NeuroRehabilitation xx (20xx) x–xx
DOI:10.3233/NRE-230216
IOS Press

Cognitive and brain connectivity trajectories in critically ill COVID-19 patients

Daniela Ramos-Usuga[a,b,1], Antonio Jimenez-Marin[a,b,1], Alberto Cabrera-Zubizarreta[c], Itziar Benito-Sanchez[b], Diego Rivera[d,e], Endika Martínez-Gutiérrez[a,f], Elena Panera[g], Victoria Boado[g], Fermín Labayen[g], Jesus M. Cortes[a,h,i,2] and Juan C. Arango-Lasprilla[i,j,2,*]

[a]*Biocruces-Bizkaia Health Research Institute, Barakaldo, Spain*
[b]*Biomedical Research Doctorate Program, University of the Basque Country (UPV/EHU), Leioa, Spain*
[c]*Osatek, Vitoria-Gasteiz, Spain*
[d]*Department of Health Sciences, Public University of Navarre, Pamplona, Spain*
[e]*Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain*
[f]*Dipartamento Interateneo di Fisica, National Institute for Nuclear Physics – Bari, Bari, Italy*
[g]*Intensive Care Unit, Cruces University Hospital, Barakaldo, Spain*
[h]*IKERBASQUE, The Basque Foundation for Science, Bilbao, Spain*
[i]*Department of Cell Biology and Histology, University of the Basque Country (UPV/EHU), Leioa, Spain*
[j]*Department of Psychology, Virginia Commonwealth University, Richmond, VA, USA*

Received 7 September 2023
Accepted 5 January 2024

Abstract.

BACKGROUND: Multiple Organ failure (MOF) is one of the main causes of admission to the Intensive Care Unit (ICU) of patients infected with COVID-19 and can cause short- and long-term neurological deficits.

OBJECTIVE: To compare the cognitive functioning and functional brain connectivity at 6–12 months after discharge in two groups of individuals with MOF, one due to COVID-19 and the other due to another cause (MOF-group), with a group of Healthy Controls (HC).

METHODS: Thirty-six participants, 12 from each group, underwent a neuropsychological and neuroimaging assessment at both time-points. Functional connectivity of the resting-state networks was compared between COVID-19 and HC while controlling for the effect of MOF. The association between functional connectivity and neuropsychological performance was also investigated.

RESULTS: Compared to the HC, COVID-19 group demonstrated hypoconnectivity between the Default Mode Network and Salience Network. This pattern was associated with worse performance on tests of attention and information processing speed, at both time-points.

CONCLUSION: The study of the association between cognitive function and brain functional connectivity in COVID-19 allows the understanding of the short- and long-term neurological alterations of this disease and promotes the development of intervention programs to improve the quality of life for this understudied population.

Keywords: COVID-19, neuropsychology, cognition, neuroimaging, intensive care, brain

[1]Equal first author contribution.

[2]Equal last author contribution.

*Address for correspondence: Juan Carlos Arango-Lasprilla, PhD, Department of Psychology, Virginia Commonwealth University, 907 Floyd Ave, Richmond, VA 23284, USA, Tel.: +34 68888406. E-mails: jcarangolasp@vcu.edu and jcalasprilla@gmail.com.

1. Introduction

According to the World Health Organization (WHO) (World Health Organization, s. f.), coronavirus disease 2019 (COVID-19) is an infectious disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2). Since SARS CoV-2 invades the cell through the binding of its spike (S) protein to the angiotensin-converting enzyme 2 (ACE2) receptor, the presence of this receptor in the cells of certain organs leads to their dysfunction (Mokhtari et al., 2020). In fact, ACE2 is most expressed in lung epithelial cells (Hamming et al., 2004), which explains why the respiratory system is heavily affected by the virus (Hamming et al., 2004). After SARS CoV-2 infection, the immune system is activated, giving rise, in the most severe cases, to an uncontrolled inflammatory response known as a cytokine storm, which causes damage to healthy tissues and organs. In turn, this situation can lead to multiple organ failure (MOF) (Mokhtari et al., 2020; Moore & June, 2020), which is characterized by the functional decline of more than one vital organic system, such as the respiratory, cardiovascular, and nervous systems (Marshall, 2001).

Although respiratory problems are among the most evident symptoms of COVID-19, according to recent studies, between 35–85% of patients infected with SARS CoV-2 present alterations of the nervous system, in both the peripheral (PNS) and central (CNS) nervous systems (Nolen et al., 2022). PNS alterations tend to be the mildest and include mainly anosmia, ageusia, and myalgia (Montalvan et al., 2020; Pezzini & Padovani, 2020), whereas CNS lesions can lead to cognitive problems and serious diseases, including loss of consciousness, ischemic cerebrovascular disease, encephalopathy, acute necrotizing hemorrhagic encephalopathy, encephalitis, meningitis, epileptic seizures, and demyelinating diseases such as Guillain-Barré syndrome (Ahmed et al., 2020; Mishra & Banerjea, 2020; Montalvan et al., 2020; Pezzini & Padovani, 2020).

Regarding cognitive problems, the most commonly reported are mental fatigue, slow information processing, difficulty concentrating and remembering, confusion, and disorientation (Hugon et al., 2021; Mahase, 2020). Although these symptoms may disappear soon after recovery from the illness, many people may continue to experience symptoms for more than two months after infection. The presence of these cognitive problems, along with other long-term physical (e.g., fatigue, breath and digestive problems) and emotional (e.g., anxiety, depression, apathy) symptoms has been termed as "long COVID" (Hugon et al., 2021; Mahase, 2020), which is linked with diminished well-being and quality of life (Méndez et al., 2021).

The literature related to long-term cognitive alterations is extensive; however, most of the findings report the alterations through case studies (Meo et al., 2021) and from a purely diagnostic perspective, with few studies evaluating the alterations through neuropsychological tests. For example, Ferrucci et al. (2022) performed a neuropsychological evaluation on 53 patients at both five and 12 months after being discharged from the hospital. The authors found that 63.2% of the participants performed poorly on at least one of the tests. Although scores improved at one year, processing speed and verbal and spatial memory continued to be affected. Poletti et al. (2022) reported similar results, finding that a high percentage of patients with COVID-19 obtained low scores in at least one cognitive domain at one month (79%) and at 3–6 months (75%) post-discharge. In addition, when compared to healthy controls (HC), patients with COVID-19 performed worse on tests of processing speed and motor coordination. On the other hand, Zhou et al. (2020) found that, in comparison with the HCs, their sample of 29 patients obtained worse results in tests that evaluated sustained attention. Miskowiak et al. (2021) also concluded that between 59–65% of their sample of participants with COVID-19 showed cognitive alterations, mainly in verbal memory and executive functions, approximately 3–4 months after hospital discharge. More recently, Schild et al. (2023), in their cross-sectional study, found that of the 52 patients with post-COVID-19 syndrome they evaluated, more than half had a neurocognitive disorder, with alterations in memory and executive functions (60.7%), attention (51.6%), language (35.5%), and perceptual and motor skills (29%).

