Cortical thickness alterations and systemic inflammation define long-COVID patients with cognitive impairment

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ABSTRACT

As the heterogeneity of symptoms is increasingly recognized among long-COVID patients, it appears highly relevant to study potential pathophysiological differences along the different subtypes. Preliminary evidence suggests distinct alterations in brain structure and systemic inflammatory patterns in specific groups of long-COVID patients.

To this end, we analyzed differences in cortical thickness and peripheral immune signature between clinical subgroups based on 3 T-MRI scans and signature inflammatory markers in n = 120 participants comprising healthy never-infected controls (n = 30), healthy COVID-19 survivors (n = 29), and subgroups of long-COVID patients with (n = 26) and without (n = 35) cognitive impairment according to screening with Montreal Cognitive Assessment. Whole-brain comparison of cortical thickness between the 4 groups was conducted by surface-based morphometry.

We identified distinct cortical areas showing a progressive increase in cortical thickness across different groups, starting from healthy individuals who had never been infected with COVID-19, followed by healthy COVID-19 survivors, long-COVID patients without cognitive deficits (MoCA ≥ 26), and finally, long-COVID patients exhibiting significant cognitive deficits (MoCA < 26). These findings highlight the continuum of cortical thickness alterations associated with COVID-19, with more pronounced changes observed in individuals experiencing cognitive impairment (p < 0.05, FWE-corrected). Affected cortical regions covered prefrontal and temporal gyri, insula, posterior cingulate, parahippocampal gyrus, and parietal areas. Additionally, we discovered a distinct immunophenotype, with elevated levels of IL-10, IFNγ, and sTREM2 in long-COVID patients, especially in the group suffering from cognitive impairment.

We demonstrate lingering cortical and immunological alterations in healthy and impaired subgroups of COVID-19 survivors. This implies a complex underlying pathomechanism in long-COVID and emphasizes the necessity to investigate the whole spectrum of post-COVID biology to determine targeted treatment strategies targeting specific sub-groups.

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1. Introduction

While the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is now transformed into an endemic with inferior cases of critical illness (Biancolella et al., 2022), the socio-economic burden of long-lasting post-viral symptoms, also known as long-COVID or post-COVID or Post-Acute COVID-19 Syndrome (PACS), is developing into another major health crisis (Ghanemi et al., 2021). Long-COVID patients are severely affected by a diverse multi-systemic syndrome comprising cognitive deficits, fatigue, post-exertional malaise, and depressed mood as major neuropsychiatric complaints (Peter et al., 2022). Surprisingly, clinical routine diagnostics hardly ever reveal any damage or dysfunction in the peripheral or central nervous system in long-COVID patients after a mild case of Coronavirus disease 2019 (COVID-19) (Fleischer et al., 2022) leading to patients being misdiagnosed and directed to psychosomatic treatments. Case-control neuroimaging studies such as the eminent UK Biobank study (Douaud et al., 2022) demonstrated alterations of cortical thickness in healthy subjects after survival of COVID-19. Additionally, there is accumulating evidence suggesting metabolic, functional and structural alterations of brain physiology of long-COVID patients affecting systems critical for cognition and emotion processing as well (Zawilska and Kuczynska, 2022). Our own group, in line with other pilot studies in long-COVID patients (Lu et al., 2020; Hafiz et al., 2022) demonstrated higher gray matter volume (GMV) in long-COVID patients in a pattern of bilateral fronto-temporal areas, insula, hippocampus, amygdala, basal ganglia, and thalamus compared to healthy controls. (Besteher et al., 2022) Pathophysiological mechanisms might be lingering neuroinflammation (Altmann et al., 2023) with infiltration by immune cells and accumulation of tissue water (Erickson et al., 2023) as well as compensatory neuroplasticity and increase of synaptic connections (Gellrich et al., 2018; Curtis et al., 2007). There is still considerable heterogeneity between the results obtained in brain imaging studies. Part of this might be due to the high diversity of investigated samples regarding main symptoms, recovery time, as well as age and sex profiles of study cohorts. Since, however, not all variance can be attributed to methodological differences and clinical experience clearly suggests the existence of subtypes, this has led scientists around the world to investigate biological subtypes of long-COVID (Pfaff et al., 2023).

Another emerging feature in long-COVID research is the long-lasting persistence of systemic elevated levels of C-reactive protein and immune perturbations (Benedetti et al., 2021; Kappelmann et al., 2021). Several studies intended to characterize the immunopathogenesis related to long-COVID (Altmann et al., 2023; Schultheiss et al., 2022; Etter et al., 2022). Most of these results point toward a long-term disruption of adaptive immunity, and altered systemic immune response. The heterogeneity of reports on immune perturbations might be a consequence of irregular subtyping. Unfortunately, study methodologies are inconsistent with apparent differences regarding co-medication of patients, timepoint of blood sampling and recovery time. Often, these features are not reported in detail. Most studies lack a control group with COVID-19 survivors without any clinical long-COVID symptoms for comparison, although these also represent a subtype of long-COVID biology. Unless the whole spectrum of long-COVID biology and mediators of different clinical courses are understood, targeted treatment remains elusive.

To address this gap in the literature, we aimed to investigate alterations of cortical thickness in particular long-COVID patient groups stratified according to their degree of cognitive impairment and healthy controls with and without previous SARS-CoV-2 infection. Based on our own recent report about higher gray matter volumes (GMV) and evidence of lingering systemic and neuroinflammation, we first hypothesized higher cortical thickness in patients stratified according to their cognitive symptom severity compared to healthy participants in areas relevant to cognition and emotion processing. Secondly, we expected lingering inflammation reflected by signature inflammatory peripheral markers after COVID-19 to be more pronounced in patients with cognitive impairment.

2. Methods

2.1. Participants and assessment

Patients were recruited from the post-COVID outpatient clinic of the Department of Internal Medicine IV (Infectiology) and the Department of Neurology of Jena University Hospital. There they had undergone verification of their post-COVID condition (via real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) at the time of acute infection) and anamnesis covering timepoint and severity of their COVID-19 symptoms according to WHO by a board-certified physician. Inclusion criteria did not define limits regarding specific symptoms or recovery time to be able to cover the whole spectrum of long-COVID symptomatology. None of the participants had a history of DSM-5 disorders, as determined by careful screening via MINI Interview through a trained rater according to DSM-5 (Sheehan et al., 1998). They also had no history of major neurological or unmedicated medical conditions, or of psychiatric history in first-degree relatives. Exclusion criteria for all participants were: exclusion criteria for an MRI scan, neurological conditions and unmedicated internal medical conditions, especially chronic inflammatory conditions, and a history of or current substance abuse disorder. All participants completed the Multiple-Choice Vocabulary Test B (MWT-B), which is available in German (Antretter et al., 2013), to estimate crystallized IQ and confirm the inclusion criterion of IQ higher 80.

All participants gave written informed consent to participate in the study. The study protocol was approved by the local Ethics Committee of Jena University Medical School.

From April 2021 to June 2022, we included 61 long-COVID patients, 29 healthy survivors with a detected COVID-19 without development of long-COVID syndrome and 30 healthy controls without known and serologically evident prior COVID-19. The groups were matched regarding age and sex. We further divided the group of long-COVID patients in two subgroups depending on their result in the neurocognitive screening via Montreal Cognitive Assessment (MoCA) based on established criteria. There were 26 patients with a MoCA score below 26 (mild to moderate cognitive impairment) and 35 patients with a score of 26 or higher (no cognitive impairment) (Malek-Ahmadi et al., 2014; Nasreddine et al., 2005). Patient subgroups showed no significant differences regarding age and sex. A summary of demographic characteristics of groups is given in Table 1.

