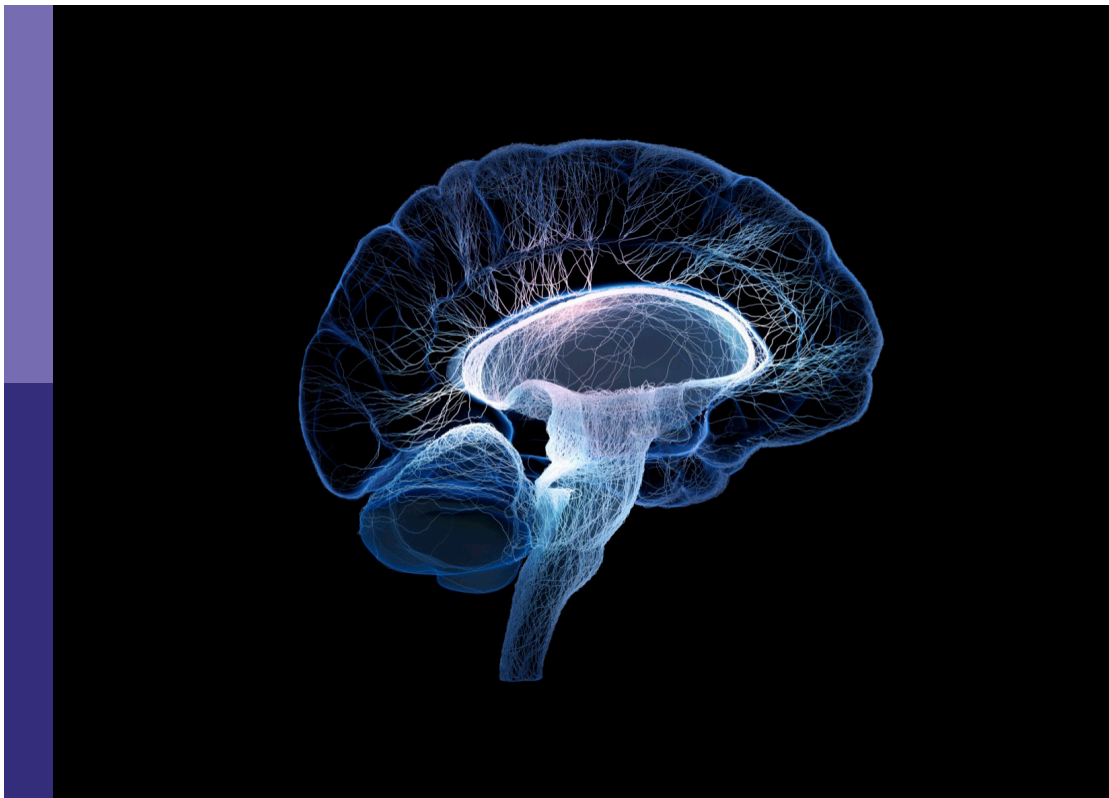
frontiers | Research Topics

SARS-CoV-2 in neurodegenerative diseases

Edited by
Gonzalo Emiliano Aranda-Abreu, David Pozo,
Alfonso Carreon-Rodriguez and Sonia Zuñiga

Published in
Frontiers in Neuroscience
Frontiers in Neurology
Frontiers in Immunology



ISSN 1664-8714
ISBN 978-2-8325-4381-8
DOI 10.3389/978-2-8325-4381-8

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

SARS-CoV-2 in neurodegenerative diseases

Topic editors

Gonzalo Emiliano Aranda-Abreu — Universidad Veracruzana, Mexico
David Pozo — University of Seville, Spain
Alfonso Carreon-Rodriguez — National Institute of Public Health, Mexico
Sonia Zuñiga — National Center for Biotechnology, Spanish National Research Council (CSIC), Spain

Citation

Aranda-Abreu, G. E., Pozo, D., Carreon-Rodriguez, A., Zuñiga, S., eds. (2024). *SARS-CoV-2 in neurodegenerative diseases*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4381-8

Table of contents

frontiers | Frontiers in Neuroscience

TYPE Editorial
PUBLISHED 16 January 2024
DOI 10.3389/fnins.2024.1360234

OPEN ACCESS

EDITED AND REVIEWED BY
Einar M. Sigurdsson,
New York University, United States

*CORRESPONDENCE
Gonzalo Emiliano Aranda-Abreu
garanda@uv.mx

RECEIVED 22 December 2023
ACCEPTED 08 January 2024
PUBLISHED 16 January 2024

CITATION
Aranda-Abreu GE, Carreón-Rodriguez A, Zuñiga S and Pozo D (2024) Editorial: SARS-CoV-2 in neurodegenerative diseases. *Front. Neurosci.* 18:1360234. doi: 10.3389/fnins.2024.1360234

Editorial: SARS-CoV-2 in neurodegenerative diseases

Gonzalo Emiliano Aranda-Abreu[1]*, Alfonso Carreón-Rodriguez[2], Sonia Zuñiga[3] and David Pozo[4]

[1]Instituto de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Mexico, [2]Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico, [3]National Center for Biotechnology, Spanish National Research Council (CSIC), Madrid, Spain, [4]Department of Medical Biochemistry, Molecular Biology and Immunology, Faculty of Medicine, University of Seville, Seville, Spain

KEYWORDS

SARS-CoV-2, neurodegeneration, brain, disease, neuroinflammation

Editorial on the Research Topic
SARS-CoV-2 in neurodegenerative diseases

In the month of March in the year 2020, an alarming pandemic triggered by the SARS-CoV-2 virus, which leads to the development of the notorious COVID-19 disease, was officially declared. The consequences of this coronavirus have been utterly devastating, resulting in the unfortunate demise of countless individuals due to the severe deterioration experienced both in the pulmonary as well as systemic aspects of their health. Regrettably, it has been observed that the elderly population and those individuals with underlying health conditions, commonly referred to as comorbidities, have proven to be the most susceptible and vulnerable to the detrimental effects of this virus. Astonishingly, statistics have estimated that an astonishing number exceeding 70 million people across the globe have been diagnosed with the SARS-CoV-2 virus, including individuals diagnosed with neurodegenerative diseases such as Alzheimer's, who happen to be at the highest risk for both hospitalization and mortality.

The mechanisms and pathways through which the virus infiltrates and impacts the nervous system have been subjects of extensive research and scientific inquiry. One of the primary proposed pathways suggests that the virus is capable of directly infecting neurons, thereby triggering a cascading sequence of events that ultimately leads to the induction of various inflammatory agents. Interestingly, these inflammatory agents are generated as a result of the systemic inflammation that transmits to the brain. Consequently, it becomes increasingly evident that individuals diagnosed with neurodegenerative diseases must be given heightened attention and care, as there is a growing body of evidence suggesting that the presence of the coronavirus can significantly exacerbate the progression and severity of such diseases. Consequently, it becomes absolutely imperative to actively seek and develop effective therapeutic interventions and treatment modalities specifically tailored for individuals diagnosed with neurodegenerative diseases, with the ultimate goal of mitigating and decelerating the adverse effects caused by the SARS-CoV-2 virus.

The COVID-19 pandemic has presented an unparalleled array of challenges, the implications of which on the central nervous system have emerged as a critical area of investigation. Within this editorial, we will delve into a plethora of studies that shed light on the intricate and interconnected nature of the relationship between the SARS-CoV-2 virus and the alterations it induces within the cerebral realm.

The following articles are part of this topic.

1. *Brain Cortical Alterations in COVID-19 Patients with Neurological Symptoms* (Sanabria-Diaz et al.).

   Studies examining cortical alterations in COVID-19 patients with neurological symptoms reveal the diverse range of manifestations the virus can induce within the brain. From changes in connectivity to cognitive impairments, understanding these alterations is crucial for guiding clinical interventions.
2. *Risk and Prognostic Factors for SARS-CoV-2 Infection in a Spanish Multiple Sclerosis Population during the First 5 Waves* (Pilo De La Fuente et al.).

   The intersection of SARS-CoV-2 and multiple sclerosis in the Spanish population highlights the need to identify risk and prognostic factors. This knowledge is essential for tailoring prevention and treatment strategies in this specific cohort.
3. *SARS-CoV-2, Long COVID, Prion Disease, and Neurodegeneration* (Zhao et al.).

   The intricate relationship between SARS-CoV-2, long COVID, prion diseases, and neurodegeneration poses urgent questions. How does the virus influence prion pathways, and what are the implications for long-term neurodegeneration?
4. *Late Neurological Consequences of SARS-CoV-2 Infection: New Challenges for the Neurologist* (Korchut and Rejdak).

   Understanding the late neurological consequences of SARS-CoV-2 infection presents novel challenges for neurologists. From persistent symptoms to cognitive issues, specialized attention and adaptive management strategies are required.
5. *SARS-CoV-2-Specific Antibody Responses Following BNT162b2 Vaccination in Individuals with Multiple Sclerosis Receiving Different Disease-Modifying Treatments* (Lambrianides et al.).

   The variability in SARS-CoV-2-specific antibody responses post-BNT162b2 vaccination in multiple sclerosis patients prompts questions about the efficacy of diverse disease-modifying treatments.
6. *Adamantanes for the Treatment of Neurodegenerative Diseases in the Presence of SARS-CoV-2* (Butterworth).

   Exploring adamantanes as a potential treatment for neurodegenerative diseases in the context of SARS-CoV-2 underscores the need for innovative and multifaceted therapeutic strategies.
7. *The Link between SARS-CoV-2-Related Microglial Reactivity and Astrocyte Pathology in the Inferior Olivary Nucleus* (Madden et al.).

   Investigating the connection between SARS-CoV-2-related microglial reactivity and astrocyte pathology in specific brain regions provides unique insights into the mechanisms behind neurological manifestations of the virus.
8. *COVID-19: A Modern Trigger for Guillain-Barré Syndrome, Myasthenia Gravis, and Small Fiber Neuropathy* (Gomez et al.).

   The association between COVID-19 and neuromuscular syndromes raises questions about pathogenesis and necessitates heightened vigilance in affected patients.
9. *Long-Lasting Neutralizing Antibodies and T Cell Response After the Third Dose of mRNA Anti-SARS-CoV-2 Vaccine in Multiple Sclerosis* (Maglione et al.).

   The persistence of neutralizing antibodies and T cell responses after the third dose of mRNA anti-SARS-CoV-2 vaccine underscores the importance of tailored vaccination strategies for individuals with multiple sclerosis.
10. *The Determinants of COVID-Induced Brain Dysfunctions After SARS-CoV-2 Infection in Hospitalized Patients* (Yasir et al.).

    Identifying the determinants of COVID-induced brain dysfunctions after SARS-CoV-2 infection in hospitalized patients is crucial for enhancing care and rehabilitation.

In conclusion, the COVID-19 pandemic has brought about a myriad of challenges, particularly in relation to the central nervous system. The intricate connections between the SARS-CoV-2 virus and cerebral alterations necessitate comprehensive investigations in order to enhance our understanding of the underlying mechanisms and develop effective interventions. By addressing the various aspects discussed in this editorial, we can work toward mitigating the impact of COVID-19 on the central nervous system and improving patient outcomes.

Author contributions

GA-A: Writing – original draft, Writing – review & editing. AC-R: Writing – original draft, Writing – review & editing. SZ: Writing – original draft, Writing – review & editing. DP: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

frontiers | Frontiers in Neuroscience

TYPE Opinion
PUBLISHED 27 September 2022
DOI 10.3389/fnins.2022.1002770

OPEN ACCESS

EDITED BY
Alfonso Carreon-Rodriguez,
National Institute of Public Health, Mexico

REVIEWED BY
Mohammadmahdi Sabahi,
Hamadan University of Medical Sciences, Iran

*CORRESPONDENCE
Walter J. Lukiw
wlukiw@lsuhsc.edu

SPECIALTY SECTION
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

RECEIVED 25 July 2022
ACCEPTED 07 September 2022
PUBLISHED 27 September 2022

CITATION
Zhao Y, Jaber VR and Lukiw WJ (2022) SARS-CoV-2, long COVID, prion disease and neurodegeneration. *Front. Neurosci.* 16:1002770. doi: 10.3389/fnins.2022.1002770

SARS-CoV-2, long COVID, prion disease and neurodegeneration

Yuhai Zhao[1,2], Vivian R. Jaber[2] and Walter J. Lukiw[2,3,4]*

[1]Department of Cell Biology and Anatomy, Louisiana State University Health Sciences Center, New Orleans, LA, United States, [2]LSU Neuroscience Center, LSU Health Sciences Center, New Orleans, LA, United States, [3]Department of Ophthalmology, LSU Health Sciences Center, New Orleans, LA, United States, [4]Department of Neurology, LSU Health Sciences Center, New Orleans, LA, United States

KEYWORDS

Creutzfeldt-Jacob disease (CJD), long COVID-19, 'S1' spike protein, SARS-CoV-2, prion disease (PrD), Alzheimer's disease (AD), miRNA-146a, miRNA-155

Introduction

On the last day of the year 2019 a novel *Betacoronavirus* (2019-nCov), now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and causing the highly transmissible and lethal pneumonia COVID-19 was first reported in Wuhan, Hubei Province in Central China (Huang et al., 2020; Fu et al., 2022; Lu and Sun, 2022). Since then ongoing research and long-term studies of the sequelae of SARS-CoV-2 infection have indicated that post-infection, recovery from COVID-19 and/or COVID-19 aftermath is associated with long-term physiological and neurological deficits known generically as "long COVID" (Roy et al., 2021; Ahmad et al., 2022; Baazaoui and Iqbal, 2022). Multiple independent epidemiological and clinical studies further indicate that SARS-CoV-2 infection and "long COVID" strongly correlate with the onset of progressive neurological disturbances that include Alzheimer's disease (AD), prion disease (PrD) and other neurodegenerative disorders. These are apparent: **(i)** especially in aged and/or senile COVID-19 patients; **(ii)** in patients experiencing overlapping or inter-current illnesses that include heart disease, diabetes, hypertension, neuropsychiatric and other age-related neurological disorders; and **(iii)** in those COVID-19 patients who have experienced a particularly virulent and/or a near fatal episode of SARS-CoV-2 infection (Farheen et al., 2021; Flud et al., 2022; Fu et al., 2022). Conversely, increasing numbers of epidemiological studies suggest that elderly people with neurological deficits commonly observed in AD are highly vulnerable to SARS-CoV-2 infection, and especially the development of more severe forms of COVID-19 disease (Chiricosta et al., 2021; Hsu et al., 2021; Fu et al., 2022). The recent finding that the SARS-CoV-2 "S1" spike protein essential for viral infectivity contains prion-like domains associated with immune-evasion and the promotion of protein aggregation and aggregate "seeding" is particularly intriguing (Baazaoui and Iqbal, 2022; Bernardini et al., 2022; Tetz and Tetz, 2022). Based on these and other very recent findings this "Opinion" paper will: **(i)** address our current understanding of the emerging role of SARS-CoV-2 infection with "long COVID" with special reference to AD and PrD; **(ii)** will review the latest findings of the SARS-CoV-2 "S1" spike protein and its preferred interaction with the ubiquitous

angiotensin converting enzyme-2 (ACE2) receptor (ACE2R); and **(iii)** will highlight the interplay of the molecular biology and neuropathology of SARS-CoV-2 with the unusual and immune-evasive character of prion neurobiology, AD and PrD.

SARS-CoV-2, "long COVID" and neurological disease

The SARS-CoV-2 virus that causes COVID-19 disease is a highly contagious pathogen that continues to impact human health around the globe and is responsible for one of the worst pandemics in recorded human history. Of the ∼600 million people that have been infected about half of all COVID-19 patients exhibit the symptomology of "long COVID" and many experience some type of lingering neurological complications including, prominently, "brain fog," confusion, impaired consciousness, deficits in cognition and memory, encephalopathy, encephalitis and/or cerebrovascular deficits (Mao et al., 2020; Roy et al., 2021; Ahmad et al., 2022; Baazaoui and Iqbal, 2022; Visco et al., 2022; https://www.worldometers.info/coronavirus/coronavirus-death-toll/; https://www.science.org/content/article/what-causes-long-covid-three-leading-theories?cookieSet=1; https://www.forbes.com/sites/joshuacohen/2022/06/22/dutch-research-on-long-covid-shows-50-of-study-partic-ipants-have-1-or-more-symptoms-3-months-after-becoming-infected-with-coronavirus/?sh$=$45228b705a6a; last accessed 29 August 2022). Up to ∼45% of COVID-19 patients develop a mild-to-severe encephalopathy and encephalitis due to complications arising from viral-induced "***cytokine storm***," elevated inflammatory signaling and/or anti-neural autoimmunity, sometimes referred to as "***cytokine storm syndrome***" (Mao et al., 2020; Vigasova et al., 2021; Baazaoui and Iqbal, 2022; Piekut et al., 2022). As is consistently observed in AD brain, the pro-inflammatory cytokines interleukin-1beta (IL-1β), IL-8, IL-18, the interleukin-1 receptor antagonist (IL-1RA) and serum neurofilament light (NF-L) chain protein, each a biomarker for all-cause pro-inflammatory neurodegeneration are positively associated with COVID-19 disease severity and are predictors of long-term outcome (Mao et al., 2020; Krey et al., 2021; Zetterberg and Schott, 2022). SARS-CoV-2 infected patients with existing AD are invariably associated with more severe complications of COVID-19 including increased morbidity and mortality (Mao et al., 2020; Krey et al., 2021; Chung et al., 2022; Guasp et al., 2022; Zetterberg and Schott, 2022). Depending upon COVID-19 disease course and post-infection severity multiple epidemiological studies indicate that about ∼30–35% of all COVID-19 patients experience lasting neurological and neuropsychiatric symptoms ranging from relatively minor effects such as "***brain fog***" to more severe neurological complications. A pre-existing diagnosis of AD predicts the highest risk of COVID-19 infection yet identified, with the highest mortality among the most elderly AD patients (Song et al., 2021; Zhao et al., 2021; Ahmad et al., 2022; Baazaoui and Iqbal, 2022; Choe et al., 2022; Chung et al., 2022; Flud et al., 2022; Lingor et al., 2022; Visco et al., 2022). Interestingly, viral and/or other microbial infections, including SARS-CoV-2 invasion of the human brain and CNS, have long been known to contribute, intensify, propagate and/or augment the same neuropathological and pro-inflammatory neurodegenerative changes as is observed over the entire AD continuum from the earliest detectable forms of mild cognitive impairment (MCI) to the more severe terminal stages of AD (see below; Chiricosta et al., 2021; Ciaccio et al., 2021; Lingor et al., 2022; Lukiw et al., 2022; Piekut et al., 2022; Sirin et al., 2022; Szabo et al., 2022; Zhao and Lukiw, 2022).

The SARS-CoV-2 "S1" spike protein, the ACE2R and amyloidogenesis

The SARS-CoV-2 virus possesses an unusually large, positive-sense single-stranded RNA (ssRNA) genome of about ∼29,903 nucleotides (nt) packaged into a nucleocapsid core within a ∼100 nm diameter virion particle that possesses a compact spherical lipoprotein envelope (SARS-CoV-2 isolate Wuhan-Hu-1, National Center for Biological Information (NCBI) GenBank Accession No. NC_045512.2; last accessed 29 August 2022; Ke et al., 2020; Sah et al., 2020; Wu et al., 2020; Mousavizadeh and Ghasemi, 2021; Zhao and Lukiw, 2022). Extending outward and decorating the surface of the SARS-CoV-2 lipoprotein envelope are the 672 amino acid homotrimeric 'S1' spike glycoproteins that play essential roles in ACE2R-recongition, viral attachment, fusion and entry into host cells to initiate SARS-CoV-2 infection (Duan et al., 2020; Ke et al., 2020; Zhao and Lukiw, 2022). Interestingly: **(i)** the ACE2R, normally a ubiquitously expressed zinc-containing metallo-carboxypeptidase (EC 3.4.17.23) surface receptor glycoprotein of the renin-angiotensin system (RAS) that has a role in the regulation of blood pressure is up-regulated in limbic regions of AD-affected brain (Ding et al., 2021; Zhao and Lukiw, 2022); **(ii)** the SARS-CoV-2 'S1' spike protein is absolutely essential in ACE2R recognition and viral entry (Hill et al., 2021; Letarov et al., 2021; Palacios-Rápalo et al., 2021); **(iii)** the main antigen used as a target in COVID-19 vaccines is a lipid nanoparticle enclosing an RNA sequence encoding the full length SARS-CoV-2 'S1' spike protein, since blocking 'S1' spike entry into host cells will prevent the initiation of SARS-CoV-2 infection (Actis et al., 2021; Dai and Gao, 2021); **(iv)** variations in the prion-like domains of the 'S1' spike protein differs among SARS-CoV-2 variants thus modulating 'S1' affinity for the ACE2R (Shahzad and Willcox, 2022; Tetz and Tetz, 2022); **(v)** SARS-CoV-2 'S1' spike protein binds to the aggregation-prone glycosoaminoglycan heparin and heparin binding proteins (HBP) including amyloid-beta (Aβ) peptides, α-synuclein, tau and prion proteins and TDP-43 thus

facilitating viral infection while accelerating the coalescence and aggregation of multiple pathological amyloidogenic proteins in the brain and CNS (Idrees and Kumar, 2021; Paiardi et al., 2022); **(vi)** targeting the interaction of SARS-CoV-2 'S1' spike protein with these brain-enriched proteins may be a useful strategy to reduce pro-inflammatory aggregation processes that may limit the neurodegenerative disease process in COVID-19 patients (Clausen et al., 2020; Paiardi et al., 2022); and **(vii)** AD and COVID-19 infection share several important risk factors and comorbidities that include gender, aging, oxidative stress, hypertension, diabetes, APOE4 expression and up-regulation of the same families of inducible microRNAs (miRNAs), systemic inflammation and neuro-inflammation and/or the massive cytokine signaling disruptions referred to as the "***cytokine storm***" (Mao et al., 2020; Ciaccio et al., 2021; Vigasova et al., 2021). Interestingly just as the ACE2R is the most important cell surface receptor for SARS-CoV-2, elevated ACE2R expression appears to impose a significant risk factor for SARS-CoV-2 transmission in AD patients resulting in a higher viral load in AD-affected brain, and this may explain the high prevalence of SARS-CoV-2 infection among AD patients at any stage of the disease (Lim et al., 2020; Ding et al., 2021; Hill et al., 2021; Zhao et al., 2021; Shen et al., 2022; Zhao and Lukiw, 2022; Table 1).

TABLE 1 *Human neurological diseases and/or syndromes associated with 'long COVID'*; recent reports of age-related, progressive, terminal and/or incapacitating neurological disorders associated with SARS-CoV-2 infection and COVID-19; the majority of these most recent reports involve COVID-19 with the neurodegenerative disorders AD, PrD (primarily Creutzfeldt-Jakob disease; CJD); and/or the onset of visual system disturbances (alphabetically ordered; Hill et al., 2021; Hixon et al., 2021; Oldfield et al., 2021; Zhou et al., 2021; Ahmad et al., 2022; Baazaoui and Iqbal, 2022; Flud et al., 2022; Lukiw, 2022; Piekut et al., 2022; Piras et al., 2022; Visco et al., 2022).

