BRAIN
ORIGINAL ARTICLE

Persistent white matter changes in recovered COVID-19 patients at the 1-year follow-up

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There is growing evidence that severe acute respiratory syndrome coronavirus 2 can affect the CNS. However, data on white matter and cognitive sequelae at the 1-year follow-up are lacking. Therefore, we explored these characteristics in this study.

We investigated 22 recovered coronavirus disease 2019 (COVID-19) patients and 21 matched healthy controls. Diffusion tensor imaging, diffusion kurtosis imaging and neurite orientation dispersion and density imaging were performed to identify white matter changes, and the subscales of the Wechsler Intelligence scale were used to assess cognitive function. Correlations between diffusion metrics, cognitive function and other clinical characteristics were then examined. We also conducted subgroup analysis based on patient admission to the intensive care unit.

The corona radiata, corpus callosum and superior longitudinal fasciculus had a lower volume fraction of intracellular water in the recovered COVID-19 group than in the healthy control group. Patients who had been admitted to the intensive care unit had lower fractional anisotropy in the body of the corpus callosum than those who had not. Compared with the healthy controls, the recovered COVID-19 patients demonstrated no significant decline in cognitive function. White matter tended to present with fewer abnormalities for shorter hospital stays and longer follow-up times.

Lower axonal density was detected in clinically recovered COVID-19 patients after 1 year. Patients who had been admitted to the intensive care unit had slightly more white matter abnormalities. No significant decline in cognitive function was found in recovered COVID-19 patients. The duration of hospital stay may be a predictor for white matter changes at the 1-year follow-up.

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Received April 23, 2021. Revised October 13, 2021. Accepted October 27, 2021. Advance access publication December 16, 2021

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Keywords: recovered COVID-19 patients; white matter changes; cognitive function; 1-year follow-up; intensive care unit

Abbreviations: COVID-19 = coronavirus disease 2019; DKI = diffusion kurtosis imaging; DTI = diffusion tensor imaging; NODDI = neurite orientation dispersion and density imaging; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SLF = superior longitudinal fasciculus; TBSS = tract-based spatial statistics

Introduction

The coronavirus disease 2019 (COVID-19) pandemic has posed great challenges worldwide, including diagnosis, treatment and post-infection care for survivors. Although substantial progress has been made in addressing the acute effects of COVID-19, the long-term health consequences of recovered patients remain unknown. As the population of recovered COVID-19 patients continues to grow, increasing attention has been given to post-infection care. It is well known that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) attacks the lungs, subsequently causing viral pneumonia, but it also affects the CNS through direct and/or indirect impacts.[1–3] Neurological manifestations, such as encephalitis, cerebral haemorrhage and impaired consciousness,[1] and neuroimaging findings, such as cerebrovascular disease, perfusion abnormalities and white matter changes,[4] have been detected in the acute and subacute stages of the disease. However, patients without these manifestations have also demonstrated persistent CNS abnormalities after recovery.[5] Therefore, detecting and evaluating these changes is clinically vital, and a deeper investigation into the sequelae of COVID-19 can inform individual-based medical care for recovered patients. Additionally, patients admitted to the intensive care unit (ICU) have different imaging manifestations in the acute stage and worse cognitive outcomes after discharge than patients who had never been admitted to the ICU.[6,7] Therefore, we also conducted a comparison between patients who had or had not been admitted to the ICU.

Diffusion tensor imaging (DTI), an imaging modality based on a simplistic model of brain microstructure, is the most common diffusion model used to evaluate white matter integrity. The DTI model assumes simple Gaussian diffusion through the brain microstructure. Diffusion kurtosis imaging (DKI),[8] an advanced diffusion MRI technique based on the theory of non-Gaussian diffusion, is considered to better reflect diffusion in biological tissues, especially in brain areas with high tissue heterogeneity. However, the DTI and DKI models are both based on the 'signal representations' approach, which lacks specificity and can only provide an indirect characterization of the microstructure. Neurite orientation dispersion and density imaging (NODDI), based on the 'tissue model', is a more advanced multicompartment diffusion model.[9,10] NODDI can directly measure properties in three microstructural environments, namely intracellular, extracellular and free water environments, which makes it possible to estimate biologically relevant parameters. Several studies have reported white matter changes in recovered COVID-19 patients,[5,11] indicating that these patients present with persistent white matter abnormalities. However, the status and changes in white matter in recovered COVID-19 patients after 1 year remain unknown, and white matter changes evaluated by DKI and NODDI models have not yet been reported. Tract-based spatial statistics (TBSS)[12] is a whole-brain analysis that combines the strengths of voxel-based analyses and tractography-based analyses. It overcomes the alignment and smooth kernel problems of voxel-based morphometry and improves the sensitivity, objectivity and interpretability of the analysis of multisubject diffusion imaging studies. Therefore, we used this tool to investigate changes in white matter.

In this context, the purposes of this study were to assess the long-term change in white matter by using these three diffusion models, to assess cognitive function in recovered COVID-19 patients and to investigate correlations with clinical characteristics in an attempt to explain the mechanisms underlying the abnormalities observed at the 1-year follow-up.