2.2. Magnetic resonance imaging (MRI)

Subjects underwent high–resolution T1-weighted MRI on a 3 Tesla Siemens Prisma fit scanner (Siemens, Erlangen, Germany) using a standard quadrature head coil and an axial 3-dimensional magnetization

Table 1
Demographic data. M – Mean, SD – standard deviation, BMI – body mass index.

| | Long-COVID Group | | | |
| | healthy controls | survivors | patients with MoCA $\geq$ 26 | patients with MoCA < 26 |
	M (SD)	M (SD)	M (SD)	M (SD)
Age (Years)	42.0 (11)	41.9 (13)	42.0 (11)	49.1 (14)
Education (Years)	11.0 (1)	11.6 (1)	11.2 (1)	11.1 (1)
BMI (kg/m²)	25.8 (4)	24.3 (4)	25.8 (4)	26.5 (5)
Time since infection (months)	–	8 (5.8)	9.7 (3.8)	10.3 (4.5)
	N	N	N	N
Total	30	29	35	26
Female	13	17	23	14
Male	17	12	12	12

prepared rapid gradient echo (MP-RAGE) sequence (TR 2400 ms, TE 2.22 ms, α 8°, 208 contiguous sagittal slices, FoV 256 mm, voxel resolution 0.8 × 0.8 × 0.8 mm; acquisition time 6:38 min). All subjects gave their consent for the native brain MRI scan after consultation of a licensed radiologist. The scan was part of an MRI protocol of 60 min total duration. All scans were checked to exclude imaging artefacts.

2.3. Surface-based morphometry

To investigate cortical grey matter, we performed surface-based morphometry (SBM) with the CAT 12 toolbox (Computational Anatomy Toolbox 12) of the Structural Brain Mapping group, Jena University Hospital, Jena, Germany, which is implemented in SPM12 (Statistical Parametric Mapping, Institute of Neurology, London, UK). All T1-weighted images were corrected for bias – field inhomogeneities, then boundaries between grey matter, white matter and cerebrospinal fluid were defined and spatially normalized using the DARTEL algorithm (Ashburner, 2007). The segmentation process was further extended by accounting for partial volume effects (Tohka et al., 2004), applying adaptive maximum a posteriori estimations (Rajapakse et al., 1997). After pre-processing and in addition to visual checks for artefacts all scans passed an automated quality check protocol. Scans were smoothed with a Gaussian kernel of 12 mm (FWHM). Subcortical grey matter and white matter alterations were not analysed.

2.4. Blood collection and serum levels of inflammatory markers

For the assessment of inflammatory parameters, a standardized blood extraction was performed on all participants within a designated time frame between 7:30–9:00 a.m. at the day of MRI scanning. 7.5 mL serum gel monovettes (Sarstedt) were used and 30 min after extraction, centrifugation was carried out at 1000 g/rct for 20 min. The samples were divided into aliquots of 0.25 mL each and frozen at $-80°$ C. Frozen samples were then transferred to our cooperating institute in Magdeburg, where serum was thawed at $4°$ C followed by a centrifugation step at 400 g for 10 min to remove any debris. The quantification of multiple key inflammatory mediators including IL-10, IFN-γ, IL-6, TNF-α, CXCL10, sTREM2, sTREM-1, CCL2 (MCP-1), IL-18, BDNF, VEGF, β-NGF, sRAGE, CX3CL1, α-synuclein, G-CSF, IFNα2, IL-2, IL-7, IL-1RA and CXCL8 was performed using the LEGENDplex^{TM} multiplex bead-based assay (BioLegend, 741091, 740795, 741197) following manufacturer's instructions as previously described (Garza et al., 2023).

2.5. Statistics

2.5.1. Imaging and cognitive symptoms

Statistical analyses of SBM were carried using the general linear model (GLM) approach implemented in SPM12. We performed an ANOVA including the four groups to test for a group effect on cortical thickness. Age and sex were used as nuisance variables (in order to remove variance related to these potentially confounding variables). We performed a whole-brain analysis investigating both positive and negative effects of group. Post-hoc we performed pairwise 2-sample t-tests in SPM to test for differences of cortical thickness between two specific groups. Age, sex and time since infection were used as nuisance variables for all tests. As a non-parametrical statistic, we applied threshold-free cluster enhancement (TFCE) with 5000 permutations (Spisak et al., 2019) to all analyses and corrected for multiple comparisons via family wise error method (FWE) at p < 0.05. Anatomical labelling of clusters was performed using Desikan-Killiany DK40 Atlas (Desikan et al., 2006). To explore the relationship of clinical and biological features with the differences in cortical thickness we extracted adjusted raw values of maximum thickness (vertex of strongest effect size) of the significant clusters to R. In a next step, we assessed the relationship of MoCA scores with group and cortical thickness (averaged across clusters). Note that for this analysis, all long-COVID patients (PC

≥ 26 and PC < 26) were summarized into one group. We conducted a multiple linear regression with the syntax: MoCA ~ cortical thickness * cohort in R, version 4.2.2 (2022–10-31). Data visualizations were created using ggplot, version 3.4.2.

2.5.2. Immunological markers

Statistical analysis and data visualization of serum cytokines were conducted using GraphPad Prism 9. Assessment of data distribution revealed non-normality as shown by D'Agostino-Pearson and Shapiro-Wilk tests. Therefore, group differences were evaluated using Kruskal-Wallis test followed by Dunn's multiple comparisons test. An alpha value of $p < 0.05$ was used for all statistical tests. Thus, p values ≤ 0.05 were considered statistically significant and marked with asterisks as follows: * for $p \leq 0.05$; ** for $p \leq 0.001$; *** for $p \leq 0.0001$. Data are presented as individual values and bar charts display the mean with its corresponding standard error of the mean (SEM). To control for effects of age and sex we performed a linear regression analysis.

3. Results

3.1. Group differences of cortical thickness

We found several significant clusters ($p < 0.05$, FWE-corrected) with increasing cortical thickness moving from entirely healthy, never infected controls to healthy COVID-19 survivors to long-COVID patients with normal MoCA scores (MoCA ≥ 26) to long-COVID patients with relevant cognitive deficits (MoCA < 26). Clusters of significantly higher cortical thickness were covering prefrontal and temporal gyri, insula, posterior cingulate and parahippocampal gyrus as well as superior parietal areas (Fig. 1, Table 2). Values of cortical thickness at the peak vertex were subsequently extracted for the 4 clusters of relevant size of $k_E > 100$ for further analyses.

### 3.2. Results of group differences in pair-wise t-tests

All participant groups having been previously infected with SARS-CoV-2 had significantly increased cortical thickness compared to healthy, never infected controls. Affected cortical areas were small and only right-hemispheric in healthy survivors, some more extensive in long-COVID patients without cognitive impairment spanning right temporo-parietal areas and most pronounced in long-COVID patients with cognitive impairment spanning left and right prefronto-temporal areas. Moreover, the long-COVID patient groups showed significant cortical thickness alterations compared to the healthy survivors in small occipito-parietal clusters (Fig. 2, Table 3).

3.3. Relationship between MoCA, cohort and cortical thickness

This regression model revealed only an effect of group ($F(2) = 8.52$, $p < 0.001$), but neither of cortical thickness ($F(1) = 2.12$, $p = 0.149$), nor their interaction ($F(2) = 0.15$, $p = 0.857$). Pairwise post-hoc tests revealed significant differences between MoCA scores of the long-COVID and the two healthy control groups (HC_N vs. long-COVID: t(69.7) = 4.53, $p < 0.001$; HC_S vs. long-COVID: t(54.1) = 2.61, $p = 0.012$), while the two HC groups did not differ (HC_N vs. HC_S: t(52.8) = 1.23, $p = 0.199$). Age, sex and time since infection were used as nuisance variables. Mean MoCA scores were lower in the long-COVID group (M = 25.9) compared to HC_S (M = 27.2) and HC_N (M = 28.0). Thus, while the long-COVID group showed lower cognitive performance than the healthy groups, this was not associated with cortical thickness values.