Neurological disorder	Reference
Alzheimer's disease (AD)	Hill et al., 2021
	Chiricosta et al., 2021
	Ciaccio et al., 2021
	Ding et al., 2021
	Zhao et al., 2021
	Shen et al., 2022
	Zhao and Lukiw, 2022
Epilepsy	Roy et al., 2021
Multiple sclerosis (MS)	Muñoz-Jurado et al., 2022
Prion disease (PrD)	Bernardini et al., 2022
	Ciolac et al., 2021
	Kuvandik et al., 2021
	Lukiw et al., 2022
	Olivo et al., 2022
	Shahzad and Willcox, 2022
	Szabo et al., 2022
	Tayyebi et al., 2022
	Tetz and Tetz, 2022
	Young et al., 2020
Visual system disturbances	Hill et al., 2021
	Hixon et al., 2021
	Tisdale et al., 2021
	Zhao et al., 2021
	Lukiw, 2022
	Piras et al., 2022

SARS-CoV-2, prion neurobiology and prion disease

Human prion diseases (PrD) represent an expanding spectrum of progressive, and fatal neurodegenerative disorders affecting about one person in every one million per year worldwide, of which 80–95% are sporadic Creutzfeldt-Jacob disease (CJD) and the remainder representing genetic and/or familial CJD cases (Geschwind, 2015; Ayers et al., 2020). PrD infections are characterized by transmissibility, progressive neurological deficits caused by the accumulation of and aggregation of a misfolded "scrapie" isoform (PrPSc) from the native cellular prion protein (PrPc); and the rapid development of a progressive systemic inflammation very similar in nature to AD (Holmes et al., 2010; Ayers et al., 2020). A number of interesting associations are being made between SARS-CoV-2 infection and prion neurobiology and PrD: **(i)** several recent reports link multiple aspects of the 'S1' spike protein structure and function, immunology and epidemiology with PrD, prion-like spread and prion neurobiology (Letarov et al., 2021; Baazaoui and Iqbal, 2022; Paiardi et al., 2022; Shahzad and Willcox, 2022). Because 'S1' spike proteins support heparin and HBP interacions that promote the aggregation of Aβ peptides, α-synuclein, tau and prion proteins, SARS-CoV-2 infection itself may exacerbate the formation of amyloid peptide-enriched aggregates that support pro-inflammatory neurodegeneration, neuronal cell death and AD- and/or PrD-type change (Idrees and Kumar, 2021; Paiardi et al., 2022). 'S1' spike proteins containing 'prion-like' domains in free form may also play a role in systemic amyloidogenesis that in turn supports systemic inflammation, and the formation of pathogenic pro-inflammatory lesions in the brain and CNS (Letarov et al., 2021; Baazaoui and Iqbal, 2022; Shahzad and Willcox, 2022; Tetz and Tetz, 2022). Prion-like domains are known to self-associate, aggregate with other prion-like and HBP domains and amyloids, α-synuclein, tau and other prion proteins and contribute to protein-misfolding diseases that include AD and PrD infection (Holmes et al., 2010; Geschwind, 2015; Ayers et al., 2020); and **(ii)** there are several recent case studies of patients developing PrD and or exacerbating the neuropathology of PrDs such as CJD in conjunction with SARS-CoV-2 infection. Schmahmann's laboratory described a 60 yr old male patient whose first manifestations of CJD occurred in

tandem with symptomatic onset of COVID-19. Quantification of a panel of the patient's systemic inflammatory mediators and biomarkers (including increased secretion of IL-1 and TNF) in response to the SARS-CoV-2-mediated-hastening of CJD pathogenesis suggested a significant relationship between host immune-responses to SARS-CoV-2 and an acceleration of inflammatory neurodegenerative cascades characteristic of CJD infection (Young et al., 2020). Olivo et al. described the case of a 70-year-old man with seizures and a rapidly evolving CJD during an acquired SARS-CoV-2 co-infection, again supporting the concept that CJD during SARS-CoV-2 infection is characterized by an accelerated progression of CJD (Olivo et al., 2022). Bernardini et al. (2022) recently described a ∼40 year old male COVID-19 patient who developed CJD 2 months after COVID-19 onset with presenting symptoms of visuospatial deficits, hallucinations, ataxia and diffuse myoclonus-and their study concluded that the short interval between SARS-CoV-2 respiratory and CJD neurological symptoms was indicative of a causal relationship between a COVID-mediated neuroinflammatory state, protein misfolding and subsequent aggregation of PrPc into PrPSc, and emphasized the role of SARS-CoV-2 as an significant viral initiator of neurodegeneration (Bernardini et al., 2022). These developing molecularly- and clinically-evidenced associations between CJD and SARS-CoV-2 infection underscores an overlapping pathological link between PrD and COVID-19 both involving a systemic inflammation, a progressive and insidious lethal neurodegeneration and a potential acceleration of prion-like protein spread following SARS-CoV-2 viral invasion (Pogue and Lukiw, 2021; Song et al., 2021; Baazaoui and Iqbal, 2022).

Another interesting link between SARS-CoV-2 infection, PrD and the development of inflammatory neurodegeneration are the effects of these infections and their pathophysiological consequences on the abundance, speciation and complexity of a small family of inducible pathological microRNAs (miRNAs). These include, predominantly, the NF-kB (p50/p65)-sensitive miRNA-146a-5p and miRNA-155-5p and others (Zhao et al., 2020; Pogue and Lukiw, 2021; Pinacchio et al., 2022). A large amount of work has focused on the 22 nucleotide brain-enriched miRNA-146a-5p found to be significantly up-regulated in 10 known forms of PrD of both rodents and humans including CJD, in AD and in other sporadic and progressive age-related neurological disorders, and after infection by at least 18 neurotropic DNA and/or RNA viruses, including SARS-CoV-2, that infect the human brain, CNS, immune, lymphatic and hepatic, respiratory and/or circulatory systems (Pogue and Lukiw, 2021; Roganović, 2021; Pinacchio et al., 2022). Interestingly, the ACE2R recognized by the SARS-CoV-2 'S1' spike protein is up-regulated by miRNA-146a and the many types of PrD and viral infections that induce miRNA-146a-5p and/or miRNA-155 are all associated with a advancing and insidious systemic inflammation and specific neurological disease symptoms and/or syndromes that are progressive, age-related, insidious, incapacitating and invariably fatal. Despite an apparent lack of nucleic acids in prions, both DNA- and RNA-containing viruses, along with prions, significantly and progressively induce miRNA-146a and/or miRNA-155 in the infected host, but whether this represents part of the host's adaptive immunity, innate-immune response or a mechanism to enable the invading prion or virus a successful infection remains incompletely understood (Ayers et al., 2020; Carlson and Prusiner, 2021; Pogue and Lukiw, 2021; Roganović, 2021; Pinacchio et al., 2022; Zhao and Lukiw, 2022).

The multi-system and neurological impact of SARS-CoV-2 infection

It is important to emphasize that COVID-19 disease typically presents as an unusually rapid onset, highly transmissible viral pneumonia, and that SARS-CoV-2 infection initially requires a critical interaction between the viral 'S1' spike protein of SARS-CoV-2 and the surface membrane-exposed ACE2R. Some of the highest ACE2R densities have been found in the cholesterol- and sphingolipid-enriched lipid raft domains of multiple epithelial and endothelial cells of the human respiratory tract, however ACE2R has been identified on every human host cell type so far analyzed except for enucleated red blood cells (Hill et al., 2021; Palacios-Rápalo et al., 2021; Zhao et al., 2021; Kirtipal et al., 2022; Lukiw et al., 2022). ACE2R is abundantly detected in all cell types of the whole brain, CNS, neurovasculature, choroid plexus and the visual tracts extending from the retina to the occipital lobe that involve multiple visual processing and neuro-ophthalmic signaling pathways (Hill et al., 2021; Hixon et al., 2021; Zhao et al., 2021; Lukiw, 2022; Piras et al., 2022). Human vision and visual processing is negatively impacted by SARS-CoV2 infection (Hill et al., 2021; Hixon et al., 2021; Tisdale et al., 2021; Lukiw, 2022). Interestingly, the highest ACE2R expression yet described in the human CNS has been localized to the neurons of the medulla oblongata and pons in the brainstem, containing the Botzinger neuron complex and the brain's medullary respiratory centers, and this may in part explain the vulnerability of most SARS-CoV-2 infected patients to serious respiratory distress (Zhao et al., 2021; Lukiw et al., 2022; Molina-Molina and Hernández-Argudo, 2022). Besides the ubiquity and presence of the ACE2R on every human host cell type, all neural cell and tissue systems are linked together by a neural syncytium, a continuous intercellular networking system along which viruses may translocate (Kiyoshi and Zhou, 2019). Viruses such as SARS-CoV-2 also appear to utilize exosome- and vesicle-mediated transport mechanisms in systemic viral proliferation (Saheera et al., 2020; Eymieux et al., 2021; Visco et al., 2022). Using these various strategies for viral spread and means of translocation: **(i)** the SARS-CoV-2 virus has an enormous potential to infect, damage and/or destroy

almost every cell, tissue type and organ system within the human host; and **(ii)** to induce a serious multi-organ system failure with highly interactive respiratory, cardiovascular, dermatologic, endocrine, gastrointestinal, hematologic, immunological, pulmonary, renal and/or neuro-ophthalmic, neurological or psychiatric complications across multiple human populations in diverse global environments (Mercatelli and Giorgi, 2020; Flud et al., 2022; Kirtipal et al., 2022; Rodriguez-Rivas et al., 2022; Visco et al., 2022).

Discussion

It has been just over ∼30 months since SARS-CoV-2 viral infection and COVID-19 disease were first described. SARS-CoV-2 infections are currently responsible for a serious and disturbing global pandemic in which just under ∼600 million people have been infected and about ∼6.5 million have died (https://www.worldometers.info/coronavirus/coronavirus-death-toll/; last accessed 29 August 2022). Over this relatively brief period of time about 40–60% of all "recovered" COVID-19 patients have experienced some type of ill-defined, wide-ranging and highly variable neurological complication and exhibit the symptomology of "long COVID." Just as is the case for other incompletely characterized neurotrophic viral infections there are unexpected, unpredicted and sometimes alarming neurological and other sequelae to SARS-CoV-2-based viral infection. These include: **(i)** a pathological association with AD and novel onset human PrD; **(ii)** the recognition of self-associating prion-like viral domains in the SARS-CoV-2 'S1' spike protein driving amyloidogenesis and neurotoxic aggregate formation; and **(iii)** the persistent emergence of novel SARS-CoV-2 viral strains highly resistant to natural host immune responses and the anti-'S1' spike glycoprotein-based vaccines and vaccination strategies (Oldfield et al., 2021; Bernardini et al., 2022; Rodriguez-Rivas et al., 2022; Shahzad and Willcox, 2022). Existing and ongoing research have uncovered significantly overlapping pathological neurology and neurochemistry and the involvement of multiple physiological systems in the complex and highly interactive disease mechanisms that define "***long COVID***," PrD, neurodegeneration and SARS-CoV-2 neurobiology (Ritchie et al., 2020; Lingor et al., 2022; Lukiw et al., 2022; Olivo et al., 2022; Shahzad and Willcox, 2022; Visco et al., 2022). As in global pandemic infections of the past it is our opinion that we should anticipate additional unexpected associations of brain and CNS disease-linked mechanisms and pathways between SARS-CoV-2-mediated viral infection and other categories of age-related, immune-evasive pro-inflammatory forms of neurodegeneration. Importantly, the SARS-CoV-2 'S1' spike proteins contain both self-associating "prion-like" regions, amyloid peptide-binding and other domains that appear to play roles in pathological "seeding," amyloidogenesis and/or spreading that supports the formation of pathogenic lesions in the brain and CNS which contribute to pro-inflammatory neurodegeneration, neural cell atrophy and/or neuronal cell death (Tavassoly et al., 2020a,b; Lukiw et al., 2022; Tetz and Tetz, 2022).

The observed association between the more severe forms of COVID-19 and progressive neurodegenerative disorders that include AD and PrD at the molecular-genetic level are fascinating in that: **(i)** each disorder is a noteworthy example of a highly virulent, immune-evasive and often lethal neurotropic entity; and **(ii)** each of these pathogenic types are difficult to characterize, diagnose and treat, and possess unexpected characteristics, persistence and disease modalities (Baazaoui and Iqbal, 2022; Bernardini et al., 2022). The long-term effects and impact of COVID-19 disease infection and/or re-infection with the most recently identified SARS-CoV-2 strains including the SARS-CoV-2 '***Omicron stealth variants***' BA.5 and/or B.1.1.529 (https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html; last accessed 29 August 2022) are not yet known. They are however sure to open new and intriguing chapters in the study of viral neurology, the epidemiology and neurobiology of these highly transmissible zoonotic entities, and multiple, highly interactive aspects of the human neurological and systemic pathophysiology associated with SARS-CoV-2 infection and neurodegeneration, especially in cases involving the elderly and in immunologically compromised human populations.

Author contributions

YZ, VJ, and WL collected, analyzed, summarized the literature, and formulated an overall opinion on this contemporary topic. WL wrote the article. All authors contributed to the article and approved the submitted version.

Funding

Research on viral infection of the human brain with focus on HSV-1, SARS-CoV-2 and prion disease, single-stranded RNA (ssRNA) including SARS-CoV-2, miRNA, miRNA-mRNA and ssRNA-mRNA interactions involving neuronal atrophy, altered synaptic signaling, AD innate-immune response and neuro-inflammation was supported through Translational Research Initiative Grants from LSUHSC, The Brown Foundation, Joe and Dorothy Dorsett Innovation in Science Healthy Aging Award and NIA Grants AG18031 and AG038834.

Acknowledgments

Sincere thanks are extended to Drs. P.N. Alexandrov and J.M. Hill for human brain tissue samples and/or extracts, unpublished data, SARS-CoV-2, angiotensin converting

enzyme-2 (ACE2) receptor (ACE2R) DNA and cDNA probes, recombinant ACE2 and ACE2R protein control standards and initial data interpretation and to A.I. Pogue and D. Guillot for expert technical assistance, bioinformatics and statistics. This communication is dedicated to Drs P.N. Alexandrov and J.M. Hill for their many years of scientific work on altered ACE2 and ACE2R signaling in AD and neurodegenerative disease. Research on viral infection of the human brain with focus on HSV-1, SARS-CoV-2 and prion disease, single-stranded RNA (ssRNA) including SARS-CoV-2, miRNA, miRNA-mRNA and ssRNA-mRNA interactions involving neuronal atrophy, altered synaptic signaling, AD innate-immune response and neuro-inflammation was supported through Translational Research Initiative Grants from LSUHSC, The Brown Foundation, Joe and Dorothy Dorsett Innovation in Science Healthy Aging Award and NIA Grants AG18031 and AG038834.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Actis, G. C., Ribaldone, D. G., and Pellicano, R. (2021). COVID vaccine's hot problems: erratic serious blood clotting, ill-defined prion-like reactogenicity of the spike, unclear roles of other factors. *Minerva Med.* 112, 695–697. doi: 10.23736/S0026-4806.21.07769-7

Ahmad, S. J., Feigen, C. M., Vazquez, J. P., Kobets, A. J., and Altschul, D. J. (2022). Neurological sequelae of COVID-19. *J. Integr. Neurosci.* 21, 77. doi: 10.31083/j.jin2103077

Ayers, J. I., Paras, N. A., and Prusiner, S. B. (2020). Expanding spectrum of prion diseases. *Emerg. Top Life Sci.* 4, 155–167. doi: 10.1042/ETLS20200037

Baazaoui, N., and Iqbal, K. (2022). COVID-19 and neurodegenerative diseases: prion-like spread and long-term consequences. *J. Alzheimers Dis.* 18. doi: 10.3233/JAD-220105

Bernardini, A., Gigli, G. L., Janes, F., Pellitteri, G., Ciardi, C., Fabris, M., et al. (2022). Creutzfeldt-Jakob disease after COVID-19: infection-induced prion protein misfolding? A case report. *Prion.* 16, 78–83. doi: 10.1080/19336896.2022.2095185

Carlson, G. A., and Prusiner, S. B. (2021). How an infection of sheep revealed prion mechanisms in Alzheimer's disease and other neurodegenerative disorders. *Int. J. Mol. Sci.* 22, 4861. doi: 10.3390/ijms22094861

Chiricosta, L., Gugliandolo, A., and Mazzon, E. (2021). SARS-CoV-2 exacerbates beta-amyloid neurotoxicity, inflammation and oxidative stress in Alzheimer's disease patients. *Int. J. Mol. Sci.* 22, 13603. doi: 10.3390/ijms222413603

Choe, K., Park, H. Y., Ikram, M., Lee, H. J., Park, T. J., Ullah, R., et al. (2022). Systematic review of the common pathophysiological mechanisms in covid-19 and neurodegeneration: The role of bioactive compounds and natural antioxidants. *Cells.* 11, 1298. doi: 10.3390/cells11081298

Chung, S. J., Chang, Y., Jeon, J., Shin, J. I., Song, T. J., and Kim, J. (2022). Association of Alzheimer's disease with COVID-19 susceptibility and severe complications: a nationwide cohort study. *J. Alzheimers Dis.* 87, 701–710. doi: 10.3233/JAD-220031

Ciaccio, M., Lo Sasso, B., Scazzone, C., Gambino, C. M., Ciaccio, A. M., Bivona, G., et al. (2021). COVID-19 and Alzheimer's disease. *Brain Sci.* 11, 305. doi: 10.3390/brainsci11030305

Ciolac, D., Racila, R., Duarte, C., Vasilieva, M., Manea, D., Gorincioi, N., et al. (2021). Clinical and radiological deterioration in a case of Creutzfeldt-Jakob Disease following SARS-CoV-2 Infection: hints to accelerated age-dependent neurodegeneration. *Biomedicines.* 9, 1730. doi: 10.3390/biomedicines9111730

Clausen, T. M., Sandoval, D. R., Spliid, C. B., Pihl, J., Perrett, H. R., Painter, C. D., et al. (2020). SARS-CoV-2 infection depends on cellular heparan sulfate and ACE2. *Cell.* 183, 1043–1057.e15. doi: 10.1016/j.cell.2020.09.033

Dai, L., and Gao, G. F. (2021). Viral targets for vaccines against COVID-19. *Nat. Rev. Immunol.* 21, 73–82. doi: 10.1038/s41577-020-00480-0

Ding, Q., Shults, N. V., Gychka, S. G., Harris, B. T., and Suzuki, Y. J. (2021). Protein expression of angiotensin-converting enzyme 2 (ACE2) is upregulated in brains with Alzheimer's disease. *Int. J. Mol. Sci.* 22, 1687. doi: 10.3390/ijms22041687

Duan, L., Zheng, Q., Zhang, H., Niu, Y., Lou, Y., and Wang, H. (2020). The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: implications for the design of spike-based vaccine immunogens. *Front. Immunol.* 11, 576622. doi: 10.3389/fimmu.2020.576622

Eymieux, S., Uzbekov, R., Rouillé Y., Blanchard, E., Hourioux, C., Dubuisson, J., et al. (2021). Secretory vesicles are the principal means of SARS-CoV-2 egress. *Cells.* 10, 2047. doi: 10.3390/cells10082047

Farheen, S., Agrawal, S., Zubair, S., Agrawal, A., Jamal, F., Altaf, I., et al. (2021). Patho-physiology of aging and immune-senescence: possible correlates with comorbidity and mortality in middle-aged and old COVID-19 patients. *Front. Aging.* 2, 748591. doi: 10.3389/fragi.2021.748591

Flud, V. V., Shcherbuk, Y. A., Shcherbuk, A. Y., Leonov, V. I., and Al-Sahli, O. A. (2022). Neurological complications and consequences of new coronavirus COVID-19 infection in elderly and old patients. *Adv. Gerontol.* 35, 231–242. doi: 10.34922/AE.2022.35.2.007

Fu, Y. W., Xu, H. S., and Liu, S. J. (2022). COVID-19 and neurodegenerative diseases. *Eur. Rev. Med. Pharmacol Sci.* 26, 4535–4544. doi: 10.26355/eurrev_202206_29093

Geschwind, M. D. (2015). Prion diseases. *Continuum.* 21, 1612–1638. doi: 10.1212/CON.0000000000000251

Guasp, M., Muñoz-Sánchez, G., Martínez-Hernández, E., Santana, D., Carbayo, Á., and Naranjo, L. (2022). CSF biomarkers in COVID-19 associated encephalopathy and encephalitis predict long-term outcome. *Front. Immunol.* 13, 866153. doi: 10.3389/fimmu.2022.866153

Hill, J. M., Clement, C., Arceneaux, L., and Lukiw, W. J. (2021). Angiotensin converting enzyme 2 (ACE2) expression in the aged brain and visual system. *J. Aging. Sci.* 9, 7.001. doi: 10.21203/rs.3.rs-296818/v1

Hixon, A. M., Thaker, A. A., and Pelak, V. S. (2021). Persistent visual dysfunction following posterior reversible encephalopathy syndrome due to COVID-19. *Eur. J. Neurol.* 28, 3289–3302. doi: 10.1111/ene.14965

Holmes, C., Cunningham, C., and Perry, V. H. (2010). Systemic inflammation and disease progression in Alzheimer disease. *Neurology.* 74, 1157–1158. doi: 10.1212/WNL.0b013e3181d5df7f

Hsu, J. T., Tien, C. F., Yu, G. Y., Shen, S., Lee, Y. H., Hsu, P. C., et al. (2021). The effects of $A\beta_{1-42}$ binding to the SARS-CoV-2 spike protein

S1 subunit and angiotensin-converting enzyme 2. *Int. J. Mol. Sci.* 22, 8226. doi: 10.3390/ijms22158226

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., and Hu, Y. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Idrees, D., and Kumar, V. (2021). SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration. *Biochem. Biophys. Res. Commun.* 554, 94–98. doi: 10.1016/j.bbrc.2021.03.100

Ke, Z., Oton, J., Qu, K., Cortese, M., Zila, V., McKeane, L., et al. (2020). Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature.* 588, 498–502. doi: 10.1038/s41586-020-2665-2

Kirtipal, N., Kumar, S., Dubey, S. K., Dwivedi, V. D., Gireesh Babu, K., Malý, P., and Bharadwaj S. (2022). Understanding on the possible routes for SARS CoV-2 invasion via ACE2 in the host linked with multiple organ damage. *Infect. Genet. E*99, 105254. doi: 10.1016/j.meegid.2022.105254

Kiyoshi, C. M., and Zhou, M. (2019). Astrocyte syncytium: a functional reticular system in the brain. *Neural. Regen. Res.* 14, 595–596. doi: 10.4103/1673-5374.247462

Krey, L., Huber, M. K., Höglinger, G. U., and Wegner, F. (2021). Can SARS-CoV2 infection lead to neurodegeneration and Parkinson's disease? *Brain. Sci.* 11, 1654. doi: 10.3390/brainsci11121654

Kuvandik, A., Ozcan, E., Serin, S., and Sungurtekin, H. (2021). Creutzfeldt-Jakob disease after the COVID-19 vaccination. *Turk J. Intensive Care.* doi: 10.4274/tybd.galenos.2021.91885

Letarov, A. V., Babenko, V. V., and Kulikov, E. E. (2021). Free SARS-CoV-2 spike protein S1 particles may play a role in the pathogenesis of COVID-19 infection. *Biochemistry.* 86, 257–261. doi: 10.1134/S0006297921030032

Lim, K. H., Yang, S., Kim, S. H., and Joo, J. Y. (2020). Elevation of ACE2 as a SARS-CoV-2 entry receptor gene expression in Alzheimer's disease. *J. Infect.* 81, e33–e34. doi: 10.1016/j.jinf.2020.06.072

Lingor, P., Demleitner, A. F., Wolff, A. W., and Feneberg, E. (2022). SARS-CoV-2 and neurodegenerative diseases: what we know and what we don't. *J. Neural. Transm.* 129:1155–1167. doi: 10.1007/s00702-022-02500-w

Lu, Y., and Sun, Z. (2022). SARS-CoV-2 molecular diagnostics in China. *Clin. Lab. Med.* 42, 193–201. doi: 10.1016/j.cll.2022.03.003

Lukiw, W. J. (2022). David Hunter Hubel, the 'Circe effect', and SARS-CoV-2 infection of the human visual system. *Front. Biosci.* 27, 7. doi: 10.31083/j.fbl2701007

Lukiw, W. J., Jaber, V. R., Pogue, A. I., and Zhao, Y. (2022). *SARS-CoV-2 invasion and pathological links to prion disease. Biomolecules.* (In press). doi: 10.3390/biom12091253

Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., and He, Q. (2020). Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol.* 77:683–690. doi: 10.1001/jamaneurol.2020.1127

Mercatelli, D., and Giorgi, F. M. (2020). Geographic and genomic distribution of SARS-CoV-2 mutations. *Front. Microbiol.* 11, 1800. doi: 10.3389/fmicb.2020.01800

Molina-Molina, M., and Hernández-Argudo, M. (2022). Respiratory consequences after COVID-19: outcome and treatment. *Rev. Esp. Quimioter.* 35, 67–72. doi: 10.37201/req/s01.16.2022

Mousavizadeh, L., and Ghasemi, S. (2021). Genotype and phenotype of COVID19: their roles in pathogenesis. *J. Microbiol. Immunol. Infect.* 54, 159–163. doi: 10.1016/j.jmii.2020.03.022

Muñoz-Jurado, A., Escribano, B. M., Agüera, E., Caballero-Villarraso, J., Galván, A., and Túnez, I. (2022). SARS-CoV-2 infection in multiple sclerosis patients: interaction with treatments, adjuvant therapies, and vaccines against COVID-19. *J. Neurol.* 269, 4581–4603. doi: 10.1007/s00415-022-11237-1

Oldfield, P. R., Hibberd, J., and Bridle, B. W. (2021). How does severe acute respiratory syndrome-coronavirus-2 affect the brain and its implications for the vaccines currently in use. *Vaccines.* 10, 1. doi: 10.3390/vaccines10010001

Olivo, S., Furlanis, G., Buoite Stella, A., Fabris, M., Milanic, R., Zanusso, G., et al. (2022). Rapidly evolving Creutzfeldt-Jakob disease in COVID-19: from early status epilepticus to fatal outcome. *Acta Neurol. Belg.* 19:1–4. doi: 10.1007/s13760-022-02023-x

Paiardi, G., Richter, S., Oreste, P., Urbinati, C., Rusnati, M., and Wade, R. C. (2022). The binding of heparin to spike glycoprotein inhibits SARS-CoV-2 infection by three mechanisms. *J. Biol. Chem.* 298, 101507. doi: 10.1016/j.jbc.2021.101507

Palacios-Rápalo, S. N., Jesús-González, L. A. D., Cordero-Rivera, C. D., FarfanMorales, C. N., Osuna-Ramos, J. F., et al. (2021). Cholesterol-rich lipid rafts as platforms for SARS-CoV-2 entry. *Front. Immunol.* 12, 796855. doi: 10.3389/fimmu.2021.796855