Materials and methods

This study was approved by the ethics committee of the Second Xiangya Hospital of Central South University. All participants provided written informed consent in accordance with the Declaration of Helsinki.

Participants

In total, 237 recovered COVID-19 patients were recruited from the First Hospital of Changsha. The inclusion criteria for the recovered COVID-19 group were as follows: (i) a diagnosis of COVID-19 according to the guidelines of the National Health Commission[13] and a discharge date between February and April 2020; (ii) age >18 years; and (iii) willingness and ability to undergo brain MRI scanning. The exclusion criterion was a structural abnormality on traditional neuroimaging except for white matter hyperintensity. Age-, sex- and education-matched healthy controls were recruited, and participants with severe psychiatric disease (e.g. schizophrenia or depression), severe somatic disease (e.g. diabetes, uncontrolled hypertension or heart disease), drug abuse, history of traumatic brain injury or surgery, or brain structural abnormality (e.g. encephalomalacia foci, brain infections or neoplasms) on neuroimaging were excluded, except for mild-moderate white matter hyperintensity. Among 237 discharged patients, 23 volunteered to participate in our research and one patient was excluded because he did not undergo an MRI scan. Finally, 22 recovered COVID-19 patients and 21 healthy controls were included. A flow chart of patient inclusion is shown in Fig. 1.

All participants underwent psychiatric evaluations via face-to-face interviews conducted by trained medical staff. Information on the following clinical characteristics was collected: age; sex; education; history of sojourn; clinical type (National Health Commission guidelines: mild, moderate or severe); hospitalization days and the presence of fever, cough or gastrointestinal symptoms. Four inflammatory markers were also collected: erythrocyte sedimentation rate (ESR); C-reactive protein (CRP); neutrophil/lymphocyte ratio (NLR) and systemic immune-inflammation index (SII) (SII = platelets × neutrophils/

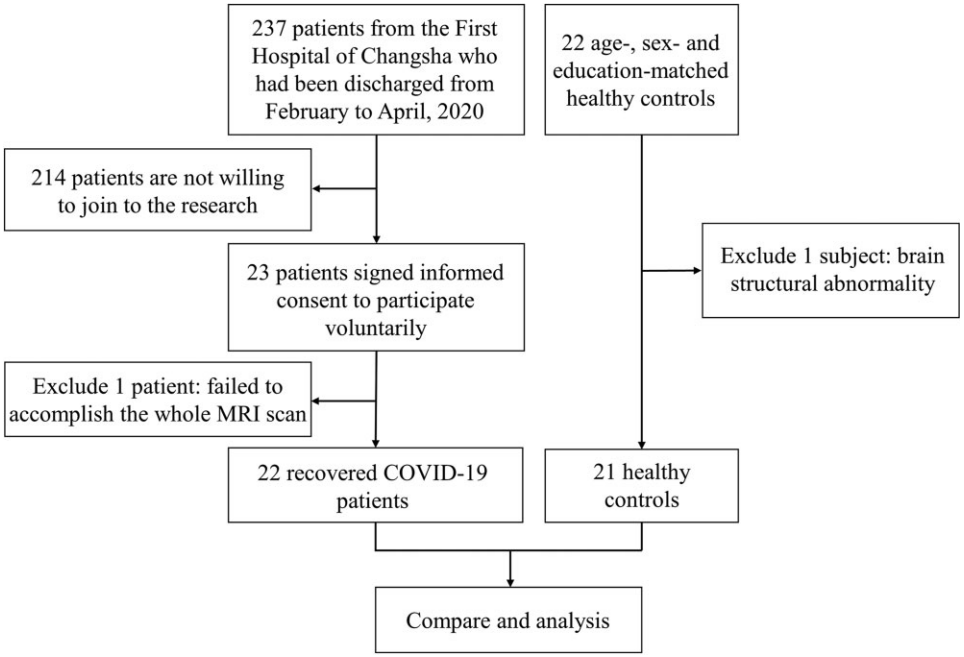

Figure 1 Flow chart of the study.

lymphocytes).[14] Baseline clinical characteristics and inflammatory markers were used for further analysis in this study. The demographic characteristics and neuropsychological tests of the recovered COVID-19 patients and healthy controls are presented in Table 1. The clinical features of the recovered COVID-19 patients are presented in Table 2. The demographic and clinical characteristics of the ICU and non-ICU groups are presented in Table 3. The median interval time from discharge to MRI scan was 351.5 days.

