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

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Running title: WM changes in recovered COVID-19 patients
Abstract

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 one-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 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 one 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 one-year follow-up.

**Keywords:** recovered COVID-19 patients; white matter changes; cognitive function; one-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; TBSS = tract-based spatial statistics
Introduction

The coronavirus disease 2019 (COVID-19) pandemic has posed great challenges worldwide, including diagnosis, treatment, and postinfection 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 postinfection 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. Neurological manifestations, such as encephalitis, cerebral hemorrhage, and impaired consciousness, and neuroimaging findings, such as cerebrovascular disease, perfusion abnormalities, and white matter (WM) changes, 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. 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. 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 WM integrity. The DTI model assumes simple Gaussian diffusion through the brain microstructure. Diffusion kurtosis imaging (DKI), 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 WM changes in recovered COVID-19 patients,\(^5,11\) indicating that these patients present with persistent WM abnormalities. However, the status and changes in WM in recovered COVID-19 patients after one year remain unknown, and WM 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 WM.

In this context, the purposes of this study were to assess the long-term change in WM 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 one-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: (1) a diagnosis of COVID-19 according to the guidelines of the National Health Commission and a discharge date between February and April 2020; (2) age greater than 18 years; and (3) willingness and ability to undergo brain MRI scanning. The exclusion criterion was a structural abnormality on traditional neuroimaging except for WM hyperintensity. Age-, sex- and education-matched healthy controls (HCs) were recruited, and subjects 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 WM 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 HCs were included. A flowchart of patient inclusion is shown in Figure 1.

All subjects 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/lymphocytes). 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 HCs 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, Erlangen, Germany) with a 32-channel head coil. All subjects 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 T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), 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 (TR)/echo time (TE) = 5400/92 ms, field of view (FOV) = 224 × 224 mm, 112 × 112 matrix, 40 slices, 2 × 2 × 3 mm³ voxels, bandwidth = 1654 Hz/pixel, b = 1000/2000 s/mm², 64 diffusion-weighting directions at each b value, and 10 b0 scans. T1WI, T2WI, FLAIR, MPRAGE and SWI were independently reviewed by two neuroradiologists with
more than ten 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 5 cognitive tests. (1) The logical memory (LM) test, a measure of verbal episodic memory\textsuperscript{15}: the tester read a sentence made up of multiple
corresponding symbols and then they were instructed to match the correct
symbols to the corresponding numbers in two minutes. The total score was the number of
correctly matched symbols, and a higher score indicated better performance in the assessment.
(2) The digit symbol substitution test (DSST), which has been frequently used to assess participants’
processing speed, sustained attention and working memory.\textsuperscript{16,17} The patients were shown 9
numbers and their corresponding symbols and then they were instructed to match the correct
(3) 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 subject 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?” (4) The Digit
span (DS) task, a verbal attention and working memory task that has been widely used in
cognitive assessment.\textsuperscript{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).\textsuperscript{20} In our study, the
DS task was presented as sequences of digits of increasing length, ranging from 2 to 9 numbers.
(5) The word fluency test (WFT): in one minute, the subjects were asked to name as many
animals as possible. The subjects completed these neuropsychological tests on the same day as
the MRI scan.
**Image analysis**

Image processing included initial preprocessing and diffusion metric computations. Prior to preprocessing, each subject’s diffusion images were visually inspected to verify that they were free from major artifacts (e.g., head motion). Motion, eddy current artifacts, 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 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 subject’s b0 image. Then, four DTI metrics (fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and mean diffusivity (MD)) were calculated by the FSL *dtifit* command. DKI model fitting was performed using DKE (version 2.6.0), and the mean kurtosis (MK) was calculated. Three NODDI parameters (orientation dispersion index (ODI), 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 white-matter skeleton was extracted in the standard Montreal Neurological Institute (MNI) space to minimize the partial volume effects in a finite imaging resolution. The WM skeleton included only voxels in the center of WM tracts and excluded edge voxels, which may be contaminated with signals from the nearby anatomy. Within the WM 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 utilized to obtain a corrected $p$ value. WM 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 (ROI) analysis**

To produce aggregate results at the subject level, post hoc ROI analyses were performed. For each subject, the mean of each diffusion metric was computed in the regions that tested as significant with TBSS. For between-group differences, a boxplot was used with subjects’ means plotted based on their group membership. The anatomical interpretation of the ROI 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 analyzed using IBM SPSS Statistics 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 upon 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) HCs. A comparison of the characteristics between the two groups is presented in Table 1. There were no statistically significant differences between the patients and HCs 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 (MODS), 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: 8 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 (CR) (anterior and superior part), genu of the corpus callosum (CC), and superior longitudinal fasciculus L (SLF) (Figure 2 A). The results based on ROIs that were significant in the TBSS analyses are shown in Figure 2 B.