Piekut, T., Hurła, M., Banaszek, N., Szejn, P., Dorszewska, J., and Kozubski, W. (2022). Infectious agents and Alzheimer's disease. *J. Integr. Neurosci.* 21, 73. doi: 10.31083/j.jin2102073

Pinacchio, C., Scordio, M., Santinelli, L., Frasca, F., Sorrentino, L., Bitossi, C., et al. (2022). Analysis of serum microRNAs and rs2910164 GC single-nucleotide polymorphism of miRNA-146a in COVID-19 patients. *J. Immunoassay Immunochem.* 43, 347–364. doi: 10.1080/15321819.2022.2035394

Piras, M., Cau, F., Manchia, M., Paribello, P., Saba, L., and Suri, J. S. G., et al. (2022). Strong ACE-2 expression in the choroidal vessels: do high choroid plexuses serve as a gateway for SARS-CoV-2 infection on the human brain? *Eur. Rev. Med. Pharmacol. Sci.* 26, 3025–3029. doi: 10.26355/eurrev_202204_28633

Pogue, A. I., and Lukiw, W. J. (2021). microRNA-146a-5p, neurotropic viral infection and prion disease (PrD). *Int. J. Mol. Sci.* 22, 9198. doi: 10.3390/ijms22179198

Ritchien, K., Chan, D., and Watermeyer, T. (2020). The cognitive consequences of the COVID-19 epidemic: Collateral damage? *Brain Commun.* 2, fcaa069. doi: 10.1093/braincomms/fcaa069

Rodriguez-Rivas, J., Croce, G., Muscat, M., and Weigt, M. (2022). Epistatic models predict mutable sites in SARS-CoV-2 proteins and epitopes. *Proc. Natl. Acad. Sci. USA.* 119, e2113118119. doi: 10.1073/pnas.2113118119

Roganović, J. R. (2021). microRNA-146a and−155, upregulated by periodontitis and type 2 diabetes in oral fluids, are predicted to regulate SARS-CoV-2 oral receptor genes. *J. Periodontol.* 92, 35–43. doi: 10.1002/JPER.20-0623

Roy, D., Ghosh, R., Dubey, S., Dubey, M. J., Benito-León, J., and Kanti Ray, B. (2021). Neurological and neuropsychiatric impacts of COVID-19 pandemic. *Can. J. Neurol. Sci.* 48, 9–24. doi: 10.1017/cjn.2020.173

Sah, R., Rodriguez-Morales, A. J., Jha, R., Chu, D. K. W., Gu, H., and Peiris, M. (2020). Complete genome sequence of a 2019 novel coronavirus (SARSCoV-2) strain isolated in Nepal. *Microbiol. Resour. Announc.* 9, e169–e220. doi: 10.1128/MRA.00169-20

Saheera, S., Potnuri, A. G., and Krishnamurthy, P. (2020). Nano-vesicle mis-communication in senescence-related pathologies. *Cells.* 9, 1974. doi: 10.3390/cells9091974

Shahzad, S., and Willcox, M. (2022). The possible role of prion-like viral protein domains on the emergence of novel viruses as SARS-CoV-2. *J. Mol. Evol.* 90, 227–230. doi: 10.1007/s00239-022-10054-4

Shen, W. B., Logue, J., Yang, P., Baracco, L., Elahi, M., Reece, E. A., et al. (2022). SARS-CoV-2 invades cognitive centers of the brain and induces Alzheimer's-like neuropathology. *bioRxiv.* doi: 10.1101/2022.01.31.478476

Sirin, S., Nigdelioglu Dolanbay, S., and Aslim, B. (2022). The relationship of early- and late-onset Alzheimer's disease genes with COVID-19. *J. Neural. Transm.* 129:847–859. doi: 10.1007/s00702-022-02499-0

Song, E., Zhang, C., Israelow, B., Lu-Culligan, A., Prado, A. V., Skriabine, S., et al. (2021). Neuroinvasion of SARS-CoV-2 in human and mouse brain. *J. Exp. Med.* 218, e20202135. doi: 10.1101/2020.06.25.169946

Szabo, M. P., Iba, M., Nath, A., Masliah, E., and Kim, C. (2022). Does SARS-CoV-2 affect neurodegenerative disorders? TLR2, a potential receptor for SARS-CoV-2. *Exp. Mol. Med.* 54:447–454. doi: 10.1038/s12276-022-00755-7

Tavassoly, O., Safavi, F., and Tavassoly, I. (2020a). Heparin-binding peptides as novel therapies to stop SARS-CoV-2 cellular entry and infection. *Mol. Pharmacol.* 98, 612–619. doi: 10.1124/molpharm.120.000098

Tavassoly, O., Safavi, F., and Tavassoly, I. (2020b). Seeding brain protein aggregation by SARS-CoV-2 as a possible long-term complication of COVID-19 infection. *ACS Chem. Neurosci.* 11, 3704–3706. doi: 10.1021/acschemneuro.0c00676

Tayyebi, G., Malakouti, S. K., Shariati, B., and Kamalzadeh, L. (2022). COVID-19-associated encephalitis or Creutzfeldt-Jakob disease: a case report. *Neurodegener. Dis. Manag.* 12, 29–34. doi: 10.2217/nmt-2021-0025

Tetz, G., and Tetz, V. (2022). Prion-like domains in spike protein of SARS-CoV-2 differ across its variants and enable changes in affinity to ACE2. *Microorganisms.* 10, 280. doi: 10.3390/microorganisms10020280

Tisdale, A. K., and Dinkin, M., Chwalisz,. BK. (2021). Afferent and efferent neuro-ophthalmic complications of Coronavirus disease 19. *J. Neuroophthalmol.* 41, 154–165. doi: 10.1097/WNO.0000000000001276

Vigasova, D., Nemergut, M., Liskova, B., and Damborsky, J. (2021). Multi-pathogen infections and Alzheimer's disease. *Microb. Cell. Fact.* 20, 25. doi: 10.1186/s12934-021-01520-7

Visco, V., Vitale, C., Rispoli, A., Izzo, C., Virtuoso, N., and Ferruzzi, G. J. (2022). Post-COVID-19 syndrome: involvement and interactions between respiratory, cardiovascular and nervous systems. *J. Clin. Med.* 11, 524. doi: 10.3390/jcm11030524

Wu, F., Zhao, S., Yu, B., Chen, Y. M., Wang, W., and Song, Z. G. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*. 579, 265–269. doi: 10.1038/s41586-020-2008-3

Young, M. J., O'Hare, M., Matiello, M., and Schmahmann, J. D. (2020). Creutzfeldt-Jakob disease in a man with COVID-19: SARS-CoV-2-accelerated neurodegeneration? *Brain Behav. Immun.* 89, 601–603. doi: 10.1016/j.bbi.2020.07.007

Zetterberg, H., and Schott, J. M. (2022). Blood biomarkers for Alzheimer's disease. *Acta Neurol. Scand*. 146:51–55. doi: 10.1111/ane.13628

Zhao, Y., Jaber, V., Alexandrov, P. N., Vergallo, A., Lista, S., Hampel, H., et al. (2020). microRNA-based biomarkers in Alzheimer's disease (AD). *Front. Neurosci.* 14, 585432. doi: 10.3389/fnins.2020.585432

Zhao, Y., Li, W., and Lukiw, W. J. (2021). Ubiquity of the SARS-CoV-2 receptor ACE2 and upregulation in limbic regions of Alzheimer's disease brain. *Folia Neuropathol.* 59, 232–238. doi: 10.5114/fn.2021.109495

Zhao, Y., and Lukiw, W. J. (2022). SARS-CoV-2 neuroinvasion, inflammatory neurodegeneration and Alzheimer's disease. *Front. Cell Neurosci.* 16, 937961. doi: 10.3389/fncel.2022.937961

Zhou, Y., Xu, J., Hou, Y., Leverenz, J. B., Kallianpur, A., Mehra, R., et al. (2021). Network medicine links SARS-CoV-2/COVID-19 infection to brain microvascular injury and neuroinflammation in dementia-like cognitive impairment. *Alzheimers Res. Ther*. 13, 110. doi: 10.1186/s13195-021-00850-3

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 19 October 2022
DOI 10.3389/fneur.2022.1001429

OPEN ACCESS

EDITED BY
David Pozo,
University of Seville, Spain

REVIEWED BY
Alessia Bianchi,
University of Palermo, Italy
Jan Hendrik Schaefer,
Goethe University Frankfurt, Germany

*CORRESPONDENCE
Belén Pilo De La Fuente
belenpf@gmail.com

SPECIALTY SECTION
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

RECEIVED 23 July 2022
ACCEPTED 30 August 2022
PUBLISHED 19 October 2022

CITATION
Pilo De La Fuente B, González Martín-Moro J, Martín Ávila G, Méndez Burgos A, Ramos Barrau L, Thuissard I, Torrejón Martín M and Aladro Benito Y (2022) Risk and prognostic factors for SARS-CoV-2 infection in Spanish population with multiple sclerosis during the first five waves. *Front. Neurol.* 13:1001429. doi: 10.3389/fneur.2022.1001429

Risk and prognostic factors for SARS-CoV-2 infection in Spanish population with multiple sclerosis during the first five waves

Belén Pilo De La Fuente[1,2]*, Julio González Martín-Moro[3,4], Guillermo Martín Ávila[1], Alejandro Méndez Burgos[1], Laura Ramos Barrau[2], Israel Thuissard[2], Marta Torrejón Martín[1] and Yolanda Aladro Benito[1,2]

[1]Department of Neurology, Getafe University Hospital, Madrid, Spain, [2]Faculty of Biomedical and Health Sciences, European University of Madrid, Madrid, Spain, [3]Department of Ophthalmology, Hospital Universitario del Henares, Madrid, Spain, [4]Faculty of Medicine, Universidad Francisco de Vitoria, Madrid, Spain

Background: Data on coronavirus disease 2019 (COVID-19) incidence in patients with multiple sclerosis (MS) during the first wave have been published but are scarce for the remaining waves. Factors associated with COVID-19 infection of any grade are also poorly known. The aim of this study was to analyze the incidence, clinical features, and risk factors for COVID-19 infection of any grade in patients with MS (pwMS) during waves 1–5.

Methods: This study prospectively analyzes the cumulative incidence of COVID-19 from the first to the fifth waves by periodic case ascertainment in pwMS followed at the University Hospital of Getafe (UHG). Global and stratified cumulative incidence was calculated. Logistic regression models were used to estimate the weight of selected variables as risk and prognostic factors.

Results: We included 431 pwMS, of whom 86 (20%) were infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The overall cumulative incidence of confirmed cases was similar to that of Madrid (13,689 vs. 13,307 per 100,000 habitants) but 3 times higher during the first wave and slightly lower from the second to the fifth waves. The majority (86%) of pwMS developed mild forms of COVID-19. Smoking was the only factor associated with a decreased risk of SARS-CoV2 infection of any grade [odds ratio (OR) 0.491; 95% CI 0.275–0.878; $p = 0.017$]. Risk factors associated with severe forms were Expanded Disability Severity Scale (EDSS) ≥3.5 (OR 7.569; 95% CI 1.234–46.440) and pulmonary disease (OR 10.763; 95% CI 1.27–91.254).

Conclusion: The incidence of COVID-19 was similar in this MS cohort to the general population. Smoking halved the risk of being infected. Higher EDSS and pulmonary comorbidity were associated with an increased risk of severe forms.

KEYWORDS

incidence, multiple sclerosis, COVID-19, severity, coronavirus

Introduction

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, China (1). Since then, it has spread rapidly around the world causing significant morbidity and mortality. Patients with multiple sclerosis (pwMS) represent a population of particular interest in this pandemic context due to the nature of their disease and the use of a wide range of immunologically active drugs (2). On the one hand, disease-modifying therapies (DMTs) have immunosuppressive effects that could hamper an effective immune response to the infection (3, 4). On the other hand, immunosuppression could offer protection by downregulating hyperinflammation and the cytokine storm associated with coronavirus disease 2019 (COVID-19) (5–7).

The Multiple Sclerosis International Federation has made recommendations regarding the risk of COVID-19 in pwMS, with a specific statement on DMTs (Multiple Sclerosis International Federation, 2021, https://www.msif.org). In addition, national MS societies have published guidelines that stratify the risk of the accepted treatments (8, 9).

Very few population-based studies on the incidence of COVID-19 in pwMS compared to the general populations have been published (10). Two recent studies, from Scotland and Brazil, have reported similar incidences of infection in pwMS and the general population (11, 12).

Older age, male sex, comorbidities (e.g., obesity, diabetes, hypertension, cardiovascular, and pulmonary disease), non-ambulatory status, and progressive forms have been suggested as risk factors for severe forms of COVID-19 (3, 13, 14). However, risk factors for developing any degree of SARS-CoV-2 infection in pwMS are unknown. Therefore, assessing the risk of COVID-19 in these patients is an important public health issue (15).

The purpose of this study was to compare the cumulative incidence of COVID-19 in pwMS and in the general population of Madrid from the first to fifth waves and compare it to the cumulative incidence in the general population of Madrid region and determine risk and prognostic factors associated with infection in these patients.

Methods

Study design and population

We conducted an ambispective (retrospective during the first wave, and prospective from second to fifth waves) observational study on all pwMS and other demyelinating disorders currently followed at the University Hospital of Getafe (UHG). UHG is a Public Health Hospital located in the south of the community of Madrid with an assigned population of 226,666 habitants (https://www.comunidad.madrid/hospital/getafe). It has a multiple sclerosis (MS) expertise unit since more than 20 years ago.

All patients with the following diagnosis were included: radiologically isolated syndrome (RIS), clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), or primary progressive MS (PPMS).

The sources of COVID-19 ascertainment were (a) asking patients during their scheduled MS consultation, usually every 6 months; and (b) periodic review of all medical documentation (neurological, emergency, and primary treating physician's reports) existing in the Public Health System since 1 March 2020. All Public Hospitals in Madrid Community are connected to each other and to the primary treating physicians through a well-developed computerized network. In this network, all emergency care reports, as well as all testing for SARS-CoV2 performed in the Public Health System, and primary treating physician's reports are available. A final review of medical reports of all patients was performed at the close of the study on 28 September 2021.

The study adhered to the Declaration of Helsinki principles was approved by the Getafe University Hospital Ethics committee and did not receive any financial support.

Selected variables

We collected patients' baseline characteristics on 1 March 2020: Expanded Disability Severity Scale (EDSS) score at baseline, number of relapses in the previous year, radiological activity (defined as the presence of new T2 lesions and/or T1 gadolinium-enhanced lesions), current DMT use, current lymphocyte count, and comorbidities (hypertension, diabetes, dyslipidemia, smoking, cardiovascular, and pulmonary disease).

Lymphopenia was defined as grade 1: absolute lymphocytes count (ALC) of 800–999/μl; grade 2: ALC of 500–799/μl; grade 3: ALC of 200–499/μl; and grade 4: ALC of <200/μl.

Disease-modifying therapies were grouped according to potential infection risk (no risk: interferon beta and glatiramer acetate; low risk: teriflunomide, azathioprine, dimethyl fumarate, and natalizumab; intermediate or high risk: fingolimod, anti-CD20 therapies, cladribine, and alemtuzumab), as proposed in previous studies (14, 16).

Following the European Center for Disease Prevention and Control Guidance, patients with fever, dyspnea, cough, or sudden onset of anosmia, ageusia, or dysgeusia after February 2020 were considered possible cases; those with clinical criteria, radiological criteria (ground-glass opacities), or an epidemiological link were defined as probable cases and those with a positive SARS-CoV-2 laboratory test [polymerase chain reaction (PCR) or rapid nucleocapsid protein antigen detection (RAD)] in nasopharyngeal swab or with demonstrated SARS-CoV-2 antibodies (IgG or IgM) in a blood sample) were established as confirmed cases (*Case Definition of Coronavirus*

2019 (COVID-19) as of 3 December 2020. https://Www.Ecdc.Europa.Eu/En/Covid-19/Surveillance/Case-Definition. Accessed 27 November 2021., n.d.). Patients were reclassified according to the COVID-19 case definition in confirmed or suspected (which included possible and probable) cases.

Regarding COVID-19, we collected symptoms and laboratory and radiological results. To study the severity of infection, we used the COVID-19 severity score proposed by Louapre et al. (14) based on a 7-point ordinal scale in which 1 indicated that the patient was not hospitalized and had no limitations on activities; 2 indicated that the patient was not hospitalized but had a limitation on activities; 3 indicated that the patient was hospitalized but did not require supplemental oxygen; 4 indicated that the patient was hospitalized and required supplemental oxygen; 5 indicated that the patient was hospitalized and received non-invasive ventilation or high-flow oxygen; 6 indicated that the patient was hospitalized and received invasive mechanical ventilation or extracorporeal membrane oxygenation; and 7 indicated death. The mild disease was defined as patients who did not require hospitalization (Louapre severity scores of 1 and 2), and moderate-severe disease was considered when patients were hospitalized (Louapre severity scores of 3–7).

The date of diagnosis was also collected and the first five waves in Spain were included until 28 September 2021 (first wave from 1 March 2020 to 30 June 2020; second wave from 1 July 2020 to 1 December 2020; third wave from 2 December 2020 to 9 March 2021; fourth wave from 10 March 2021 to 22nd June 2021; fifth wave from 23 June 2021 to 28 September 2021).

In addition, the SARS-CoV-2 vaccination history was reviewed since the COVID-19 vaccination campaign in Spain began on 1 January 2021 (vaccine brand and date of vaccination if applicable were recorded).

Statistical analysis

Baseline data were compared between patients who were infected with SARS-CoV-2 (COVID+) and those who were not (COVID–). Group comparisons were performed using chi-square (χ^2) (or Fisher's exact test) for categorical data and Student's *t*-test (or Mann–Whitney *U* test) for continuous data. Any two-sided $p < 0.05$ was considered statistically significant.

Univariate and multivariate logistic regression models were developed to assess the association between demographic and clinical characteristics with SARS-CoV-2 infection and with the severity of COVID-19 (mild vs. moderate-severe). Age, sex, MS phenotype (relapsing vs. progressive), EDSS, clinical and radiological activity, DMT level, and comorbidities were entered into the model. Variables with *p*-values ≤ 0.1 in the univariate analysis were entered into the multivariate model and those with *p*-values ≤ 0.05 were retained. Results were expressed as odds ratios (OR) and 95% CIs.

A Cox model was developed to assess the association between demographic and clinical characteristics with SARS-CoV-2 infection.

Data were analyzed using the Statistical Package for Social Sciences, version 22.0 (IBM SPSS, Inc., Chicago, IL, USA).

Given that Getafe belongs to the community of Madrid, we used the Madrid population as a reference. Cumulative incidence in Madrid was extracted from the official epidemiologic database from the city generated by a local health system, which is updated weekly (https://www.comunidad.madrid/covid-19). As at the beginning of the pandemic diagnostic capacity was very limited, first-wave data were less valuable. For this reason, the Madrid health council analyzed separately the first and posterior waves. Global cumulative incidence until 28 September 2021 has been calculated in the MS cohort and in the general Madrid population only with confirmed cases (suspected cases were not registered in Madrid).

For second and posterior waves, cumulative incidence was adjusted by sex and age to the Madrid population. Data series of Madrid confirmed cases (positive PCR, antigen test, or antibody test) were analyzed independently and compared with data obtained from the MS cohort.

Epidemiological analysis of the data was done using Excel (Microsoft 365 MSO version 2201).

Results

On 28 September 2021, 431 pwMS-related disorders were followed at UHG. The baseline characteristics of the cohort are summarized in Table 1 (in global population, as well as in COVID+ and COVID–). Briefly, 299 (69.4%) of pwMS were women. The median age was 47.1 years (range 18–81.9), median disease duration was 12.3 years (range 0–47.9), and median EDSS score was 2 (range 0–9.5). A total of 85 (19.7%) received intermediate or high-risk DMT,88 (20.4%) had progressive forms of the disease, and 264 (61.3%) had no evidence of disease activity (NEDA-3). Comorbidities and basal lymphocyte count of the cohort are detailed in Table 1. A lower percentage of smoking patients was found in COVID+ population (21.3 vs. 35.5% in patients with COVID, $p = 0.017$). No other significant differences were found.

Characteristics of SARS-CoV-2 infection in the MS cohort

On 28 September 2021, 86 (20%) pwMS had suffered from COVID-19 since the beginning of the pandemic. Figure 1

TABLE 1 Demographic, clinical characteristics, comorbidities, and basal lymphocyte count of pwMS-related disorders followed in University Getafe Hospital, as of 28 September 2021.

Characteristics	Global population ($n = 431$)	COVID+ population ($n = 86$)	COVID- population ($n = 345$)	*p*-value
Female, *n* (%)	299 (69.4)	62 (72.1)	237 (68.7)	*0.602*
Age at pandemic onset, median (range)	47.1 (19–81.9)	46 (19–80.1)	47.6 (19–81.9)	*0.485*
Disease duration, y, median (range)	12.3 (0–47.9)	12.4 (0–47.9)	12.2 (0–47.2)	*0.501*
Last EDSS score, median (range)	2 (0–9.5)	2 (0–9.5)	2 (0–9.5)	*0.719*
Disease-modifying treatment, *n* (%)				*0.945*
- No treatment	138 (32)	30 (34.9)	108 (31.3)	
- No risk treatment	98 (22.7)	19 (22.1)	79 (22.9)	
- Low-risk treatment	110 (25.5)	21 (24.4)	89 (25.8)	
- Moderate-high risk treatment	85 (19.7)	16 (18.6)	69 (20)	
MS type, *n* (%)				*0.631*
- RRMS	321 (74.5)	69 (80.2)	252 (73)	
- SPMS	66 (15.3)	12 (14)	54 (15.7)	
- PPMS	22 (5.1)	3 (3.5)	19 (5.5)	
- CIS	14 (3.2)	2 (2.3)	12 (3.5)	
- RIS	8 (1.9)	0 (0)	8 (2.3)	
Disease activity, *n* (%)				
- patients with MS relapses	51 (11.8)	9 (10.4)	42 (12.2)	*0.852*
- patients with EDSS progression	73 (16.9)	16 (18.4)	(16.5)	*0.632*
- patients with radiological activity	109/413 (27)	20/82 (24.4)	89/331 (26.9)	*0.678*
- patients with NEDA-3	264 (61.3)	47 (54.7)	217 (62.9)	*0.174*
Comorbidities, *n* (%)				
- Smoking	134/410 (31.1)	17/80 (21.3)	117/330 (35.5)	*0.017*
- Hypertension	71 (16.5)	11 (12.8)	60 (17.4)	*0.335*
- Diabetes	18 (4.2)	2 (2.3)	16 (4.6)	*0.546*
- Cardiovascular disease	11 (2.6)	3 (3.5)	8 (2.3)	*0.465*
- Pulmonary disease	30 (7)	7 (8.1)	23 (6.7)	*0.637*
Basal lymphocyte count, *n* (%)				*0.163*
- No lymphopenia	364/429 (84.8)	69 (80.2)	295/343 (86)	
- Lymphopenia grade 1	30/429 (7)	7 (8.1)	23/343 (6.7)	
- Lymphopenia grade 2	24/429 (5.6)	6 (7)	18/343 (5.2)	
- Lymphopenia grade 3	11/429 (2.6)	4 (4.7)	7/343 (2)	
- Lymphopenia grade 4	0 (0)	0 (0)	0 (0)	

EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; CIS, clinically isolated syndrome; RIS, radiologically isolated syndrome; NEDA-3, no evidence of disease-activity-3.

summarizes the main symptoms, being fever (64.3%) and cough (63.9%) the most frequent ones. Of these 86 patients, 59 (68.6%) were confirmed cases and 27 (31.4%) were suspected COVID-19 cases. Figure 2 represents the number of cases of each wave, with cases broken down by the referred diagnostic criteria. Most suspected cases 24/27 (89%) belong to the first wave due to the limited availability of tests during this period. Only 12 (14%) of all cases suffered severe forms that required hospitalization, and half of them were infected during the first wave. Only one of the 431 patients died (during the second wave). None of the patients needed intensive care unit admission. Only one patient (1.2%), who was infected during the second wave (1.2%), required high-flow oxygen. Figure 3 represents the evolution of severity along different waves.

One (1.2%) patient had COVID-19 twice (during first and second waves). Between January 2021 (when the vaccination campaign started in Madrid) and 28 September 2021, 88.4% (375/424) of pwMS were completely vaccinated. Four (4.7%) patients had COVID-19 after vaccination, and one of them had 2 weeks after the first dose. None of them received moderate or high-risk treatment (one had no treatment, two had interferons, and one had glatiramer acetate).