MRI acquisition

All MRI data were acquired on a 3-T MRI scanner (MAGNETOM Skyra, Siemens Healthcare) with a 32-channel head coil. All participants were placed in a supine position with a headset or foam padding between their head and the head coil to minimize head motion. The MRI scanning sequences included T_1-weighted imaging, T_2-weighted imaging, fluid-attenuated inversion recovery (FLAIR) imaging, three-dimensional magnetization-prepared rapid acquisition gradient echo (3D MPRAGE) imaging, susceptibility weighted imaging (SWI) and diffusion MRI. Diffusion MRI was acquired with the following parameters: repetition time/echo time = 5400/92 ms, field of view = 224 × 224 mm, 112 × 112 matrix, 40 slices, $2 \times 2 \times 3\ mm^3$ voxels, bandwidth = 1654 Hz/pixel, b = 1000/2000 s/mm^2, 64 diffusion-weighting directions at each b-value and 10 b0 scans. T_1-weighted imaging, T_2-weighted imaging, FLAIR, MPRAGE and SWI were independently reviewed by two neuroradiologists with >10 years of experience in neuroimaging to check for structural abnormalities. Any disagreement between the two observers was resolved by consensus.

Neuropsychological test acquisition

All participants completed the following five cognitive tests. (i) The logical memory (LM) test, a measure of verbal episodic memory,[15] where the tester read a sentence made up of multiple words, and the participants repeated it immediately (LM-A) and after 30 min (LM-B). (ii) The digit symbol substitution test (DSST), which has been frequently used to assess participants' processing speed, sustained attention and working memory.[16,17] The patients were shown nine numbers and their corresponding symbols and then they were instructed to match the correct symbols to the corresponding numbers in 2 min. The total score was the number of correctly matched symbols, and a higher score indicated better performance in the assessment. (iii) The Knowledge subscale of the Wechsler Intelligence scale, which primarily measures the participant's breadth of knowledge, ability to learn and accept, and ability to understand daily things. The participant was asked a number of common-sense questions, such as 'which season of the year has the longest days?' and 'what time of day has the shortest shadow?'. (iv) The digit span (DS) task, a verbal attention and working memory task that has been widely used in cognitive assessment.[18,19] The DS task consists of two parts: repeating digit sequences in the order presented and in reverse order (forward digit span, FDS and backward digit span, BDS, which assess visual and visuospatial sequence representation, respectively).[20] In our study, the DS task was presented as sequences of digits of increasing length, ranging from 2 to 9. (v) The word fluency test (WFT): in 1 min, the participants were asked to name as many animals as possible. The participants completed these neuropsychological tests on the same day as the MRI scan.

Image analysis

Image processing included initial preprocessing and diffusion metric computations. Before preprocessing, each participant's diffusion images were visually inspected to verify that they were free from major artefacts (e.g. head motion). Motion, eddy current artefacts and geometric distortions were corrected using the eddy command provided in the FMRIB Software Library (FSL).[21] Using an in-house MATLAB script, the transformation matrices, output from the eddy command, were used to rotate the corresponding

Table 1 Demographic and neuropsychological tests of recovered COVID-19 patients and healthy controls

	Patients	Healthy controls	$t/Z/\chi^2$	P
n	22	21		
Sex	M:11; F:11	M:5; F:16	3.154	0.076
Age (years)	54.14 ± 9.76	49.14 ± 12.44	−1.468	0.15
Education (years)	12 (12; 16)	12 (10.5; 16)	−1.163	0.87
Neuropsychological tests				
LM-A	7.15 ± 2.76	6.81 ± 3.16	−0.367	0.716
LM-B	5.5 (3.25; 8.75)	5 (3; 8)	−0.618	0.536
DSST	71.50 ± 21.26	75.38 ± 24.73	0.538	0.594
Knowledge subscale of Wechsler Intelligence scale	18 (14.25; 22.5)	14 (12; 21)	−1.848	0.065
FDS	11.5 (11; 13)	12 (10.5; 13)	−0.623	0.533
BDS	7 (5; 8.5)	6 (4.5; 8)	−0.567	0.57
WFT	20.67 ± 6.49	20.18 ± 7.66	−0.194	0.848

BDS = backward digit span; DS = digit span task; DSST = digital symbol substitution test; FDS = forward digit span; LM = logical memory task; WFT = word fluency test.

Table 2 Clinical characteristics of recovered COVID-19 patients

	Recovered COVID-19 patients	
Clinical type		
Moderate	10/22	
Severe	12/22	
Hospitalization days	14.5 (11.75; 28.75)	
Follow-up days	351.5 (329.75; 357.25)	
	Acute stage	**Follow-up days**
Neurological symptoms		
Fatigue	8 (36.36%)	5 (22.73%)
Headache	1 (4.55%)	5 (22.73%)
Myalgia	4 (18.18%)	5 (22.73%)
Smell loss	9 (40.91%)	2 (9.09%)
Taste loss	8 (36.36%)	2 (9.09%)
Inflammatory markers		
ESR (mm/h)	51.23 ± 25.82	12.60 ± 9.06
CRP (mg/l)	21.47 (11.25; 41.48)	2.64 ± 2.15
NLR	2.64 (2.01; 3.88)	2.19 (1.67; 3.03)
SII	385.71 (260.19; 750.53)	477.75 (279.23; 551.98)

CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; NLR = neutrophil/lymphocyte ratio; SII = systemic immune-inflammation index.