In addition, several neuroimaging studies have shown that these neurocognitive alterations seem to be related to structural lesions and metabolic changes in the brain, mainly in white matter (Huang et al., 2022). Likewise, some studies have found that structural and functional connectivity is also altered in these patients. For example, Fischer et al. (2022) compared the functional and structural connectivity of 11 patients with disorder of consciousness (DoC) due to COVID-19, 18 patients with DoC due to severe traumatic brain injury (TBI), and 14 HC. Results indicated that compared to HC, patients with COVID-19

showed reduced intra-network connectivity within the default mode network (DMN) and reduction in connectivity between the DMN and the salience network (SN). Similarly, Díez-Cirarda et al. (2022) conducted a cross-sectional study of functional and structural neuroimaging combined with a neuropsychological evaluation of 86 patients with long COVID and 36 HC, finding alterations mainly in attention and memory, as well as hypoconnectivity between the left and right parahippocampal gyrus and between the left cerebellum III and left and right frontal superior orbital cortex. With the exception of these two studies, (Díez-Cirarda et al., 2022) most neuroimaging research (including studies that combine this tool with neuropsychological assessment; Cecchetti et al., 2022; Klinkhammer et al., 2021) focuses on the evaluation of structural or metabolic changes in the brain of patients with COVID-19 (Hafiz et al., 2022; Huang et al., 2022; Meo et al., 2021), not including the assessment of functional connectivity. As such, the majority of recent literature in this area is affected by two major limitations, a cross-sectional research design, and lack of inclusion of other cognitive measures.

The use of functional connectivity, and specifically, the fMRI-resting state has demonstrated multiple clinical and research advantages as a useful tool for the evaluation of various CNS diseases (Dennis & Thompson, 2014; Palacios et al., 2013). For this reason, the use of these neuroimaging techniques and their combination with a neuropsychological examination could represent an advance in the understanding of the neurocognitive consequences of people who been diagnosed with COVID-19. In addition, considering that many of these patients experience long-term sequelae of COVID-19 infection, longitudinal studies are necessary to determine long-term effects. For these reasons, the objective of this longitudinal study is to compare the cognitive functioning and functional connectivity of a group of patients with MOF due to COVID-19 (COVID, study group), a group of patients with MOF due to a cause other than COVID-19 (MOF, positive control group), and Healthy Controls (HC, negative control group) at 6 and 12 months after discharge from the Intensive Care Unit (ICU).

2. Methods

2.1. Participants

The sample was composed by 12 patients who suffered MOF after COVID-19 infection (COVID-19 group) and 12 MOF patients without COVID-19 infection (MOF group) who had been treated at the ICU of the Cruces University Hospital, as well as 12 HC, mainly relatives of the patients, matched with them by age, sex, and years of education.

The inclusion and exclusion criteria for MOF patients were the following: a) age between 18 and 65 years old; b) report of MOF due to respiratory failure, cardiogenic shock, or septic shock; c) no structural brain injury detected in T1 (no T1-BD); d) no history of developmental problems, learning disabilities, or neurological or psychiatric conditions prior to admission to the ICU; e) no cerebral hypoxia during ICU admission; f) no previous chronic organ injury that might alter functional connectivity; g) no history of daily consumption and/or use of an illicit substance or other medications that may impact cognitive functioning; h) SOFA score ≥ 4 for at least 48 hours during ICU admission, implying an associated mortality rate of at least 20%; i) MMSE score ≥ 23; and j) no contraindications for magnetic resonance imaging (i.e., morbid obesity, pacemaker, metal prostheses, or pregnancy); k) no severe visual and/or hearing deficit at the time of the evaluation; and l) ability to read and write at the time of evaluation. For COVID-19 participants, the inclusion criteria were the same as the aforementioned criteria for MOF plus additional criteria of m) diagnosed COVID-19 infection demonstrable by positive PCR test; and n) not having received any COVID-19 vaccine.

The inclusion criteria and exclusion for the HC were the following: a) age between 18 and 65 years old; b) no history of developmental problems, learning disabilities, or neurological or psychiatric conditions prior to admission to the ICU; c) no history of daily consumption and/or use of an illicit substance or other medications that may impact cognitive functioning; d) MMSE score ≥ 23; e) scored ≤ 4 on the Patient Health Questionnaire–9 for assessing depression; f) no contraindications for magnetic resonance imaging; g) no severe visual and/or hearing deficit at the time of the evaluation; and h) ability to read and write at the time of evaluation.

2.2. Measures

2.2.1. Screening tests

- The Sequential Organ Failure Assessment (SOFA) was used to assess the severity of MOF, quantifying the extent of organ dysfunction in patients after the failure (Jones et al., 2009)

- The Mini-Mental State Examination (MMSE) was included to assess the overall cognitive status of participants.

2.2.2. Neuropsychological evaluation

The assessment protocol included the following neuropsychological tests:

- Rey-Osterrieth Complex Figure (ROCF; Rey, 2009)
- Hopkins Verbal Learning Test-Revised (HVLT-R; Benedict et al., 1998)
- Stroop color and word test (Golden, 2010)
- Trial Making Test (TMT; Reitan & Wolfson, 1985)
- Brief Test of Attention (BTA; Schretlen et al., 1996)
- Symbol Digit Modalities Test (SDMT; Smith, 2013)
- Modified Wisconsin Card Sorting Test (M-WCST; Schretlen, 1997)
- Verbal Fluency Test (VFT; Reitan & Wolfson, 1985)
- Boston Naming Test (BNT; Kaplan et al., 2005)

2.2.3. Neuroimaging

2.2.3.1. Imaging acquisition. The brain MRIs were acquired in a Philips 3-Tesla Achieva dStream MRI scanner with a 32-channel head coil and included the following sequences:

- Anatomical data: High resolution T1 images were acquired with a 3D Turbo Field Echo (TFE): repetition time TR = 7.4 ms, echo time TE = 3.4 ms, inversion time IT = 850 ms, voxel size = $1.1 \times 1.1 \times 1.2\,mm^3$, slice thickness = 1.2 mm, field of view FOV = $250 \times 250\,mm^2$, 300 contiguous sagittal slices covering the entire brain and brainstem.
- Resting state functional data: A session with a total duration of 7.40 minutes was acquired, using SENSE (with a factor of 2.2) the following parameters: 214 whole-brain gradient echo echo-planar images with TR/TE = 2100/27 ms, FOV = $240 \times 240\,mm^2$, voxel size = $3 \times 3 \times 3\,mm^3$, 80×80 matrix, slice thickness of 3 mm, 45 axial slices, interleaved in ascending order.

2.2.3.2. Imaging preprocessing. The resting-state fMRI (rs-fMRI) images were preprocessed using the CONN toolbox v21a (Whitfield-Gabrieli & Nieto-Castanon, 2012). The default pipeline was used only modifying the outlier-frame criteria, marking as outlier such frames with FD > 0.5. The voxel dimensions were also modified to $3 \times 3 \times 3\,mm^3$, and the spatial smoothing were modified to 6 mm full width at half maximum (FWHM). For nuisance regression and temporal filtering, the chosen option was to do both steps simultaneously. The confounds removed were scrubbing, 12 movement parameters, 5 CSF components, and 5 WM components.

