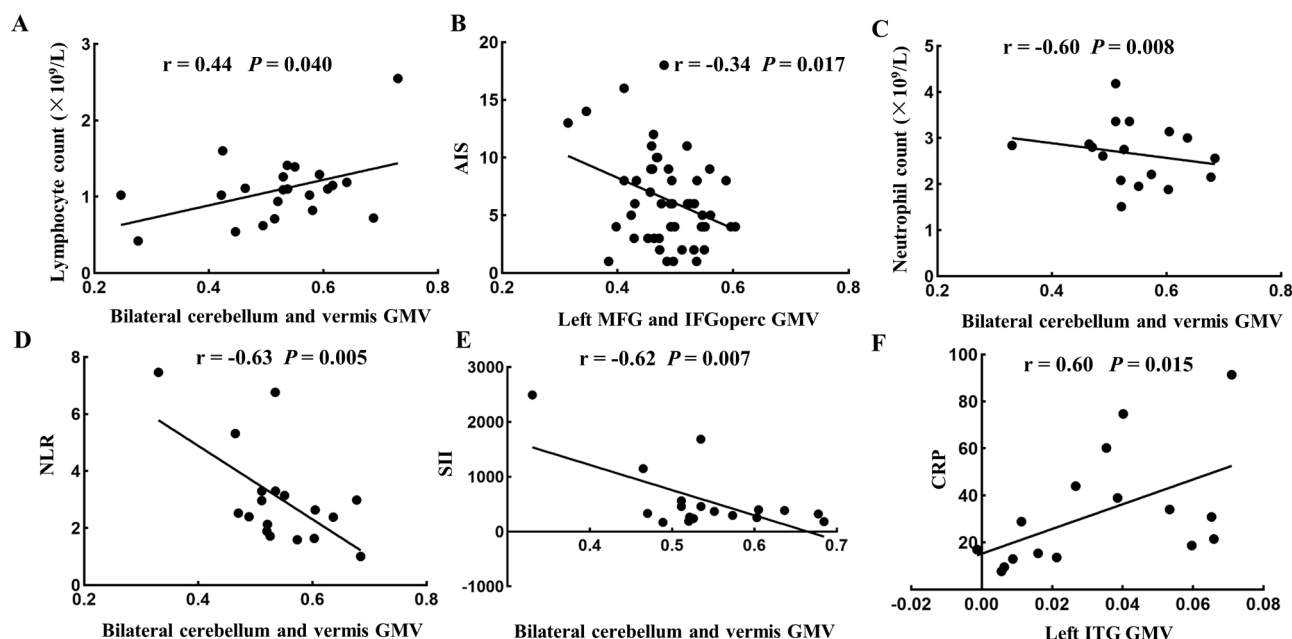


Fig. 4. Correlation analyses results.

(A, B) The GMV in the bilateral cerebellum and vermis was positively correlated with the lymphocyte count, and the GMV in the left MFG and IFGoperc was negatively correlated with AIS in COVID-19_{one}. (C, D, E) In COVID-19_{two}, the GMV in the bilateral cerebellum and vermis was negatively correlated with the neutrophil count ($P_{FDR} = 0.040$), NLR ($P_{FDR} = 0.025$), and SII ($P_{FDR} = 0.035$). (F) The difference in GMV in the left ITG between the two groups was positively correlated with CRP. GMV, gray matter volume. COVID-19_{one}, patients one-year post-recovery after SARS-CoV-2 infection. COVID-19_{two}, patients two years post-recovery after SARS-CoV-2 infection. AIS, Athens Insomnia Scale. MFG, middle frontal gyrus. IFGoperc, inferior frontal gyrus of operculum. MTG, middle temporal gyrus. ITG, inferior temporal gyrus. NLR, neutrophil/lymphocyte ratio. SII, systemic immune-inflammation index. CRP, C-reactive protein.

decreased GMV was correlated with AIS score and inflammatory markers.

4.1. Neuropsychiatric and clinical sequelae

Compared with HCs, COVID-19_{one} showed significantly severe insomnia, but insomnia was not significantly different in COVID-19_{two}. A previous study found that 74.8% of patients with COVID-19 had sleep disturbances, which was the highest among uninfected people, health care workers, and infected people (Alimoradi et al., 2021). Furthermore, insomnia was still a common symptom at follow-up six months (Huang et al., 2021), which was consistent with our findings in COVID-19_{one}. Financial and other stress, side effects of anti-inflammatory drugs, or other physical discomforts (such as headache, cough, etc.) may be the cause of insomnia (Alimoradi et al., 2021; Altena et al., 2020). After removing these possible causes, insomnia improved significantly in COVID-19_{two}. However, changes in anxiety, depression and cognitive function scores in patients with COVID-19 recovery were not found.