3.4. Peripheral cytokines

Our results revealed heterogeneity in the cytokines levels within the selected cohort. Table 4 provides a complete overview of the median concentrations and interquartile range (IQR) of the cytokines measured.
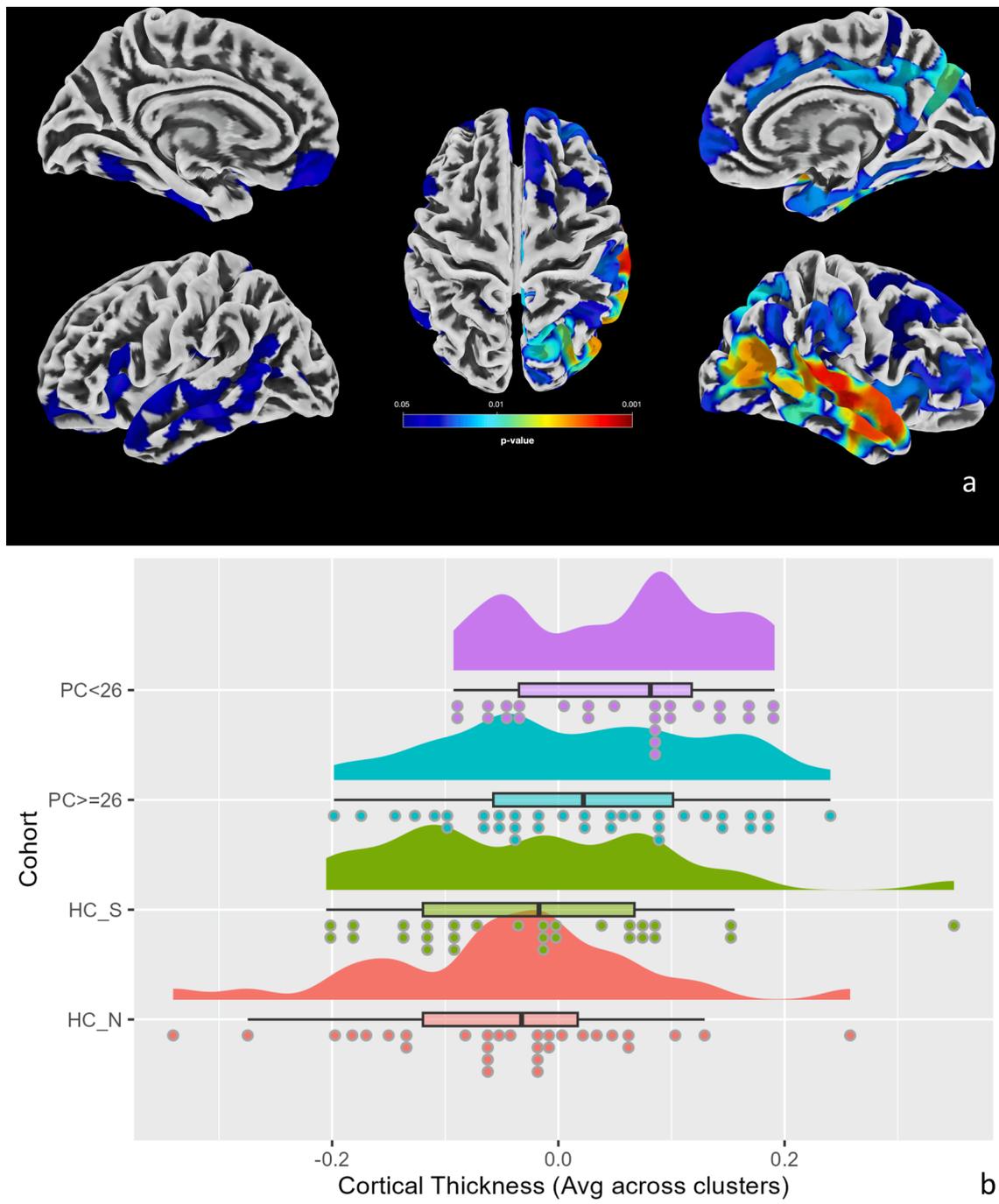
Fig. 1. a) Results of comparison of cortical thickness ($p < 0.05$, FWE-corrected) across groups presented as surface overlays. Marked clusters show areas of cortical thickness significantly increasing across groups moving from healthy to long-COVID patients with cognitive impairment: healthy controls (HC) < survivors (HC_S) < long-COVID patients with MoCA ≥ 26 (PC ≥ 26) < long-COVID patients with MoCA < 26 (PC < 26). Color bar represents *p*-value. b) Rain Cloud Plot depicting the distribution of cortical thickness values across groups, values extracted from the peak vertex per cluster and averaged across the four significant clusters.

Fig. 3 illustrates the observed group comparison for each cytokine. Contrary to our initial hypothesis and previously published data on post-COVID patients, we did not observe elevated levels of canonical pro-inflammatory cytokines, such as IL-6 and TNFα. However, the analysis revealed a significant increase in serum levels of IFNγ in long-COVID patients with cognitive impairment according to MoCA when compared to the long-COVID group with normal MoCA scores ($p = 0.0150$). Additionally, we observed elevated levels of IL-10 in long-COVID patients with cognitive impairment against the non-infected controls ($p = 0.0143$). Furthermore, across all SARS-CoV-2 infected groups, there were notably increased levels of sTREM2, which were

significantly different in the long-COVID patient group with cognitive impairment in comparison with healthy controls ($p = 0.0248$).

The linear regression on age and sex revealed no correlations with sex and cytokine levels. Regarding age, there was a significant role of age in sTREM2 across all participants (suppl. Fig. 1).

4. Discussion

4.1. Cortical thickness alterations

We identified bilateral cortical thickness alterations in several areas

Table 2

Clusters of significantly increasing thickness across all groups (healthy controls < survivors < long-COVID patients with MoCA ≥ 26 < post-COVID patients with MoCA < 26). TFCE – threshold-free cluster enhancement.