2.2.3.3. Imaging statistical considerations.

- Multiple comparisons correction: For any of the image analyses, statistics was assessed independently for all the number of voxels. To correct for multiple comparisons, a two-sided parametric correction was applied using a voxel threshold p-uncorrected < 0.05 and cluster threshold p-FDR < .05.
- Hypothesis contrasts for voxel brain morphometry differences: For addressing the morphological differences, the contrasts used were [1 –1] (COVID-19 > HC) and [–1 1] (COVID-19 < HC).
- Hypothesis contrasts for functional connectivity differences: For addressing the connectivity differences, the contrasts used were [1 –1 0] (COVID-19 > HC controlling by the effect of MOF) and [–1 1 0] (COVID-19 < MOF controlling by the effect of MOF). This ensured that any possible differences observed between groups may be due to the COVID-19 condition and not because participants in the COVID-19 group had also experienced MOF.

Functional connectivity and neuropsychological performance association: For the association with neuropsychological composites, the contrast used were [0 1 0 0 0] (group, composite, age, years of education, and SOFA). The reason for including SOFA variable as covariate is because in (Jimenez-Marin et al., 2020) authors reported an association between SOFA and brain connectivity in critically ill patients and may impact to the association between neuropsychological performance and connectivity. In this case, composites were calculated with z-scores of the tests computed using the mean and standard deviation of each single group, as this association was performed in each group separately.

2.3. Procedure

The study began in 2018, recruiting patients who had suffered a MOF for respiratory failure, cardiogenic shock, or septic shock. In 2020, with the beginning of COVID-19, the sample was expanded with patients admitted to the ICU for MOF due to SARS CoV-2 infection. Therefore, none of these COVID-19 patients received vaccination. All patients and HC underwent neuropsychological assessments and brain MRI evaluations at 6 and 12 months after ICU discharge. The study was approved by the Ethical Committee of the Cruces University Hospital (Code CEIC E16/52) and was performed in accordance with the Helsinki Declaration. All participants gave their written informed consent.

2.4. Data analyses

2.4.1. Descriptive analyses and neuropsychological performance

Means and standard deviations of demographic and clinical variables were calculated. Performance differences on each neuropsychological test were assessed by One-Way ANOVA. To determine differences between specific groups, post hoc tests (Tukey-Kramer when there was a normal distribution or Games-Howell when there was not a normal distribution) were run. Effect sizes were also assessed using eta-squared ($\eta 2$). Furthermore, each test was grouped into a composite (e.g., executive functioning, language), which was calculated by the sum of the z-scores of the tests based on the means and standard deviations of the three groups together, then divided by the square root of the number of tests included in each composite. Group comparisons among composites were performed using One-Way ANOVA. All the analyses were run in Matlab 2021b and SPSS Statistics 20 (The MathWorks, Inc.)

2.5. Imaging analyses

2.5.1. Structural abnormalities

Voxel-based morphometry (VBM) was applied with FSL-VBM (Douaud et al., 2007), FSL version 6.0.1, an optimized VBM protocol carried out with FSL tools. The modulated gray matter images resulting from the tool were then smoothed with an isotropic Gaussian kernel of full width at half maximum of 9.42 mm ($\sigma = 4$ mm). Final images were used for group comparison.

2.5.2. Functional connectivity analyses

Connectivity maps per each subject for eight resting state networks (RSN) included in CONN were generated using the seed-based-correlation (SBC) analyses (Nieto-Castanon, 2020). The seeds were obtained by averaging the fMRI time-series across all regions within each RSN. As a result, we obtained for each subject a corresponding brain map for each RSN. These maps were next used for group comparisons and for the association between brain functional connectivity and cognitive performance as represented by each composite. For all the details for assessing the statistical significance, see the supplementary material.

3. Results

3.1. Descriptive analyses

The mean age for the sample ranged from 54.83 to 55.50, and the mean of the years of education ranged from 12.17 to 13.42. Moreover, more than half of the sample in all three groups were males. There were no significant differences in terms of age, sex, or years of education between the HC and COVID-19 groups, nor between the MOF and COVID-19 groups. During ICU admission, the mean SOFA scores of the MOF and COVID-19 groups were 9.42 and 6.83, respectively. On the other hand, the mean MMSE scores of HC, MOF, and COVID-19 groups were 29.22, 28.83, and 28.81, respectively. There were no significant differences in SOFA and MMSE across the three groups.

3.2. Neuropsychological performance

The neuropsychological assessment at 6 months from ICU discharge revealed that the COVID-19 group generally performed worse across all tests compared to HC and MOF group, although exhibiting fewer pronounced distinctions in comparison to this last group. The One-Way ANOVAs shown a marginally significant differences in HVLT-R Recall ($F = 3.1$; $p = 0.05$), with the Tukey post hoc showing significant differences between COVID-19 group and HC ($p > 0.05$). Additionally, the effect size was large ($\eta^2 = 0.16$). At 12 months after ICU discharge, there were no significant differences between groups, although scores on most tests were also lower in the COVID-19 group compared to the other two groups.

3.3. Brain morphology

Structural brain comparisons using voxel-based morphometry provided no significant differences between the COVID-19 and HC groups.

3.4. Functional connectivity

Group differences were assessed in the functional connectivity of eight well-known RSNs following an image preprocessing pipeline detailed in Fig. 1. After multiple comparison correction, the only network with significant differences in the contrasts COVID-19 > HC controlling for MOF, and COVID-19 < HC controlling for MOF in both time-points (e.g., 6 and 12 months) was the salience network (see Fig. 2, brain plot). These significant connectivity alterations consisted of a combination of decreased positive correlations from the salience region Anterior Cingulate Cortex (ACC) and decreased negative correlations between the salience node and DMN in the angular gyrus, precuneus, ACC, and superior frontal gyrus. For each of the clusters resulting from the group comparisons (represented in different colors in Fig. 2), we obtained its median value in the SBC map for each participant and compared the different groups. Notably, the boxplots illustrate a general trend, indicating that both positive connections (yellow cluster) and negative connections (green, purple, red, and blue clusters) increased from HC to MOF and further from MOF to COVID-19, demonstrating a progressive decrease in connectivity from healthy to MOF and from MOF to COVID-19.

3.5. Association between functional connectivity and neuropsychological performance

The SBC maps of the salience network were used for voxel-based associations with the four different composites. After multiple comparison correction, significant associations surviving at the two time points only existed for the attention domain (Fig. 3), in a cluster of voxels located in the superior frontal gyrus. At + 12 m time point, a second cluster also emerged in the angular gyrus (colored in red in Fig. 3). The two clusters are part of the DMN. The scatterplots in Fig. 3 represent the association between median values of salience connectivity and the attention score for both time-points. Negative correlations ($r = –0.83$, $p = .002$ at 6 months; $r = –.92$, $p < 0.001$ at 12 months) were found for the COVID-19 group, whereas no associations were found in those regions in the MOF and HC groups. The negative correlations indicated that the patients with the highest attention scores had a relatively more negative correlation between salience and DMN.

4. Discussion

The present longitudinal study had as objectives: 1) to compare the cognitive functioning of the COVID-19, MOF, and HC groups through a neuropsychological battery; 2) to assess the functional connectivity differences between the COVID-19 and HC groups, correcting for the MOF group, and 3) to assess the association between cognitive functioning and functional connectivity across the three groups.