Compared with the hospitalization group, the COVID-19_{one} group had significantly less fever, fatigue, smell and taste disturbances, persistent myalgia, cough, and dyspnea, and increased headache, expectoration, and chest tightness. Previous studies found that 66% of COVID-19-infected patients had full recovery of smell and taste disturbances after six months (Teaima et al., 2022), and fatigue, myalgia, chest tightness and dyspnea were the main clinical symptoms after one year and two years (Huang et al., 2022a; 2021; Zhang et al., 2021), which was consistent with our findings. Compared with COVID-19_{one}, COVID-19_{two} showed common headache, fatigue, dyspnea, and chest pain, and cough symptoms continued to improve. According to previous evidence provided by SARS, neurological and respiratory symptoms may persist for several years (Lam et al., 2009; Hui et al., 2005), which might explain the changes in clinical symptoms in our study.

4.2. GMV recovered

Compared with HCs, the GMV in the left MFG and IFGoperc decreased in COVID-19_{one} and was negatively correlated with AIS scores that were significantly higher than those of HCs. However, compared with HCs, COVID-19_{two} had no significantly reduced GMV in the left frontal lobe and no significant difference in AIS scores. A study reported that the frontal lobe played an important role in the hyperarousal process (Cheng et al., 2022). Therefore, we considered that the reduction in the left frontal lobe may be related to insomnia symptoms in COVID-19_{one}. With insomnia symptoms continuously improving, the GMV of the left frontal lobe undergoes reversible changes. Meyer et al. concluded that in most patients with COVID-19 recovery, frontoparietal-dominant cortical dysfunction was fully reversible (Meyer et al., 2022).

4.3. GMV decreased or aggravated

In our cross-sectional study, GMV in the cerebellum and vermis was decreased in both COVID-19_{one} and COVID-19_{two} compared with HCs. The former was positively correlated with the lymphocyte counts, and the latter was negatively correlated with the neutrophil count, NLR, and SII. In our longitudinal study, there was no significant difference in GMV in the cerebellum and vermis between COVID-19_{one} and COVID-19_{two}, indicating that the virus's effect on the cerebellum and vermis gray persisted. Our results were consistent with the findings of a six-month follow-up PET study, which found decreased metabolism in both cerebellar hemispheres in seven patients who recovered from COVID-19 (Kas et al., 2021). In an autopsy study of 43 people who died of COVID-19, the activation of microglia and the infiltration of cytotoxic T lymphocytes were most pronounced in the brainstem and cerebellum (Matschke et al., 2020). In addition, severe COVID-19 patients had lower lymphocytes and higher neutrophil counts, NLR, and SII (Tan et al., 2020). Therefore, the reduction in GMV in the bilateral cerebellum and cerebellar vermis may be related to the persistent influence on gray

matter caused by neuroinflammation from viral infection.

We found SARS-CoV-2 damage to the temporal lobe GMV in both cross-sectional and longitudinal analyses. It is interesting to find that compared with HCs, COVID-19_{one} showed damage in the right MTG and ITG, but COVID-19_{two} showed damage in the left MTG. This finding suggested that changes in the right temporal lobe in COVID-19_{one} may be associated with right olfactory perception dominance (Karstensen et al., 2018). The virus is more easily transported to the temporal cortex of the "secondary olfactory cortex" through a part of the olfactory bulb in the right "primary olfactory cortex", causing damage to the right temporal lobe (Karstensen et al., 2018; Han et al., 2019). A three-month follow-up study of cerebral microstructural changes in patients with COVID-19 found that DTI differences were only present on the right side, and they also believed that the structural changes on the right side were related to right-side odor perception (Lu et al., 2020). Left-sided temporal lobe damage in COVID-19_{two} may be associated with immune-induced inflammatory storms or fever.

In the longitudinal follow-up, the GMV in the left ITG decreased and was positively correlated with CRP. The higher the levels of inflammatory markers during hospitalization, the greater the decrease in GMV in COVID-19_{two}. A previous three-month follow-up study of recovery from COVID-19 also found decreased cerebral blood flow and damage to white matter fiber tracts correlated with high levels of inflammatory factors (Qin et al., 2021). Additionally, another study of six-month recovery from COVID-19 found that GMV in the inferior/middle gyrus and fusiform gyrus was significantly lower in febrile patients than in non-febrile patients in the acute phase (Duan et al., 2021), which was consistent with our findings. However, the specific mechanism still needs to be further explored.