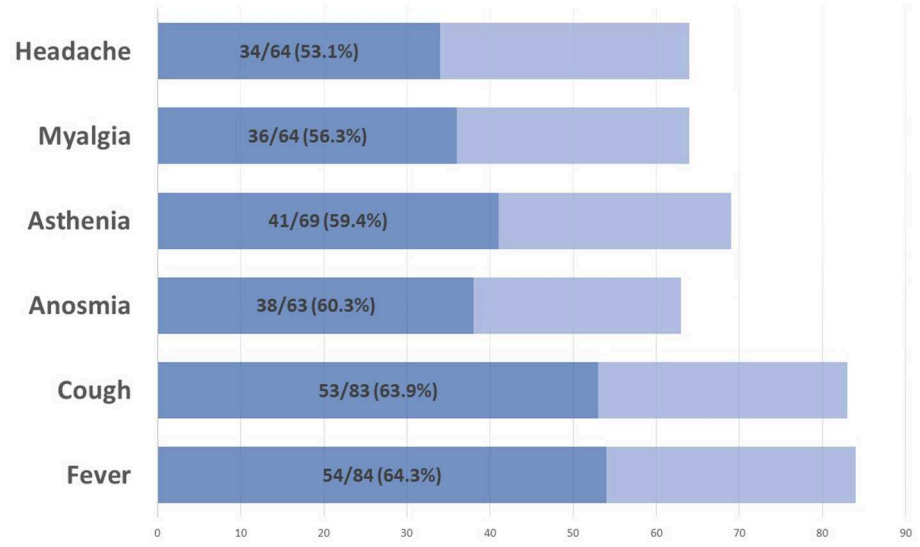


FIGURE 1
Main COVID-19 symptoms.

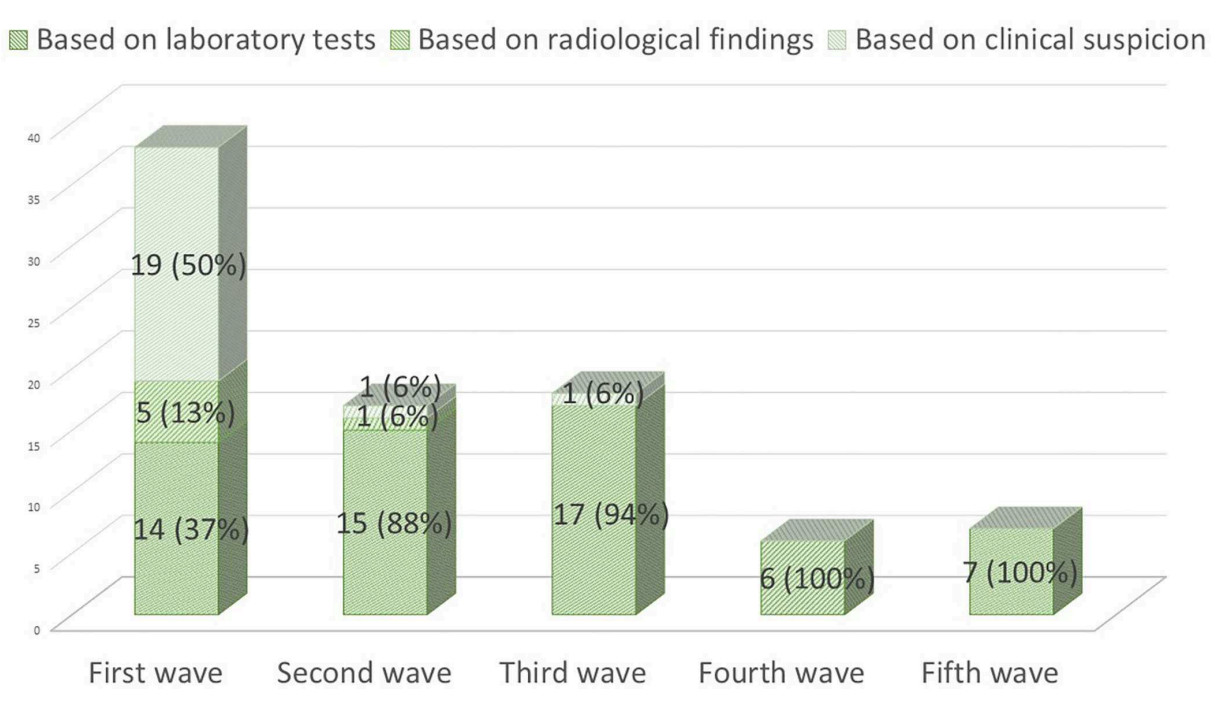


FIGURE 2
COVID-19 cases during the first five waves (1 March 2020 to 28 September 2021) and diagnostic criteria used.

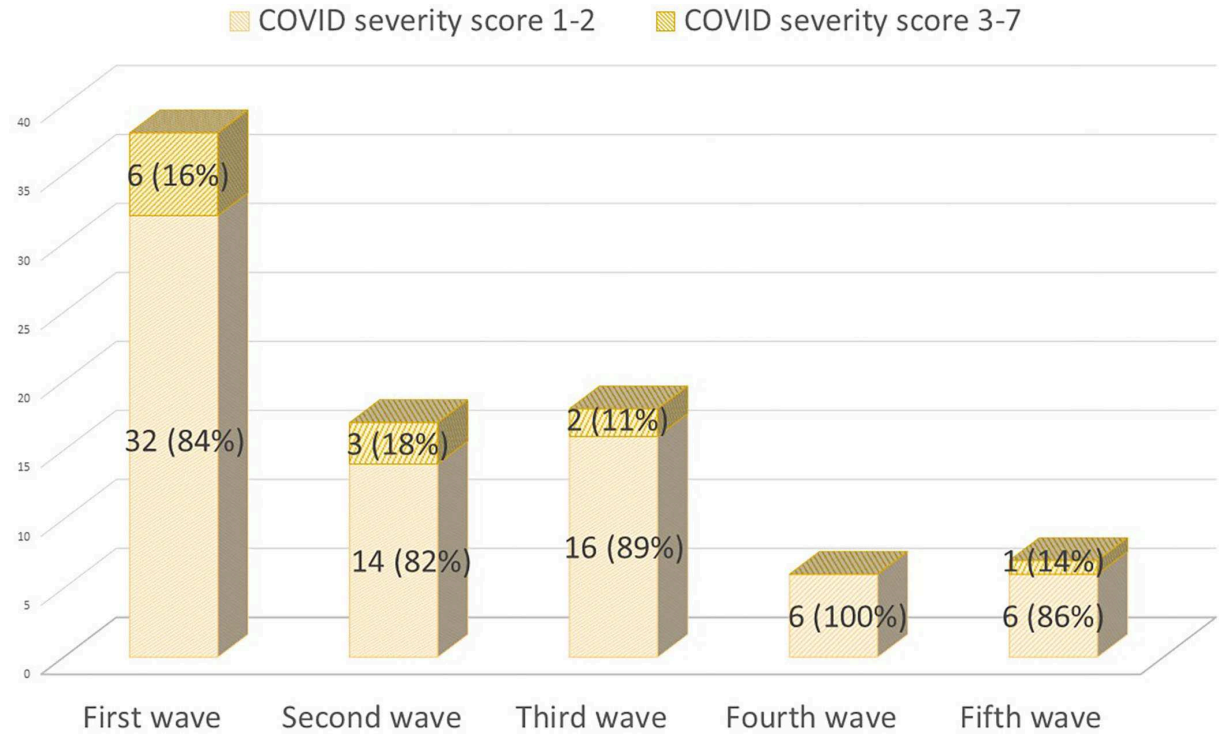


FIGURE 3
COVID-19 cases during the first five waves (1 March 2020 to 28 September 2021) classified by their severity [(14); severity scale: 1–2 mild cases; 3–7 severe cases who required hospitalization].

Cumulative incidence of SARS-CoV-2 infection

The cumulative incidence of confirmed cases in the MS cohort until 28 September 2021 (including five waves) was similar to that of the Madrid general population (13,689 per 100,000 habitants vs. 13,307 per 100,000 habitants, $p = 0.815$) (Table 2A).

During the first wave, the cumulative incidence was three times higher in the population with MS (3,248 vs. 1,029, $p < 0.001$; men 2,273 vs. 985; women 3,679 vs. 1,068) (Table 2A). During the second and posterior waves, the cumulative incidence was slightly lower in the MS cohort than in the general population but not statistically significant (10,441 vs. 12,278, $p = 0.245$) (Table 2A).

In the analysis of age and sex-adjusted cumulative incidences of confirmed cases during the second to fifth waves, significant heterogenicity was found among different age groups (Table 2B). For instance, the higher cumulative incidence was found in men aged between 25 and 44 years in the MS group (18,750 vs. 14,247 in the general population), whereas the lower cumulative incidence was observed in women aged above 65 years in the MS group (3,333 vs. 7,937 in the general population).

Variables associated with COVID-19 infection

Only smoking was associated with a decreased risk of SARS-CoV-2 infection (OR 0.491; 95% CI 0.275–0.878; $p = 0.017$). No other factors, neither demographic, clinical, disability, comorbidity, nor treatment was associated with the risk of SARS-CoV-2 infection (Figure 4).

The Cox proportional hazard regression analysis replicated the results of the logistic regression model. Again, only smoking was associated with a reduced risk of SARS-CoV-2 infection [hazard ratio (HR) 0.497; 95% CI 0.287–0.866; $p = 0.012$] with 77.2% patients with COVID in the non-smoker group and 88% patients with COVID in the smoker group as of 28 September 2021 (Figure 5).

Variables associated with severe COVID-19

Significant risk factors associated with severe forms of COVID-19 (that required hospitalization) in the univariate logistic regression models were EDSS progression (OR 4.091; 95% CI 1.099–15.226; $p = 0.036$), age (OR per 10 years 2.594;

TABLE 2A Sex-adjusted cumulative incidences of COVID-19 in our MS cohort and in Madrid population during the first wave, during second to fifth waves, and global (first to fifth waves).

Age group	Sex	Cumulative incidence in Madrid (1st wave) per 100,000 hab. (95% IC)	Cumulative incidence in MS (1st wave) per 100,000 hab. (95% IC)	Cumulative incidence in Madrid (2nd−5th waves) per 100,000 hab. (95% IC)	Cumulative incidence in MS (2nd−5th waves) per 100,000 hab. (95% IC)	Global cumulative incidence in Madrid per 100,000 hab. (95% IC)	Global cumulative incidence in MS group per 100,000 hab. (95% IC)
Total	M	985	2,273	12,373	12,121	13,358	14,394
		(974–996)	(469–6,642)	(12,335–12,411)	(6,928–19,684)	(13,319–13,398)	(8,666–22,478)
	F	1,068	3,679	12,191	9,699	13,260	13,378
		(1,057–1,079)	(1,837–6,583)	(12,155–12,228)	(6,496–13,929)	(13,323–13,298)	(9,557–18,217)
	Total	1,029	3,248	12,278	10,441	13,307	13,689
		(1,021–1,036)	(1,776–5,450)	(12,252–12,305)	(7,616–13,971)	(13,279–13.334)	(10,421–17,658)

TABLE 2B Age and sex-adjusted cumulative incidences of COVID-19 in our MS cohort and in Madrid population during second to fifth waves.

Age group	Sex	Cumulative incidence in Madrid (2nd−5th waves) per 100,000 habitants (95% IC)	Cumulative incidence in MS group (2nd−5th waves) per 10,000 habitants (95% IC)
15–24	M	18,604 (18,461–18,749)	–
	F	19,462 (19,314–19,611)	–
	Total	19,029 (18,925–19,132)	–
25–44	M	14,247 (14,172–14,323)	18,750 (8,574–35,593)
	F	14,582 (14,507–14,658)	13,008 (7,435–21,124)
	Total	14,418 (14,365–14,472)	14,620 (9,461–21,582)
45–64	M	11,401 (11,332–11,470)	8,824 (3,238–19,205)
	F	11,316 (11,250–11,381)	8,633 (4,461–15,080)
	Total	11,357 (11,309–11,404)	8,696 (5,154–13,743)
≥65	M	8,590 (8,509–8,671)	7,692 (195–42,859)
	F	7,937 (7,871–8,003)	3,333 (84–18,572)
	Total	8,208 (8,157–8,259)	4,651 (563–16,802)
Total	M	12,373 (12,335–12,411)	12,121 (6,928–19,684)
	F	12,191 (12,155–12,228)	9,699 (6,496–13,929)
	Total	12,278 (12,252–12,305)	10,441 (7,616–13,971)

95% CI 1.411–4.766; $p = 0.002$), pulmonary comorbidity (OR 5.833; 95% CI 1.120–30.375; $p = 0.036$), progressive course (OR 11.55; 95% CI 2.958–45.098; $p < 0.001$), and EDSS ≥3.5 (OR 7.867; 95% CI 2.086–29.664; $p = 0.002$) (Figure 6). In the multivariate logistic regression model, EDSS ≥ 3.5 (OR 7.569; 95% CI 1.234–46.440; $p = 0.029$), pulmonary comorbidity (OR 10.763; 95% CI 1.27–91.254; $p = 0.029$), and age (OR per 10 years 1.692; 95% CI 0.886–3.219; $p = 0.113$) were retained (Figure 6).

Discussion

This observational study of COVID-19 incidence in a cohort including all pwMS followed in a hospital of Madrid has many strengths. First, case ascertainment bias is likely to be small because it is based on a systematic review of all patients in the cohort. They were regularly interrogated about their COVID-19 experience during their medical visits and, aside from patients' anamnesis, primary physician's medical, emergency, and hospitalization reports were reviewed periodically until 28 September 2021, to detect unreported mild cases and to integrate all cases up to that date. Second, this analysis has incorporated data until 28 September 2021, including the first five waves, which enables us to analyze the pandemic evolution in our MS cohort with more accurate data due to the higher percentage of confirmed cases and its chronological comparison with the vaccination campaign in Madrid.

We found a cumulative incidence of confirmed cases in the MS cohort until 28 September 2021, similar to that of the Madrid population, higher during the first wave and lower in the second and subsequent waves. Sepúlveda et al. also found an incidence of confirmed COVID-19 similar to that of the Barcelona population (16) and a 2-fold higher cumulative incidence when all cases (confirmed and suspected) were included. Nevertheless, their data are restricted to the first wave until 18 June 2020. In this period, we found a 3-fold higher cumulative incidence than that observed in the Madrid population, including only confirmed cases. These differences may be explained by the different case ascertainments, based on questionnaires completed by patients vs. systematic and periodic

Factors	UNIVARIATE OR (95% CI)	p value	MULTIVARIATE OR (95% CI)	p value
Male	0.849 (0.503 - 1.433)	0.541		
Relapse previous year	0.843 (0.394 - 1.807)	0.661		
Radiological activity	0.877 (0.501 - 1.535)	0.646		
EDSS progression	1.155 (0.626 - 2.132)	0.645		
Disease activity	1.407 (0.873 - 2.268)	0.161		
Age (per 10 y)	0.932 (0.761 - 1.138)	0.489		
Smoking	0.491 (0.275 - 0.878)	**0.017**	0.491 (0.275 - 0.878)	0.017
Hypertension	0.697 (0.349 - 1.391)	0.305		
Diabetes	0.490 (0.110 - 2.171)	0.347		
Cardiovascular comorbidity	1.523 (0.395 - 5.864)	0.541		
Pulmonary comorbidity	1.241 (0.514 - 2.994)	0.632		
Progressive course	0.801 (0.433 - 1.481)	0.479		
EDSS >3	0.989 (0.580 - 1.686)	0.968		
Basal lymphocytes	1.000 (0.999 - 1.000)	0.882		
Treatment				
No (ref)				
No risk treatment	0.866 (0.455 - 1.648)	0.661		
Low risk treatment	0.849 (0.455 - 1.586)	0.608		
Moderate-high risk treatment	0.835 (0.424 - 1.644)	0.602		

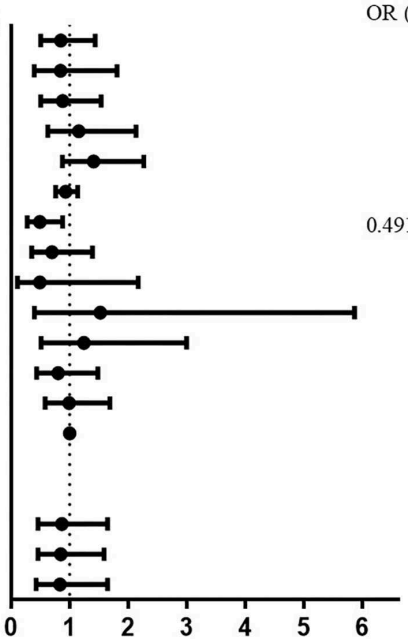


FIGURE 4
Risk factors of SARS-CoV-2 infection (univariate and multivariate analyses).

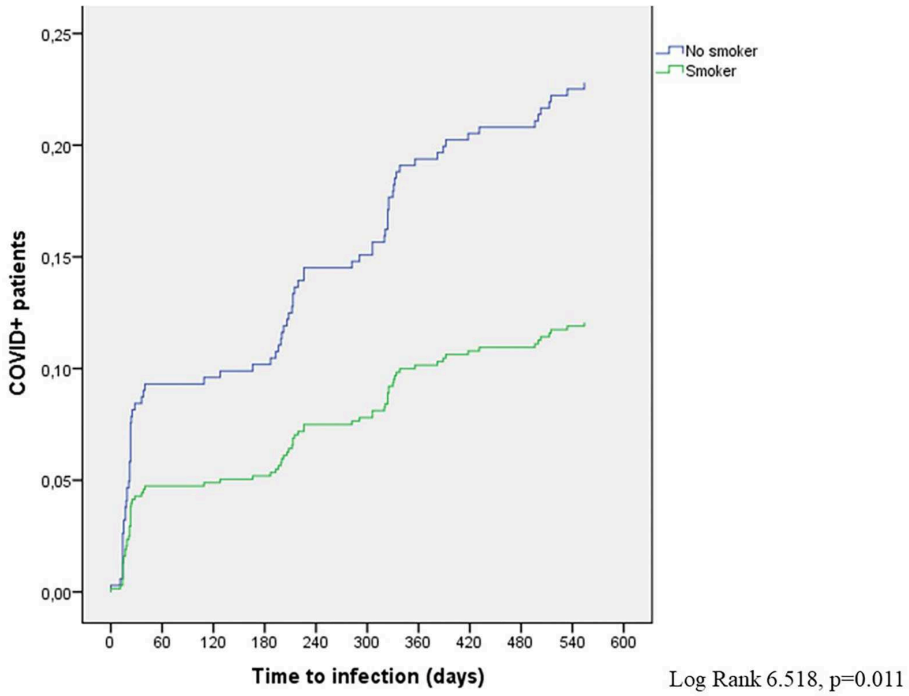


FIGURE 5
COVID+ patients in the smoker and non-smoker subgroups (Cox model).

10.3389/fneur.2022.1001429

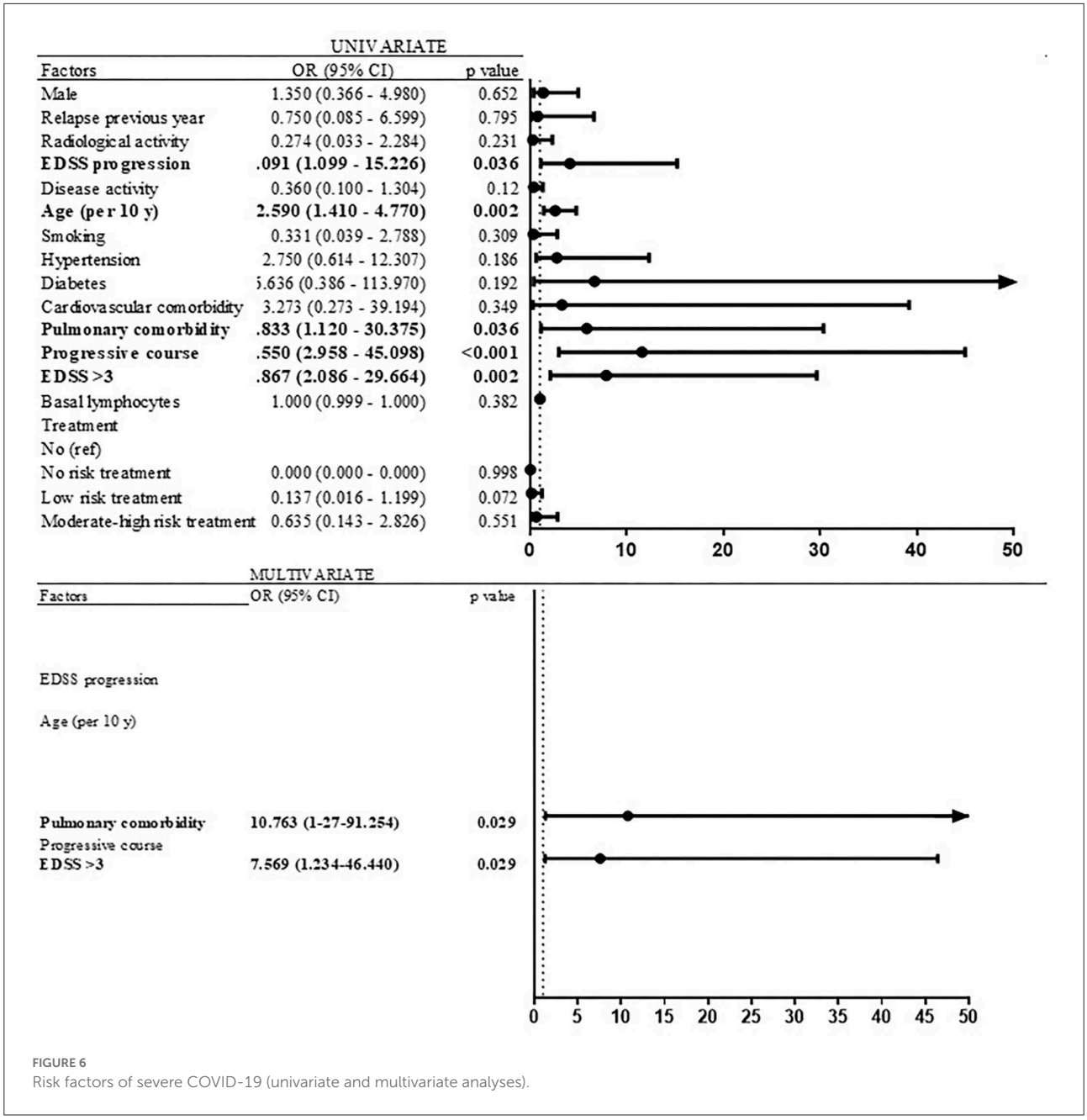


FIGURE 6
Risk factors of severe COVID-19 (univariate and multivariate analyses).

reviews of medical records and direct interviews with patients in our study.

Crescenzo et al. reported a frequency measure of confirmed COVID-19 in pwMS 2.5 times higher than that reported in the inhabitants of the Veneto region (1 vs. 0.4%) (17). Infection rates during the first wave are not comparable for multiple reasons, and the pressure of care and availability of diagnostic tests differ in each country and regions of each country. The higher incidence of confirmed infection in MS cohorts could be explained in part by increased surveillance and testing to optimize the surveillance of patients, theoretically more susceptible to severe infection.

In the analysis of the waves' evolution since the beginning of the pandemic, we can observe a progressive reduction in the proportion of suspected cases and a progressive increase in the percentage of confirmed cases that can account for the lack of diagnostic tests during the first months and its posterior progressive availability. During the first wave, only 37% of our patients had a positive confirmatory laboratory test, data slightly inferior to the percentages observed in other series: 45.3% (39

out of 86 patients) in the Dutch cohort (13) and 42.1% (146 out of 347) in the French registry (14). The different methodology used (register vs. systematic review of a cohort) and the scarce availability of tests during that period (in which laboratory tests were usually reserved for more severe cases) may account for the differences observed.

In addition, the analyses of the waves' evolution show a progressive downward trend in the number of infected patients, more pronounced from the third wave onward, coinciding with the beginning of the vaccination campaign in Madrid. All approved vaccines have demonstrated to be effective in reducing the risk of COVID, especially the risk of severe COVID-19 and hospitalization, leaving no doubts about the risk/benefit ratio of vaccination in the current pandemic (18). Our findings also support these recommendations. In our cohort, 88.4% of pwMS have been vaccinated, similar to the 86.7% vaccination rate of the general population in Madrid until 28 September 2021. In Spain, the vaccination has had good acceptance, with high vaccination rates since the beginning of the campaign.

