Supplementary Information

Contains: Supplementary Methods, Supplementary Figures 1 and 2, Supplementary Tables 1-4

Selective visuoconstructional impairment following mild COVID-19 with inflammatory and neuroimaging correlation findings.

JJ de Paula et al.

Corresponding author:

Prof. Marco A. Romano-Silva. Faculdade de Medicina da UFMG. Av Alfredo Balena 190. 30130-100. Belo Horizonte. Brazil. Contact number: 55-31-9-8399-7175. E-mail: romano-silva@ufmg.br

Supplementary Methods

Participants

Inclusion criteria: adults with past mild COVID-19 cases in the last year, age between 18 and 60 years, and confirmed diagnosis of SARS-CoV-2 by RT-PCR. Exclusion criteria: self-reported history of autoimmune disease, previous chronic mental disorders or neurological diagnosis, history of recurrent infections, substance abuse, previous brain surgery, and endotracheal or orotracheal intubation during hospitalization/treatment of SARS-CoV-2. After the previous screening we assessed 196 COVID-19 patients. During our analysis we excluded 4 patients from the neuropsychology subsample. One had a non-disclosed diagnosis of Tourette Syndrome, two were psychologists with previous knowledge of the adopted cognitive tests and one individual was excluded due to the presence of incidental brain lesions detected at MRI scanning during the research. The final sample had 192 participants (Table 1). From this point on our sample was stratified in three subsamples (neuropsychology, neuroimage and immunology) in order to maximize the number of participants for each method adopted in the study.

Neuropsychological tests were available for 191 participants (one patient was unable to perform the tests due to anxiety symptoms). Neuroimaging data was available for 166 participants - excluding five as previously mentioned - other 26 images were excluded due to technical problems during data acquisition (6 MRI datasets and 20 FDG-PET datasets), which led to a final subsample of neuroimage data of 135 participants. Lastly, immunological data was acquired for 100 participants which had both neuropsychological and neuroimaging data.

Participants' sociodemographic and the self-reported symptoms during the COVID-19 infection characteristics are shown on Table 1. We found no statistically significant differences between the subsamples in terms of age (χ^2=1.08, p=0.982), education (χ^2=0.253, p=0.993), sex (χ^2=0.06, p=0.970) or socioeconomic classification (χ^2=1.09, p=0.895).

Cognitive assessment

DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure-Adult [1].

This is a self-reported measure of mental health based on the most recent classification of the American Psychiatric Association. The adult version consists of 23 questions of 13 psychiatric domains. We scored the scale according to the DSM-5 guidelines, and classified the participants in "normal" ou "in risk for mental disorder" in each of the 13 domains. We also created a general psychopathology measure using the total score of the scale, in which higher scores represent worse mental health.

Self Reporting Questionnaire - SRQ-20 [2].

The SRQ-20 is a brief scale for assessment of non-psychotic psychiatric symptoms. Its 20 questions refer mainly to symptoms of depression, anxiety and somatic disorders. Scores range from 0 to 20 and higher scores represent worse mental health. The cutoff 7 ("normal") / 8 ("clinical") shows a high sensitivity and specificity for mental disorders, and was used to classify our participants.

AD8 Screening Questionnaire [3].

This is a brief measure designed to detect cognitive changes possibly related to neurocognitive disorders. The AD8 consists of 8 questions referring to different aspects of cognitive impairment, including problems of judgment, memory and impairment in activities of daily living.

The Rey-Osterrieth Complex Figure Test - ROCF [4].

This is a task in which examinees are asked to copy and recall a complex geometric figure. This task involves multiple cognitive processes including visuoconstruction, planning, visuospatial processing and memory. We used a copy, immediate recall (3 minutes) and delayed recall (30 minutes). Results from copy and each memory recall range from 0 to 36, higher scores represent better performance.

Modified version of the switching verbal fluency test - msVFT [5].

A variation of the traditional category fluency test designed to emphasize the cognitive flexibility assessment. We used the categories animals and then fruits, each in 60 seconds. Each correct word is scored as a point. The patient then is required to produce pairs of words (one animal and one fruit), focusing on the assessment of cognitive flexibility. For the latter procedure we score the total of correct pairs. Higher scores in this test represents better performance.

Trail Making Test [6].

A classical test designed to assess psychomotor speed, attention and executive functions. uThe version used is composed of two parts: "A" and "B", in which the participant must visually search and connect numbers (A) and numbers-letters (B). The test is scored based on the total time dispensed in each part.