diffusion-weighting directions to match the rotation of the brain image during the motion correction process. Then, the b0 images were extracted, and nonbrain voxels were masked out by applying the FSL bet command to the participant's b0 image. Then, four DTI metrics (fractional anisotropy, radial diffusivity, axial diffusivity and mean diffusivity) were calculated by the FSL dtifit command. DKI model fitting was performed using DKE (v.2.6.0), and the mean kurtosis was calculated. Three NODDI parameters [orientation dispersion index, volume fraction of intracellular water (V_{ic}) and volume fraction of the isotropic diffusion compartment (V_{iso})] were calculated using the open-source tool AMICO (https://github.com/daducci/AMICO).[22]

TBSS analysis

TBSS was performed using the FSL toolbox, TBSS. A common whole-brain while-matter skeleton was extracted in the standard Montreal Neurological Institute space to minimize the partial volume effects in a finite imaging resolution. The white matter skeleton included only voxels in the centre of white matter tracts and excluded edge voxels, which may be contaminated with signals from the nearby anatomy. Within the white matter skeleton, nonparametric permutation-based statistics were performed using the FSL randomize command for voxelwise statistical analyses, and age was used as a covariant in this study. Threshold-free cluster enhancement[23] and 5000 permutations were used to obtain a corrected *P*-value. White matter voxels were considered significant at a corrected *P*-value <0.05 after being adjusted for multiple comparisons by controlling the familywise error (FWE) rate.

Post hoc region of interest analysis

To produce aggregate results at the participant level, *post hoc* region of interest analyses were performed. For each participant, the mean of each diffusion metric was computed in the regions that tested as significant with TBSS. For between-group differences, a box plot was used with participants' means plotted based on their group membership. The anatomical interpretation of the region of interest was based on the 'JHU ICBM-DTI-81 White-Matter Labels' provided in FSL after skeletonization.

Statistical analysis

The demographic and clinical characteristics and aspects of the neuropsychological data were analysed using IBM SPSS

Table 3 Demographic and clinical characteristics of ICU and non-ICU patients

	ICU	Non-ICU	$t/Z/\chi^2$	P
n	8	14		
Sex	M:5; F:3	M:6; F:8		0.659
Age (years)	55.88 ± 10.789	53.14 ± 9.396	0.622	0.541
Education (years)	14 (9.75; 16)	12 (12; 16)	−0.178	0.858
Neurological symptoms (acute stage)	4/8	9/14		0.662
Inflammatory markers				
ESR (mm/h)	49.50 ± 31.204	52.00 ± 25.159	−1.154	0.88
CRP (mg/l)	43.97 (18.73; 74.71)	14.49 (7.68; 30.89)	−2.239	0.025*
NLR	3.15 (2.13; 6.76)	2.58 (1.89; 3.34)	−0.671	0.502
SII	385.71 (295.73; 1683.71)	364.45 (238.91; 685.34)	−0.821	0.412
Hospitalization days	20.5 (11.25; 38.5)	14 (12; 25)	−0.617	0.537
Neuropsychological tests				
LM-A	7.00 ± 2.449	7.21 ± 2.966	−0.155	0.878
LM-B	6.83 ± 3.656	5.36 ± 3.104	0.926	0.367
DSST	75.83 ± 18.946	69.64 ± 22.589	0.586	0.565
Knowledge subscale of Wechsler Intelligence scale	16.33 ± 4.676	19.14 ± 4.521	−1.261	0.223
FDS	11.5 (11; 13.25)	11.5 (10; 13)	−0.589	0.556
BDS	6.5 (5.5; 7.75)	7 (5; 9)	−0.126	0.9
WFT	14.67 ± 7.638	22.17 ± 5.540	−1.965	0.071
White matter hyperintensity (Fazekas scale)				
0/1/2	3/3/2	5/8/1	1.657	0.597

BDS = backward digit span; CRP = C-reactive protein; DS = digit span task; DSST = digital symbol substitution test; ESR = erythrocyte sedimentation rate; FDS = forward digit span; ICU = intensive care unit; LM = logical memory task; NLR = neutrophil/lymphocyte ratio; SII = systemic immune-inflammation index; WFT = word fluency test.
*$P < 0.05$.

Table 4 Anatomical regions of tract-based spatial statistics results

	Cluster index	Anatomical regions	Voxels	Min P	X	Y	Z
V_{ic}	1	Corona radiata (anterior and superior part) L and R Genu of corpus callosum	3435	0.042	83	151	67
V_{ic}	2	SLF L	564	0.046	126	127	97

V_{ic} = volume fraction of intracellular water.