In our cohort, no differences were found in demographic characteristics, MS clinical profile, MS activity, DMT, and basal lymphocyte count between COVID+ and COVID− subgroups. Zabalza et al. through an email survey with 758 valid respondents determined that age, contact with a confirmed case, residence in Barcelona, MS duration, and time on CD20 treatment were independent factors for presenting COVID-19 in a multivariable model (19). In our series, only smoking was associated with SARS-CoV-2 infection, as a protective factor. In the literature, data on the relationship between smoking and COVID-19 are contradictory and inconclusive (20). Smoking is well-established as having an adverse impact on lung health, and some authors have described that patients with a smoking history have a higher likelihood of developing more severe symptoms of COVID-19 and worse in-hospital outcomes than non-smokers (21). In contrast, the prevalence of current smokers among hospitalized patients with COVID-19 has been reported consistently lower than that observed in the general population (22, 23) and, consequently, some authors conclude that current smokers appear to be at a reduced risk of SARS-CoV-2 infection, compared with non-smokers (22–24). The association between tobacco and SARS-CoV-2 infection seems to be complex. On the one hand, tobacco may worsen the prognosis of those patients with a long history of smoking but may behave as a protective factor in patients with a short history of tobacco smoking who have not yet developed lung pathology. Although the mechanism involved in this protective factor is poorly understood, Polverino et al. postulated that cigarette smoke or nicotine stimulation may modify angiotensin-converting enzyme 2 (ACE2) expression (22). It cannot be ruled out a direct effect of tobacco smoke. The oxidizing effect of tobacco smoke or the heat emitted by the cigarette could also have a viricidal effect. In any case, this association may not imply a true or causal relationship, and smoking is not advocated as a prevention or treatment of COVID-19 (24).

In our cohort, EDSS $\geq$3.5 and pulmonary comorbidity were associated with more severe forms. In the North American Registry, increased disability (defined as non-ambulatory) was independently associated with hospitalization, as well as age, black race, cardiovascular disease, diabetes, and obesity (25). During the first wave, Loonstra et al. reported that among the Dutch population, pwMS with COVID-19 who were hospitalized were older, were more often male, and had secondary-progressive MS, higher EDSS score, and more comorbidity compared to nonhospitalized patients (13). They did not find any association between severity of COVID-19 and low lymphocyte count, as we did not find (13). In the French Covisep registry, Louapre et al. detected that age, EDSS $\geq$ 6, and obesity were independent variables for severe COVID-19 forms in the multivariate logistic regression model (14).

A total of 12 (13.9%) patients were hospitalized in our series, whereas Sahraian et al. reported 25% (2) and Parrotta reported 24% (3) of COVID hospitalization rate in pwMS. Our data are closer to the admission rate in the general population of Madrid, where 13.85% of infected patients required hospitalization as of 28 September 2021. Nevertheless, this data depend on the diagnostic capacity of each region and should be viewed with caution. Landtblom et al. also reported a similar risk of more severe COVID-19 outcomes in pwMS compared to the general population in the Swedish MS registry (SMSreg) (10).

In our cohort, one (1.2% of total and 1.7% of confirmed cases) patient died. This percentage is similar to 1.54% reported by Sormani et al. in the Italian Register (15) but lower than 2.3% (5/219) reported by Moreno-Torres et al. (26) and 3.5% observed in the French Covisep registry (14). The only death observed in our cohort took place during the second wave, whereas French Register published data only until 21 May 2020 and Italian Register published data only until 10 September 2020. Therefore, these results are not fully comparable. Our death rate is also lower than that observed in the general population of Madrid until 28 September 2021 (2.79% of infected patients died). These differences may be explained by the fact that the rates have not been stratified by age and sex. Higher COVID mortality rates have been observed in older population, while MS predominantly affects women aged between 20 and 40 years.

Our study has several limitations. Due to its observational nature, some data were missing and could not be analyzed. Another problem was the lack of access to testing in our area during the first coronavirus wave (as all over the world). The inclusion of all COVID-19 cases (possible, probable, and confirmed) in the study of risk factor associated with infection and with severe forms makes these results less reliable. Finally, as a consequence of its unicentric design, the sample size is limited. Larger studies with more extensive populations may better elucidate other COVID implications in the population with MS.

Conclusion

This study provides several important observations. The cumulative incidence of confirmed COVID-19 in pwMS is similar to that of the general population, with a higher incidence during the first wave and a lower incidence during the second to fifth waves. Only smoking was associated with the risk of having COVID-19 as a protective factor. Other comorbidities, MS clinical profile, and DMT were not risk factors. The clinical outcome of pwMS with COVID-19 is good, with 86% mild forms. Higher EDSS and pulmonary comorbidities were associated with severe forms of COVID-19.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

BP and YA: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft preparation, and writing—review editing. JG: formal analysis, methodology, visualization, writing—original draft preparation, and writing—review editing. GM and AM: data curation and software. LR: conceptualization and data curation. IT: formal analysis and methodology. MT: data curation. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Wang L, Wang Y, Ye D, Liu Q. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. *Int J Antimicrob Agents*. (2020) 55:105948. doi: 10.1016/j.ijantimicag.2020.105948

2. Sahraian MA, Azimi A, Navardi S, Ala S. Evaluation of the rate of COVID-19 infection, hospitalization and death among Iranian patients with multiple sclerosis coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company ' s public news and information. Mult *Scler Relat Disord*. (2020) 46:102472. doi: 10.1016/j.msard.2020.102472

3. Parrotta E, Kister I, Charvet L, Sammarco C, Saha V, Charlson RE, et al. COVID-19 outcomes in MS: observational study of early experience from NYU Multiple Sclerosis Comprehensive Care Center. *Neurol Neuroimmunol neuroinflamm*. (2020) 7:e835.doi: 10.1212/NXI.0000000000000835

4. Sormani MP. An Italian programme for COVID-19 infection in multiple sclerosis. *Lancet Neurol*. (2020) 19:481–2. doi: 10.1016/S1474-4422(20)30147-2

5. Aguirre C, Meca-Lallana V, Barrios-Blandino A, del Río B, Vivancos J. Covid-19 in a patient with multiple sclerosis treated with natalizumab: may the blockade of integrins have a protective role? *Mult Scler Relat Disord*. (2020) 44:102250. doi: 10.1016/j.msard.2020.102250

6. Hung IF-N, Lung K-C, Tso EY-K, Liu R, Chung TW-H, Chu M-Y, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet*. (2020) 395:1695–704. doi: 10.1016/S0140-6736(20)31042-4

7. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0

8. Costa-Frossard L, Moreno-Torres I, Meca-Lallana V, García-Domínguez JM. EMCAM (Multiple Sclerosis Autonomous Community of Madrid) document for the management of patients with multiple sclerosis during the SARS-CoV-2 pandemic. *Rev Neurol*. (2020) 70:329–40. doi: 10.33588/rn.7009.2020155

9. Giovannoni G. Anti-CD20 immunosuppressive disease-modifying therapies and COVID-19. *Mult Scler Relat Disord*. (2020) 41:102135. doi: 10.1016/j.msard.2020.102135

10. Landtblom AM, Berntsson SG, Boström I, Iacobaeus E. Multiple sclerosis and COVID-19: the Swedish experience. *Acta Neurol Scand*. (2021) 144:229–35. doi: 10.1111/ane.13453

11. Fernandes PM, O'Neill M, Kearns PKA, Pizzo S, Watters C, Baird S, et al. Impact of the first COVID-19 pandemic wave on the Scottish Multiple Sclerosis Register population. *Wellcome Open Res*. (2020) 5:1–12. doi: 10.12688/wellcomeopenres.16349.1

12. Ferreira LC, Sousa NA de C, Ferreira MLB, Dias-Carneiro RPC, Mendes MF, Piccolo AC, et al. Incidence and clinical outcome of Coronavirus disease 2019 in a cohort of 11,560 Brazilian patients with multiple sclerosis. *Mult Scler J*. (2021). 27:1615–9. doi: 10.1177/1352458520978354

13. Loonstra FC, Hoitsma E, van Kempen ZLE, Killestein J, Mostert JP. COVID-19 in multiple sclerosis: the Dutch experience. *Mult Scler J*. (2020) 26:1256–60. doi: 10.1177/1352458520942198

14. Louapre C, Collongues N, Stankoff B, Giannesini C, Papeix C, Bensa C, et al. Clinical characteristics and outcomes in patients with coronavirus disease 2019 and multiple sclerosis. *JAMA Neurol*. (2020) 77:1079–88. doi: 10.1001/jamaneurol.2020.2581

15. Sormani MP, De Rossi N, Schiavetti I, Carmisciano L, Cordioli C, Moiola L, et al. Disease-modifying therapies and coronavirus disease 2019 severity in multiple sclerosis. *Ann Neurol*. (2021) 89:780–9. doi: 10.1002/ana.26028

16. Sepúlveda M, Llufriu S, Martínez-Hernández E, Català M, Artola M, Hernando A, et al. Incidence and impact of COVID-19 in MS: a survey from a Barcelona MS Unit. *Neurol Neuroimmunol neuroinflamm*. (2021) 8:1–8. doi: 10.1212/NXI.0000000000000954

17. Crescenzo F, Marastoni D, Bovo C, Calabrese M. Frequency and severity of COVID-19 in multiple sclerosis: a short single-site report from northern Italy. *Mult Scler Relat Disord*. (2020) 44:102372. doi: 10.1016/j.msard.2020.102372

18. Toscano S, Chisari CG, Patti F. Multiple sclerosis, COVID-19 and vaccines: making the point. *Neurol Ther*. (2021) 10:627–49. doi: 10.1007/s40120-021-00288-7

19. Zabalza A, Cárdenas-Robledo S, Tagliani P, Arrambide G, Otero-Romero S, Carbonell-Mirabent P, et al. COVID-19 in multiple sclerosis patients: susceptibility, severity risk factors and serological response. *Eur J Neurol*. (2021) 28:3384–95. doi: 10.1111/ene.14690

20. Shastri MD, Shukla SD, Chin W, Kc R, Dua K, Patel RP, et al. Smoking and COVID-19 : what we know so far. *Respir Med*. (2021) 176:106237. doi: 10.1016/j.rmed.2020.106237

21. Reddy RK, Charles WN, Sklavounos A, Dutt A, Seed PT, Khajuria A. The effect of smoking on COVID-19 severity: a systematic review and meta-analysis. *J Med Virol*. (2021) 93:1045–56. doi: 10.1002/jmv.26389

22. Polverino F. Cigarette smoking and COVID-19: a complex interaction. *Am J Respir Crit Care Med*. (2020) 202:471–2. doi: 10.1164/rccm.202005-1646LE

23. Simons D, Shahab L, Brown J, Perski O. The association of smoking status with SARS-CoV-2 infection, hospitalization and mortality from COVID-19: a living rapid evidence review with Bayesian meta-analyses (version 7). *Addiction*. (2021) 116:1319–68. doi: 10.1111/add.15276

24. Tsigaris P, Teixeira Da Silva JA. Smoking prevalence and COVID-19 in Europe. *Nicotine Tob Res*. (2020) 22:1646–9. doi: 10.1093/ntr/ntaa121

25. Salter A, Fox RJ, Newsome SD, Halper J, Li DKB, Kanellis P, et al. Outcomes and risk factors associated with SARS-CoV-2 infection in a North American registry of patients with multiple sclerosis. *JAMA Neurol*. (2021) 78:699–708. doi: 10.1001/jamaneurol.2021.0688

26. Moreno-Torres I, Meca Lallana V, Costa-Frossard L, Oreja-Guevara C, Aguirre C, Alba Suárez EM, et al. Risk and outcomes of COVID-19 in patients with multiple sclerosis. *Eur J Neurol*. (2021) 28:3712–21. doi: 10.1111/ene.14990

frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 20 October 2022
DOI 10.3389/fnins.2022.992165

OPEN ACCESS

EDITED BY
Kumar Vaibhav,
Augusta University, United States

REVIEWED BY
Pallavi Shrivastava,
LSU Health Sciences Center New Orleans, United States
Andrea Dressing,
University of Freiburg Medical Center, Germany

*CORRESPONDENCE
Gretel Sanabria-Diaz
gretel.sanabriadiaz@unibas.ch

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Translational Neuroscience, a section of the journal Frontiers in Neuroscience

RECEIVED 12 July 2022
ACCEPTED 28 September 2022
PUBLISHED 20 October 2022

CITATION
Sanabria-Diaz G, Etter MM, Melie-Garcia L, Lieb JM, Psychogios M-N, Hutter G and Granziera C (2022) Brain cortical alterations in COVID-19 patients with neurological symptoms. *Front. Neurosci.* 16:992165. doi: 10.3389/fnins.2022.992165

Brain cortical alterations in COVID-19 patients with neurological symptoms

Gretel Sanabria-Diaz[1,2,3*†], Manina Maja Etter[4,5†], Lester Melie-Garcia[1,2,3], Johanna M. Lieb[6], Marios-Nikos Psychogios[6], Gregor Hutter[4,5] and Cristina Granziera[1,2,3]

[1]Translational Imaging in Neurology (ThINK) Basel, Department of Biomedical Engineering, Faculty of Medicine, University Hospital Basel, University of Basel, Basel, Switzerland, [2]Department of Neurology, University Hospital Basel, Basel, Switzerland, [3]Research Center for Clinical Neuroimmunology and Neuroscience Basel (RC2NB), University Hospital Basel, University of Basel, Basel, Switzerland, [4]Brain Tumor Immunotherapy Lab, Department of Biomedicine, University Hospital Basel, University of Basel, Basel, Switzerland, [5]Division of Neurosurgery, University Hospital Basel, Basel, Switzerland, [6]Department of Neuroradiology, Clinic of Radiology and Nuclear Medicine, University Hospital Basel, Basel, Switzerland

Background: Growing evidence suggests that the central nervous system is affected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), since infected patients suffer from acute and long-term neurological sequelae. Nevertheless, it is currently unknown whether the virus affects the brain cortex. The purpose of this study was to assess the cortical gray matter volume, the cortical thickness, and the cortical surface area in a group of SARS-CoV-2 infected patients with neurological symptoms compared to healthy control subjects. Additionally, we analyzed the cortical features and the association with inflammatory biomarkers in the cerebrospinal fluid (CSF) and plasma.

Materials and methods: Thirty-three patients were selected from a prospective cross-sectional study cohort during the ongoing pandemic (August 2020–April 2021) at the university hospitals of Basel and Zurich (Switzerland). The group included patients with different neurological symptom severity (Class I: nearly asymptomatic/mild symptoms, II: moderate symptoms, III: severe symptoms). Thirty-three healthy age and sex-matched subjects that underwent the same MRI protocol served as controls. For each anatomical T1w MPRAGE image, regional cortical gray matter volume, thickness, and surface area were computed with FreeSurfer. Using a linear regression model, cortical measures were compared between groups (patients vs. controls; Class I vs. II–III), with age, sex, MRI magnetic field strength, and total intracranial volume/mean thickness/total surface area as covariates. In a subgroup of patients, the association between cortical features and clinical parameters was assessed using partial correlation adjusting for the same covariates. *P*-values were corrected using a false discovery rate (FDR).

Results: Our findings revealed a lower cortical volume in COVID-19 patients' orbitofrontal, frontal, and cingulate regions than in controls ($p < 0.05$).

Regional gray matter volume and thickness decreases were negatively associated with CSF total protein levels, the CSF/blood-albumin ratio, and CSF EN-RAGE levels.

Conclusion: Our data suggest that viral-triggered inflammation leads to neurotoxic damage in some cortical areas during the acute phase of a COVID-19 infection in patients with neurological symptoms.

KEYWORDS

SARS-CoV-2, COVID-19, gray matter volume (GMV), cortical thickness, surface area, neurological symptoms

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to affect millions of people worldwide. After 2 years of the pandemic, current evidence suggests that the virus directly or indirectly impacts the brain (Kremer et al., 2020; Ladopoulos et al., 2021; Manca et al., 2021; Pajo et al., 2021). Frequently, patients who suffer from a mild to a severe infection show neurological manifestations (García-Azorín et al., 2021; Meppiel et al., 2021; Taquet et al., 2021), which are described in 30–80% of hospitalized patients (Helms et al., 2020; Kotfis et al., 2020).

However, the underlying mechanisms leading to brain alterations and cognitive impairment remain unknown. There is some evidence pointing to a viral neurotropism (Paterson et al., 2020; de Erausquin et al., 2021), virus-induced inflammatory state (refereed as a cytokine storm) (Deleidi and Isacson, 2012; Butowt et al., 2021; Yang et al., 2021) and systemic post-infectious inflammation (McQuaid et al., 2021). Nevertheless, the virus' presence in the human brain has still to be demonstrated (von Weyhern et al., 2020; Brady et al., 2021; McQuaid et al., 2021).

Previous studies during a SARS-CoV-2 chronic phases and after infection revealed an increase in gray matter volume (GMV) in brain regions (Lu et al., 2020) and the frontotemporal network (Duan et al., 2021). Regarding SARS-CoV-2-infected patients with neurological symptoms, it has been reported that cortical thickness (CTh) decreases in frontal and limbic areas (Qin et al., 2021). Furthermore, a recent longitudinal population study using pre- and post-COVID MRI scans from 401 SARS-CoV-2 -infected patients and 384 healthy controls reported cortical thickness reduction in the orbitofrontal cortex and parahippocampal gyrus, more pronounced gray matter tissue damage in regions that were functionally connected to the primary olfactory cortex, and a greater reduction in global brain size after SARS-CoV-2 infection (Douaud et al., 2021). Nevertheless, this study mainly focused on patients with mild infection (Douaud et al., 2021). These findings suggest a link between SARS-CoV-2 infection and brain morphometric alterations. However, they applied a single morphometric approach based on GMV or CTh analysis in mildly affected patients without neurological complications during the post-infection phase.

Instead, in this study, we investigated the characteristics of the cortex of COVID-19 patients depicting different severity stages of neurological symptoms and compared the findings to healthy subjects by using a multi-morphometric approach. It is well-documented that CTh, surface area (SA), and GMV capture different underlying morphological processes (Dickerson et al., 2009; Panizzon et al., 2009; Lemaitre et al., 2012). The implementation of this approach may offer insights into which feature is more pertinent to detecting cortical alterations in neurologically compromised patients. Additionally, we investigated the association between the morphometric measures in COVID-19 patients with neurological symptoms and body fluids measures related to (i) infection, (ii) organ damage, and (iii) concomitant hyperinflammatory response.

Materials and methods

Participants

Data were obtained from a prospective, two-center, cross-sectional study (clinicaltrials.gov NCT04472013, IRB approval EKNZ 2020-01503) including COVID-19 patients from August 2020 to April 2021 at the Swiss University Hospitals of Basel and Zurich. Participants remained anonymous, and written consent was given by the patients or a legal representative. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; GMV, cortical gray matter volume; CTh, cortical thickness; SA, cortical surface area; FDR, false discovery rate; CSF, Cerebrospinal fluid; TIV, Total Intracranial Volume; TRANCE, tumor necrosis factor-related activation-induced cytokine; EN-RAGE, receptor for advanced glycation end-products binding protein; OPG, osteoprotegerin.

study included a neurological examination, lumbar puncture, blood withdrawal for cerebrospinal fluid (CSF) and plasma soluble protein analysis, cranial MRI, or CT scan. Neurological examination was performed using the National Institutes of Health Stroke Scale (NIHSS). Glasgow Coma Scale (GCS) was used in sedated patients.

Thirty-two patients underwent contrast-enhanced brain MRI imaging. Due to logistic challenges, staffing, and medical surveillance issues during the COVID-19 pandemic, five patients underwent cranial computed tomography (CT) instead of brain MRI. In contrast, one patient was imaged with a brain MRI and cranial CT.

For this study, the SARS-CoV-2 infection cohort was selected retrospectively based on the following inclusion criteria: (1) age > 18 years, (2) SARS-CoV-2 infection confirmed by reverse transcriptase PCR (rRT-PCR) testing, and (3) a 3D high-resolution T1-weighted MRI sequence of the whole brain at the time of their positive SARS-CoV-2 qRT-PCR test (**Table 1**). The exclusion criteria were (1) SARS-CoV-2 RT-PCR-negative testing and (2) pregnancy. Retrospectively, biobanked age- and sex-matched healthy individuals (n = 33) with no neurological pre-existing risk factor other than headache served as controls (**Table 1**).

Additional information about pre-existing risk factors and clinical indications for the brain MRI study of patients can be found in **Supplementary Tables 2**, **3**. Patients with strokes were not included in our sample. As expected, several comorbidities characterized the group of hospitalized SARS-CoV-2 infected patients.

Enrolled patients (n = 33) were subdivided into three Neuro-COVID severity classes, referred to as Class I (n = 16), II (n = 10), or III (n = 7). The neurological symptoms in Class I involved mild signs/symptoms such as anosmia, ageusia, headache, and dizziness. Patients in Class II showed moderate signs/symptoms (e.g., mono/para/quadriparesis, fatigue), whereas Class III represented those with severe signs/symptoms such as seizures, and cognitive impairment (Fotuhi et al., 2020; Etter et al., 2022). Patients with pre-existing illnesses in their past medical history were included. Based on the sample size per Class, we considered the following groups for further analyses (**Table 2**).

TABLE 1 Demographics, clinical, and paraclinical characteristics of COVID-19 patients and the control group.

	Patients	Controls	p-value
Numbers of subjects	33	33	na
Age	50.45 ± 19.13	51.64 ± 23.25	0.78
Sex, Male/Female (M/F)	13/20	12/21	0.80
Neuro-COVID class	I (n = 15), II (n = 8), III (n = 5)	na	na
CSF leukocytes (mmol/l)	n = 16 (4.38 ± 3.83)	na	na
CSF lactate (mmol/l)	n = 15 (1.89 ± 0.52)	na	na
CSF protein total (mmol/l)	n = 16 (345.41 ± 186.37)	na	na
CSF blood albumin ratio	n = 14 (6.50 ± 4.61)	na	na
CSF glucose (mmol/l)	n = 15 (4.54 ± 1.33)	na	na
Plasma TRANCE (pg/mL)	n = 20 (2.74 ± 0.93)	na	na
Plasma EN-RAGE (pg/mL)	n = 20 (3.30 ± 1.37)	na	na
CSF OPG (pg/mL)	n = 18 (9.55 ± 0.68)	na	na
CSF TRANCE (pg/mL)	n = 18 (-0.31 ± 0.29)	na	na
CSF EN-RAGE (pg/mL)	n = 18 (0.43 ± 0.74)	na	na
Weight (kg)	69.06 ± 11.62	72.79 ± 15.85	0.44
Height (meters)	1.70 ± 0.10	1.69 ± 0.10	0.73
MRI magnetic field strength	1.5 T (n = 29) 3 T (n = 4)	1.5 T (n = 11) 3 T (n = 22)	na
TIV (cm^3)	1520.03 ± 179.37	1523.14 ± 146.27	0.86
Mean cortical thickness (mm^3)	2.34 ± 0.15	2.39 ± 0.16	0.27
Surface area (mm^2)	1596.4 ± 198.25	1655.79 ± 163.27	0.10

Data are shown as Mean ± SD and/or n (sample size). P-values are obtained by independent-test, Pearson chi^2, and Non-parametric Mann-Whitney U test when a variable is not normally distributed (Shapiro-Wilk $P < 0.05$), or there is no homogeneity of variances (Levene's test). n, sample size; sd, standard deviation; CSF, Cerebrospinal fluid; TIV, Total Intracranial Volume; TRANCE, tumor necrosis factor-related activation-induced cytokine; EN-RAGE, receptor for advanced glycation end-products binding protein; OPG, osteoprotegerin; na, not applicable; cm, centimeters; mm, millimeters; kg, kilograms; mmol/l, millimole per liters; pg/mL, picograms per milliliter; M, male; F, female; I, NeuroCOVID Class 1; II, NeuroCOVID Class 2; III, NeuroCOVID Class 3.

Biomarker measurements in cerebrospinal fluid

In a subset of patients, CSF and blood examinations were performed simultaneously during the acute phase of COVID-19, on an average latency period of 3.5 days (range: 1–12 days) after the first positive SARS-CoV-2 qRT-PCR test result. Measures in the CSF included the number of leukocytes, levels of lactate and total protein, and CSF/blood-albumin-ratio (Gabay and Kushner, 2008). Additionally, chemokines, soluble cell membrane proteins, and cytokines, were measured using the Olink 96 target

TABLE 2 Demographics, clinical, and paraclinical characteristics of Neuro-COVID Class I and Class II–III groups.