4.4. Potential reasons for GMV recovery coexisting with injury

There are many possible mechanisms of the sequelae of the nervous system, but the exact mechanism is not clear. It may be related to the damage of neurogenesis and neuroblast formation, activation of microglia and astrocytes, reduction of oligodendrocytes, and dysregulation of myelin by neuro-inflammatory response (Spudich and Nath, 2022; Fernández-Castañeda et al., 2022). In our study, we found that changes in GMV varied in different brain regions, which may be related to different mechanisms of viral infection. We identified the differences in brain areas by GMV and then correlated GMV with the behavioral scales and inflammatory markers during hospitalization, which may help us explore the underlying causes. The GMV in the left frontal lobe was significantly different in COVID-19_{one} compared with the GMV in HCs and was negatively correlated with AIS. However, the GMV in the left frontal lobe was not significantly different in COVID-19_{two} compared with HCs, and the insomnia symptoms significantly improved. We suspect that improvements in GMV in the left frontal lobe are associated with insomnia. Considering that other brain areas with GMV decreases were associated with inflammatory markers during hospitalization, we believe that the decreased GMV may be related to inflammation. However, a larger sample size is needed to test these conjectures in the future.

4.5. Limitations

This study has several limitations. We did not collect MR data during hospitalization due to the lack of awareness of the neurological damage caused by the virus in the early stages of the outbreak and concerns about nosocomial transmission. The HCs were imaged only at a single time point, and we will improve this in subsequent studies. Since blood collection is an invasive test, we did not collect blood from HCs, COVID-19_{one}, or COVID-19_{two}. Therefore, it is not possible to compare the differences in inflammatory indicators cross-sectionally and longitudinally or to dynamically observe the changes in inflammatory indicators and electrolytes in COVID-19_{one} and COVID-19_{two}. In addition, our study

had a small sample size and a high loss rate of follow-up, which may lead to data bias, so studies with larger samples are needed to verify the results. This study only focused on the changes in brain structure in COVID-19 survivors followed up for two years, and we will continue to study the changes in function and cerebral blood flow in future work.

5. Conclusions

GMV recovery (left frontal and temporal lobes) coexisted with injury (cerebellum, vermis and right temporal lobes), and the dynamic changes in GMV were associated with AIS and inflammatory factors. This study may shed some light on the dynamic changes in brain structure and the possible predictors that may be related to gray matter changes in COVID-19_{two}.

Funding

This study was supported by Hunan Provincial Natural Science Foundation for Excellent Young Scholars (grant number 2022JJ20089), the Research Project of Postgraduate Education and Teaching Reform of Central South University (grant number 2021JGB147, 2022JGB117), the Clinical Research Center For Medical Imaging In Hunan Province (grant number 2020SK4001), the science and technology innovation program of Hunan Province (grant number 2021RC4016) and the Emergency Project of Pneumonia Epidemic of novel coronavirus infection in Hunan Province (grant number 2020SK3006).

CRedit authorship contribution statement

Yanyao Du: Data curation, Formal analysis, Visualization, Writing – original draft. **Wei Zhao:** Visualization, Writing – original draft, Writing – review & editing. **Sihong Huang:** Formal analysis. **Yijie Huang:** Data curation. **Yanjing Chen:** Data curation. **Huiting Zhang:** Writing – review & editing. **Hu Guo:** Writing – review & editing. **Jun Liu:** Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors have no conflict of interest.