Statistics v.24.0. Unpaired two-sample *t*-tests, chi-square tests and Kruskal–Wallis tests were performed for age, sex and education. In addition, the Kruskal–Wallis test and unpaired two-sample *t*-tests were performed for neuropsychological tests. The correlations between diffusion parameters and neuropsychological test scores were evaluated by partial correlations, using age, sex and education as covariates. In the recovered COVID-19 group, Spearman correlations were evaluated between diffusion parameters, cognitive function, inflammatory markers, hospitalization days and follow-up days. Correlations were corrected for multiple comparisons using an FWE correction.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

Demographic and clinical characteristics

The study included 22 (male: 11; female: 11) recovered COVID-19 patients and 21 (male: 5; female: 16) healthy controls. A comparison of the characteristics between the two groups is presented in Table 1. There were no statistically significant differences between the patients and healthy controls with regard to sex ratio, age or education, justifying their use as the experimental group and control group, respectively. Two patients had complications: one had sepsis and multiple organ dysfunction syndrome, and the other had acute respiratory distress syndrome (ARDS). The clinical features of the recovered COVID-19 group are presented in Table 2, which indicates that 14/22 (63.64%) patients had neurological symptoms in the acute stage. At the time of scanning, 9/22 (40.91%) patients had neurological symptoms. The mean ESR was 51.23 mm/h, the median CRP was 21.47 mg/l, NLR was 2.64, SII was 385.71 and the length of hospitalization was 14.5 days. The inflammatory markers 3 months after discharge from the hospital are displayed in the follow-up column in Table 2 and indicate that these data had returned to normal.

The recovered COVID-19 patients were divided into two subgroups: eight patients who had been hospitalized in the ICU were placed in the ICU group, and the remaining 14 patients, who had never been hospitalized in the ICU, were placed in the non-ICU group. The demographic and clinical characteristics of the two groups are presented in Table 3. Except for CRP ($P = 0.025$), the other demographic and clinical features demonstrated no significant differences between the groups.

Diffusion metrics

The TBSS analyses revealed a lower V_{ic} value in the patients than in the controls; further details of the significant results are shown in Table 4. Abnormal diffusion metrics were detected in the following regions: bilateral corona radiata (anterior and superior part), genu of the corpus callosum and superior longitudinal fasciculus L (SLF) (Fig. 2A). The results based on regions of interest that were significant in the TBSS analyses are shown in Fig. 2B.