	Class I	Class II–III	*p*-value
Numbers of subjects	16	17	na
Age	47.87 ± 21.27	47.22 ± 15.22	0.96
Sex, male/female (M/F)	6/9	4/9	0.61
CSF leukocytes (mmol/l)	n = 8 (3.63 ± 3.54)	*n* = 6 (4 ± 2.53)	0.65
CSF lactate (mmol/l)	*n* = 9 (1.68 ± 0.21)	*n* = 6 (1.86 ± 0.53)	0.72
CSF protein total (mmol/l)	*n* = 9 (293.56 ± 80.53)	*n* = 6 (354.50 ± 188.20)	0.40
CSF blood albumin ratio	*n* = 9 (5.03 ± 1.84)	*n* = 4 (7.20 ± 4.73)	0.60
CSF glucose (mmol/l)	*n* = 9 (4.21 ± 1.33)	*n* = 6 (4.70 ± 1.50)	0.55
Plasma TRANCE (pg/mL)	*n* = 11 (3.32 ± 0.75)	*n* = 7 (2.06 ± 0.55)	**0.001**
Plasma EN-RAGE (pg/mL)	*n* = 11 (2.65 ± 1.39)	*n* = 7 (4.12 ± 0.98)	**0.003**
CSF OPG (pg/mL)	*n* = 10 (9.31 ± 0.64)	*n* = 6 (9.79 ± 0.68)	0.18
CSF TRANCE (pg/mL)	*n* = 10 (-0.39 ± 0.31)	*n* = 6 (-0.27 ± 0.26)	0.47
CSF EN-RAGE (pg/mL)	*n* = 10 (0.33 ± 0.26)	*n* = 6 (0.12 ± 0.42)	0.22
Weight (kg)	64.73 ± 10.66	69.92 ± 7.45	0.22
Height (meters)	1.70 ± 0.09	1.68 ± 0.09	0.42
MRI magnetic field strength	1.5 T (*n* = 15)	1.5 T (*n* = 9) 3 T (*n* = 4)	**0.02**
TIV (cm^3)	1516.44 ± 160.10	1478.65 ± 195.50	0.58
Mean cortical thickness (mm^3)	2.37 ± 0.13	2.36 ± 0.17	0.88
Surface area (mm^2)	1650.3 ± 161.20	1592.94 ± 244.39	0.46

Data are shown as Mean ± SD and/or n (sample size). *P*-values were obtained by independent-test, Pearson chi^2, and Non-parametric Mann-Whitney *U* test when a variable was not normally distributed (Shapiro-Wilk $P < 0.05$), or there was no homogeneity of variances (Levene's test). n, sample size; sd, standard deviation; CSF, Cerebrospinal fluid; TIV, Total Intracranial Volume; TRANCE, tumor necrosis factor-related activation-induced cytokine; EN-RAGE, receptor for advanced glycation end-products binding protein; OPG, osteoprotegerin; na, not applicable; cm, centimeters; mm, millimeters; kg, kilograms; mmol/l, millimole per liters; pg/mL, picograms per milliliter; M, male; F, female; I, Neuro-COVID Class 1; II, Neuro-COVID Class 2; III, Neuro-COVID Class 3. Bold indicates *p*-values < 0.05.

neurology[1] and Olink 96 target inflammation[2] panels. Based on previous reports of associations with a SARS-CoV-2 infection (Etter et al., 2022; Jarius et al., 2022), we selected five cytokines for our correlation analysis (plasma-TRANCE, plasma-EN-RAGE, CSF-OPG, CSF-TRANCE, and CSF-EN-RAGE). For further information about the CSF and blood-derived measures and their clinical significance, see **Supplementary Table 1**.

Imaging protocols

3D high-resolution T1-weighted anatomical images were acquired using two different MRI scanners: Scanner 1: 1.5 Tesla Siemens Avanto Fit and Scanner 2: 3 Tesla Siemens Skyra. A Magnetization Prepared—RApid Gradient Echo (MPRAGE) pulse sequence covering the whole brain was used in both MRI scanners with the following parameters. Scanner 1: 160 contiguous slices of 1 mm thickness in sagittal orientation; in-plane FOV = 256 × 256 mm^2, and matrix size 256 × 256 yielding an in-plane spatial resolution of 1 × 1 mm^2 and voxel size of 1 × 1 × 1 mm^3. The echo (TE), repetition (TR), and inversion (TI) times were set to TE/TR/TI = 2.8 ms/2,400 ms/900 ms with a flip angle FA = 8°. Scanner 2: A 160 contiguous slices of 1 mm thickness in sagittal orientation; in-plane FOV = 256 × 240 mm^2, and matrix size 256 × 240 yielding an in-plane spatial resolution of 1 × 1 mm^2 and voxel size of 1 × 1 × 1 mm^3. The echo, repetition, and inversion times were set to TE/TR/TI = 2.98 ms/2,300 ms/900 ms with a flip angle FA = 9°.

Imaging analysis

Cortical reconstruction and volumetric segmentation were performed with Freesurfer.[3] The technical details of these procedures are described in prior publications (Dale and Sereno, 1993; Fischl et al., 1999, 2002, 2004; Fischl and Dale, 2000). Briefly, the preprocessing steps include motion correction, intensity normalization, Talairach registration, skull stripping, subcortical white matter segmentation, tessellation of the gray matter/white matter boundary, topology correction, surface deformation to end with a surface 3D model of the cortex by means of intensity and continuity information.

Regional gray matter volume, cortical thickness, and surface area computations

Applying FreeSurfer's pipeline, local CTh and SA were calculated at each vertex. Each cortical segmentation was

1 https://www.olink.com/products-services/target/neurology-panel/

2 https://www.olink.com/products-services/target/inflammation/

3 http://surfer.nmr.mgh.harvard.edu/

visually checked for inaccuracies and manually corrected. CTh was calculate as the shortest distance between the GM/WM boundary and pial surface at each vertex across the cortical mantle, measured in millimeters (mm). GMV was calculated with FreeSurfer's automated procedure for volumetric measures (Fischl et al., 2002, 2004).

In addition, global brain measures (mean CTh and total SA) were computed using the FreeSurfer (Fischl et al., 2002). The total intracranial volume (TIV) estimation was based on the sum of resulting raw values for gray matter, white matter, and CSF derived from the SPM 12 (Statistical Parametric Mapping) (Ashburner and Friston, 2005).

We selected 34 gyral-based regions of interest per hemisphere, according to the Desikan-Killiany atlas (Desikan et al., 2006). For each of the 68 bilateral cortical regions, FreeSurfer calculates (i) the average CTh (in mm), (ii) total cortical SA of the pial (in mm^2), and (iii) the cortical GMV (in mm^3).

Statistical analysis

All variables' normal distributions and equality of variances were assessed using Shapiro-Wilk tests and Levene's tests. As appropriate, clinical and demographic variables were compared between groups with an independent *t*-test, Mann-Whitney, or Chi-square tests.

Regional morphometric measures were compared between groups (patients vs. controls) and Neuro-COVID Classes (Class I vs. Class II-III) using a linear regression model. The covariates were age, gender, age × gender interaction, MRI magnetic field strength, and global measures (TIV/mean CTh/total SA). False discovery rate (FDR) correction was used to adjust for multiple comparisons by the number of structures.

The association between morphometric brain descriptors and biological parameters was assessed using a partial correlation and adjusted for age, sex, age × sex interaction, MRI magnetic field strength, and global brain measure (TIV/mean CTh/total SA). The resulting values were corrected for multiple comparisons using FDR correction.

The statistical analysis was performed using the JASP[4] and MATLAB software ("partialcorri.m" function).[5]

Results

Sample description statistics

The groups did not significantly differ in age, gender, education, or global measures (TIV, Mean CTh, SA, Global gray matter, Global white matter, and total CSF) (**Table 1**). However, there was a trend in patients showing lower values in all global measures compared to controls (**Table 1**). Regarding the body-fluid measures, 25% of the patients showed increased leukocytes levels (4/16), 33% abnormal lactate levels (5/15), 12.5% increased protein levels (2/16), 46.7% increased glucose levels (7/15), and 21.4% an increased CSF/blood-albumin ratio (3/14).

The Neuro-COVID Class I and II-III did not differ significantly in age, gender, education, or global measures (TIV, Mean CTh, SA, Global gray matter, Global white matter, and total CSF) (**Table 2**). However, the Classes were statistically different in plasma TRANCE levels, plasma EN-RAGE levels, and MRI magnetic field applied to acquire 3D T1 images (all $p < 0.05$) (**Table 2**).

Morphometric differences between the SARS-CoV-2 and controls group

Three cortical regions showed different GMV between SARS-CoV-2 infected patients and controls. Patients exhibited lower GMV values in the right rostral anterior cingulate (controls mean = 1.90; patients mean = 0.38; *p*-corrected = 0.04), left medial orbitofrontal (controls mean = 5.08; patients mean = 0.84; *p*-corrected = 0.04), and left superior frontal regions (controls mean = 22.01; patients means = 3.75; *p*-corrected = 0.04). There were no significant differences in CTh and SA between groups after FDR correction. However, patients showed a trend to lower values compared to the control group (see **Supplementary Tables 4–6**).

When the uncorrected *p*-values were analyzed, 12 regions were found to have lower GMV in COVID-19 patients, and 50% were located in frontal structures. The rest belonged to limbic, parietal, and temporal regions (**Supplementary Table 5**).

For a complete list of group contrast and regional *p*-values, see **Supplementary Tables 4–6**.

Brain regional morphometric differences between the Class I and Class II–III patients

After multiple comparison corrections, the Neuro-COVID Class I and II-III did not significantly differ in GMV, CTh, and SA. However, several regions showed a GMV decrease in patients with moderate-severe neurological symptoms compared to the mild ones before FDR correction (posterior cingulate, rostral anterior cingulate, precuneus, inferior parietal, pars orbital, and middle temporal) (*p*-uncorrected < 0.05) (**Supplementary Table 7**). The same patterns were observed for CTh and SA in Class II-III compared to Class I (**Supplementary Tables 7–9**).

4 https://jasp-stats.org/

5 https://www.mathworks.com/

Regional gray matter volume and cortical thickness associated with clinical variables in SARS-CoV-2 infected patients

In a subgroup of patients, we studied the association between regional GMV, CTh, and SA with CSF and blood measures linked to infectious and inflammatory processes (CSF leukocytes, CSF lactate, CSF total protein, CSF/blood-albumin-ratio, CSF/plasma EN-RAGE levels, CSF/plasma OPG levels, and CSF/plasma TRANCE levels).

Figure 1 shows 23 brain regions where GMV was negatively correlated with the CSF leukocyte count, CSF lactate, CSF total protein, CSF/blood-albumin-ratio, and CSF EN-RAGE levels after FDR correction ($p < 0.05$) (**Supplementary Table 10**). A higher CTh in six cortical regions (frontal, orbitofrontal, and temporal cortices) was found to be associated with higher CSF EN-RAGE levels and CSF/blood-albumin-ratio levels in all regions but one, where we measured a negative correlation (**Figure 1** and **Supplementary Table 11**). SA did not correlate with any biological variable.

Between the statistically significant associations, we found three regions previously reported with lower GMV in patients compared to controls: right rostral anterior cingulate, left medial orbitofrontal, and left superior frontal (subsection "Morphometric differences between the SARS-CoV-2 and controls group"). They were negatively correlated with the CSF/blood-albumin-ratio and CSF EN-RAGE levels in the patients subgroup with a CSF study (**Figure 2**).

Discussion

In this study, SARS-CoV-2-infected patients with neurological symptoms ranging from mild to severe exhibited lower cortical volume in the orbitofrontal, frontal, and cingulate cortex compared to healthy controls. Besides, in those patients, a lower regional cortical GMV and CTh were associated with an increase in total protein CSF levels, CSF/blood-albumin ratio, and the levels of an inflammatory cytokine named EN-RAGE.

Recent studies reported structural, metabolic, and functional alterations in the frontal regions during the acute, subacute, and long-COVID phases (Anzalone et al., 2020; Duan et al., 2021; Guedj et al., 2021; Hosp et al., 2021; Kas et al., 2021). Unlike those studies, our work focused on hospitalized patients with neurological symptoms. Lower cortical GMV in the orbitofrontal cortex vs. controls was not an unexpected result in this patient group. This cortical area is a secondary olfactory cortex and part of a possible direct SARS-CoV-2 central nervous system invasion pathway (Baig and Sanders, 2020; Meinhardt et al., 2020; Bougakov et al., 2021).

Our results—especially the finding of a reduction in CTh and GMV in the orbitofrontal and cingulate cortex—confirm and extend very recent findings that were reported in a large cohort of SARS-CoV-2 infected patients obtained from the UK Biobank (Douaud et al., 2021): in fact, they demonstrated that the same regions that were altered in mildly infected patients are altered in patients with a severe form of the disease, pointing at a relationship between SARS-CoV-2 infection and gray matter alterations.

On the other hand, volumetric changes in the gray matter within the cingulate gyrus have also been described in post-COVID patients, which correlated to the loss of smell during the acute phase (Lu et al., 2020). In this respect, the vulnerability of the cingulate cortex to COVID-19 has been linked to its rich concentration of angiotensin-converting enzyme 2 (ACE-2), where the virus binds through its spike protein (Ibi et al., 2019; Ni et al., 2020). Interestingly, both regions, the orbitofrontal and cingulate cortex play essential roles in different cognitive functions such as attention, motivation, decision making, and conflict-error monitoring, which are impaired in COVID-19 patients (Ibi et al., 2019; Lu et al., 2020).

As to the possible mechanisms leading to lower GMV in these areas, this may be due to direct viral damage, especially to the olfactory cortex (Gori et al., 2020; Meinhardt et al., 2020). In addition, other possible causes might have contributed to these findings, such as a reduced oxygen supply to the brain (Solomon et al., 2020), post-infectious inflammation, cytokine-related hyperinflammation, and complications due to a coagulopathic state (Koralnik and Tyler, 2020; Pezzini and Padovani, 2020). Furthermore, without pre-COVID MRI studies, we cannot exclude that prior anatomical characteristics (i.e., atrophy) might be implicated in our results.

Another factor influencing our findings is the heterogeneity of the cohort's pre-existing risk factors. In hospitalized SARS-CoV-2 infected patients, this is not unexpected. They were affected by several of the 24 demographic and health risk factors categories associated with severe disease outcomes (https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html, Updated June 15, 2022). Indeed, these risk factors may alter the brain's gray matter volume and cortical thickness.

We considered the sample characteristics, including the pre-existing risk factors heterogeneity, as a strength of our study. The patient group we enrolled in this study represents the real-world characteristics of severely infected COVID-19 patients during the recent pandemic.

Favoring the hypothesis that a virus-triggered inflammatory process may represent the underlying cause of the observed cortical changes, we found a strong association between the GMV and CTh of frontal, orbitofrontal, temporal, parietal, and limbic cortices and an increase in the CSF/blood-albumin-ratio, CSF total protein levels, and CSF EN-RAGE levels. Our results are in line with a comprehensive study on the CSF profile in patients with COVID-19 and neurological symptoms (Jarius et al., 2022). The authors found a persistently elevated

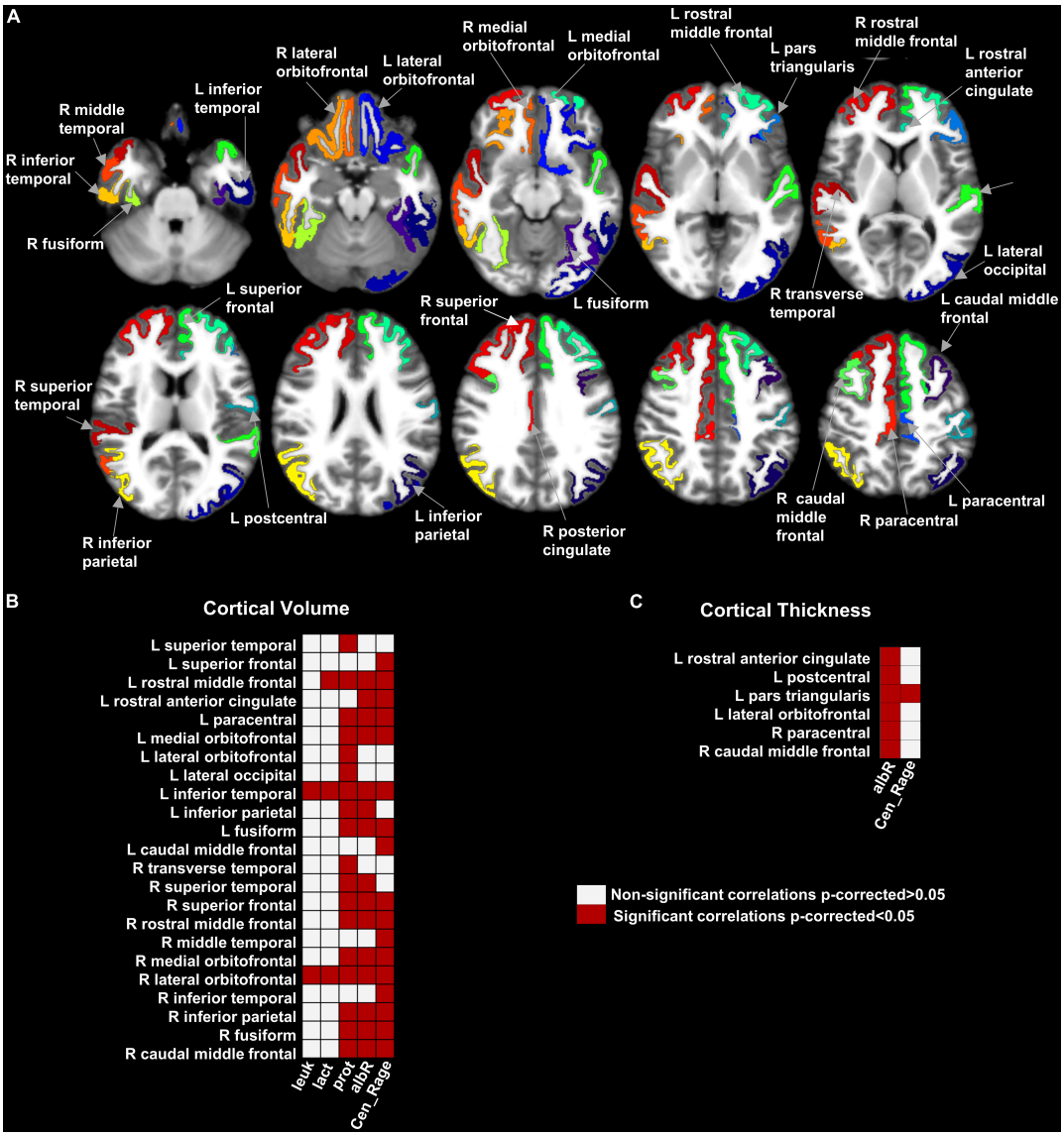


FIGURE 1
Map of the brain regions with a significant association between gray matter volume, cortical thickness, and clinical variables in hospitalized SARS-CoV-2 patients with neurological symptoms/signs. Panel **(A)** shows the 29 brain regions with significant correlation values for GMV and cortical thickness after multiple comparison corrections (False Discovery Rate- FDR). Panels **(B,C)** shows the matrices representing the association significance (significant p-corrected < 0.05 in red squares). CSF, Cerebrospinal fluid; leuk, leukocytes; lact, lactate; prot, protein; albR, Albumin CSF-blood ratio; Cen_Rage: CSF EN-RAGE, extracellular receptor for advanced glycation end-products binding protein; R, right; L, left.

CSF/blood-albumin ratio, CSF total protein levels, CSF lactate levels, and inflammatory cytokines in COVID-19 patients.

An increase in the CSF/blood-albumin ratio points toward an impaired blood-brain barrier. This finding has been frequently reported in COVID-19 patients with neurological symptoms (Jarius et al., 2022). It has been related to several mechanisms such as a direct viral infection, non-specific inflammatory damage (Perico et al., 2020; Varga et al., 2020; Huang et al., 2021), a virus-induced anti-endothelial auto-immunity (Shi et al., 2022), and hypoxia-related alterations (Ackermann et al., 2020; Buja et al., 2020). However, an increase in the CSF/blood-albumin-ratio might also be caused by changes in the CSF production and resorption (Pellegrini, 2020; McMahon et al., 2021) or viral infection of the choroid plexus (Yang et al., 2021). Similarly, an increase in CSF total protein levels is often found in COVID-19 patients and interpreted as an indirect sign of an inflammatory response (Tandon et al., 2021).

Further, our study revealed that an increase in CSF EN-RAGE levels is associated with a widespread decrease in cortical GMV. EN-RAGE is a cytokine involved in an

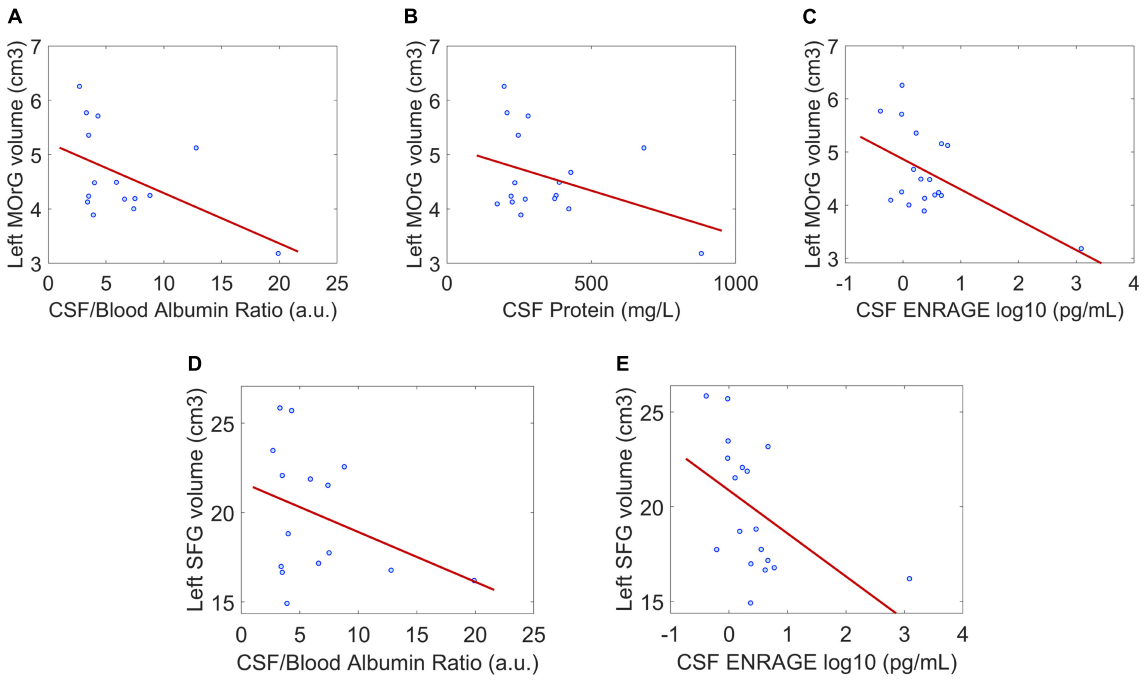


FIGURE 2
Correlation between regional gray matter volume and CSF clinical parameters in regions where gray matter volume between patients and controls was significantly different. **(A–C)** The negative associations between gray matter volume of the left medial orbitofrontal gyrus and CSF/blood Albumin Ratio, CSF protein, and CSF EN-RAGE. **(D,E)** The negative associations between gray matter volume of the left superior frontal gyrus and CSF/blood Albumin Ratio, CSF EN-RAGE. $P < 0.05$ is considered significant. Dot blue represents an individual patient's measure. MOrG, medial orbitofrontal gyrus; SFG, superior frontal gyrus; cm^3, centimeters cubic; a.u, arbitrary unit; pg/mL, picograms per milliliter.

inflammatory cascade, leading to accelerated atherosclerosis (Ligthart et al., 2014). Increased CSF EN-RAGE levels may cause vascular alterations and consequently affect different organs, particularly in severe infections (Huang et al., 2020; Choudhary et al., 2021; Thepmankorn et al., 2021). Whether these factors contributed to the association of decreased GMV and CTh remains unknown. Future studies should investigate potential causal effects relating the production of EN-RAGE to cortical damage.

Limitation

Our study has some limitations, encompassing the small sample size, the cross-sectional design, and the absence of pre-infection MRIs. Moreover, the lack of CSF and blood assessments in healthy subjects and some patients have undoubtedly limited the results' interpretation and statistical power. Future studies should also be powered to consider the influence of comorbidities on brain alterations in neurologically affected SARS-CoV-2 infected patients. These comorbidities may modulate host-viral interactions, immunological and inflammatory responses, thereby contributing to different outcomes in COVID-19 patients.

In this study, we did not include a control group of patients with non-COVID-19 pneumonia, a condition that may lead to GMV alterations in several brain regions due to the decreased oxygenation to the cortex (Zhang et al., 2013; Harch and Fogarty, 2017). Future work should address these potential confounding factors and aim to elucidate whether the lack of adequate brain oxygenation rather than direct inflammatory/viral-triggered processes are responsible for neurodegenerative processes in this patient population. A voxel-wise/vertex-wise analysis will be implemented in future work to detect subtle morphological variations due to COVID-19.

Finally, follow-up data may allow exploring which cortical changes are associated with the acute disease or Post-COVID Syndrome. Whether the observed cortical alterations represent a consequence of viral infection is still to be determined. Future longitudinal studies should help elucidate the underlying causal mechanisms and clinical impact of these findings.

Conclusion

In this study, using a multi-morphometric approach, we found that the gray matter volume in fronto-orbital and

cingulate regions is affected in SARS-CoV-2 infected patients with neurological symptoms. The regional cortical GMV measure revealed brain changes in patients during the acute phase of COVID-19 infectious not shown by the cortical thickness and surface area measurements. The widespread cortical volumetric changes appear to be related to the infectious process and concomitant hyperinflammatory response represented by the CSF total protein values, CSF/blood-albumin ratio, and CSF EN-RAGE levels. Our results highlight the importance of considering cortical alteration patterns, particularly volumetric patterns, during the clinical assessment of neurologically affected COVID-19 patients. The study of this measure may improve clinical management and future cognitive rehabilitation programs.