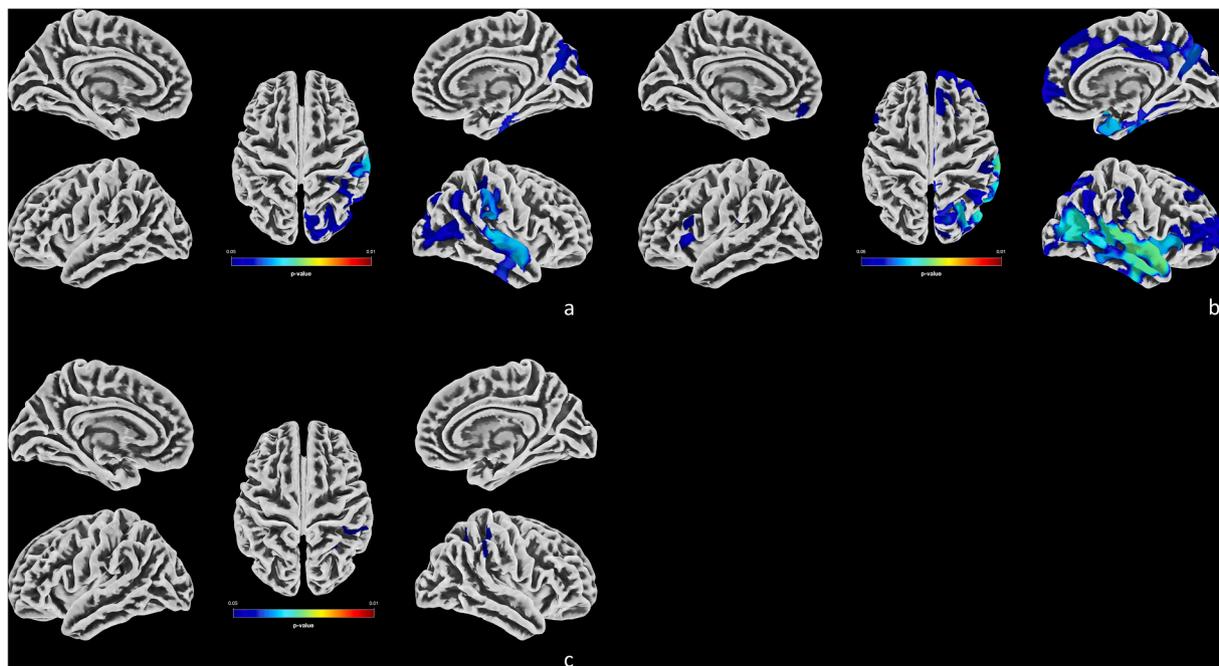| contrast	hemisphere	cluster size	overlap region	p-value	TFCE	peak cluster level		
						mm	mm	mm
healthy controls < survivors < post-COVID patients with MoCA ≥ 26 < post-COVID patients with MoCA < 26	left	1800	superiortemporal middletemporal inferiorparietal inferiortemporal supramarginal transversetemporal insula	0.026	1776.61	−66	−27	−12
		1081	insula parsopercularis lateralorbitofrontal parstriangularis supramarginal postcentral precentral parsorbitalis	0.027	1745.79	−31	22	4
		551	fusiform inferiortemporal lingual	0.034	1393.97	−48	−25	−30
		434	medialorbitofrontal rostralmiddlefrontal lateralorbitofrontal frontalpole	0.030	1578.22	−4	48	−18
	right	13,478	inferiorparietal superiortemporal superiorfrontal supramarginal superiorparietal middletemporal precuneus rostralmiddlefrontal lateraloccipital insula inferiortemporal postcentral isthmuscingulate caudalmiddlefrontal posteriorcingulate parsopercularis cuneus parahippocampal entorhinal	0.002	7569.17	52	−18	−7

relevant for cognition and emotion processing like the prefrontal cortex, temporal gyri and insula as well as the posterior cingulate and parahippocampal gyrus with higher cortical thickness moving from healthy, never infected controls with lowest cortical thickness to healthy COVID-19 survivors to patients without and finally patients with cognitive impairment according to MoCA.

The prefrontal cortex is established as the pacemaker of cognitive control and executive function (Friedman and Robbins, 2022) and (Jobson et al., 2021; Firouzabadi et al., 2022; Pizzagalli and Roberts, 2022; Smucny et al., 2022; McWhirter et al., 2023) thus lending support to the notion that prefrontal alterations might underlie cognitive deficits in long-covid patients. The temporal lobe is involved in various cognitive functions such as long-term memory and modality-general to modality-specific processing of external and internal stimuli (Jackson et al., 2018). Insula, posterior cingulate and parahippocampal gyrus belong to different functional networks within the limbic system and are involved in emotion processing, which is also impaired in many long-COVID patients (Kubota et al., 2023).

Our results are in line with several previously published structural imaging studies, though imaging literature in long-COVID patients is heterogenous regarding direction of gray matter alterations. Most studies show a widespread fronto-temporo-parietal pattern of brain structural alterations in long-COVID strengthening the well documented fact that SARS-CoV-2 overcomes the blood–brain-barrier and might induce neuroinflammation. Erickson et al. recently showed that SARS-CoV-2 crosses the blood–brain-barrier by adsorptive transcytosis using glycoproteins and therefore affect the whole brain and not only certain areas connected to specific entryways (Erickson et al., 2023; Banks and Erickson, 2022).

Some pilot studies have documented an increase in GMV or cortical thickness in long-COVID patients compared to healthy controls (Lu et al., 2020; Hafiz et al., 2022; Besteher et al., 2022). Both are measures of brain gray matter characterizing either volume as a combination of tissue composition, effects of perfusion, thickness and gyrification, or thickness reflecting the distance between outer and inner border of the cortex at any given vertex. These measures therefore reflect different biological features, but are in general highly intercorrelated (Storsve et al., 2014), which is why both will be considered for the discussion. Lu et al. were the first to report higher bilateral GMV in olfactory cortices, hippocampi, insulas, left Rolandic operculum, left Heschl's gyrus and right cingulate gyrus after 3 months of recovery from COVID-19. These were found to be correlated with self-reported symptoms of memory loss (Lu et al., 2020). Also, Tu et al. reported increased GMV and functional activity in bilateral hippocampus and amygdala in COVID-19 survivors 3–6 months after hospital discharge compared to controls (Tu et al., 2021). Our own group reported increased GMV in bi-hemispheric fronto-temporal areas, insula, hippocampus, amygdala, basal ganglia, and thalamus in ambulatory patients reporting to a specialized post-

Fig. 2. Results of pair-wise cortical thickness comparisons ($p < 0.05$, FWE-corrected) between groups presented as surface overlays in a) healthy controls < long-COVID patients with MoCA $\geq$ 26, b) healthy controls < long-COVID patients with MoCA < 26, c) survivors < long -COVID patients with MoCA < 26.

Table 3
Clusters of pairwise differences in cortical thickness between groups. TFCE – threshold-free cluster enhancement.

contrast	hemisphere	cluster size	overlap region	p-value	TFCE	peak cluster level		
						mm	mm	mm
healthy controls < survivors	right	176	precuneus	0.047	1821.22	22	−66	20
healthy controls < long-COVID patients with MoCA $\geq$ 26	right	56	cuneus insula	0.050	1396.46	36	11	1
		2630	superiortemporal supramarginal middletemporal inferiortemporal fusiform parahippocampal postcentral transversetemporal	0.027	4062.99	54	−22	6
		1585	lateraloccipital superiorparietal	0.038	2232.26	46	−68	8
		473	inferiorparietal supramarginal	0.041	2266.54	49	−61	37
healthy controls < long-COVID patients with MoCA $\geq$ 26	left	9523	insula parsopercularis parstriangularis lateralorbitofrontal postcentral precentral supramarginal	0.023	6290.88	52	−18	−7
		38	medialorbitofrontal	0.048	1477.21	−6	48	−15
	right	9523	inferiorparietal superiortemporal	0.023	6290.88	52	−18	−7
survivors < long-COVID patients with MoCA $\geq$ 26	right	278	supramarginal postcentral	0.048	4029.66	45	−40	39
		92	superiorparietal inferiorparietal	0.049	3963.79	28	−55	54

COVID clinic after an average of 8 months after acute COVID-19 (Besteher et al., 2022). A small case-series following 4 patients up to 12 months after recovery found increased global cerebral GMV to be associated with the degree of pulmonary function damage hypothesizing gray matter to be prone to brain edema caused by hypoxia (Chen et al., 2022). These data of previous studies support our observation of long-term increased GMV and/or cortical thickness to be associated with

ongoing symptoms. This might reflect a sustained subtle disturbance of brain tissue homeostasis similarly to the acute phase. Of note, Hafiz et al. identified higher fatigue levels and higher GMV within the limbic system and basal ganglia in COVID-19 survivors compared to healthy controls already 2 weeks after hospital discharge (Hafiz et al., 2022). It should, however be acknowledged that there are also studies reporting a decrease in GMV or cortical thickness in post-COVID patients or healthy