Five Point Test - (FPT) [7].

A test designed to evaluate non-verbal fluency and requires the participant to generate a different drawing by connecting five points (as in the face of a dice). This task measures the ability to initiate and sustain the productivity

and self-monitoring, which is a way to assess the executive functions. Performance is analyzed based on the number of unique designs the participant is able to make.

Digit Span [8].

This is a task assessing operational memory and attention. First the participant repeats a crescent series of digits, starting from 2 up to 9 numbers. Following this initial step the participant is presented to the backward, similar to the first one but he must repeat digits in the inverse order.

The Logical Memory Test [9].

A memory test where a short story is read to the participant and he must remember as much information as possible, in an immediate trial (as soon as the reading is finished) and a delayed trial (about 30 minutes from the reading). Each trial is scored on 23 elements, and higher scores represent better performance.

Multiplex assay

The multiplex (Human Cytokine/Chemokine/Growth Factor 45-Plex ProcartaPlex Panel 1 - Thermo Cat. No. EPX450-12171-901), a typical kit for exploring neuroinflammation. It enables the study of immune function by analyzing 45 protein targets (GM-CSF, IFN alpha, IFN gamma, IL-1 alpha, IL-1 beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A (CTLA-8), IL-18, IL-21, IL-22, IL-23, IL-27, IL-31, LIF, SCF, TNF alpha, TNF beta, Eotaxin (CCL11), GRO alpha (CXCL1), IP-10 (CXCL10), MCP-1 (CCL2), MIP-1 alpha (CCL3), MIP-1 beta (CCL4), RANTES (CCL5), SDF-1 alpha, BDNF, EGF, FGF-2, HGF, NGF beta, PDGF-BB, PlGF-1, SCF, VEGF-A, VEGF-D) in a single well using Luminex xMAP technology.

Statistical analysis

Neuropsychological data

For each neuropsychological test we used the available Brazilian normative data, stratified usually by age and/or education, to produce adjusted Z-scores. Measures scored in time units were inverted, so negative Z-scores indicated worse performance while positive indicated better performance. We then used typical criteria for cognitive difficulties (scores -1.5 standard-deviations below the population parameters) to classify the performance of each participant in each test as “normal” or “impaired”. This corresponds to nearly the 8th percentile. Cognitive deficits should have values significantly higher than this. To double-check our classification and reduce possible bias from normative data a subset of our participants (n=49) were matched in terms of age, education and sex with a control group from another study, conducted before the COVID-19 pandemic, that was assessed using the same protocol. These controls were used in psychometric studies for these neuropsychological tests, previously conducted by our group and published in Malloy-Diniz and colleagues (2018) [10]. Chi-square and Independent samples t-tests were used to compare the neuropsychological data of COVID-19 patients and controls.

Processing and analysis of neuroimaging data

MRI brain scans were inspected by two experienced radiologists and 18F-FDG-PET scans by a nuclear physician and a radiologist. All valid FDG-PET and T1-MRI datasets were converted from DICOM format to NIfTI format using DCM2NII software (http://www.cabiatl.com/mricro/mricron/dcm2nii.html), and images were oriented manually to place the anterior commissure at the origin of the three-dimensional Montreal Neurological Institute (MNI) coordinate system. FDG-PET images were co-registered to the T1-MRI datasets of the same individual, using PMOD™ version 3.4 (PMOD Technologies Ltd., Zurich, Switzerland), and corrected for partial volume effects (PVE) to avoid the confounding influence of regional brain atrophy (Thomas et al, 2011) and high white matter uptake (Matsubara et al, 2016). PVE correction on the co-registered FDG-PET images was conducted through the Meltzer method [11] fully implemented in PVElab software (http://pveout.ibb.cnr.it/PVEOut_Software.htm) [12].

Using Statistical Parametric Mapping (SPM) v12 (Wellcome Trust Center of Neuroimaging, London, United Kingdom), running in MATLAB R2012a (MathWorks, Sherborn, Massachusetts), the T1-MRI datasets of each individual were segmented into gray matter, white matter and cerebrospinal fluid using the unified segmentation model [13]. Subsequently, a custom template was created using Diffeomorphic Anatomical Through Exponentiated Lie Algebra [14]. This template was normalized into MNI space, and these parameters were applied to the separated gray matter, white matter and PVE-corrected FDG-PET images in order to achieve spatial normalization to MNI space [13]. Finally, the spatially normalized gray matter, white matter and PVE-corrected FDG-PET images were smoothed with a Gaussian filter of 8-mm at FWHM.