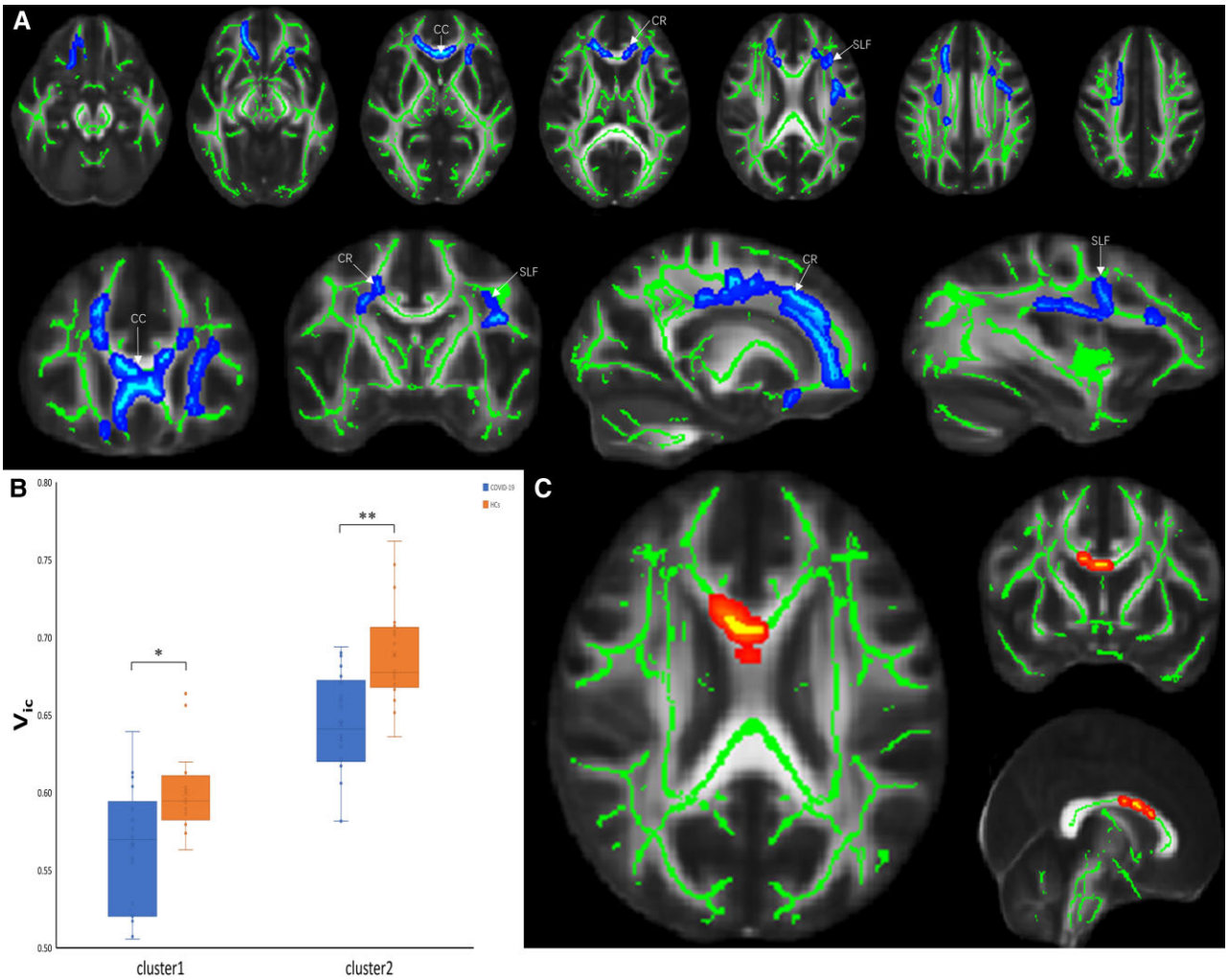

Figure 2 Results of TBSS analysis and *post hoc* regions of interest analysis. (**A**) TBSS results for V_{ic} between recovered COVID-19 patients and healthy controls (HCs). The TBSS analyses revealed decreased V_{ic} in patients than in controls. Green represents white matter skeleton. Blue-light blue represents areas of significant differences. Blue represents higher V_{ic}, and light blue represents lower V_{ic}. These tracts are named after significant fibre tracts in Table 4. (**B**) *Post hoc* region of interest (ROI) analysis results. Clusters are significant tracts in TBSS. The blue boxes represent recovered COVID-19 group, and the orange boxes represent healthy controls. Cluster 1 of recovered COVID-19 group: median = 0.570, interquartile interval (IQR) = 0.072, minimum = 0.506, maximum = 0.639; Cluster 2 of recovered COVID-19 group: median = 0.641, interquartile interval = 0.052, minimum = 0.582, maximum = 0.694; Cluster 1 of healthy controls: median = 0.595, IQR = 0.028, minimum = 0.563, maximum = 0.664; Cluster 2 of healthy controls: median = 0.677, IQR = 0.039, minimum = 0.635, maximum = 0.762. (**C**) TBSS results of fractional anisotropy (FA) between ICU and non-ICU patients. The TBSS analyses revealed decreased fractional anisotropy in ICU patients than in non-ICU patients. Significant voxels are on the body of the corpus callosum (CC). Green represents white matter skeleton. Red and yellow represent areas of significant differences. Red represents higher fractional anisotropy and yellow represents lower fractional anisotropy. *$P < 0.005$, **$P < 0.001$; CR = corona radiata; V_{ic} = volume fraction of intracellular water.

The TBSS analyses revealed a lower fractional anisotropy in the ICU group than in the non-ICU group. The body of the corpus callosum (150 voxels) was significantly different between these two subgroups (Fig. 2C).

Neuropsychological test results and correlation analysis

The entire neuropsychological test datasets were lost for two recovered COVID-19 patients. The WFT data were lost in five other recovered COVID-19 patients and 4 healthy controls. Cognitive function as assessed by the subscales of the Wechsler Intelligence scale was not significantly different either between recovered COVID-19 patients and healthy controls or between the ICU and non-ICU groups (Tables 1 and 3).

Within the COVID-19 group, V_{ic} of cluster 1 was negatively correlated with length of hospitalization ($P = 0.014$, $r = -0.407$) and positively correlated with days of follow-up ($P = 0.011$, $r = 0.419$). V_{ic} of cluster 2 was negatively correlated with length of hospitalization ($P = 0.011$, $r = -0.419$) and positively correlated with days of follow-up ($P = 0.007$, $r = 0.442$) (Table 5). The Spearman correlations in the COVID-19 group are presented in Fig. 3. However, after multiple comparison corrections, no significant correlation remained within this group.

Discussion

In the present study, we comprehensively investigated white matter changes in recovered COVID-19 patients at the 1-year follow-up using conventional DTI metrics and DKI and NODDI models. To the best of our knowledge, this is the first study to

investigate white matter changes at the 1-year follow-up. Our results showed that recovered COVID-19 patients had lower V_{ic} values than healthy controls 1 year after recovery. Additionally, patients who were admitted to the ICU had slightly more white matter abnormalities. Compared with healthy controls, recovered COVID-19 patients showed no significant decline in cognitive function. Finally, white matter tended to present with fewer abnormalities for shorter hospital stays and longer follow-up times.

V_{ic}, a potential proxy for axonal density measurements, may be explained by oedema and axonal beading followed by apoptosis.[24] In our study, V_{ic} was significantly lower in the recovered COVID-19 patients than in the healthy controls, indicating the existence of microstructural changes at the 1-year follow-up, despite the patients having clinically recovered and presenting with normal conventional MRI findings. Among the eight diffusion parameters, only V_{ic} showed statistical significance after correction for multiple comparisons, indicating that white matter microstructural changes in these patients may be subtle and that NODDI was a better diffusion model for demonstrating these subtle changes in white matter. The subtle changes may be related to the fact that the target of SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2),[25] which is mainly distributed in vascular endothelial cells and smooth muscle cells[26]; vessels in the white matter are relatively sparse. Compared with other relatively short-term follow-up studies,[5,27] which reported more diffusion parameter abnormalities and/or larger significant brain regions, the results in the present study indicate that white matter changes are a dynamic process and that the white matter eventually returns to normal. Decreased fractional anisotropy indicates the chaotic dispersion of water in white matter fibre bundles and usually represents unhealthy, structurally disordered white matter fibres in ICU patients.[28,29] The significant brain regions included only 150 voxels in the body of the corpus callosum, showing that the white matter difference between the ICU group and non-ICU group was very subtle. Additionally, these findings indicate that the white matter abnormalities in recovered COVID-19 patients impacted all