Table 4
Circulating cytokine levels in healthy controls, survivors, long-COVID and long-COVID with cognitive impairment cohorts. Values are presented in pg / mL, showing median and inter-quartile range (IQR) per analyte within each group. Statistical evaluation was performed using Kruskal-Wallis test.

	healthy controls	survivors	Long-COVID MoCA ≥ 26	MoCA < 26	
n	*30*	*29*	*35*	*26*	ANOVA
	pg/mL (m. IQR)				*p-value*
IL-10	5.71 (4.7)	8.08 (8.0)	7.69 (7.7)	11.18 (10.47)	*0.0103*
IFN-γ	17.71 (43.7)	25.54 (38.49)	17.28 (21.25)	37.7 (35.49)	*0.0146*
IL-6	3.062 (2.32)	5.128 (3.40)	3.259 (4.81)	4.731 (3.85)	*0.1563*
TNFα	21.6 (28.29)	30.61 (35.32)	26.59 (33.14)	41.36 (31.22)	*0.1655*
CXCL10	58.21 (43.52)	62.43 (67.87)	60.79 (56.43)	91.23 (67.54)	*0.1331*
TGF-β1	87.05 (53.79)	86.74 (71.33)	97.19 (52.14)	96.4 (62.79)	*0.8418*
sTREM2	309.6 (89.2)	363.1 (247.3)	358.6 (229.1)	445.3 (318.8)	*0.0051*
sTREM1	96.77 (68.72)	82.12 (101.54)	107 (95.78)	91.63 (97.29)	*0.6760*
MCP-1	63.61 (27.82)	61.7 (29.23)	55.54 (23.29)	50.43 (22.42)	*0.7233*
IL-18	139.5 (104.05)	135 (78.5)	141.9 (60.8)	137.7 (73.18)	*0.7694*
BDNF	1993 (2054)	2014 (1594)	2645 (2027)	2589 (1957)	*0.4681*
VEGF	69.4 (28.21)	71.32 (57.89)	80.43 (43.93)	69.96 (38.05))	*0.3822*
β-NGF	2.62 (4.28)	4.95 (4.87)	3.89 (6.27)	4.67 (6.69)	*0.2367*
sRAGE	33.37 (291.23)	33.37 (375.23)	33.37 (148.33)	33.37 (126.63)	*0.6061*
CX3CL1	374.5 (390.2)	419.3 (709.6)	533.7 (604.6)	504.4 (513.9)	*0.2326*
α-synuclein	28,185 (11288)	27,814 (7576)	25,388 (10726)	25,090 (16012)	*0.6312*
G-CSF	90.92 (53.13)	103.5 (67.48)	98.46 (45.13)	121.6 (67.65)	*0.1322*
IFN-α2	4.63 (7.57)	6.67 (6.82)	6.26 (5.61)	7.01 (3.84)	*0.4749*
IL-2	6.60 (3.49)	6.40 (1.82)	6.85 (2.45)	7.84 (3.23)	*0.2986*
IL-7	23.73 (16.13)	23.94 (14.02)	20.94 (10.46)	25.87 (12.79)	*0.5325*
IL-1RA	64.4 (93.03)	74.38 (74.34)	65.75 (55.33)	91.89 (58.41)	*0.1052*
CXCL8	17.63 (21.51)	14.95 (8.13)	14.39 (9.60)	22.35 (12.25)	*0.0784*

MoCA: Montreal Cognitive Assessment.
HC_N: healthy controls without prior SARS-CoV-2 infection
HC_S: healthy COVID-19 survivors.
PC: Long-COVID patients.
PC ≥ 26: Long-COVID patients with a result equal or higher than 26 points in the MoCA.
PC < 26: Long-COVID patients scoring less than 26 points in the MoCA.

COVID-19 survivors compared to non-infected controls. The most eminent study is the longitudinal thickness analysis in the UK Biobank sample, reporting cortical thinning in survivors of COVID-19. Since cortical thickness is highly dependent on age, it is noteworthy that the sample was 10 years older on average than our cohort and that cortical alterations were not connected to clinical symptom severity. Therefore, the study has established an intriguing link between survival of systemic viral infections and premature brain aging, but no insight into brain structural correlates in symptomatic long-COVID patients (Douaud et al., 2022). Another contributor to the differences in results on brain structural alterations in long-COVID patients is the severity of acute COVID-19 in the patients assessed in the different studies. In some

studies neurocognitive symptoms and disability connected to regional cortical thinning is pronounced in patients hospitalized during acute COVID-19, especially in those requiring oxygen supplementation (Duan et al., 2021; Sanabria-Diaz et al., 2022). Our cohort consisted of patients with mild ambulatory acute disease, similarly to the vast majority of symptomatic long COVID patients. The differences in results obtained from patients with different acute COVID-19 severity point towards different biological pathways to long-COVID symptomatology.

Another potential underlying mechanism might be compensation for loss or dysfunction of cellular components caused by the acute infection. This might result in a long-lasting process of shaping new interneural connections and recruiting stem cells (Curtis et al., 2007; Karstensen et al., 2018). Since our sample of long-COVID patients consisted mainly of those with a mild acute phase, major tissue damage is not very likely. Another more suitable explanation for our results might be lingering systemic and neuroinflammation leading to tissue swelling and migration of immune cells, which might then be detected as an increase in cortical thickness. Although there is a lack of brain imaging studies in comparable systemic infectious conditions to support this hypothesis, there is some evidence of regional cortical thickness increase in amygdala and hippocampus in limbic encephalitis (Harms et al., 2023), bipolar disorder (Poletti et al., 2019) and recovery in traumatic brain injury (Dall'Acqua et al., 2017), that might be connected to acute or subacute neuroinflammatory processes. To elucidate this mechanism, we investigated markers of inflammation in our sample.

4.2. Immunological alterations

Recent reports indicate an increase of canonical pro-inflammatory cytokines such as IL-6 and TNFα, accompanied by elevated levels of pleiotropic IL-10, described as a distinguishing factor of hyperinflammation during severe SARS-CoV-2 infection (Carlini et al., 2023). Following the symptomatic resolution of the acute phase, the inflammatory changes have been described to return to normal levels in most survivors with mild-to-moderate disease. Nonetheless, a persistent immunological dysfunction after the infection remains prevalent in a subgroup of patients, which is associated with certain neurological or psychiatric symptoms leading to a long-COVID diagnosis (Ceban et al., 2022).

Previous studies have detected diverse results on the serum concentration of TNFα, IL-1b, and IL-6 during ongoing long-COVID (Yin et al., 2023). These cytokines can be secreted by the peripheral immune system, as well as in the CNS by microglia, astrocytes, and infiltrating monocytes (Gabay, 2006; Kummer et al., 2021; Riazi et al., 2008). In our cohort, the expressions of IL-6 and TNFα in long-COVID patients were not significantly altered. This might be due to the timepoint of the assessment, which was on average at 9.8 months after infection with a wide range from 1 to 25 months, whereas in the work of Schultheiß et al. the assessment was performed at maximum not later than 17 months after the infection. Also, the applied treatment strategies are not in all studies disclosed, which might cause the variations (Schultheiss et al., 2022).