Using the voxel-based morphometry (VBM) statistical approach, we investigated the presence of linear correlations between regional GM/WM volumes and scores on each neuropsychological test for which COVID-19 patients presented significantly poorer performance relative to controls. The GLM was used, based on random Gaussian field theory [15], entering age, sex and education (years) as confounding variables. The total intracranial volume (TIV) was included as an additional covariate (MATLAB get_totals script) (http://www.cs.ucl.ac.uk/staff/g.ridgway/vbm/get_totals.m). Only voxels with values above an absolute GM and WM threshold of 0.05 entered these VBM analyses.

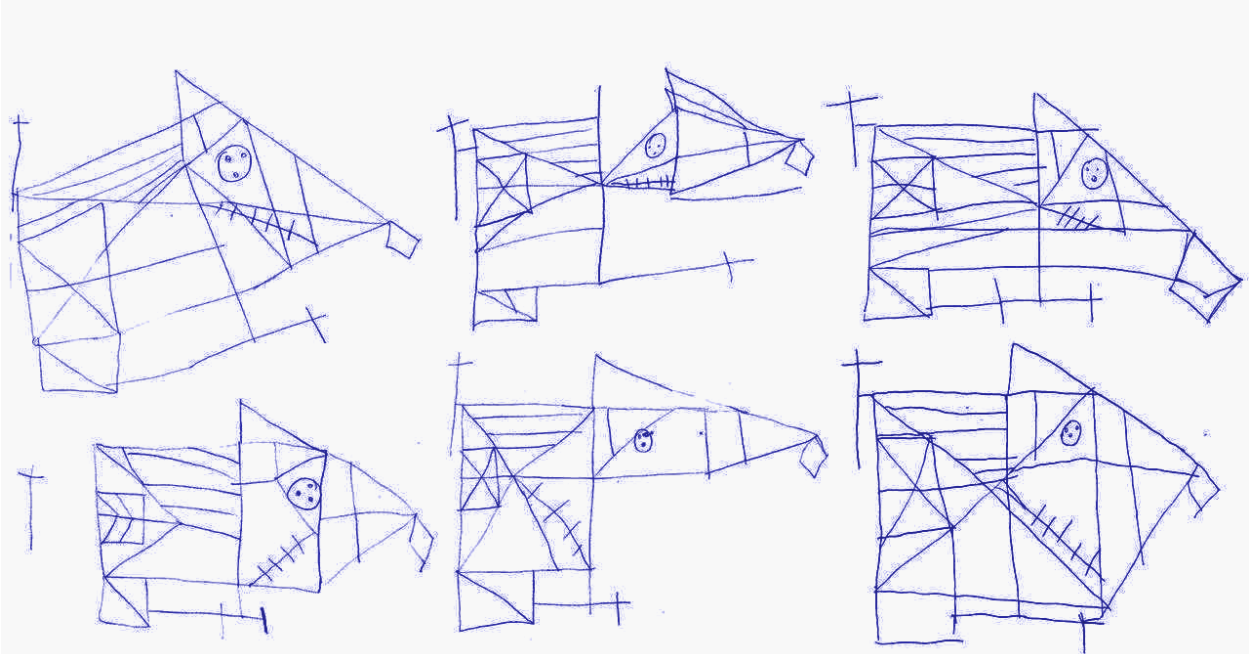
The same voxelwise approach was used to investigate the presence of significant linear correlations between regional glucose metabolism (using the PVE-corrected FDG-PET datasets) and scores on any neuropsychological test(s) for which COVID-19 patients presented poorer performance as compared to controls. To ensure the FDG-PET analysis only included voxels mapping cerebral tissue, a default threshold of 0.5 of the mean tracer uptake inside the brain was selected. Global uptake differences between brain scans were adjusted using the “proportional scaling” SPM12 option, with age, sex and education included as confounding factors.

In all analyses above, resulting statistics at each voxel were transformed to Z-scores and displayed as SPMs into standardized space, at a threshold of $p < 0.0005$ (two-tailed), corresponding to a Z score > 3.29, and an extent threshold of 10 contiguous voxels. These maps were then inspected for the presence of significant voxel clusters of positive or negative correlations with neuropsychological test results.