References

- Cagnazzo, F., Arquiza, C., Derraz, I., et al., 2021. Neurological manifestations of patients infected with the SARS-CoV-2: a systematic review of the literature. *J. Neurol.* 268 (8), 2656–2665. <https://doi.org/10.1007/s00415-020-10285-9>.
- Caruso, D., Guido, G., Zerinian, M., et al., 2021. Post-acute sequelae of COVID-19 Pneumonia: six-month chest CT follow-up. *Radiology* 301 (2), E396–E405. <https://doi.org/10.1148/radiol.2021210834>.
- Zhang, H.G., Dagliati, A., Shakeri Hossein Abad, Z., et al., 2022. International electronic health record-derived post-acute sequelae profiles of COVID-19 patients. *NPJ Digit. Med.* 5 (1), 81. <https://doi.org/10.1038/s41746-022-00623-8>.
- Al-Aly, Z., Xie, Y., Bowe, B., 2021. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature* 594 (7862), 259–264. <https://doi.org/10.1038/s41586-021-03553-9>.
- Mao, L., Jin, H., Wang, M., et al., 2020. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol.* 77 (6), 683–690. <https://doi.org/10.1001/jamaneurol.2020.1127>.
- Varatharaj, A., Thomas, N., Ellul, M.A., et al., 2020. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. *Lancet Psychiatry* 7 (10), 875–882. [https://doi.org/10.1016/S2215-0366\(20\)30287-X](https://doi.org/10.1016/S2215-0366(20)30287-X).
- Yang, R.C., Huang, K., Zhang, H.P., et al., 2022. SARS-CoV-2 productively infects human brain microvascular endothelial cells. *J. Neuroinflammation* 19 (1), 149. <https://doi.org/10.1186/s12974-022-02514-x>.
- Bourgonje, A.R., Abdulle, A.E., Timens, W., et al., 2020. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J. Pathol.* 251 (3), 228–248. <https://doi.org/10.1002/path.5471>.
- Speranza, E., Williamson, B.N., Feldmann, F., et al., 2021. Single-cell RNA sequencing reveals SARS-CoV-2 infection dynamics in lungs of African green monkeys. *Sci. Transl. Med.* 13 (578), eabe8146. <https://doi.org/10.1126/scitranslmed.abe8146>.
- Huang, L., Li, X., Gu, X., et al., 2022a. Health outcomes in people 2 years after surviving hospitalisation with COVID-19: a longitudinal cohort study. *Lancet Respir. Med.* 10 (9), 863–876. [https://doi.org/10.1016/S2213-2600\(22\)00126-6](https://doi.org/10.1016/S2213-2600(22)00126-6).