Firstly, the COVID-19 group performed worse at 6 months after ICU discharge across almost all neuropsychological tests compared to the other two groups, although significant differences were only found in the HVLT-R Recall compared to the HC group. At 12 months there were no significant differences in any of the tests, although the means of the COVID-19 group continued being, in general, lower than those of the other two groups. It should be noted that the effect size of the HVLT-R Recall was large ($\eta^2 = 0.16$).

Previous literature has shown that even months after hospital discharge, patients infected with SARS CoV-2 present cognitive alterations related to memory (Díez-Cirarda et al., 2022; Ferrucci et al., 2022; Miskowiak et al., 2021; Schild et al., 2023, 2023), attention and processing speed (Ferrucci et al., 2022, 2022; Poletti et al., 2022; Schild et al., 2023, 2023; Zhou et al., 2020), executive functions (Miskowiak et al., 2021; Schild et al., 2023, 2023), language (Schild et al., 2023) and motor skills (Poletti et al., 2022; Schild et al., 2023). In the present study, significant differences have only been found in one HVLT-R score that measures verbal learning and memory, which partially agrees with previous studies (Díez-Cirarda et al., 2022; Ferrucci et al., 2022; Miskowiak et al., 2021; Schild et al., 2023). Although it is true that it is only a score, the effect size has been large, so one could speculate that, if the sample size were increased, the differences would be more evident and significant, even in the rest of the scores. Furthermore, it should be noted that the evaluation protocols of the present study and that of Díez-Cirarda et al. (2022) share five neuropsychological tests, and that the means of COVID-19 patients from both studies are similar. Although the two samples may vary in

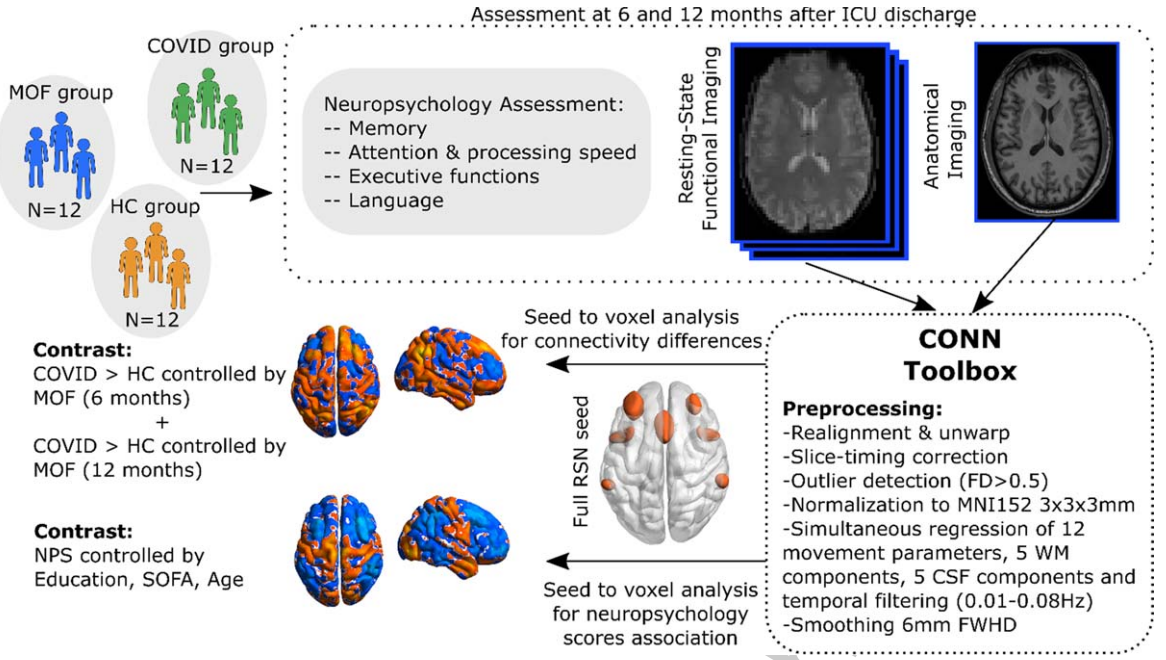

Fig. 1. Resting-state fMRI preprocessing and analysis pipeline. Note: Double acquisition is needed: High resolution anatomical images (T1) and functional images at rest. Following state-of-the-art CONN pipeline of neuroimaging preprocessing, time-series of the blood oxygenation level dependent (BOLD) signal were obtained for each voxel. Using as seeds the average functional dynamics across all the voxels belonging to each resting state network, and applying seed to voxel analysis (a.k.a. seed-based correlation), we built functional connectivity brain maps for each RSN, used for group comparison and for assessing the association with neuropsychological measures.

many aspects, they are Spanish patients who have been hospitalized for COVID-19. One of the factors that most influences the variability of results in research is the type of tests selected to evaluate the participants, therefore, it would be recommended to establish a specific neuropsychological evaluation protocol for patients with COVID-19 who present cognitive complaints, in order to compare the results of the different studies. This is especially relevant since it is a syndrome that is still very unknown and still requires further research.

The results of the functional connectivity demonstrated an atypical connectivity pattern in the COVID-19 group compared to the HC group in both 6 and 12 months, characterized by hypoconnectivity of the SN to one of its nodes (ACC) and with the DMN to four of its nodes (angular gyrus, precuneous, ACC, and superior frontal gyrus). These findings are in agreement to those of Fischer et al. (2022) who found that the functional connectivity of patients with COVID-19 was significantly lower than that of HC patients both within the DMN and between the DMN and the NS. In addition, Díez-Cirarda et al. (2022) also found hypoconnectivity between the left and right parahippocampal gyrus and between the left cerebellum III and the left and right frontal superior orbital cortex. The literature on functional connectivity in populations with neurological disorders is varied, as some studies report hyperconnectivity (Baker et al., 2014; Whitfield-Gabrieli et al., 2009), whereas others find hypoconnectivity (Calhoun, 2009; Dennis & Thompson, 2014; Palacios et al., 2017) in these clinical populations. However, a recent study by Schultz et al. (2017) evaluated people with preclinical profiles of Alzheimer's disease (AD) with fMRI and PET-tau, finding hyperconnectivity between the DMN and SN in participants with low TAU levels, yet hypoconnectivity among participants with higher TAU levels. According to the authors, the initial hyperconnectivity could be a compensatory mechanism in the early phases of a brain disorder, such as AD, which would later lead to a decrease in functional connectivity once these compensatory mechanisms are saturated by neuronal loss. Future studies are needed that include larger sample sizes and that evaluate the trajectories of functional connectivity from the acute phase to more chronic states of the disease, which thus may be able to replicate the pattern of findings in the present study and those of Fischer et al. (2022).