The Shapiro-Wilk Test was performed to define the normality distribution of data, which indicated non-parametric distribution. Mann-Whitney U Test was employed and correlation analyses were performed through Spearman rank for non-parametric observations. Z-scores at the Rey-Osterrieth Complex Figure (ROCF) test were compared with the Kolmogorov-Smirnov statistical test. $P < 0.05$ was considered statistically significant. Tests were performed using SPSS 25.0 and GraphPad Prism.

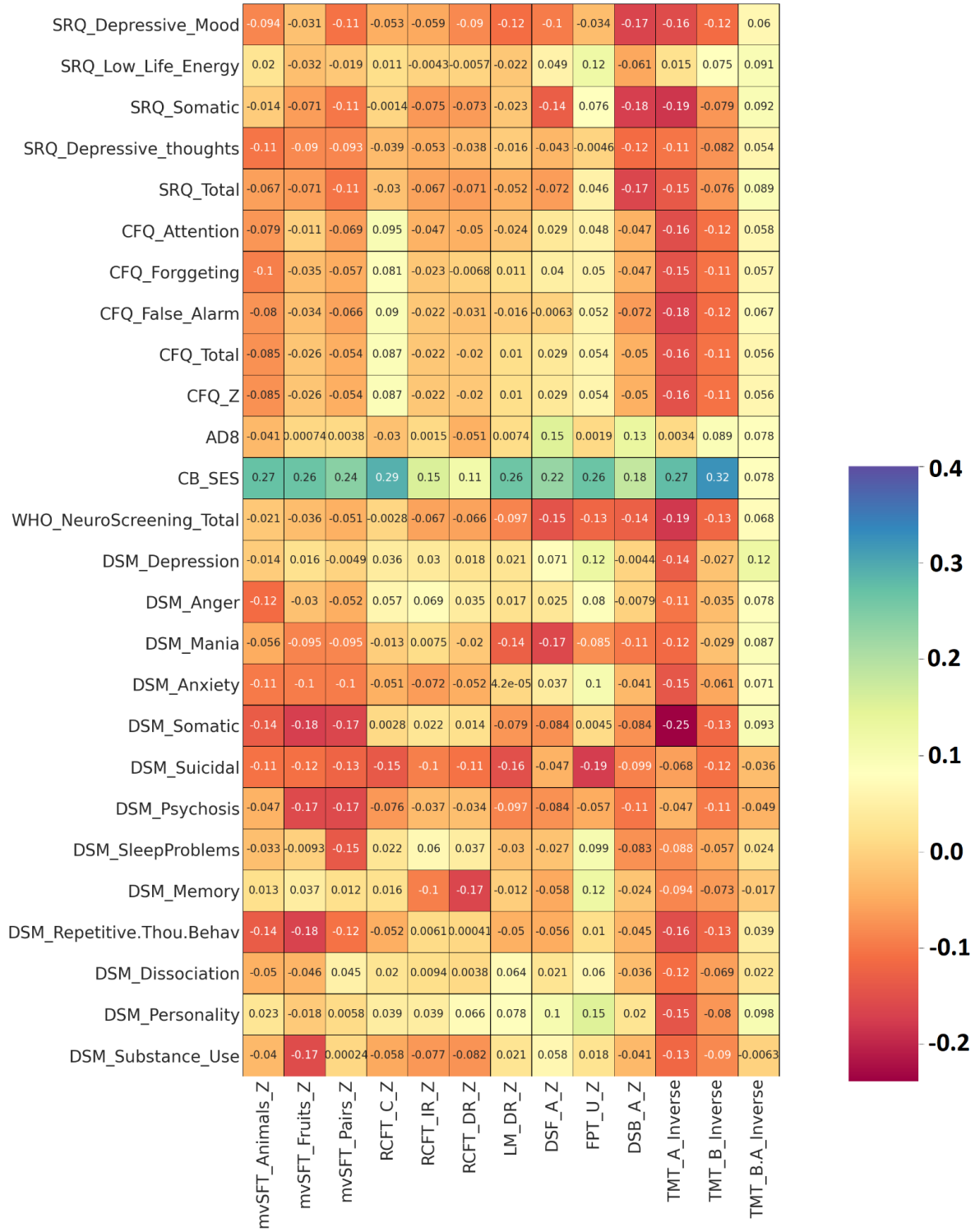
Grouping was performed with the hierarchical clustering algorithm parameterized with Spearman's correlation and complete linkage, implemented in GenePattern [16]. Biomarkers were also clustered with the same parameterization, based on their expression values in the individuals.