The TBSS analyses revealed a lower FA in the ICU group than in the non-ICU group. The body of the CC (150 voxels) was significantly different between these two subgroups (Figure 2 C).

### Neuropsychological test results and correlation analysis

The entire neuropsychological test datasets were lost for 2 recovered COVID-19 patients. The WFT data were lost in 5 other recovered COVID-19 patients and 4 HCs. Cognitive function as assessed by the subscales of the Wechsler Intelligence scale was not significantly different either between recovered COVID-19 patients and HCs or between the ICU and non-ICU groups (Table 1 and Table 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 ($p = 0.011$, $r = \ldots$)
-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 Figure 3. However, after multiple comparison correction, no significant correlation remained within the this group.

**Discussion**

In the present study, we comprehensively investigated WM changes in recovered COVID-19 patients at the one-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 WM changes at the one-year follow-up. Our results showed that recovered COVID-19 patients had lower $V_{ic}$ values than HCs one 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 edema and axonal beading followed by apoptosis. In our study, $V_{ic}$ was significantly lower in the recovered COVID-19 patients than in the HCs, indicating the existence of microstructural changes at the one-year follow-up, despite the patients having clinically recovered and presenting with normal conventional MRI findings. Among the 8 diffusion parameters, only $V_{ic}$ showed statistical significance after correction for multiple comparisons, indicating that WM microstructural changes in these patients may be subtle, and indicated that NODDI was a better diffusion model for demonstrating these subtle changes in WM. The subtle changes may be related to the fact that the target of SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2), which is mainly distributed in vascular endothelial cells and smooth muscle cells; vessels in the...
WM 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 WM changes are a dynamic process and that the WM eventually returns to normal. Decreased FA indicates the chaotic dispersion of water in WM fiber bundles and usually represents unhealthy, structurally disordered WM fibers in ICU patients.²⁸,²⁹ The significant brain regions included only 150 voxels in the body of the CC, showing that the WM difference between the ICU group and non-ICU group was very subtle. Additionally, these findings indicate that the WM abnormalities in recovered COVID-19 patients impacted all COVID-19 subjects 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 WM manifestations than mild patients at the 3-month follow-up.⁵ No study has yet compared WM integrity between ICU patients and non-ICU patients after the one-year follow-up. Our results indicated that the impact of severe illness on ICU patients may gradually decrease over time.

The CR, CC and SLF were the main areas with abnormal fibers presented in our results. Although WM is not the key target of neurotropic viruses, these connecting fibers could act as channels for intracranial viral transmission.¹¹ The CR consists of a large number of projection fibers that connect the cortex to the brainstem and the thalamus in both an afferent and efferent manner.³⁰ The CC connects the bilateral cerebral hemispheres and communicates between brain regions with powerful parallel fibers. The CC 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 fiber tract that travels in discrete fascicles, leading to distant cortical areas in the same hemisphere.³¹ The CR and SLF are important components of the connecting fibers with the CC, which play a
key role in commissural fibers. These factors make them potential targets after viral infection.

Additionally, abnormalities of these tracts have been found in previous relatively short follow-up studies. 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.

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 to date we have been unable to obtain baseline or short-term neuropsychological test data. Several studies have shown cognitive impairment in COVID-19 patients; 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. 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 HCs 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 WM to recover; in COVID-19 patients, the changes in WM tended to be reversible and showed constant recovery over a long period of time. However, WM abnormalities were not related to inflammatory markers, which is inconsistent with previous studies, possibly because the
mechanism of persistent WM changes is not caused by inflammatory storms but by other causes, such as acute hypoxic-ischemic 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 subjects who volunteered to participate and did not make subjective choices through researchers. We used multiple diffusion models and metrics to more comprehensively display WM changes using voxel-based methods. Strict statistical analysis and correction were also performed. However, more patients and HCs should be recruited in future studies to test and clarify the results of the present research. Second, the patients in our study had no prior 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, WM hyperintensity is a common condition in elderly individuals, but moderate-severe WM hyperintensity could influence white matter integrity. We counted the degree and number of patients with WM hyperintensity according to the modified version of the Fazekas scale 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 subjects to overcome this limitation. Last, we used only diffusion imaging to explore WM changes in a single center, and multimodal imaging and multicenter 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 one year. ICU patients had slightly more white matter abnormalities. However, inflammatory storms were not the main cause of these WM
changes after one year of recovery. The duration of hospital stay may be a predictor for white matter changes at the one-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) 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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Figure legends

**Figure 1** Flowchart of the study.

**Figure 2** Results of TBSS analysis and post hoc ROI analysis. (A) Tract-based spatial statistics (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 fiber 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 HCs. Cluster 1 of recovered COVID-19 group: median = 0.570, interquartile interval = 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 HCs: median = 0.595, interquartile interval = 0.028, minimum = 0.563, maximum = 0.664; Cluster 2 of HCs: median = 0.677, interquartile interval = 0.039, minimum = 0.635, maximum = 0.762. (C) TBSS results of FA between intensive care unit (ICU) and non-ICU patients. The TBSS analyses revealed decreased FA in ICU patients than in non-ICU patients. Significant voxels are on the body of the CC. Green represents white matter skeleton. Red-yellow represents areas of significant differences. Red represents higher FA, and yellow represents lower FA. *$p < 0.005$, **$p < 0.001$; CC, Corpus Callosum; CR, Corona Radiata; FA, fractional anisotropy; SLF, Superior Longitudinal Fasciculus; $V_{ic}$, volume fraction of intracellular water.