Table 5 Correlation results

	X	Y	r	P
Spearman correlation	Hospitalization days	V_{ic} (cluster 1)	−0.407	0.014*
		V_{ic} (cluster 2)	−0.419	0.011*
	Follow-up days	V_{ic} (cluster 1)	0.419	0.011*
		V_{ic} (cluster 2)	0.442	0.007*

V_{ic} = volume fraction of intracellular water.
*$P < 0.05$.

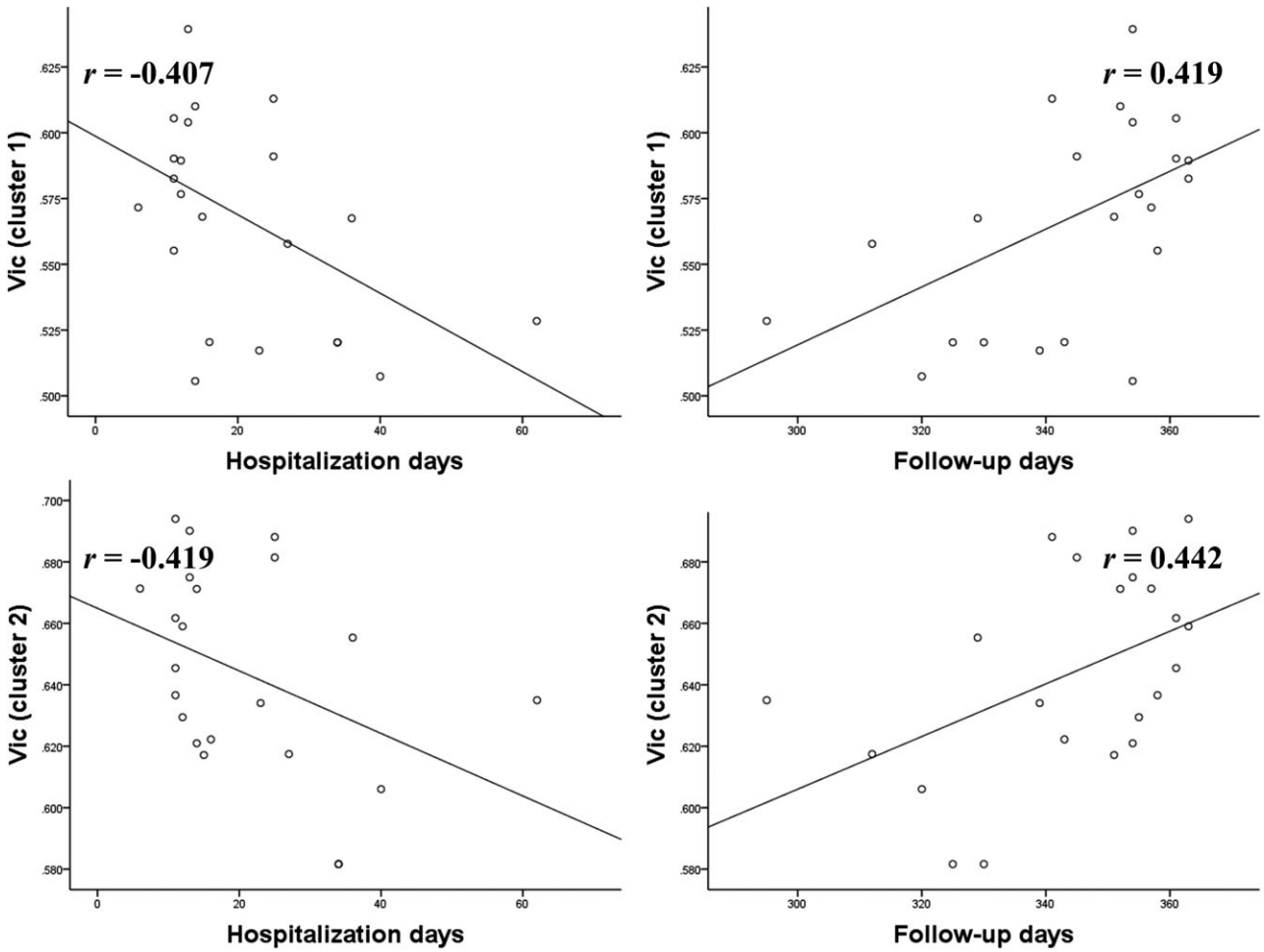

Figure 3 Spearman correlations results. V_{ic} correlated negatively with hospitalization days and correlated positively with follow-up days. V_{ic} = volume fraction of intracellular water.