Functional enrichment analysis was performed with the Ingenuity Pathway Analysis (IPA) software (Qiagen). The interaction network was built from the list of differentially expressed biomarkers and using the Connect function restricted to interactions experimentally observed or predicted with high confidence. All other parameters were set to default. Next, the most connected and informative componentes of the Ingenuity Toxicity List (clinical pathology endpoints) and Canonical Pathways were added with the Overlay function.



Supplementary Figure 1. Examples of impaired performance in Rey-Osterrieth Complex Figure Test copy by COVID-19 patients.

Supplementary Figure 2. Heatmap correlation of psychiatric symptoms, sociodemographic factors and neuropsychological tests.



Spearman correlation was performed using the Pandas library in Python3. The correlation product was plotted on a heatmap using the SeaBorn library. The y-axis of the image shows attributes of the questionnaires considered for the correlation analysis, and the x-axis shows the results of neuropsychological tests. No strong correlations were found, except a few trends regarding socioeconomic status according to the Criteria Brazil of economic classification [17].

Supplementary Table 1: sociodemographic aspects of the COVID-19 patients (n=192)

Sociodemographic measures			n	%
Age (group)	20 - 29		39	20%
	30 - 39		75	39%
	40 - 49		46	2%
	50 - 59		32	17%
Sex	Male		55	29%
	Female		137	71%
Education (highest degree)	High		65	34%
	College		127	66 %
SES (classification)	Low		18	10%
	Medium		114	62%
	High		52	28%
Access to private healthcare	Yes		133	72%
Hospitalization due to COVID-19	Yes		11	6%
Self-reported COVID-19 symptoms	Fever		79	41%
	Headache		147	77%
	Chills		87	45%
	Dry cough		105	55%
	Sore throat		77	40%
	Myalgia		131	68%
	Shortness of breath		63	33%
	Ageusia		112	58%
	Anosmia		123	64%
	Diarrhea		72	37%
	Nausea		58	30%
	Vomit		30	16%
	Other symptoms		42	22%

Supplementary Table 2. Full description of the study sample (n=191) and the subsamples which went to neuroimaging (n=135) and immunological (n=100) assessment.

		Neuropsychology		Neuroimaging		Immunological	
		n	%	n	%	n	%
Age (group)	20 - 29	39	20%	25	19%	23	23%
	30 - 39	75	39%	53	39%	36	36%
	40 - 49	46	24%	34	25%	23	23%
	50 - 59	32	17%	23	17%	19	19%
Sex	Male	39	26%	32	25%	25	25%
	Female	111	74%	96	75%	76	75%
Education (highest degree)	High	65	34%	47	35%	35	35%
	College	127	66%	88	65%	66	65%
SES (classification)	Low	18	10%	18	13%	12	12%
	Medium	114	62%	78	58%	61	60%
	High	52	28%	38	28%	28	28%
Private healthcare	Yes	133	72%	101	75%	79	78%
Hospitalization	Yes	11	6%	9	7%	5	5%
Self-reported COVID-19 symptoms	Fever	79	41%	59	44%	42	42%
	Headache	147	77%	109	81%	78	77%
	Chills	87	45%	68	50%	53	52%
	Dry cough	105	55%	78	58%	55	54%
	Sore throat	77	40%	55	41%	44	44%
	Myalgia	131	68%	99	73%	72	71%
	Shortness of breath	63	33%	46	34%	33	33%
	Ageusia	112	58%	82	61%	60	59%
	Anosmia	123	64%	89	66%	68	67%
	Diarrhea	72	38%	56	41%	40	40%
	Nausea	58	30%	45	33%	35	35%
	Vomit	30	16%	27	20%	22	22%
	Other	42	22%	38	28%	32	32%

Supplementary Table 3. Screening of psychiatric symptoms by the DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure-Adult in a sample of 192 mild COVID-19 patients.

Symptom	**n**	**%**
Depression	94	49%
Anger	90	47%
Mania	51	27%
Anxiety	101	53%
Somatic problems	84	44%
Suicidal tougths	25	13%
Psychosis	20	10%
Sleep problems	96	50%
Memory problems	63	33%
Repetitive thoughts	36	19%
Dissociation	60	31%
Personality disorders	46	24%
Substance use	89	47%

Supplementary Table 4. Proteins measured with multiplex assay without differences between groups with/without visuoconstructional deficit.