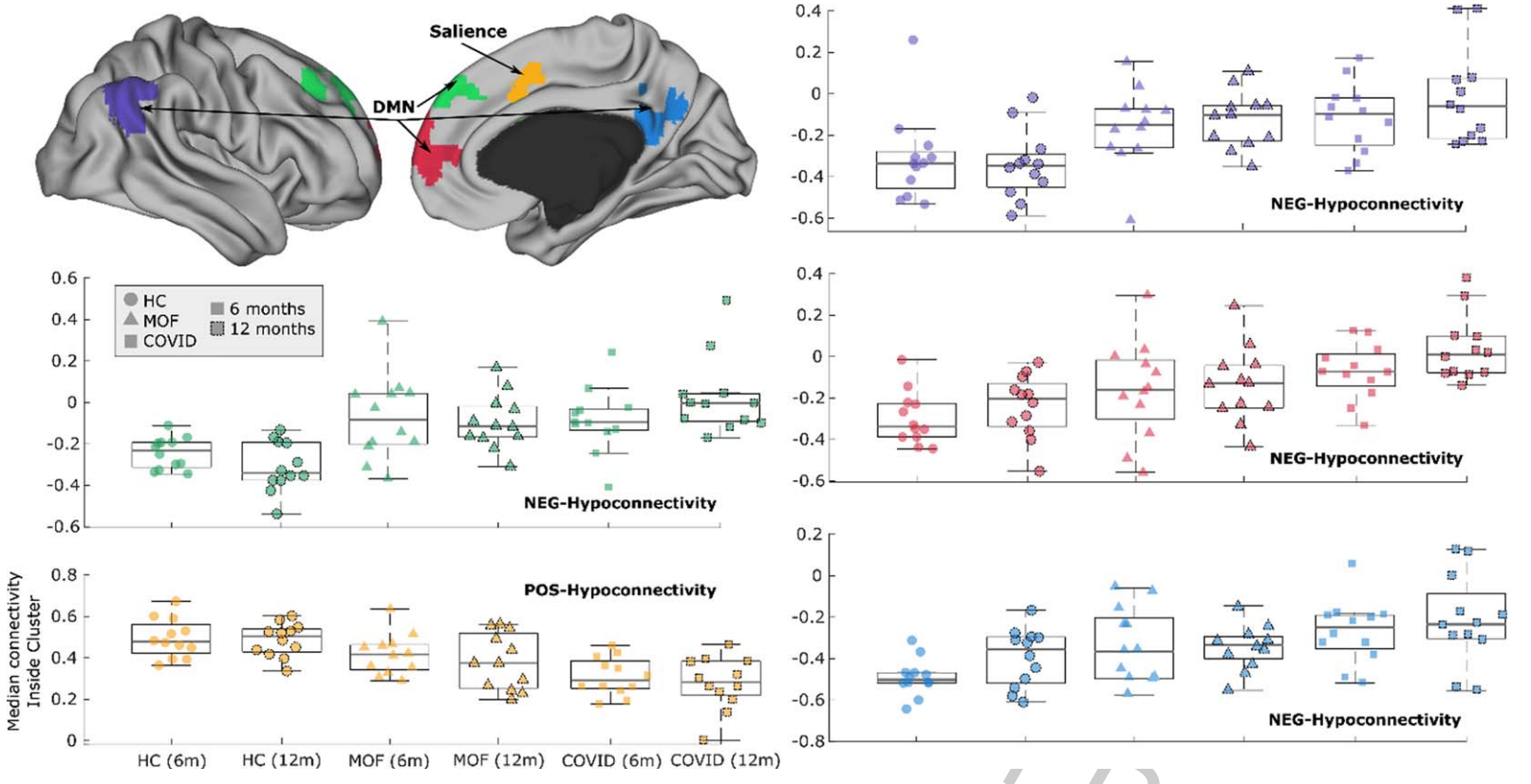

Fig. 2. Salience connectivity alterations in critically ill COVID-19 patients. Note: Statistically significant connectivity alterations not present in MOF were found in a cluster inside the salience network (yellow) and in several clusters belonging to the DMN (purple, green, red, and blue). For each participant, the median connectivity within each cluster was calculated and represented in the boxplots (different colors refer to different clusters). Each dot represents a different participant, being circles for the HC group, triangles for the MOF group, and squares for the COVID-19 group. No line around the shapes represents the 6 months' median connectivity, and the dotted line represents the one at 12 months. NEG-Hypoconnectivity refers to hypoconnectivity occurring in negative functional connections (green, purple, red, and blue), while POS-Hypoconnectivity indicates hypoconnectivity in positive functional connections (yellow).

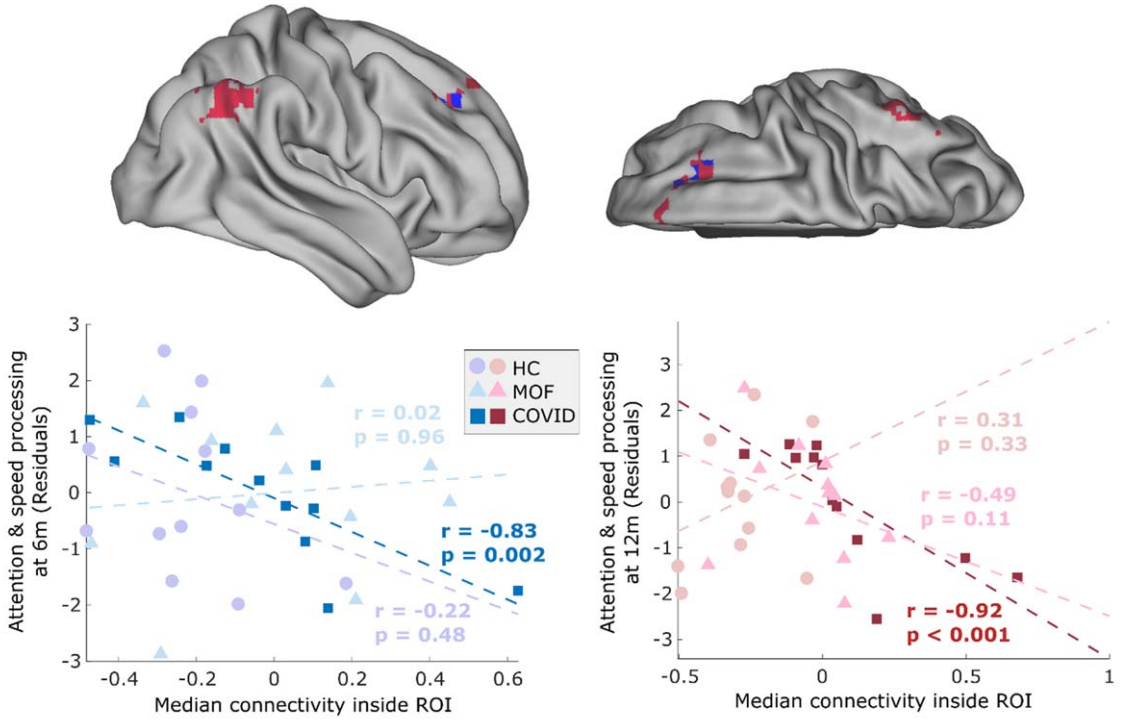

Fig. 3. Association between functional connectivity of the salience network and cognitive performance in critically ill COVID-19 patients. Note: The association between the salience connectivity was correlated with the attention and processing speed domain at 6 (blue clusters) and 12 months (red clusters) after ICU discharge. The two scatterplots show that statistically significant associations were only found in the COVID-19 group.