COVID-19 participants not just those who stayed in the ICU with severe illness. However, the imaging manifestations of ICU and non-ICU patients are different in the acute stage,[6] and severe patients have shown worse white matter manifestations than mild patients at the 3-month follow-up.[5] No study has yet compared white matter integrity between ICU patients and non-ICU patients after the 1-year follow-up. Our results indicated that the impact of severe illness on ICU patients may gradually decrease over time.

The corona radiata, corpus callosum and SLF were the main areas with abnormal fibres presented in our results. Although white matter is not the key target of neurotropic viruses, these connecting fibres could act as channels for intracranial viral transmission.[11] The corona radiata consists of a large number of projection fibres that connect the cortex to the brainstem and the thalamus in both an afferent and efferent manner.[30] The corpus callosum connects the bilateral cerebral hemispheres and communicates between brain regions with powerful parallel fibres. The corpus callosum is a vulnerable target, and damage to this region has been found in the acute phase and during follow-up.[6,27] The SLF is a long association fibre tract that travels in discrete fascicles, leading to distant cortical areas in the same hemisphere.[31] The corpus callosum, corona radiata and SLF are important components of the connecting fibres with the, which play a key role in commissural fibres. These factors make them potential targets after viral infection. Additionally, abnormalities of these tracts have been found in previous relatively short follow-up studies.[5,11,27] At the same time, the significant brain voxels were primarily anterior brain regions, which may be related to the high density of ACE2 in the frontal cortex.[32,33]

No significant decline in cognitive function was found in recovered COVID-19 patients in our research. In accordance with previous studies, our patients may undergo a process of cognitive decline and recovery. If baseline and short-term follow-up cognitive function can be obtained, this conjecture can be better supported, but so far we have been unable to obtain baseline or short-term neuropsychological test data. Several studies have shown cognitive impairment in COVID-19 patients[14,34,35]; however, these studies represent relatively short-term research. Additionally, previous long-term studies have shown that complications such as delirium and ARDS have an impact on patients' long-term cognitive function.[7,36,37] However, only two patients in our study had COVID-19 complications, which may be the reason why we obtained negative results. Furthermore, the cognitive function was relatively low in the healthy controls compared to COVID-19 patients. Years and quality of education may be the most likely cause.

The white matter in the COVID-19 patients tended to present with fewer abnormalities for shorter hospital stays and longer follow-up times. We can roughly link the hospitalization stay with the severity of the illness and conclude that a more serious condition correlates with greater microstructural changes. Additionally, the duration of follow-up represents the time for the white matter to recover; in COVID-19 patients, the changes in white matter tended to be reversible and showed constant recovery over a long period of time. However, white matter abnormalities were not related to inflammatory markers, which is inconsistent with previous studies, possibly because the mechanism of persistent white matter changes is not caused by inflammatory storms but by other causes, such as acute hypoxic-ischaemic changes.

There were several limitations in the present study. First, our study had a small sample size. To improve the reliability of the results, we included participants who volunteered to participate and did not make subjective choices through researchers. We used multiple diffusion models and metrics to more comprehensively display white matter changes using voxel-based methods. Strict statistical analysis and correction were also performed. However, more patients and healthy controls should be recruited in future studies to test and clarify the results of the present research. Second, the patients in our study had no previous brain MRI scans because they had demonstrated no severe neurological manifestations. Therefore, we could not obtain the patients' baseline imaging status or assess dynamic changes during the follow-up period. However, we will conduct follow-up observations on these patients to explore long-term dynamic changes in the future. Third, white matter hyperintensity is a common condition in elderly individuals,[38] but moderate-severe white matter hyperintensity could influence white matter integrity.[39] We counted the degree and number of patients with white matter hyperintensity according to the modified version of the Fazekas scale[40] to compare the constituent ratios of the two groups before the analysis. There was no significant difference in the constituent ratio between the two groups ($P = 0.609$). We will attempt to include more participants to overcome this limitation. Last, we used only diffusion imaging to explore white matter changes in a single centre, and multimodal imaging and multicentre studies should be combined in future studies.

In conclusion, lower axonal density with no significant decline in cognitive function were discovered in recovered COVID-19 patients after 1 year. ICU patients had slightly more white matter abnormalities. However, inflammatory storms were not the main cause of these white matter changes after 1 year of recovery. The duration of hospital stay may be a predictor for white matter changes at the 1-year follow-up.

Funding

This study was funded by the Innovative Major Emergency Project Funding against New Coronavirus Pneumonia in Hunan Province (2020SK3014), Key Emergency Project of Pneumonia Epidemic of Novel Coronavirus Infection (2020SK3006), Clinical Research Center for Medical Imaging in Hunan Province (2020SK4001), National Natural Science Foundation of China (82102157), Hunan Provincial Natural Science Foundation of China (2021JJ40895), Science and Technology Innovation Program of Hunan Province (2020SK53423), Leading talents of scientific and technological innovation in Hunan Province in 2021 (2021RC4016) and Key R&D Project of Science and Technology Department of Hunan Province (2022SK2047).

Competing interests

The authors report no competing interests.

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