**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.
Table 1 Demographic and neuropsychological tests of recovered COVID-19 patients and HCs

|                  | PT  | HCs | t/Z/x² | p   |
|------------------|-----|-----|--------|-----|
| N                | 22  | 21  |        |     |
| Sex              | M:11; F:11 | M:5; F:16 | 3.154 | 0.076 |
| Age(y)           | 54.14 ± 9.76 | 49.14 ± 12.44 | -1.468 | 0.15  |
| Education(y)     | 12 (12; 16) | 12 (10.5; 16) | -1.163 | 0.87  |
| Neuropsychological tests | | | | |
| LM               | 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) | -0.367 | 0.57  |
| WFT              | 20.67 ± 6.49 | 20.18 ± 7.66 | -0.194 | 0.848 |

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.
### Table 2 Clinical characteristics of recovered COVID-19 patients

| Clinical type       | Recovered COVID-19 patients |
|---------------------|-----------------------------|
| Moderate            | 10/22                       |
| Severe              | 12/22                       |
| Hospitalization days| 14.5 (11.75; 28.75)         |
| Follow-up days      | 351.5 (329.75; 357.25)      |

| Neurological symptoms | Acute stage | Follow-up days |
|-----------------------|-------------|----------------|
| 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 | Acute stage | Follow-up days |
|----------------------|-------------|----------------|
| 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) |

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; SII, systemic immune-inflammation index.
Table 3: Demographic and clinical characteristics of ICU and non-ICU patients

|                        | ICU       | Non-ICU   | t/Z/x²   | p       |
|------------------------|-----------|-----------|----------|---------|
| N                      | 8         | 14        |          |         |
| Sex                    | M:5; F:3  | M:6; F:8  |          | 0.659   |
| Age (y)                | 55.88 ± 10.789 | 53.14 ± 9.396 | 0.622   | 0.541   |
| Education (y)          | 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 ± 3.636 | 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 |

*p < 0.05.

ICU, intensive care unit; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; SII, systemic immune-inflammation index; 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.
| Cluster index | Anatomical regions                                      | Voxels | Min p | X   | Y   | Z   |
|---------------|---------------------------------------------------------|--------|-------|-----|-----|-----|
| $V_{ic}$ 1    | Corona radiata (anterior and superior part) L & R       | 3435   | 0.042 | 83  | 151 | 67  |
|               | Genu of corpus callosum                                 |        |       |     |     |     |
| $V_{ic}$ 2    | Superior longitudinal fasciculus L                      | 564    | 0.046 | 126 | 127 | 97  |

$V_{ic}$, volume fraction of intracellular water
# Table 5 Correlation results

| X                      | Y                        | r   | p     |
|------------------------|--------------------------|-----|-------|
| Spearman correlation   | Hospitalization days     | -0.407 | 0.014* |
|                        | $V_v$ (cluster 1)        |     |       |
|                        | $V_v$ (cluster 2)        | -0.419 | 0.011* |
| Follow-up days         | $V_v$ (cluster 1)        | 0.419  | 0.011* |
|                        | $V_v$ (cluster 2)        | 0.442  | 0.007* |

* $p < 0.05$.

$V_v$, volume fraction of intracellular water
Figure 1

237 patients from the First Hospital of Changsha who had been discharged from February to April, 2020

22 age-, sex- and education-matched healthy controls

214 patients are not willing to join to the research

23 patients signed informed consent to participate voluntarily

Exclude 1 subject: brain structural abnormality

Exclude 1 patient: failed to accomplish the whole MRI scan

22 recovered COVID-19 patients

21 healthy controls

Compare and analysis
Figure 2
Figure 3
Figure 4

Data acquisition

Demographic and clinical characteristics
MRI

Neuropsychological test

Data processing

Imaging preprocessing
DTI
DKI
NODDI
TBSS

Randomize

Post hoc ROI analysis

Statistical analysis

COVID-19 vs HCs
V_tr ↓

ICU vs non-ICU
FA ↓

Results
Huang et al. reveal persistent white matter microstructural changes in recovered COVID-19 patients at one-year follow-up, with slightly more abnormalities seen in patients who were admitted to the ICU. Severity of illness in the acute stage may predict white matter integrity one year after recovery.