Our results highlight that long-COVID patients with cognitive impairment exhibit increased IFNγ levels, a pro-inflammatory cytokine known for its crucial role in protection against infection. IFNγ is mainly produced by activated T cells, natural killer cells, as well as by innate lymphoid cells and plays a critical role in mediating host defense against various pathogens. However, excessive production of IFNγ can lead to neuroinflammation and neuronal damage (French et al., 2021; Haruwaka et al., 2019; Steffen et al., 2022). These changes can create a proinflammatory environment that primes microglia to be reactive and aid in the inflammation in a positive loop (Melief et al., 2012). A recent proteomics study even described a subgroup of long-COVID patients with upregulated IFNγ signaling and NF-κB signaling as well as neutrophil activation driving persistent inflammation as a relevant diagnostic and therapeutic feature (Talla et al., 2023).
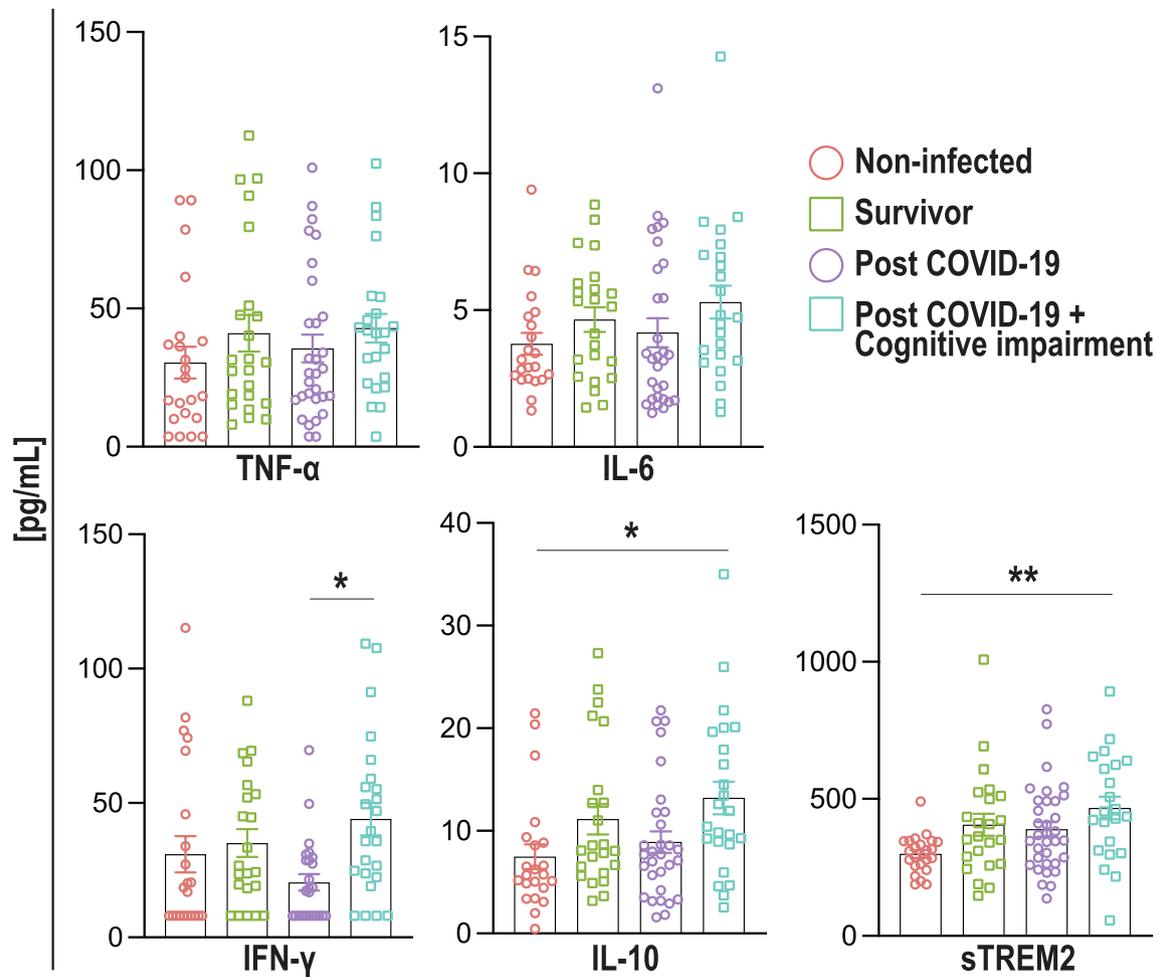
Fig. 3. Circulating cytokine changes in non-infected healthy controls, survivors, post-COVID19, and Post-COVID19 with cognitive impairment groups. Levels of TNF-α, IL-6, IFNγ, IL-10 and sTREM2 detected in serum are shown as individual values in pg / mL. Bar charts display the mean with standard error of the mean (SEM). Statistical evaluation was performed using Kruskal-Wallis test followed by Dunn's multiple comparisons test. P values: * for $p \leq 0.05$; ** for $p \leq 0.001$; *** for $p \leq 0.0001$.

Interestingly, a significant increase in the serum levels of IL-10 was also detected in the group of long-COVID patients with cognitive impairments. The dysregulation of IL-10 in long-COVID may contribute to a sustained inflammatory state, perpetuating tissue damage and symptom severity. Both findings are consistent with our imaging results, which displayed increased cortical thickness in long-COVID patients with cognitive impairment compared to the long-COVID group without cognitive impairment, demonstrating a link between the observed peripheral inflammation and cortical thickness modifications.

In addition, we assessed the levels of sTREM2 (soluble triggering receptor expressed on myeloid cells 2), a soluble form of the TREM2 receptor primarily expressed on microglia. TREM2 plays a crucial role in modulating the inflammatory response, and its involvement in neurodegenerative disorders and neuroinflammatory conditions has been suggested by several studies, indicating its significance in CNS pathology (Colonna, 2023; Deming et al., 2018; Guerreiro et al., 2013; Jonsson et al., 2013). Of note, it is imperative to acknowledge that our findings revealed levels of peripheral sTREM2 only due to constraints inherent to our study, namely, the unattainability of CSF or microglia directly. However, recent work by Popovacki et al, show a positive correlation between CSF and plasma sTREM2 levels in patients with MCI and AD (Spanic Popovacki et al., 2023). Therefore, we speculate about the potential CNS relevance of these peripheral findings. Our results revealed increased sTREM2 levels in the serum of long-COVID patients with cognitive impairment when compared to healthy controls. This finding

suggests a corresponding shift in microglial reactivity in response to neuronal degeneration, which aligns with previous studies demonstrating an association between sTREM2 levels and neuronal injury markers in early-stage Alzheimer's Disease (AD) (Liu et al., 2018; Suarez-Calvet et al., 2016; Zhao et al., 2022). Therefore, the upregulation of sTREM2 detected in the long-COVID patient group with cognitive impairment may represent a similar pattern of microglial reactivity and neuronal degeneration with the involvement of neuroinflammatory mechanisms in this specific population. Further investigation is imperative to validate these observations and ascertain the specificity of our peripheral findings in the context of microglial alterations in TREM2 expression during long-COVID.

4.3. Limitations and conclusions

Although we were careful with the selection and characterization of our sample, some limitations must be addressed accordingly. It has to be noted that our sample size is still too small to reflect the whole spectrum of biological consequences of surviving COVID-19. We therefore focused on cognitive symptom severity to stratify the patient group. The broad spectrum of long-COVID symptoms and considerable interindividual variation of symptom severity might have caused us to be unable to find a significant association of MoCA values with cortical thickness or cytokine levels.

We corrected for age and sex to address the confounders most

influential on brain structure. Sex was also not significantly correlated with cytokine levels or sTREM2. In line with previous studies, age was correlated with sTREM2 in the overall sample (Henjum et al., 2016). As the long-COVID group with cognitive impairment was not significantly older than the other groups, we argue that the sTREM2 upregulation appears to be an effect of long-COVID, because increased thickness in this subgroup is rather an effect of inflammation or compensation than of aging. Cortical atrophy and volume decrease are brain markers for aging (Gaser et al., 2013) and especially in mild cognitive impairment or dementia, a specific and very different pattern is affected (Ries et al., 2008).