Finally, when studying the relationship between cognitive functioning and functional connectivity, the connectivity between the SN and certain areas of the DMN (angular gyrus and superior frontal gyrus) was correlated with the attention domain and information processing speed in the COVID-19 group at both 6 months and 12 months. The patients who had a more negative connectivity between SN and DMN had better scores. This association was not present in the HC and MOF groups. The SN, also known as the ventral attention network (VAN), is comprised mainly by the anterior insula and the anterior cingulate cortex. The VAN is involved in the detection and integration of outgoing stimuli, attention, and memory, including exchanging information with other networks on a large scale, which ultimately makes it possible to initiate behavior in accordance with the highlighted stimuli (Menon & Uddin, 2010). VAN and DMN have been shown to have a mutual negative correlation in healthy populations (Gopinath et al., 2015; Martinez-Gutierrez et al., 2022; Uddin, 2015; Uddin et al., 2009), in agreement with our findings showing that, the patients with better attention functioning have consistently higher VAN-DMN negative correlations.

To date, only the cross-sectional study by Díez-Cirarda et al. (2022) has examined both cognitive functioning and functional connectivity in the resting state among patients with COVID-19. Their results indicated that hypoconnectivity between the bilateral frontal superior orbital cortex and the cerebellum was associated with poorer performance on tests of learning and memory. Both neuropsychological assessment and neuroimaging are increasingly used tools in the field of neuroscience research for the study of neurological diseases and in the clinic for evaluation and diagnostic purposes. For this reason, the combined use of these tools is essential in future longitudinal studies with people with COVID-19.

4.1. Limitations

The results of this study must be considered while taking into account several limitations. First, the

study sample size is small. Future research should include larger sample sizes in a replication of study findings. Second, the neuropsychological evaluation included measures of processing speed and attention, learning and memory, executive functioning, and language; however, it is possible that participants also experienced problems in other areas of cognitive functioning that were not evaluated, such as perception, motor skills, or orientation. It is recommended that future studies conduct more comprehensive evaluations. Third, it was not possible to determine if all individuals who participated had the same type of variant of COVID-19. As has been reported in the literature, there are some COVID-19 variants that have been associated with the presence of milder symptoms (Menni et al., 2022), and for this reason, future studies should try to homogenize the groups according to the variants that caused the infection to assess whether the profiles of cognitive functioning and/or functional connectivity varies between groups. Likewise, this study did not assess if the patients with COVID-19 had been infected for the first time or if they previously been diagnosed with the disease. This is important, as some studies have found that COVID-19 reinfections may increase the severity of lung, heart, and CNS problems, and even the risk of death (Bowe et al., 2022). Fourth, the patients with COVID-19 who participated in this study did not received any dose of the COVID-19 vaccine, which is relevant given that various studies have found that the number of vaccine doses received significant reduced symptoms in those subsequently infected people (Barda et al., 2021), which could also be associated with a neuropsychological and functional connectivity profile different from that found in this study. Finally, we did not acquire neuroimaging sequences for determining the presence of microhemorrhages, previously reported in other studies on COVID-19, which may affect brain connectivity.

5. Conclusion

To the best of the authors' knowledge, this is the first study to merge neuropsychological assessment and functional connectivity examination longitudinally in critically ill COVID-19 patients, all of whom have experienced multi-organ failure (MOF). The main result is that patients with COVID-19 present a different functional connectivity pattern than healthy participants, characterized by hypoconnectivity between the DMN and SN, which is associated with worse scores on attention and information processing speed both at 6 and 12 months after discharge from the ICU.

There is an extensive literature on COVID-19, particularly on the neurological alterations that some patients present. However, most research has been based on case studies, research with a cross-sectional design, and neuroimaging limited to the study of brain structure. Given that functional connectivity may provide relevant information on the neurological alterations of this disease, the present study employed a longitudinal design to study the neuropsychological functioning and the brain connectivity while also including two control groups (HC and MOF not due to COVID-19) with which to compare the outcomes of COVID-19 patients. COVID-19 continues to be a global public health emergency, and its long-term consequences are largely unknown. Longitudinal, multidisciplinary research is essential to understand the nature of the disease and its sequelae to improve prevention, diagnosis, and treatment and minimize disease burden in the global population.

Acknowledgments

The authors have no acknowledgments.

Declaration of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Funding

D.R.U was supported by a predoctoral fellowship from the Basque Government (PRE_2019_1_0164).

A.J.M was supported by a predoctoral fellowship from the Basque Government (PRE_2019_1_0070).

J.M.C. was funded by Ikerbasque: The Basque Foundation for Science and from the Department of Economic and Infrastructure Development of the Basque Country (Elkartek Program, KK-2021/00009).

References

Ahmed, M. U., Hanif, M., Ali, M. J., Haider, M. A., Kherani, D., Memon, G. M., Karim, A. H., & Sattar, A. (2020).

Neurological Manifestations of COVID-19 (SARS-CoV-2): A Review. *Frontiers in Neurology*, *11*, 518. https://doi.org/10.3389/fneur.2020.00518

Baker, J. T., Holmes, A. J., Masters, G. A., Yeo, B. T. T., Krienen, F., Buckner, R. L., & Öngür, D. (2014). Disruption of Cortical Association Networks in Schizophrenia and Psychotic Bipolar Disorder. *JAMA Psychiatry*, *71*(2), 109. https://doi.org/10.1001/jamapsychiatry.2013.3469

Barda, N., Dagan, N., Cohen, C., Hernán, M. A., Lipsitch, M., Kohane, I. S., Reis, B. Y., & Balicer, R. D. (2021). Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: An observational study. *The Lancet*, *398*(10316), 2093-2100. https://doi.org/10.1016/S0140-6736(21)02249-2

Benedict, R. H. B., Schretlen, D., Groninger, L., & Brandt, J. (1998). Hopkins Verbal Learning Test – Revised: Normative Data and Analysis of Inter-Form and Test-Retest Reliability. *The Clinical Neuropsychologist*, *12*(1), 43-55. https://doi.org/10.1076/clin.12.1.43.1726

Bowe, B., Xie, Y., & Al-Aly, Z. (2022). Acute and postacute sequelae associated with SARS-CoV-2 reinfection. *Nature Medicine*, *28*(11), 2398-2405. https://doi.org/10.1038/s41591-022-02051-3

Calhoun, V. D. (2009). Functional brain networks in schizophrenia: A review. *Frontiers in Human Neuroscience*, *3*. https://doi.org/10.3389/neuro.09.017.2009

Cecchetti, G., Agosta, F., Canu, E., Basaia, S., Barbieri, A., Cardamone, R., Bernasconi, M. P., Castelnovo, V., Cividini, C., Cursi, M., Vabanesi, M., Impellizzeri, M., Lazzarin, S. M., Fanelli, G. F., Minicucci, F., Giacalone, G., Falini, A., Falautano, M., Rovere-Querini, P., … Filippi, M. (2022). Cognitive, EEG, and MRI features of COVID-19 survivors: A 10-month study. *Journal of Neurology*, *269*(7), 3400-3412. https://doi.org/10.1007/s00415-022-11047-5