- Huang, L., Yao, Q., Gu, X., et al., 2021a. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet* 398 (10302), 747–758. [https://doi.org/10.1016/S0140-6736\(21\)01755-4](https://doi.org/10.1016/S0140-6736(21)01755-4).
- Tian, T., Wu, J., Chen, T., et al., 2022. Long-term follow-up of dynamic brain changes in patients recovered from COVID-19 without neurological manifestations. *JCI Insight* 7 (4), e155827. <https://doi.org/10.1172/jci.insight.155827>.
- Qin, Y., Wu, J., Chen, T., et al., 2021. Long-term microstructure and cerebral blood flow changes in patients recovered from COVID-19 without neurological manifestations. *J. Clin. Investig.* 131 (8), e147329. <https://doi.org/10.1172/JCI147329>.
- Du, Y.Y., Zhao, W., Zhou, X.L., et al., 2022. Survivors of COVID-19 exhibit altered amplitudes of low frequency fluctuation in the brain: a resting-state functional magnetic resonance imaging study at 1-year follow-up. *Neural Regen. Res.* 17 (7), 1576–1581. <https://doi.org/10.4103/1673-5374.327361>.
- Huang, S., Zhou, Z., Yang, D., et al., 2022b. Persistent white matter changes in recovered COVID-19 patients at the 1-year follow-up. *Brain* 145 (5), 1830–1838. <https://doi.org/10.1093/brain/awab435>.
- Duan, K., Premi, E., Pilotto, A., et al., 2021. Alterations of frontal-temporal gray matter volume associate with clinical measures of older adults with COVID-19. *Neurobiol. Stress* 14, 100326. <https://doi.org/10.1016/j.ynstr.2021.100326>.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., et al., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23 (Suppl 1), S208–S219. <https://doi.org/10.1016/j.neuroimage.2004.07.051>.
- Douaud, G., Mackay, C., Andersson, J., et al., 2009. Schizophrenia delays and alters maturation of the brain in adolescence. *Brain* 132 (Pt 9), 2437–2448. <https://doi.org/10.1093/brain/awp126>.
- Alimoradi, Z., Broström, A., Tsang, H.W.H., et al., 2021. Sleep problems during COVID-19 pandemic and its' association to psychological distress: a systematic review and meta-analysis. *EclinicalMedicine* 36, 100916. <https://doi.org/10.1016/j.eclinm.2021.100916>.
- Huang, C., Huang, L., Wang, Y., et al., 2021b. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 397 (10270), 220–232. [https://doi.org/10.1016/S0140-6736\(20\)32656-8](https://doi.org/10.1016/S0140-6736(20)32656-8).
- Altena, E., Baglioni, C., Espie, C.A., et al., 2020. Dealing with sleep problems during home confinement due to the COVID-19 outbreak: practical recommendations from a task force of the European CBT-I Academy. *J. Sleep Res.* 29 (4), e13052. <https://doi.org/10.1111/jsr.13052>.
- Teaima, A.A., Salem, O.M., Teama, M., et al., 2022. Patterns and clinical outcomes of olfactory and gustatory disorders in six months: prospective study of 1031 COVID-19 patients. *Am. J. Otolaryngol.* 43 (1), 103259. <https://doi.org/10.1016/j.amjoto.2021.103259>.
- Zhang, X., Wang, F., Shen, Y., et al., 2021. Symptoms and health outcomes among survivors of COVID-19 infection 1 year after discharge from hospitals in Wuhan, China. *JAMA Netw. Open* 4 (9), e2127403. <https://doi.org/10.1001/jamanetworkopen.2021.27403>.
- Lam, M.H., Wing, Y.K., Yu, M.W., et al., 2009. Mental morbidities and chronic fatigue in severe acute respiratory syndrome survivors: long-term follow-up. *Arch. Intern. Med.* 169 (22), 2142–2147. <https://doi.org/10.1001/archinternmed.2009.384>.
- Hui, D.S., Wong, K.T., Ko, F.W., et al., 2005. The 1-year impact of severe acute respiratory syndrome on pulmonary function, exercise capacity, and quality of life in a cohort of survivors. *Chest* 128 (4), 2247–2261. <https://doi.org/10.1378/chest.128.4.2247>.
- Cheng, Y., Xue, T., Dong, F., et al., 2022. Abnormal functional connectivity of the salience network in insomnia. *Brain Imaging Behav.* 16 (2), 930–938. <https://doi.org/10.1007/s11682-021-00567-9>.
- Meyer, P.T., Hellwig, S., Blazhenets, G., Hosp, J.A., 2022. Molecular imaging findings on acute and long-term effects of COVID-19 on the brain: a systematic review. *J. Nucl. Med.* 63 (7), 971–980. <https://doi.org/10.2967/jnumed.121.263085>.
- Kas, A., Soret, M., Pyatigorskaya, N., et al., 2021. The cerebral network of COVID-19-related encephalopathy: a longitudinal voxel-based 18F-FDG-PET study. *Eur. J. Nucl. Med. Mol. Imaging* 48 (8), 2543–2557. <https://doi.org/10.1007/s00259-020-05178-y>.
- Matschke, J., Lütgehetmann, M., Hagel, C., et al., 2020. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 19 (11), 919–929. [https://doi.org/10.1016/S1474-4422\(20\)30308-2](https://doi.org/10.1016/S1474-4422(20)30308-2).
- Tan, L., Wang, Q., Zhang, D., et al., 2020. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. *Signal Transduct. Target. Ther.* 5 (1), 33. <https://doi.org/10.1038/s41392-020-0148-4>.
- Karstensen, H.G., Vestergaard, M., Baaré, W.F.C., et al., 2018. Congenital olfactory impairment is linked to cortical changes in prefrontal and limbic brain regions. *Brain Imaging Behav.* 12 (6), 1569–1582. <https://doi.org/10.1007/s11682-017-9817-5>.
- Han, P., Zang, Y., Akshita, J., Hummel, T., 2019. Magnetic resonance imaging of human olfactory dysfunction. *Brain Topogr.* 32 (6), 987–997. <https://doi.org/10.1007/s10548-019-00729-5>.
- Lu, Y., Li, X., Geng, D., et al., 2020. Cerebral micro-structural changes in COVID-19 patients—an MRI-based 3-month follow-up study. *EclinicalMedicine* 25, 100484. <https://doi.org/10.1016/j.eclinm.2020.100484>.
- Spudich, S., Nath, A., 2022. Nervous system consequences of COVID-19. *Science* 375 (6578), 267–269. <https://doi.org/10.1126/science.abm2052>.
- Fernández-Castañeda, A., Lu, P., Geraghty, A.C., Song, E., Lee, M.H., Wood, J., et al., 2022. Mild respiratory COVID can cause multi-lineage neural cell and myelin dysregulation. *Cell* 185 (14), 2452–2468. <https://doi.org/10.1016/j.cell.2022.06.008> e16.