Although we ensured the absence of SARS-CoV-2 contact in our healthy controls via serology and thorough anamnesis, a residual risk of being infected without symptoms and losing antibodies over time remained. For case-control studies in long-COVID, it is therefore highly preferable to use legacy data from pre-pandemic controls.

Reviewing the imaging literature and our results from this study, it seems to be important to consider the clinical symptom severity as well as age, sex and stage of recovery when investigating brain structural long-term effects of long-COVID. Primarily cognitive performance appears to get worse in patients after being stressed, which is generally not established in long-COVID research yet. Although we standardized the time and processing of blood samples and the time of the following MRI, we did not include the condition of previous stress on the participants at the day of investigation.

Our findings emphasize the need to investigate the whole spectrum of post-COVID biology and determine substantial clinical sub-groups considering more aspects such as age, sex, initial COVID-19 severity, treatment, and recovery period. Furthermore, the observation that post-viral effects are detectable in clinically healthy cohorts might point to the possibility of unknown long-term effects especially in light of recent evidence on the association between viral infections and inreased long-term risk for dementia (Levine et al., 2023). Insights from our findings might thus inform future studies, that investigate immunomodulation as a treatment for specific subgroups suffering from post-infectious sequelae.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2023.11.028.

References

Altmann, D.M., Whettlock, E.M., Liu, S., Arachchillage, D.J., Boyton, R.J., 2023. The immunology of long COVID. Nat. Rev. Immunol.

Antretter, E., Dunkel, D., Haring, C., 2013. The assessment of cognitive abilities in psychiatric patients: are widely used psychological tests still up-to-date? Psychiatr. Prax. 40 (3), 120–129.

Ashburner, J., 2007. A fast diffeomorphic image registration algorithm. Neuroimage 38 (1), 95–113.

Banks, W.A., Erickson, M.A., 2022. The next chapter for COVID-19: A respiratory virus inflames the brain. Brain Behav. Immun. 101, 286–287.

Benedetti, F., Palladini, M., Paolini, M., et al., 2021. Brain correlates of depression, post-traumatic distress, and inflammatory biomarkers in COVID-19 survivors: A multimodal magnetic resonance imaging study. Brain Behav. Immun. Health, 100387.

Besteher, B., Machnik, M., Troll, M., et al., 2022. Larger gray matter volumes in neuropsychiatric long-COVID syndrome. Psychiatry Res. 317, 114836.

Biancolella, M., Colona, V.L., Mehrian-Shai, R., et al., 2022. COVID-19 2022 update: transition of the pandemic to the endemic phase. Hum. Genomics 16 (1), 19.

Carlini, V., Noonan, D.M., Abdalalem, E., et al., 2023. The multifaceted nature of IL-10: regulation, role in immunological homeostasis and its relevance to cancer, COVID-19 and post-COVID conditions. Front. Immunol. 14, 1161067.

Ceban, F., Ling, S., Lui, L.M.W., et al., 2022. Fatigue and cognitive impairment in Post-COVID-19 Syndrome: A systematic review and meta-analysis. Brain Behav. Immun. 101, 93–135.

Chen, S., Lan, Y., Li, H., et al., 2022. Relationship between Lung and Brain Injury in COVID-19 Patients: A Hyperpolarized (129)Xe-MRI-based 8-Month Follow-Up. Biomedicines. 10 (4).

Colonna, M., 2023. The biology of TREM receptors. Nat. Rev. Immunol. 1–15.

Curtis, M.A., Kam, M., Nannmark, U., et al., 2007. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 315 (5816), 1243–1249.

Dall'Acqua, P., Johannes, S., Mica, L., et al., 2017. Prefrontal Cortical Thickening after Mild Traumatic Brain Injury: A One-Year Magnetic Resonance Imaging Study. J. Neurotrauma 34 (23), 3270–3279.

Deming, Y., Li, Z., Benitez, B.A., Cruchaga, C., 2018. Triggering receptor expressed on myeloid cells 2 (TREM2): a potential therapeutic target for Alzheimer disease? Expert Opin. Ther. Targets 22 (7), 587–598.

Desikan, R.S., Segonne, F., Fischl, B., et al., 2006. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 31 (3), 968–980.

Douaud, G., Lee, S., Alfaro-Almagro, F., et al., 2022. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. Nature.

Duan, K., Premi, E., Pilotto, A., et al., 2021. Alterations of frontal-temporal gray matter volume associate with clinical measures of older adults with COVID-19. Neurobiol. Stress 14, 100326.

Erickson, M.A., Logsdon, A.F., Rhea, E.M., et al., 2023. Blood-brain barrier penetration of non-replicating SARS-CoV-2 and S1 variants of concern induce neuroinflammation which is accentuated in a mouse model of Alzheimer's disease. Brain Behav. Immun. 109, 251–268.

Etter, M.M., Martins, T.A., Kulsvehagen, L., et al., 2022. Severe Neuro-COVID is associated with peripheral immune signatures, autoimmunity and neurodegeneration: a prospective cross-sectional study. Nat. Commun. 13 (1), 6777.

Firouzabadi, F.D., Ramezanpour, S., Firouzabadi, M.D., Yousem, I.J., Puts, N.A.J., Yousem, D.M., 2022. Neuroimaging in Attention-Deficit/Hyperactivity Disorder: Recent Advances. AJR Am. J. Roentgenol. 218 (2), 321–332.

Fleischer, M., Szepanowski, F., Tovar, M., et al., 2022. Post-COVID-19 Syndrome is Rarely Associated with Damage of the Nervous System: Findings from a Prospective Observational Cohort Study in 171 Patients. Neurol. Ther. 11 (4), 1637–1657.

French, T., Israel, N., Dusedau, H.P., et al., 2021. The Immunoproteasome Subunits LMP2, LMP7 and MECL-1 Are Crucial Along the Induction of Cerebral Toxoplasmosis. Front. Immunol. 12, 619465.

Friedman, N.P., Robbins, T.W., 2022. The role of prefrontal cortex in cognitive control and executive function. Neuropsychopharmacology 47 (1), 72–89.

Gabay, C., 2006. Interleukin-6 and chronic inflammation. Arthritis Res. Ther. 8 Suppl 2 (Suppl 2):S3.

Gaser C, Franke K, Kloppel S, Koutsouleris N, Sauer H, Alzheimer's Disease Neuroimaging I. BrainAGE in Mild Cognitive Impaired Patients: Predicting the Conversion to Alzheimer's Disease. *PLoS One.* 2013;8(6):e67346.

Gellrich, J., Han, P., Manesse, C., et al., 2018. Brain volume changes in hyposmic patients before and after olfactory training. Laryngoscope 128 (7), 1531–1536.

Ghanemi, A., Yoshioka, M., St-Amand, J., 2021. Post-Coronavirus-Disease-2019 (COVID-19): Toward a Severe Multi-Level Health Crisis? Med. Sci. (basel) 9 (4).

Guerreiro, R., Wojtas, A., Bras, J., et al., 2013. TREM2 variants in Alzheimer's disease. N. Engl. J. Med. 368 (2), 117–127.

Hafiz, R., Gandhi, T.K., Mishra, S., et al., 2022. Higher Limbic and Basal Ganglia volumes in surviving COVID-negative patients and the relations to fatigue. Neuroimage Rep. 2 (2), 100095.

Harms, A., Bauer, T., Witt, J.A., et al., 2023. Mesiotemporal Volumetry, Cortical Thickness, and Neuropsychological Deficits in the Long-term Course of Limbic Encephalitis. Neurol Neuroimmunol Neuroinflamm. 10 (4).

Haruwaka, K., Ikegami, A., Tachibana, Y., et al., 2019. Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. Nat. Commun. 10 (1), 5816.