Dennis, E. L., & Thompson, P. M. (2014). Functional Brain Connectivity Using fMRI in Aging and Alzheimer's Disease. *Neuropsychology Review*, *24*(1), 49-62. https://doi.org/10.1007/s11065-014-9249-6

Díez-Cirarda, M., Yus, M., Gómez-Ruiz, N., Polidura, C., Gil-Martínez, L., Delgado-Alonso, C., Jorquera, M., Gómez-Pinedo, U., Matias-Guiu, J., Arrazola, J., & Matias-Guiu, J. A. (2022). Multimodal neuroimaging in post-COVID syndrome and correlation with cognition. *Brain*, awac384. https://doi.org/10.1093/brain/awac384

Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P. M., & James, A. (2007). Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain: A Journal of Neurology*, *130*(Pt 9), 2375-2386. https://doi.org/10.1093/brain/awm184

Ferrucci, R., Dini, M., Rosci, C., Capozza, A., Groppo, E., Reitano, M. R., Allocco, E., Poletti, B., Brugnera, A., Bai, F., Monti, A., Ticozzi, N., Silani, V., Centanni, S., D'Arminio Monforte, A., Tagliabue, L., & Priori, A. (2022). One-year cognitive follow-up of COVID-19 hospitalized patients. *European Journal of Neurology*, *29*(7), 2006-2014. https://doi.org/10.1111/ene.15324

Fischer, D., Snider, S. B., Barra, M. E., Sanders, W. R., Rapalino, O., Schaefer, P., Foulkes, A. S., Bodien, Y. G., & Edlow, B. L. (2022). Disorders of Consciousness Associated With COVID-19: A Prospective Multimodal Study of Recovery and Brain Connectivity. *Neurology*, *98*(3), e315-e325. https://doi.org/10.1212/WNL.0000000000013067

Golden, C. (2010). *Manual de test de colores y palabras*. Publicaciones de psicología aplicada: TEA Ediciones.

Gopinath, K., Krishnamurthy, V., Cabanban, R., & Crosson, B. A. (2015). Hubs of Anticorrelation in High-Resolution Resting-State Functional Connectivity Network Architecture. *Brain Connectivity*, *5*(5), 267-275. https://doi.org/10.1089/brain.2014.0323

Hafiz, R., Gandhi, T. K., Mishra, S., Prasad, A., Mahajan, V., Di, X., Natelson, B. H., & Biswal, B. B. (2022). Higher limbic and basal ganglia volumes in surviving COVID-negative patients and the relations to fatigue. *Neuroimage: Reports*, *2*(2), 100095. https://doi.org/10.1016/j.ynirp.2022.100095

Hamming, I., Timens, W., Bulthuis, M., Lely, A., Navis, G., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *The Journal of Pathology*, *203*(2), 631-637. https://doi.org/10.1002/path.1570

Huang, Y., Ling, Q., Manyande, A., Wu, D., & Xiang, B. (2022). Brain Imaging Changes in Patients Recovered From COVID-19: A Narrative Review. *Frontiers in Neuroscience*, *16*, 855868. https://doi.org/10.3389/fnins.2022.855868

Hugon, J., Msika, E.-F., Queneau, M., Farid, K., & Paquet, C. (2021). Long COVID: Cognitive complaints (brain fog) and dysfunction of the cingulate cortex. *Journal of Neurology*, *269*(1), 44-46. https://doi.org/10.1007/s00415-021-10655-x

Jimenez-Marin, A., Rivera, D., Boado, V., Diez, I., Labayen, F., Garrido, I., Ramos-Usuga, D., Benito-Sánchez, I., Rasero, J., Cabrera-Zubizarreta, A., Gabilondo, I., Stramaglia, S., Arango-Lasprilla, J. C., & Cortes, J. M. (2020). Brain connectivity and cognitive functioning in individuals six months after multiorgan failure. *NeuroImage: Clinical*, *25*, 102137. https://doi.org/10.1016/j.nicl.2019.102137

Jones, A. E., Trzeciak, S., & Kline, J. A. (2009). The Sequential Organ Failure Assessment score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. *Critical Care Medicine*, *37*(5), 1649-1654. https://doi.org/10.1097/CCM.0b013e31819def97

Kaplan, E., Goodglass, H., & Weintraub, S. (2005). *Test de Vocabulario de Boston*. Editorial Medica Paramericana, S.A.

Klinkhammer, S., Horn, J., Visser-Meily, J. M. A., Verwijk, E., Duits, A., Slooter, A. J. C., & van Heugten, C. M. (2021). Dutch multicentre, prospective follow-up, cohort study comparing the neurological and neuropsychological sequelae of hospitalised non-ICU- and ICU-treated COVID-19 survivors: A study protocol. *BMJ Open*, *11*(10), e054901. https://doi.org/10.1136/bmjopen-2021-054901

Mahase, E. (2020). Covid-19: What do we know about «long covid» *BMJ (Clinical Research Ed.)*, *370*, m2815. https://doi.org/10.1136/bmj.m2815

Marshall, J. C. (2001). The multiple organ dysfunction syndrome. En R. G. Holzheimer & J. A. Mannick (Eds.), *Surgical Treatment: Evidence-Based and Problem-Oriented*. Zuckschwerdt. https://www.ncbi.nlm.nih.gov/books/NBK6868/

Martinez-Gutierrez, E., Jimenez-Marin, A., Stramaglia, S., & Cortes, J. M. (2022). The structure of anticorrelated networks in the human brain. *Frontiers in Network Physiology*, *2*, 946380. https://doi.org/10.3389/fnetp.2022.946380

Méndez, R., Balanzá-Martínez, V., Luperdi, S. C., Estrada, I., Latorre, A., González-Jiménez, P., Feced, L., Bouzas, L.,

Yépez, K., Ferrando, A., Hervás, D., Zaldívar, E., Reyes, S., Berk, M., & Menéndez, R. (2021). Short-term neuropsychiatric outcomes and quality of life in COVID-19 survivors. *Journal of Internal Medicine*, *290*(3), 621-631. https://doi.org/10.1111/joim.13262

Menni, C., Valdes, A. M., Polidori, L., Antonelli, M., Penamakuri, S., Nogal, A., Louca, P., May, A., Figueiredo, J. C., Hu, C., Molteni, E., Canas, L., Österdahl, M. F., Modat, M., Sudre, C. H., Fox, B., Hammers, A., Wolf, J., Capdevila, J., … Spector, T. D. (2022). Symptom prevalence, duration, and risk of hospital admission in individuals infected with SARS-CoV-2 during periods of omicron and delta variant dominance: A prospective observational study from the ZOE COVID Study. *The Lancet*, *399*(10335), 1618-1624. https://doi.org/10.1016/S0140-6736(22)00327-0

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: A network model of insula function. *Brain Structure and Function*, *214*(5-6), 655-667. https://doi.org/10.1007/s00429-010-0262-0