Data availability statement

The processed data supporting the conclusions of this article will be made available by the authors, without undue reservation. Requests to access the datasets should be directed to GS-D, gretel.sanabriadiaz@unibas.ch.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Northwestern and Central Switzerland (clinicaltrials.gov, NCT04472013, IRB approval EKNZ 2020-01503). The patients/participants provided their written informed consent to participate in this study.

Author contributions

GS-D, ME, LM-G, and CG: study concepts and study design. JL, ME, M-NP, and GH: data acquisition. GS-D, ME, and LM-G: data analysis. GS-D, ME, LM-G, GH, and CG: data interpretation and manuscript editing. ME, JL, M-NP, and GH: clinical studies. GS-D and LM-G: statistical analysis. All authors: guarantors of the integrity of the study, manuscript drafting or manuscript revision for important intellectual content, approval of the final version of submitted manuscript, literature research, and agrees to ensure any questions related to the work are appropriately resolved.

Funding

The study was funded by the BOTNAR Fast Track Call Foundation grant (FTC-2020-10) awarded to GH. Other research support to GH for this project includes a Swiss National Science Foundation Professorial Fellowship (PP00P3_176974); the ProPatient Forschungsstiftung, University Hospital Basel (Annemarie Karrasch Award 2019); the Department of Surgery, University Hospital Basel, to GH. GH has equity in, and is a cofounder of Incephalo Inc. CG was supported by the Swiss National Science Foundation (SNSF) grant PP00P3_176984, the Stiftung zur Förderung der gastroenterologischen und allgemeinen klinischen Forschung and the EUROSTAR E!113682 HORIZON2020.

Acknowledgments

We want to thank the volunteers who participated in this study. We are also very grateful to the medical staff for their dedication during the SARS-CoV-2 pandemic. A shorter version of this manuscript is available in the Preprint Server of Health Sciences (medRvix) under the following title: "Brain cortical changes are related to inflammatory biomarkers in hospitalized SARS-CoV-2 patients with neurological symptoms" (Sanabria-Diaz et al., 2022). Our study sample was part of a previous publication available in the Preprint Server of Health Sciences (medRvix) under the following title: "Severe Neuro-COVID is associated with peripheral immune signatures, autoimmunity and signs of neurodegeneration: a prospective cross-sectional study" (Etter et al., 2022).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.992165/full#supplementary-material

References

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al. (2020). Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in covid-19. *N. Engl. J. Med.* 383, 120–128. doi: 10.1056/NEJMOA2015432/SUPPL_FILE/NEJMOA2015432_DISCLOSURES.PDF

Anzalone, N., Castellano, A., Scotti, R., Scandroglio, A. M., Filippi, M., Ciceri, F., et al. (2020). Multifocal laminar cortical brain lesions: A consistent MRI finding in neuro-COVID-19 patients. *J. Neurol.* 267, 2806–2809.

Ashburner, J., and Friston, K. J. (2005). Unified segmentation. *Neuroimage* 26, 839–851. doi: 10.1016/J.NEUROIMAGE.2005.02.018

Baig, A. M., and Sanders, E. C. (2020). Potential neuroinvasive pathways of SARS-CoV-2: Deciphering the spectrum of neurological deficit seen in coronavirus disease-2019 (COVID-19). *J. Med. Virol.* 92, 1845–1857. doi: 10.1002/JMV.26105

Bougakov, D., Podell, K., and Goldberg, E. (2021). Multiple neuroinvasive pathways in COVID-19. *Mol. Neurobiol.* 58:1. doi: 10.1007/S12035-020-02152-5

Brady, M., McQuaid, C., Solorzano, A., Johnson, A., Combs, A., Venkatraman, C., et al. (2021). Spike protein multiorgan tropism suppressed by antibodies targeting SARS-CoV-2. *Commun. Biol.* 4:1318. doi: 10.1038/s42003-021-02856-x

Buja, L. M., Wolf, D., Zhao, B., Akkanti, B., McDonald, M., Lelenwa, L., et al. (2020). The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): Report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. *Cardiovasc. Pathol.* 48:107233. doi: 10.1016/J.CARPATH.2020.107233

Butowt, R., Meunier, N., Bryche, B., and von Bartheld, C. S. (2021). The olfactory nerve is not a likely route to brain infection in COVID-19: A critical review of data from humans and animal models. *Acta Neuropathol.* 141, 809–822. doi: 10.1007/S00401-021-02314-2

Choudhary, S., Sharma, K., and Silakari, O. (2021). The interplay between inflammatory pathways and COVID-19: A critical review on pathogenesis and therapeutic options. *Microb. Pathog.* 150:104673. doi: 10.1016/J.MICPATH.2020.104673

Dale, A. M., and Sereno, M. I. (1993). Improved localizadon of cortical activity by combining EEG and MEG with MRI cortical surface reconstruction: A linear approach. *J. Cogn. Neurosci.* 5, 162–176. doi: 10.1162/JOCN.1993.5.2.162

de Erausquin, G. A., Snyder, H., Carrillo, M., Hosseini, A. A., Brugha, T. S., and Seshadri, S. (2021). The chronic neuropsychiatric sequelae of COVID-19: The need for a prospective study of viral impact on brain functioning. *Alzheimers Dement.* 17, 1056–1065. doi: 10.1002/ALZ.12255

Deleidi, M., and Isacson, O. (2012). Viral and inflammatory triggers of neurodegenerative diseases. *Sci. Transl. Med.* 4:121s3. doi: 10.1126/SCITRANSLMED.3003492

Desikan, R. S., Segonne, F., Fischl, B., Quinn, B. T., Dickerson, B. C., Blacker, D., et al. (2006). An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968–980. doi: 10.1016/j.neuroimage.2006.01.021

Dickerson, B. C., Feczko, E., Augustinack, J. C., Pacheco, J., Morris, J. C., Fischl, B., et al. (2009). Differential effects of aging and Alzheimer's disease on medial temporal lobe cortical thickness and surface area. *Neurobiol. Aging* 30, 432–440. doi: 10.1016/j.neurobiolaging.2007.07.022

Douaud, G., Lee, S., Alfaro-Almagro, F., Arthofer, C., Wang, C., McCarthy, P., et al. (2021). Brain imaging before and after COVID-19 in UK Biobank. *medRxiv* [Preprint]. doi: 10.1101/2021.06.11.21258690

Duan, K., Premi, E., Pilotto, A., Cristillo, V., Benussi, A., Libri, I., et al. (2021). Alterations of frontal-temporal gray matter volume associate with clinical measures of older adults with COVID-19. *Neurobiol. Stress* 14:100326. doi: 10.1016/J.YNSTR.2021.100326

Etter, M. M., Martins, T. A., Kulsvehagen, L., Pössnecker, E., Duchemin, W., Hogan, S., et al. (2022). Severe neuro-COVID is associated with peripheral immune signatures, autoimmunity and signs of neurodegeneration: A prospective cross-sectional study. *medRxiv* [Preprint]. medRxiv 2022.02.18.22271039. doi: 10.1101/2022.02.18.22271039

Fischl, B., and Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U.S.A.* 97, 11050–11055. doi: 10.1073/PNAS.200033797

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* 33, 341–355. doi: 10.1016/S0896-6273(02)00569-X

Fischl, B., Van Der Kouwe, A., Destrieux, C., Halgren, E., Ségonne, F., Salat, D. H., et al. (2004). Automatically parcellating the human cerebral cortex. *Cereb. Cortex* 14, 11–22. doi: 10.1093/CERCOR/BHG087

Fischl, B., Sereno, M. I., Tootell, R. B. H., and Dale, A. M. (1999). High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum. Brain Mapp.* 8, 272–284. doi: 10.1002/(SICI)1097-019319998:4

Fotuhi, M., Mian, A., Meysami, S., and Raji, C. A. (2020). Neurobiology of COVID-19. *J. Alzheimers Dis.* 76, 3–19. doi: 10.3233/JAD-200581

Gabay, C., and Kushner, I. (2008). Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* 340, 448–454. doi: 10.1056/NEJM199902113400607

García-Azorín, D., Sierra, Á, Trigo, J., Alberdi, A., Blanco, M., Calcerrada, I., et al. (2021). Frequency and phenotype of headache in covid-19: A study of 2194 patients. *Sci. Rep.* 11:14674. doi: 10.1038/s41598-021-94220-6

Gori, A., Leone, F., Loffredo, L., Cinicola, B. L., Brindisi, G., De Castro, G., et al. (2020). COVID-19-related anosmia: The olfactory pathway hypothesis and early intervention. *Front. Neurol.* 11:956. doi: 10.3389/FNEUR.2020.00956/BIBTEX

Guedj, E., Campion, J. Y., Dudouet, P., Kaphan, E., Bregeon, F., Tissot-Dupont, H., et al. (2021). 18F-FDG brain PET hypometabolism in patients with long COVID. *Eur. J. Nucl. Med. Mol. Imaging* 48, 2823–2833.

Harch, P., and Fogarty, E. (2017). Subacute normobaric oxygen and hyperbaric oxygen therapy in drowning, reversal of brain volume loss: A case report. *Med. Gas Res.* 7:144. doi: 10.4103/2045-9912.208521

Helms, J., Kremer, S., Merdji, H., Clere-Jehl, R., Schenck, M., Kummerlen, C., et al. (2020). Neurologic features in severe SARS-CoV-2 infection. *N. Engl. J. Med.* 382, 2268–2270. doi: 10.1056/NEJMC2008597/SUPPL_FILE/NEJMC2008597_DISCLOSURES.PDF

Hosp, J. A., Dressing, A., Blazhenets, G., Bormann, T., Rau, A., Schwabenland, M., et al. (2021). Cognitive impairment and altered cerebral glucose metabolism in the subacute stage of COVID-19. *Brain* 144, 1263–1276. doi: 10.1093/BRAIN/AWAB009

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5/ATTACHMENT/D5332CA1-83D8-4C4C-BC57-00A390BF0396/MMC1.PDF

Huang, X., Hussain, B., and Chang, J. (2021). Peripheral inflammation and blood–brain barrier disruption: Effects and mechanisms. *CNS Neurosci. Ther.* 27, 36–47. doi: 10.1111/CNS.13569

Ibi, K., Fujii, K., Kobayashi, H., Senda, M., Kitazawa, K., and Honda, A. (2019). Anterior cingulate cortex involvement in non-paraneoplastic limbic encephalitis. *Brain Dev.* 41, 735–739. doi: 10.1016/J.BRAINDEV.2019.04.006

Jarius, S., Pache, F., Körtvelyessy, P., Jelčić, I., Stettner, M., Franciotta, D., et al. (2022). Cerebrospinal fluid findings in COVID-19: A multicenter study of 150 lumbar punctures in 127 patients. *J. Neuroinflammation* 19:19.

Kas, A., Soret, M., Pyatigoskaya, N., Habert, M. O., Hesters, A., Le Guennec, L., et al. (2021). The cerebral network of COVID-19-related encephalopathy: A longitudinal voxel-based 18F-FDG-PET study. *Eur. J. Nucl. Med. Mol. Imaging* 48, 2543–2557. doi: 10.1007/S00259-020-05178-Y/TABLES/4

Koralnik, I. J., and Tyler, K. L. (2020). COVID-19: A global threat to the nervous system. *Ann. Neurol.* 88, 1–11. doi: 10.1002/ANA.25807

Kotfis, K., Williams Roberson, S., Wilson, J. E., Dabrowski, W., Pun, B. T., and Ely, E. W. (2020). COVID-19: ICU delirium management during SARS-CoV-2 pandemic. *Crit. Care* 24:375 doi: 10.1186/S13054-020-02882-X/TABLES/1

Kremer, S., Lersy, F., Anheim, M., Merdji, H., Schenck, M., Oesterlé, H., et al. (2020). Neurologic and neuroimaging findings in patients with COVID-19. *Neurology* 95, e1868–e1882. doi: 10.1212/WNL.0000000000010112

Ladopoulos, T., Zand, R., Shahjouei, S., Chang, J. J., Motte, J., Charles James, J., et al. (2021). COVID-19: Neuroimaging features of a pandemic. *J. Neuroimaging* 31, 228–243. doi: 10.1111/JON.12819

Lemaitre, H., Goldman, A. L., Sambataro, F., Verchinski, B. A., Meyer-Lindenberg, A., Weinberger, D. R., et al. (2012). Normal age-related brain morphometric changes: Nonuniformity across cortical thickness, surface area and gray matter volume? *Neurobiol. Aging* 33:617.e1-9. doi: 10.1016/j.neurobiolaging.2010.07.013

Ligthart, S., Sedaghat, S., Arfan Ikram, M., Hofman, A., Franco, O. H., and Dehghan, A. (2014). A novel inflammatory marker for incident coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* 34, 2695–2699. doi: 10.1161/ATVBAHA.114.304306

Lu, Y., Li, X., Geng, D., Mei, N., Wu, P. Y., Huang, C. C., et al. (2020). Cerebral micro-structural changes in COVID-19 patients – an MRI-based 3-month follow-up study. *EClinicalMedicine* 25:100484. doi: 10.1016/J.ECLINM.2020.100484

Manca, R., De Marco, M., Ince, P. G., and Venneri, A. (2021). Heterogeneity in regional damage detected by neuroimaging and neuropathological studies in older adults with COVID-19: A cognitive-neuroscience systematic review to inform the long-term impact of the virus on neurocognitive trajectories. *Front. Aging Neurosci.* 13:258. doi: 10.3389/FNAGI.2021.646908/BIBTEX

McMahon, C. L., Staples, H., Gazi, M., Carrion, R., and Hsieh, J. (2021). SARS-CoV-2 targets glial cells in human cortical organoids. *Stem Cell Rep.* 16, 1156–1164. doi: 10.1016/J.STEMCR.2021.01.016/ATTACHMENT/AA8B333B-E15F-472F-8F11-152E610F261C/MMC1.PDF

McQuaid, C., Brady, M., and Deane, R. (2021). SARS-CoV-2: Is there neuroinvasion? *Fluids Barriers CNS* 18:32. doi: 10.1186/S12987-021-00267-Y

Meinhardt, J., Radke, J., Dittmayer, C., Franz, J., Thomas, C., Mothes, R., et al. (2020). Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat. Neurosci.* 24, 168–175. doi: 10.1038/s41593-020-00758-5

Meppiel, E., Peiffer-Smadja, N., Maury, A., Bekri, I., Delorme, C., Desestret, V., et al. (2021). Neurologic manifestations associated with COVID-19: A multicentre registry. *Clin. Microbiol. Infect.* 27, 458–466. doi: 10.1016/J.CMI.2020.11.005/ATTACHMENT/755D3954-798E-4845-8089-DB5068B79E95/MMC2.DOCX

Ni, W., Yang, X., Yang, D., Bao, J., Li, R., Xiao, Y., et al. (2020). Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit. Care* 24:422.

Pajo, A. T., Espiritu, A. I., Apor, A. D. A. O., and Jamora, R. D. G. (2021). Neuropathologic findings of patients with COVID-19: A systematic review. *Neurol. Sci.* 42, 1255–1266.

Panizzon, M. S., Fennema-Notestine, C., Eyler, L. T., Jernigan, T. L., Prom-Wormley, E., Neale, M., et al. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cereb. Cortex* 19, 2728–2735. doi: 10.1093/cercor/bhp026

Paterson, R. W., Brown, R. L., Benjamin, L., Nortley, R., Wiethoff, S., Bharucha, T., et al. (2020). The emerging spectrum of COVID-19 neurology: Clinical, radiological and laboratory findings. *Brain* 143, 3104–3120. doi: 10.1093/BRAIN/AWAA240

Pellegrini, L. (2020). SARS-CoV-2 infects the brain choroid plexus and disrupts the blood-CSF barrier in human brain organoids. *Cell Stem Cell* 27, 951.e–961.e. doi: 10.1016/j.stem.2020.10.001

Perico, L., Benigni, A., Casiraghi, F., Ng, L. F. P., Renia, L., and Remuzzi, G. (2020). Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. *Nat. Rev. Nephrol.* 17, 46–64. doi: 10.1038/s41581-020-00357-4

Pezzini, A., and Padovani, A. (2020). Lifting the mask on neurological manifestations of COVID-19. *Nat. Rev. Neurol.* 16, 636–644. doi: 10.1038/s41582-020-0398-3

Qin, Y., Wu, J., Chen, T., Li, J., Zhang, G., Wu, D., et al. (2021). Long-term microstructure and cerebral blood flow changes in patients recovered from COVID-19 without neurological manifestations. *J. Clin. Investig.* 131:e147329. doi: 10.1172/JCI147329

Sanabria-Diaz, G., Etter, M., Med, C., Melie-Garcia, L., Lieb, J., Psychogios, M.-N., et al. (2022). Brain cortical changes are related to inflammatory biomarkers in hospitalized SARS-CoV-2 patients with neurological symptoms. *medRxiv* [Preprint]. medRxiv 2022.02.13.22270662. doi: 10.1101/2022.02.13.22270662

Shi, H., Zuo, Y., Navaz, S., Harbaugh, A., Hoy, C. K., Gandhi, A. A., et al. (2022). Endothelial cell-activating antibodies in COVID-19. *medRxiv* [Preprint]. medRxiv 2021.01.18.21250041. doi: 10.1101/2021.01.18.21250041

Solomon, I. H., Normandin, E., Bhattacharyya, S., Mukerji, S. S., Keller, K., Ali, A. S., et al. (2020). Neuropathological features of Covid-19. *N. Eng. J. Med.* 383, 989–992. doi: 10.1056/NEJMC2019373/SUPPL_FILE/NEJMC2019373_DISCLOSURES.PDF

Tandon, M., Kataria, S., Patel, J., Mehta, T. R., Daimee, M., Patel, V., et al. (2021). A comprehensive systematic review of CSF analysis that defines neurological manifestations of COVID-19. *Int. J. Infect. Dis.* 104, 390–397. doi: 10.1016/J.IJID.2021.01.002

Taquet, M., Dercon, Q., Luciano, S., Geddes, J. R., Husain, M., and Harrison, P. J. (2021). Incidence, co-occurrence, and evolution of long-COVID features: A 6-month retrospective cohort study of 273,618 survivors of COVID-19. *PLoS Med.* 18:e1003773. doi: 10.1371/JOURNAL.PMED.1003773

Thepmankorn, P., Bach, J., Lasfar, A., Zhao, X., Souayah, S., Chong, Z. Z., et al. (2021). Cytokine storm induced by SARS-CoV-2 infection: The spectrum of its neurological manifestations. *Cytokine* 138:155404. doi: 10.1016/J.CYTO.2020.155404

Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., et al. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 395, 1417–1418. doi: 10.1016/S0140-6736(20)30937-5/ATTACHMENT/9001FF44-4EF3-4AF2-952A-3D2B1116074F/MMC1.PDF

von Weyhern, C. H., Kaufmann, I., Neff, F., and Kremer, M. (2020). Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet* 395:e109. doi: 10.1016/S0140-6736(20)31282-4

Yang, A. C., Kern, F., Losada, P. M., Agam, M. R., Maat, C. A., Schmartz, G. P., et al. (2021). Dysregulation of brain and choroid plexus cell types in severe COVID-19. *Nature* 595, 565–571. doi: 10.1038/s41586-021-03710-0

Zhang, H., Wang, X., Lin, J., Sun, Y., Huang, Y., Yang, T., et al. (2013). Reduced regional gray matter volume in patients with chronic obstructive pulmonary disease: A voxel-based morphometry study. *Am. J. Neuroradiol.* 34, 334–339. doi: 10.3174/AJNR.A3235

frontiers | Frontiers in Neuroscience

TYPE Systematic Review
PUBLISHED 09 February 2023
DOI 10.3389/fnins.2023.1004957

OPEN ACCESS

EDITED BY
Gonzalo Emiliano Aranda-Abreu,
Universidad Veracruzana, Mexico

REVIEWED BY
Abhishek Arora,
All India Institute of Medical Sciences Bibinagar, India
Yifan Fu,
Beihang University, China
Marco Ruggiero,
National Coalition of Independent Scholars, United States

*CORRESPONDENCE
Agnieszka Korchut
✉ agnieszka.korchut@umlub.pl

SPECIALTY SECTION
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

RECEIVED 27 July 2022
ACCEPTED 16 January 2023
PUBLISHED 09 February 2023

CITATION
Korchut A and Rejdak K (2023) Late neurological consequences of SARS-CoV-2 infection: New challenges for the neurologist. *Front. Neurosci.* 17:1004957. doi: 10.3389/fnins.2023.1004957

Late neurological consequences of SARS-CoV-2 infection: New challenges for the neurologist

Agnieszka Korchut* and Konrad Rejdak

Department of Neurology, Medical University of Lublin, Lublin, Poland

Objective: In this study, a systematic review of the literature was performed to study the frequency of neurological symptoms and diseases in adult patients with COVID-19 that may be late consequences of SARS-CoV-2 infection.

Methods: Relevant studies were identified through electronic explorations of Scopus, PubMed, and Google Scholar. We followed PRISMA guidelines. Data were collected from studies where the diagnosis of COVID-19 was confirmed and its late neurological consequences occurred at least 4 weeks after initial SARS-CoV-2 infection. Review articles were excluded from the study. Neurological manifestations were stratified based on frequency (above 5, 10, and 20%), where the number of studies and sample size were significant.

Results: A total of 497 articles were identified for eligible content. This article provides relevant information from 45 studies involving 9,746 patients. Fatigue, cognitive problems, and smell and taste dysfunctions were the most frequently reported long-term neurological symptoms in patients with COVID-19. Other common neurological issues were paresthesia, headache, and dizziness.

Conclusion: On a global scale of patients affected with COVID-19, prolonged neurological problems have become increasingly recognized and concerning. Our review might be an additional source of knowledge about potential long-term neurological impacts.

KEYWORDS

COVID-19, SARS-CoV-2, long haul, neurological manifestation, neurological complication, neuro-COVID-19, post-COVID-19

Introduction

There is growing evidence indicating that neurological manifestations occur in patients as sequelae of COVID-19 (Misra et al., 2021). Approximately one-third of positive patients develop neurological and neuropsychiatric symptoms (Rudroff et al., 2020).

SARS-CoV-2 neurotropism has been increasingly recognized by its imaging and clinical manifestations from severe (encephalitis) to mild (hyposmia) in the literature. The neurological symptoms profile associated with COVID-19 covers symptoms of the central nervous system, peripheral nervous system, and neuromuscular disorders. The impact of SARS-CoV-2 on the nervous system is associated with the following issues: olfactory and taste disorders, Guillain–Barre syndrome (GBS), encephalopathy, neurological inflammation (myelitis, encephalitis, and meningitis), cerebrovascular diseases, seizures, cognitive impairment, myalgia, non-specific symptoms such as headache, dizziness, and fatigue, or neuropsychiatric symptoms such as anxiety, depression, psychosis, and sleep disorder (Divani et al., 2020; Collantes et al., 2021; Roy et al., 2021; Yassin et al., 2021). Most infected people develop mild to moderate illness and recover without requiring hospitalization, while others must be hospitalized.

Neurological symptoms are not necessarily correlated with the severity of COVID-19 infection, implying that different mechanisms or timing of mechanisms may be involved (Rogers et al., 2021).

These symptoms can appear in three disease periods, such as acute (parainfectious), post (postinfectious), and late infections (long-term sequelae). Datta et al. (2020) presented a theoretical timeframe for periods of SARS-CoV-2 infection: acute infection (from the onset of symptoms up to 2 weeks), post-acute infection (2 weeks after initial infection), and late sequelae (4 weeks after initial infection) (Datta et al., 2020). According to current knowledge, concerning the duration of neurological manifestation from COVID-19 symptoms onset, neurological issues can be placed in a timeframe.

Regarding cerebrovascular diseases, most manifestations occur within 21 days from COVID-19 onset, and stroke was rarely the first manifestation (Vogrig et al., 2021).

In the literature, neurological inflammation related to COVID-19 is observed as para- or postinfectious disease (Paterson et al., 2020). On average, encephalitis occurred 14.5 days after the diagnosis of COVID-19 infection (range = 10.8–18.2 days) (Siow et al., 2021).

Cases of Guillain–Barre syndrome in patients with COVID-19 have been described as a parainfectious disease (Romoli et al., 2020) or a postinfectious disease with a 2-week interval between SARS-CoV-2 and GBS infection (Palaiodimou et al., 2021).

It is known that severe acute respiratory syndrome coronavirus (SARS) and Middle East respiratory syndrome coronavirus (MERS) may have prolonged neurological impact (Ngai et al., 2010; Hosseiny et al., 2020). Emerging evidence suggests that the neuroinvasive nature of COVID-19 may be the driving force behind late neurological complications.

An increasing number of patients with COVID-19 continue to experience symptoms for months, even after recovering from mild cases of COVID-19 such as muscle pain, dizziness, headaches, fatigue, and anosmia (Wijeratne and Crewther, 2020), as well as signs and symptoms involving cognitive functions (Baig, 2020). Qin et al. (2021) found that the patients with mild- and severe-type COVID-19 with no specific neurological manifestations or obvious lesions on the conventional MRI, although recovered from pneumonia, still exhibited brain microstructure changes and a decrease in cerebral blood flow after a 3-month follow-up (Qin et al., 2021). For healthcare professionals and scientists, the prolonged neurological impact is a new challenge. In the literature, we can find different nomenclature for this phenomenon as Chronic COVID syndrome (CCS) (Baig, 2020), Post-COVID-19 Neurological Syndrome (PCNS) (Wijeratne and Crewther, 2020), Long COVID, and Long hauler COVID (Mendelson et al., 2020).