Henjum, K., Almdahl, I.S., Arskog, V., et al., 2016. Cerebrospinal fluid soluble TREM2 in aging and Alzheimer's disease. Alzheimers Res. Ther. 8 (1), 17.

Jackson, R.L., Bajada, C.J., Rice, G.E., Cloutman, L.L., Lambon Ralph, M.A., 2018. An emergent functional parcellation of the temporal cortex. Neuroimage 170, 385–399.

Jobson, D.D., Hase, Y., Clarkson, A.N., Kalaria, R.N., 2021. The role of the medial prefrontal cortex in cognition, ageing and dementia. Brain Commun. 3(3):fcab125.

Jonsson, T., Stefansson, H., Steinberg, S., et al., 2013. Variant of TREM2 associated with the risk of Alzheimer's disease. N. Engl. J. Med. 368 (2), 107–116.

Kappelmann, N., Dantzer, R., Khandaker, G.M., 2021. Interleukin-6 as potential mediator of long-term neuropsychiatric symptoms of COVID-19. Psychoneuroendocrinology 131, 105295.

Karstensen, H.G., Vestergaard, M., Baare, W.F.C., et al., 2018. Congenital olfactory impairment is linked to cortical changes in prefrontal and limbic brain regions. Brain Imaging Behav. 12 (6), 1569–1582.

Kubota, T., Kuroda, N., Sone, D., 2023. Neuropsychiatric aspects of long COVID: A comprehensive review. Psychiatry Clin. Neurosci. 77 (2), 84–93.

Kummer, K.K., Zeidler, M., Kalpachidou, T., Kress, M., 2021. Role of IL-6 in the regulation of neuronal development, survival and function. Cytokine 144, 155582.

Levine, K.S., Leonard, H.L., Blauwendraat, C., et al., 2023. Virus exposure and neurodegenerative disease risk across national biobanks. Neuron 111 (7), 1086–1093 e1082.

Liu, D., Cao, B., Zhao, Y., et al., 2018. Soluble TREM2 changes during the clinical course of Alzheimer's disease: A meta-analysis. Neurosci. Lett. 686, 10–16.

Lu, Y., Li, X., Geng, D., et al., 2020. Cerebral Micro-Structural Changes in COVID-19 Patients - An MRI-based 3-month Follow-up Study. EClinicalMedicine. 25, 100484.

Malek-Ahmadi, M., Davis, K., Belden, C.M., Sabbagh, M.N., 2014. Comparative analysis of the Alzheimer questionnaire (AQ) with the CDR sum of boxes, MoCA, and MMSE. Alzheimer Dis. Assoc. Disord. 28 (3), 296–298.

McWhirter, L., Smyth, H., Hoeritzauer, I., Couturier, A., Stone, J., Carson, A.J., 2023. What is brain fog? J. Neurol. Neurosurg. Psychiatry 94 (4), 321–325.

Melief, J., Koning, N., Schuurman, K.G., et al., 2012. Phenotyping primary human microglia: tight regulation of LPS responsiveness. Glia 60 (10), 1506–1517.

Nasreddine, Z.S., Phillips, N.A., Bedirian, V., et al., 2005. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J. Am. Geriatr. Soc. 53 (4), 695–699.

Peter, R.S., Nieters, A., Krausslich, H.G., et al., 2022. Post-acute sequelae of covid-19 six to 12 months after infection: population based study. BMJ 379, e071050.

Pfaff, E.R., Madlock-Brown, C., Baratta, J.M., et al., 2023. Coding long COVID: characterizing a new disease through an ICD-10 lens. BMC Med. 21 (1), 58.

Pizzagalli, D.A., Roberts, A.C., 2022. Prefrontal cortex and depression. Neuropsychopharmacology 47 (1), 225–246.

Poletti, S., Leone, G., Hoogenboezem, T.A., et al., 2019. Markers of neuroinflammation influence measures of cortical thickness in bipolar depression. Psychiatry Res. Neuroimaging 285, 64–66.

Rajapakse, J.C., Giedd, J.N., Rapoport, J.L., 1997. Statistical approach to segmentation of single-channel cerebral MR images. IEEE Trans. Med. Imaging 16 (2), 176–186.

Riazi, K., Galic, M.A., Kuzmiski, J.B., Ho, W., Sharkey, K.A., Pittman, Q.J., 2008. Microglial activation and TNFalpha production mediate altered CNS excitability following peripheral inflammation. PNAS 105 (44), 17151–17156.

Ries, M.L., Carlsson, C.M., Rowley, H.A., et al., 2008. Magnetic resonance imaging characterization of brain structure and function in mild cognitive impairment: a review. J. Am. Geriatr. Soc. 56 (5), 920–934.

Sanabria-Diaz, G., Etter, M.M., Melie-Garcia, L., et al., 2022. Brain cortical alterations in COVID-19 patients with neurological symptoms. Front. Neurosci. 16, 992165.

Schultheiss, C., Willscher, E., Paschold, L., et al., 2022. The IL-1?, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19. Cell Rep. Med. 3 (6).

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl 20), pp. 22–33;quiz 34–57.

Smucny, J., Dienel, S.J., Lewis, D.A., Carter, C.S., 2022. Mechanisms underlying dorsolateral prefrontal cortex contributions to cognitive dysfunction in schizophrenia. Neuropsychopharmacology 47 (1), 292–308.

Spanic Popovacki, E., Babic Leko, M., Langer Horvat, L., et al., 2023. Soluble TREM2 Concentrations in the Cerebrospinal Fluid Correlate with the Severity of Neurofibrillary Degeneration, Cognitive Impairment, and Inflammasome Activation in Alzheimer's Disease. Neurol. Int. 15 (3), 842–856.

Spisak, T., Spisak, Z., Zunhammer, M., et al., 2019. Probabilistic TFCE: A generalized combination of cluster size and voxel intensity to increase statistical power. Neuroimage 185, 12–26.

Steffen, J., Ehrentraut, S., Bank, U., et al., 2022. Type 1 innate lymphoid cells regulate the onset of Toxoplasma gondii-induced neuroinflammation. Cell Rep. 38 (13), 110564.

Storsve, A.B., Fjell, A.M., Tamnes, C.K., et al., 2014. Differential longitudinal changes in cortical thickness, surface area and volume across the adult life span: regions of accelerating and decelerating change. J. Neurosci. 34 (25), 8488–8498.

Suarez-Calvet, M., Kleinberger, G., Araque Caballero, M.A., et al., 2016. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. EMBO Mol. Med. 8 (5), 466–476.

Talla, A., Vasaikar, S.V., Szeto, G.L., et al., 2023. Persistent serum protein signatures define an inflammatory subcategory of long COVID. Nat. Commun. 14 (1), 3417.

Tohka, J., Zijdenbos, A., Evans, A., 2004. Fast and robust parameter estimation for statistical partial volume models in brain MRI. Neuroimage 23 (1), 84–97.

Tu, Y., Zhang, Y., Li, Y., et al., 2021. Post-traumatic stress symptoms in COVID-19 survivors: a self-report and brain imaging follow-up study. Mol. Psychiatry.

Yin, J.X., Agbana, Y.L., Sun, Z.S., et al., 2023. Increased interleukin-6 is associated with long COVID-19: a systematic review and meta-analysis. Infect. Dis. Poverty 12 (1).

Zawilska, J.B., Kuczynska, K., 2022. Psychiatric and neurological complications of long COVID. J. Psychiatr. Res. 156, 349–360.

Zhao, A.A., Jiao, Y., Ye, G.Y., et al., 2022. Soluble TREM2 levels associate with conversion from mild cognitive impairment to Alzheimer?s disease. J. Clin. Investig. 132 (24).