Protein	Median Normal (pg/ml)	Median Visuoconstructional deficit (pg/ml)	Mann Whitney test *P* value
MIP-1 alpha	1.84	0.69	0.251
SDF-1 alpha	29.71	21.08	0.317
IL-27	115.7	58.70	0.246
IL-1 beta	3.57	2.69	0.111
IL-2	45.61	20.37	0.168
IL-4	21.14	11.16	0.168
IL-5	24.70	15.58	0.074
IL-6	22.08	0	0.218
IL-7	0.65	0.45	0.074
IL-8	3.48	1.92	0.130
PIGF-1	1.56	1.31	0.181
IL-12p70	1.34	1.00	0.555
IL-13	19.31	8.98	0.101
IL-17A	8.17	4.15	0.153
RANTES	19.97	17.30	0.558
IFN gamma	10.21	5.51	0.140
TNF alpha	23.81	12.54	0.251
MIP-1 beta	3.75	2.67	0.088
IFN alpha	0.42	0.19	0.099
IL-9	13.05	6.89	0.089
VEGF-D	3.29	2.96	0.723
TNF beta	25.08	10.96	0.143
EGF	20.58	10.39	0.117
BDNF	0.79	0.90	0.827
GRO alpha (CXCL1)	3.50	2.24	0.081
IL-1 alpha	0.13	0.06	0.245
IL-23	125.90	80.30	0.362
IL-15	27.47	14.88	0.513
IL-18	2.39	0	0.317
IL-21	1.65	1.05	0.263
FGF-2	2.63	1.78	0.193
IL-22	19.26	8.89	0.188
PDGF-BB	31.43	14.86	0.233
VEGF-A	8.41	7.56	0.473

Supplementary References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub; 2013.
2. de Jesus Mari J, Williams P. A Validity Study of a Psychiatric Screening Questionnaire (SRQ-20) in Primary Care in the city of Sao Paulo. Br J Psychiatry. 1986;148:23–26.
3. Galvin JE, Roe CM, Powlishta KK, Coats MA, Muich SJ, Grant E, et al. The AD8: A brief informant interview to detect dementia. Neurology. 2005;65:559–564.
4. Shin M-S, Park S-Y, Park S-R, Seol S-H, Kwon JS. Clinical and empirical applications of the Rey-Osterrieth Complex Figure Test. Nat Protoc. 2006;1:892–899.
5. de Paula JJ, Paiva GC de C, Costa D de S. Use of a modified version of the switching verbal fluency test for the assessment of cognitive flexibility. Dement Neuropsychol. 2015;9:258–264.
6. Bowie CR, Harvey PD. Administration and interpretation of the Trail Making Test. Nat Protoc. 2006;1:2277–2281.
7. Strauss E, Sherman EMS, Spreen O. A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary. Oxford University Press; 2006.
8. Kessels RPC, van den Berg E, Ruis C, Brands AMA. The backward span of the Corsi Block-Tapping Task and its association with the WAIS-III Digit Span. Assessment. 2008;15:426–434.
9. Nitrini R. Immediate recall of short stories depends on educational level. Dement Neuropsychol. 2008;2:310–314.
10. Malloy-Diniz LF, Fuentes D, Mattos P, Abreu N. Avaliação Neuropsicológica - 2.ed. Artmed Editora; 2018.
11. Meltzer CC, Adelson PD, Brenner RP, Crumrine PK, Van Cott A, Schiff DP, et al. Planned ictal FDG PET imaging for localization of extratemporal epileptic foci. Epilepsia. 2000;41:193–200.
12. Rask T, Dyrby T, Comerci M, Alfano B, Quarantelli M, Berkouk K, et al. PVElab: Software for correction of functional images for partial volume errors. Neuroimage. 2004;22.
13. Ashburner J, Friston KJ. Unified segmentation. Neuroimage. 2005;26:839–851.
14. Ashburner J. A fast diffeomorphic image registration algorithm. Neuroimage. 2007;38:95–113.
15. Friston KJ, Worsley KJ, Frackowiak RS, Mazziotta JC, Evans AC. Assessing the significance of focal activations using their spatial extent. Hum Brain Mapp. 1994;1:210–220.
16. Reich M, Liefeld T, Gould J, Lerner J, Tamayo P, Mesirov JP. GenePattern 2.0. Nat Genet. 2006;38:500–501.
17. Critério Brasil - ABEP. https://www.abep.org/criterio-brasil. Accessed 12 January 2022.