Meo, S. A., Abukhalaf, A. A., Alomar, A. A., & Al-Hussain, F. (2021). Magnetic Resonance Imaging (MRI) and neurological manifestations in SARS-CoV-2 patients. *European Review for Medical and Pharmacological Sciences*, *25*(2), 1101-1108. https://doi.org/10.26355/eurrev_202101_24681

Mishra, R., & Banerjea, A. C. (2020). Neurological Damage by Coronaviruses: A Catastrophe in the Queue! *Frontiers in Immunology*, *11*, 565521. https://doi.org/10.3389/fimmu.2020.565521

Miskowiak, K. W., Johnsen, S., Sattler, S. M., Nielsen, S., Kunalan, K., Rungby, J., Lapperre, T., & Porsberg, C. M. (2021). Cognitive impairments four months after COVID-19 hospital discharge: Pattern, severity and association with illness variables. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *46*, 39-48. https://doi.org/10.1016/j.euroneuro.2021.03.019

Mokhtari, T., Hassani, F., Ghaffari, N., Ebrahimi, B., Yarahmadi, A., & Hassanzadeh, G. (2020). COVID-19 and multiorgan failure: A narrative review on potential mechanisms. *Journal of Molecular Histology*, *51*(6), 613-628. https://doi.org/10.1007/s10735-020-09915-3

Montalvan, V., Lee, J., Bueso, T., De Toledo, J., & Rivas, K. (2020). Neurological manifestations of COVID-19 and other coronavirus infections: A systematic review. *Clinical Neurology and Neurosurgery*, *194*, 105921. https://doi.org/10.1016/j.clineuro.2020.105921

Moore, J. B., & June, C. H. (2020). Cytokine release syndrome in severe COVID-19. *Science*, *368*(6490), 473-474. https://doi.org/10.1126/science.abb8925

Nieto-Castanon, A. (2020). *Handbook of functional connectivity magnetic resonance imaging methods in CONN*. Hilbert Press.

Nolen, L. T., Mukerji, S. S., & Mejia, N. I. (2022). Post-acute neurological consequences of COVID-19: An unequal burden. *Nature Medicine*, *28*(1), 20-23. https://doi.org/10.1038/s41591-021-01647-5

Palacios, E. M., Sala-Llonch, R., Junque, C., Roig, T., Tormos, J. M., Bargallo, N., & Vendrell, P. (2013). Resting-State Functional Magnetic Resonance Imaging Activity and Connectivity and Cognitive Outcome in Traumatic Brain Injury. *JAMA Neurology*, *70*(7), 845. https://doi.org/10.1001/jamaneurol.2013.38

Palacios, E. M., Yuh, E. L., Chang, Y.-S., Yue, J. K., Schnyer, D. M., Okonkwo, D. O., Valadka, A. B., Gordon, W. A., Maas, A. I. R., Vassar, M., Manley, G. T., & Mukherjee, P. (2017). Resting-State Functional Connectivity Alterations Associated with Six-Month Outcomes in Mild Traumatic Brain Injury. *Journal of Neurotrauma*, *34*(8), 1546-1557. https://doi.org/10.1089/neu.2016.4752

Pezzini, A., & Padovani, A. (2020). Lifting the mask on neurological manifestations of COVID-19. *Nature Reviews. Neurology*, *16*(11), 636-644. https://doi.org/10.1038/s41582-020-0398-3

Poletti, S., Palladini, M., Mazza, M. G., De Lorenzo, R., The COVID-19 BioB Outpatient Clinic Study group, Irene, B., Sara, B., Beatrice, B., Ceciclio, B., Stefania, C., Valentina, C., Elisa, C., Jacopo, C., Marta, C., Elena, C., Federica, C., Sarah, D., Greta, D., Camilla, D. P., … Benedetti, F. (2022). Long-term consequences of COVID-19 on cognitive functioning up to 6 months after discharge: Role of depression and impact on quality of life. *European Archives of Psychiatry and Clinical Neuroscience*, *272*(5), 773-782. https://doi.org/10.1007/s00406-021-01346-9

Reitan, R. M., & Wolfson, D. (1985). *The Halstead–Reitan Neuropsycholgical Test Battery: Therapy and Clinical Interpretation*. Neuropsychological Press.

Rey, A. (2009). *FTest de Copia y Reproducción de una Figura Compleja*. Tea Ediciones, S.A.

Schild, A.-K., Goereci, Y., Scharfenberg, D., Klein, K., Lülling, J., Meiberth, D., Schweitzer, F., Stürmer, S., Zeyen, P., Sahin, D., Fink, G. R., Jessen, F., Franke, C., Onur, O. A., Kessler, J., Warnke, C., & Maier, F. (2023). Multidomain cognitive impairment in non-hospitalized patients with the post-COVID-19 syndrome: Results from a prospective monocentric cohort. *Journal of Neurology*, *270*(3), 1215-1223. https://doi.org/10.1007/s00415-022-11444-w

Schretlen, D. (1997). *Brief test of attention professional manual*. Psychological Assessment Resources.

Schretlen, D., Bobholz, J., & Brandt, J. (1996). Development and psychometric properties of the brief test of attention. *Clin Neuropsychol*, *10*, 80-89. https://doi.org/10.1080/13854049608406666

Schultz, A. P., Chhatwal, J. P., Hedden, T., Mormino, E. C., Hanseeuw, B. J., Sepulcre, J., Huijbers, W., LaPoint, M., Buckley, R. F., Johnson, K. A., & Sperling, R. A. (2017). Phases of Hyperconnectivity and Hypoconnectivity in the Default Mode and Salience Networks Track with Amyloid and Tau in Clinically Normal Individuals. *The Journal of Neuroscience*, *37*(16), 4323-4331. https://doi.org/10.1523/JNEUROSCI.3263-16.2017

Smith, A. (2013). *Test de simbolos y digitos*. TEA Ediciones S.A.

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nature Reviews. Neuroscience*, *16*(1), 55-61. https://doi.org/10.1038/nrn3857

Uddin, L. Q., Kelly, A. M., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2009). Functional connectivity of default mode network components: Correlation, anticorrelation, and causality. *Human Brain Mapping*, *30*(2), 625-637. https://doi.org/10.1002/hbm.20531

Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity*, *2*(3), 125-141. https://doi.org/10.1089/brain.2012.0073

Whitfield-Gabrieli, S., Thermenos, H. W., Milanovic, S., Tsuang, M. T., Faraone, S. V., McCarley, R. W., Shenton, M. E., Green, A. I., Nieto-Castanon, A., LaViolette, P., Wojcik, J., Gabrieli, J. D. E., & Seidman, L. J. (2009). Hyperactivity and hyper-

connectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proceedings of the National Academy of Sciences*, *106*(4), 1279-1284. https://doi.org/10.1073/pnas.0809141106

World Health Organization. (s. f.). *Coronavirus*. Recuperado 5 de agosto de 2022, de https://www.who.int/es/health-topics/coronavirus#tab=tab_1

Zhou, H., Lu, S., Chen, J., Wei, N., Wang, D., Lyu, H., Shi, C., & Hu, S. (2020). The landscape of cognitive function in recovered COVID-19 patients. *Journal of Psychiatric Research*, *129*, 98-102. https://doi.org/10.1016/j.jpsychires.2020.06.022