This review focused on the neurological symptoms and diseases that may be late consequences of SARS-CoV-2 infection.

Methods

The databases Scopus, PubMed, and Google Scholar were reviewed before 4 June 2022. An individual database search strategy was adopted with the following variations of keywords: ("COVID-19" OR "COVID19" OR "neuro-covid 19" OR "Sars Cov-2" OR "coronavirus") AND ("long term" OR "chronic" OR "long haul*" OR "post-covid*" OR "post covid*" OR "long covid*") AND ("neurological manifestation" OR "neurological complication" OR "neurolog*"). The first step was the title screening; the second was the abstract screening, and the third step was a full-text review of the relevant information. We followed PRISMA guidelines.

The inclusion criteria

The criteria for inclusion in the publication review were as follows:

- English and German language publications, which reported post-COVID-19 neurological issues among the adult population (subjective and/or objective) and
- data were collected from studies where all the patients were confirmed with positive SARS-CoV-2 PCR or antibody test.

The time criteria for identifying neurological issues were at least 4 weeks after the initial SARS-CoV-2 infection.

The exclusion criteria

We excluded review articles and publications that relied on the analysis of neurological symptoms associated with previous outbreaks (SARS in 2003; MERS in 2012). In addition, neuropsychiatric symptoms such as anxiety, depression, psychosis, and sleep disturbances were not included. Database searches were combined and duplicates were removed.

The following variables were extracted from included studies: first author, type, year and source of publication, research country, sample size, neurological issues, methods, and time when symptoms were identified. In addition, attention was paid to the severity of the COVID-19 disease course, whose severity was measured by hospitalization status without distinction between hospitalization in the intensive care unit. Descriptive analyses were applied. The incidence of neurological issues was presented as numbers and percentages. If in the analyzed article there were more follow-up visits within the time criterion of our study, the most numerous study group was selected. However, if there was the same sample size at the follow-up visits, the last visit was selected.

Categorization of neurological symptoms

To summarize the neurological issues, we had to define a cluster of cognitive problems. Such a cluster was defined as any subjective reports of concentration, memory and attention difficulty, perceived "brain fog", disorientation/confusion, word-finding difficulty, inability to effectively multitask, and measurable cognitive impairment confirmed by a test. "Frequent," "more frequent," and "the most frequent" neurological consequences were defined to have a frequency above 5, 10, and 20%, respectively, and were reported in at least five different studies including at least 1,000 of all patients studied. The term "possible significant neurological consequences" was used if the frequency of symptoms was reported above 15% in at least three publications, with a total study group of at least 300.

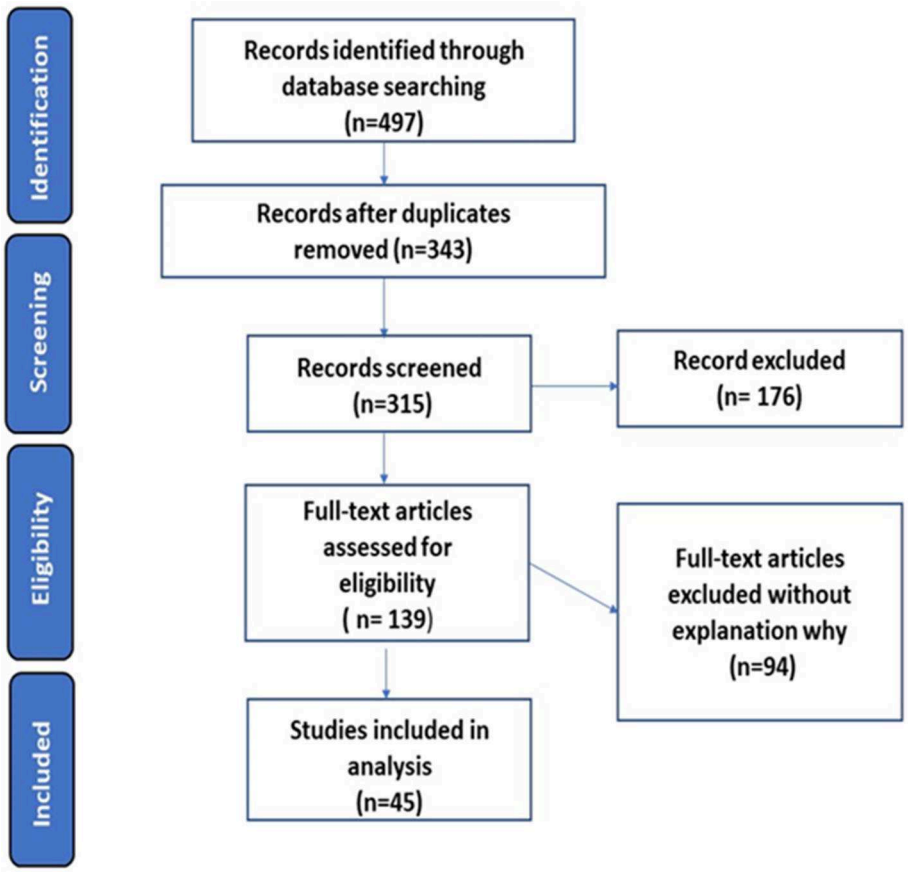


FIGURE 1
PRISMA flow diagram.

Results

Included types of publications—Short characteristic

A total of 497 articles were identified for eligible content. After excluding duplicates and screening titles and abstracts, which did not meet inclusion criteria, 139 full-text publications were assessed. From full-text publications, 94 were excluded due to the non-relevance of the investigated topic. Our review included data from 45 articles: retrospective studies (2), prospective studies (21), case reports/series (15), and cross-sectional studies (7). PRISMA flow diagram is presented in Figure 1. Features of studies included in our review are summarized in Table 1.

Characteristic of neurological issues

In our study, "the most frequent" neurological consequences were fatigue and cognitive problems. Paresthesia and altered smell/taste were classified as "more frequent" and headache and dizziness were identified as "frequent" neurological symptoms in patients with COVID-19. Myalgia and blurred vision were identified as "possible significant neurological consequences" (Table 2). Figure 2 represents the percentage distribution of neurological symptoms as late sequelae of SARS-CoV-2 infection.

Discussion

Our review presents the neurological issues that may be late consequences of SARS-CoV-2 infection. This article provides relevant information from 45 studies involving 9,746 patients. Pooled evidence showed that fatigue, cognitive problems, altered smell/taste, and paresthesia were very common neurological issues that were identified at least 4 weeks after a positive SARS-CoV-2 polymerase chain reaction (PCR) test and/or symptomatic start of confirmed SARS-CoV-2 infection. Other common neurological issues were headaches and dizziness.

Many studies reported a high rate of post-COVID-19 fatigue (Huang et al., 2020; Townsend et al., 2020; Wang et al., 2020). Fatigue is a non-specific symptom that accompanies many diseases, including infectious diseases. Of more than 6,000 studied patients from 15 different articles, more than 40% reported fatigue. It is worth mentioning that fatigue is reported in 5–45% of the general healthy population (Finsterer and Mahjoub, 2014). However, the studies included in our review did not focus on fatigue as the leading symptom. These studies evaluated various sets of symptoms with a neurological profile that accompanies the patient even for

TABLE 1 Summarized features of the included studies.

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Romero-Duarte et al. (2021)	Retrospective study	Spain	*N*: 797	H	Persistent anosmia or dysgeusia [57 (7%)] Muscular debility acquired in ICU [25 (3%)] Headache [42 (5%)] Paresthesia [27 (3%)] Movement disturbances [27 (3%)] Disorientation or confusion [21 (3%)] Vertigo [15 (2%)]	After 6 months hospital discharge, collected through clinical histories and primary care reports
Vanichkachorn et al. (2021)	Case series	USA	*N*: 100 *N*: 75	M, NH	Fatigue [80 (80%)] Headache [20 (20%)] Dizziness [19 (19%)] Paresthesia [17 (17%)] Persistent altered taste/smell [9 (9%)] Cognitive impairment [45 (45%)]	At least 4 weeks after a positive SARS-CoV-2 polymerase chain reaction (PCR) test and/or symptomatic start of confirmed SARS-CoV-2 infection, face-to-face visits and/or virtually by using either video telemedicine or telephone interactions.
Bozzali et al. (2021)	Case report	Italy	*N*: 1	NH	Focal seizures with impaired awareness	Two months after acute phase of SARS-CoV-2 infection MRI CSF analysis Blood test [18F]FDG positron emission tomography (for the exclusion of other causes)
Orr et al. (2021)	Retrospective study	Italy	*N*: 74	M	Fatigue [59 (80%)] Headache [40 (54%)] Cognitive impairment [44 (59%)] Dizziness [25 (34%)] Paresthesia [26 (35%)] Loss of taste [21 (28%)] Loss of smell [24 (32%)] Myalgia [44 (59%)]	Three follow-up visits (time relative to negative swab of SARS-CoV-2) at least a month, at least 2 months, at least 3 months, survey
			N: 154	M	Fatigue [121 (79%)] Headache [76 (49%)] Cognitive impairment [73 (47%)] Dizziness [33 (21%)] Paresthesia [48 (31%)] Loss of taste [39 (25%)] Loss of smell [42 (27%)] Myalgia [81 (53%)]	
			N: 152	M	Fatigue [113 (74%)] Headache [71 (47%)] Cognitive impairment [74 (49%)] Dizziness [34 (22%)] Paresthesia [53 (35%)] Loss of taste [41 (27%)] Loss of smell [47 (31%)] Myalgia [93 (61%)]	

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Miskowiak et al. (2021)	Prospective study	Denmark	*N*: 29	H	Cognitive impairment [17 (60%)]	3-4 months after discharge, Cognitive Impairment in Psychiatry Danish Version (SCIP-D), Trail Making Test-Part B (TMT-B), Cognitive Failures Questionnaire (CFQ)
Graham et al. (2021)	Prospective study	USA	*N*: 50	NH	Headache [32 (16%)] Brain fog [41 (20.5%)] Dizziness [20 (10%)] Numbness/tingling [29 (14.5%)] Myalgia [30 (15%)] Dysgeusia [32 (16%)] Anosmia [37 (18.5%)] Blurred vision [9 (4.5%)] Fatigue [42 (21%)] Abnormal movement [2 (1%)] Sensory dysfunction [3 (1.5%)] Cranial nerve dysfunction [5 (2.5%)] Dysarthria [2 (1%)] Dysphagia [1 (0.5%)] Short-term memory deficit1 [5 (2.5%)] Attention deficit [12 (6%)] Motor dysfunction [3 (1.5%)] Gait dysfunction [3 (1.5%)] Cerebellar dysfunction [1 (0.5%)]	More than 6 weeks from symptoms onset Evaluated for in-person visits and telemedicine 4-item recall Serial 7s
Raahimi et al. (2021)	Case report	UK	*N*: 1	H	GBS	53 days after having SARS-CoV-2 infection CSF test NCS
Carfi et al. (2020)	Case series	Italy	*N*: 143	H	Anosmia [21 (15 %)] Headache [14 (10%)] Vertigo [7 (5%)]	Mean of 60.3 (SD, 13.6) days after onset of the first COVID-19 symptom Medical assessment with detailed history and physical examination
Carvalho-Schneider et al. (2020)	Prospective study	France	*N*: 150 *N*: 116	M NH	Anosmia/ageusia [40 (27.8%)]	Two follow-up visits: 1 month and 2 months after COVID-19 symptoms onset, data collected by phone call
			N: 130 *N*: 101	M NH	Anosmia/ageusia [29 (22.7%)]	
Kayaaslan et al. (2021)	Prospective study	Turkey	*N*: 1,007 *N*: 591	M NH	Concentrations and memory deficit [163 (16.2%)] Headache [57 (5.7%)] Loss of smell [31 (3.1%)] Loss of taste [21 (2.1%)]	At least 3 months after SARS-CoV-2 infection, survey
Anaya et al. (2021)	Case series	Colombia	*N*: 100 *N*: 35	M NH	Back pain [55 (55%)] Headache [45 (45%)] Paresthesia [38 (38%)]	The median of post-COVID-19 time was 219 days (IQR: 143–258), survey

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
					Attention disorders [36 (36%)] Memory disorders [36 (36%)] Anosmia [11 (11%)] Dizziness [31 (31%)] Seizures [1 (1%)]	
Garrigues et al. (2020)	Case series	France	*N*: 120	H	Ageusia [13 (10.8%)] Anosmia [16 (13.3%)] Attention disorder [32 (26.7%)] Memory loss [41 (34.2%)]	At least 100 days after admission for COVID-19, questionnaire
Santis et al. (2020)	Prospective study	Spain	*N*: 108	NH	Headache [10 (9.3%)] Anosmia [10 (9.3%)] Dysgeusia [5 (5.6%)] Loss of memory [2 (1.9%)] Difficulty of concentrating [2 (1.9%)]	12 weeks after acute phase of SARS-CoV-2 infection, medical history and examination
Woo et al. (2020)	Cross-sectional study	Germany	*N*: 18 *N*: 7	M NH	Mild cognitive deficits [14 (78%)] Attention deficits [9 (50%)] Concentration deficits [8 (44.4%)] Short-term memory deficits [8 (44.4%)] Troubles in finding words [5 (27.8%)]	At least 20 days after recovery from SARS-CoV-2 infection, TICS-M (Modified Telephone Interview for Cognitive Status)
Leth et al. (2021)	Prospective study	Denmark	*N*: 49	H	Difficulties concentrating [19 (39%)] Impaired OMC test (*N*: 38) [8 (21%)] Paresthesia [8 (16%)] Headache [12 (24%)] Smell impairment1 [7 (35%)] Taste impairment [16 (33%)] *N*: 49 (H) Difficulties concentrating [22 (45%)] Impaired OMC test (*N*: 38) [4 (11%)] Paresthesia [13 (27%)] Headache [13 (27%)] Smell impairment [13 (27%)] Taste impairment [15 (31%)]	Two follow up visits: 6 and 12 weeks after discharge, medical history, OMC test (orientation, memory, and concentration)
Sykes et al. (2021)	Prospective study	UK	*N*: 78	H	Fatigue [26 (33.3%)] Myalgia [33 (42.3%)] Memory impairment [24 (30.8%)] Attention deficit [16 (20.5%)] Anosmia [8 (10.2%)] Cognitive impairment [4 (5.1%)] Taste deficiency [6 (7.7%)]	At least 101 days after discharge (101–125) Clinical assessment
Halpin et al. (2021)	Cross-sectional study	UK	*N*: 100	H	New or worsened concentration problem [22 (22%)] New or worsened short-term memory problem [18 (18%)]	4–8 weeks after discharge, specialist telephone screening tool

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Iqbal et al. (2021)	Cross-sectional study	Pakistan	*N*: 158	M	Loss of smell and taste [75 (47.5%)] Headache [57 (36.1%)] Brain fog [30 (19.0%)] Blurred vision [30 (19.0%)] Stroke [1 (6%)]	At least 20 days after recovery from COVID-19, questionnaire
Huang et al. (2021)	Prospective study	China	*N*: 1655	H	Fatigue or muscle weakness [1,038 (63%)] Smell disorder [176 (11%)] Taste disorder [120 (7%)] Dizziness [101 (6%)] Headache [33 (2%)]	Six months after discharge, series of questionnaires Physical examinations
Kanberg et al. (2021)	Prospective study	Sweden	*N*: 97	M	Fatigue [40 (41%)] Brain fog [29 (30%)] Changes in cognition [25 (26%)] Hyposmia [4 (4%)] Dysgeusia [5 (5%)]	Six months follow up, questionnaires
Stuby et al. (2021)	Case report	Switzerland	*N*: 1	H	Guillain–Barré syndrome (GBS)	1 month after SARS-CoV-2 infection NCS CSF analysis
Aasfara et al. (2021)	Case report	Marocco	*N*: 1	NH	Guillain–Barré syndrome (GBS) associated to a vestibulocochlear neuritis SARS-CoV-2 positive 6 weeks before (GBS)	6 weeks after a positive SARS-CoV-2 test, NCS, CSF analysis, audiometry and videonystagmography
Alemanno et al. (2021)	Prospective study	Italy	*N*: 56	H	Cognitive deficit [41 (73%)]	1 month after home discharge MoCA
Zhang et al. (2021)	Prospective study	China	*N*: 2,433	H	Fatigue [696 (27.7%)] Dizziness [82 (3.3%)] Headache [57 (2.3%)] Impaired sense of smell [32 (1.3%)]	At 1-year follow-up visit, questionnaires
Papri et al. (2021)	Case report	Bangladesh	*N*: 1	H	Guillain–Barré syndrome (GBS)	Six weeks after SARS-CoV-2 infection, NCS
Hellmuth et al. (2021)	Prospective study and two cases	USA	*N*: 14	NH	Cognitive deficits (symptoms were present for at least a median 98 days onset COVID-19)	At least a median 98 days after the onset of SARS-CoV-2 infection, medical interview
			N: 2	NH	Cognitive deficit	72 and 149 days after the onset of SARS-CoV-2 infection, California Verbal Learning Test-3 (16-word) WAIS Wechsler Adult Intelligence Scale IV Digital Span
Albu et al. (2021a)	Cross-sectional study	Spain			*N*: 30 (M) 7 (NH) Cognitive impairment [19 (63.3%)] CIP/CIM: Critical illness polyneuropathy/myopathy [7 (23.3%)]	3 months after acute phase of SARS-CoV-2 infection, Benton Temporal Orientation Test, Wechsler Adult Intelligence Scale III, Rey Auditory Verbal Learning Test, PMR task (a Spanish version of the FAS letter fluency task)

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Alvare et al. (2021)	Case report	USA			*N*: 1 (H) Extended neuralgic amyotrophy syndrome	Three weeks after discharge EMG, NCSs, and MRI.
Nakamura et al. (2021)	Case report	Japan			*N*: 1 (H) Restless legs syndrome variant	Several weeks after discharge, face-to-face interview and physical examination, colonoscopy, blood test
Poletti et al. (2021)	Cross-sectional study	Italy	*N*: 92 *N*: 122 *N*: 98	M M M	Cognitive impairment [73 (79%)] Cognitive impairment [92 (75%)] Cognitive impairment [67 (68%)]	Three follow up visits: 1-month and 2 and 3-months after discharge, Brief Assessment of Cognition in Schizophrenia (BACS), hospitalized/non hospitalized
Zhu et al. (2021)	Prospective study	China	*N*: 95	H	Hyposmia [22 (23.2%)]	At least 16 weeks after the onset of SARS-CoV-2 infection, Hyposmia Rating Scale (HRS), Brief Smell Identification Test for Chinese (B-SITC)
Boesl et al. (2021)	Prospective study	Germany	*N*: 100 *N*: 89	M NH	Cognitive impairment [72 (72%)] Fatigue [67 (67%)] Headache [36 (36%)] Hyposmia [36 (36%)] Myalgia [21 (21%)] Vertigo [20 (20%)] Limb pain [9 (9%)]	At least 12 weeks after acute phase of SARS-CoV-2 infection, questionnaires, Montreal Cognitive Assessment Scale (MoCA)
Ahmad and Salih (2021)	Case report	Iraq	*N*: 1	NH	Transverse myelitis	Two weeks after recovery from COVID-19, brain and cervical magnetic resonance imaging (MRI) CSF analysis
Frontera et al. (2021)	Prospective study	USA	*N*: 111	H	Cognitive impairment [50 (45%)] (without neurological complications during acute phase of SARS-CoV-2 infection)	Six months after discharge, Telephone MOCA (Montreal Cognitive Assessment)
			N: 90	H	Cognitive impairment [45 (50%)] (with neurological complications during acute phase of SARS-CoV-2 infection)	
Pistarini et al. (2021)	Cross-sectional study	Italy			*N*: 20 (H) Cognitive deficit [14 (70%)] (MoCA) [1 (5%)] (MMSE)	One month after SARS-CoV-2 infection, MMSE, MoCA
Park et al. (2021)	Case report	USA	*N*: 1	H	Focal seizures with impaired awareness	6 weeks after negative of SARS-CoV-2 test MRI CSF analysis Blood test EEG

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Carroll et al. (2020)	Case report	USA	*N*: 1	H	Refractory status epilepticus	6 weeks after initial infection with COVID-19 MRI CSF analysis Blood test EEG
Albu et al. (2021b)	Prospective study	Spain	*N*: 40	M	Cognitive complains [15 (37.5%)] Fatigue [35 (87.5%)]	Over 3 months after initial infection with COVID-19 Benton Temporal Orientation Test Wechsler Adult Intelligence Scale III Rey Auditory Verbal Learning Test PMR task (a Spanish version of the FAS letter fluency task)
			N: 32	M	Cognitive deficit in tests [23 (72.2%)]	
Pilotto and Cristillo (2021)	Prospective study	Italy	*N*: 165	H	Fatigue [56 (33.9%)] Memory/concentration complaints [52 (31.5%)] Myalgia [50 (30.3%)] Blurring/loss of vision [32 (19.5%)] Paresthesia [31 (18.8%)] Hyposmia/hypogeusia [27 (16.4%)] Urinary dysfunction [23 (13.9%)] Confusion [22 (13.3%)] Hypotension [20 (12.2%)] Gait disturbance [18 (10.9%)] Abnormal movements [17 (10.3%)] Headache [16 (9.7%)] Postural instability or falls [14 (8.5%)] Swallowing difficulties [10 (6.1%)]	At 6-month follow-up visits, questionnaire Neurological examination NCS MoCA
			N: 105	H	Sensor-motor polyneuropathy [2 (2%)] Cognitive impairment in test [17 (17%)] Enhanced physiological tremor [15 (15%)] Dysgeusia/hyposmia [19 (19%)]	
Garg et al. (2021)	Case report	USA	*N*: 1	H	Functional movement disorders - abnormal repetitive movement of the head	2 months after acute phase of SARS-CoV-2 infection, MRI, EEG
Rivera-Izquierdo et al. (2022)	Prospective study	Spain	*N*: 453	H	Fatigue [37 (8.2%)] Headache [13 (2.9)] Sensitivity disorders [9 (2.0)] Movement disorders [5 (1.1)] Confusion, memory loss [16 (3.5)]	3–4 months after discharge, consulted by telephone

(Continued)

10.3389/fnins.2023.1004957

TABLE 1 (Continued)

References	Type of publication	Research country	Characteristics of studies			
			Sample size (*N*)	Hospitalization status (H*/NH**/M***)	Neurological issues [*n* (%)]	Time to identified symptoms, methods
Rass et al. (2022)	Prospective study	Austria	*N*: 135 *N*: 81	M M	Hyposmia/anosmia, SS-16 < 13 [57 (45)] Cognition impairment MoCA (<26) [29 (23)] Neuropathy/myopathy [16 (12)] Muscular debility acquired in ICU [8 (6)] Symmetric axonal distal neuropathy [7 (5)] Compression neuropathy [3 (2)] GBS [1 (1)] Hyposmia/anosmia, SS-16 <13 [41 (51)] Cognition impairment MoCA (<26) [14 (18)] Neuropathy/myopathy [8 (9)] Muscular debility acquired in ICU [1 (1)] Symmetric axonal distal neuropathy [3 (4)] Compression neuropathy [3 (4)]	Two follow-up visits (3-month and 1-year), neurological examination and a standardized test battery including the assessment of hyposmia (16-item Sniffin' Sticks test), cognitive deficits (Montreal Cognitive Assessment < 26)
Bungenberg and Humkamp (2022)	Cross-sectional study	Germany	*N*: 21	H	Fatigue [13 (60%)] Cognitive problems [18 (86)] Altered smell/taste [12 (57)] Paresthesia [2 (10)] Sensory deficit [9 (43)] Impaired fine motor skills [4 (19)] Paresis [3 (14)] CIP/CIM [9 (43)] Seizures [1 (5)] Stroke/TIA [2 (10)]	The median timespan after infection was 41 weeks (range 18.14–52.29), neurological examination, common standardized neuropsychological testing battery inter alia MoCA, TAP (Test of Attentional Performance), patient-reported outcome measures (PROMs) and MRI
Ali et al. (2022)	Prospective study	USA	*N*: 27	NH	Fatigue [22 (81)] Brain fog [16 (59)] Numbness/tingling [14 (52)] Headache [13 (48)] Dysgeusia [7 (26)] Anosmia [9 (33)] Dizziness [11 (41)] Blurred vision [8 (30)]	6–9 months after their initial Neuro-COVID-19 clinic evaluation. Phone/email questionnaire
Wong-Chew et al. (2022)	Prospective study	Mexico	*N*: 928	H	Fatigue [232 (25)] Headache [158 (17)] Lack of concentration [91 (9.8)] Loss of memory [78 (8.4)] Bradyphrenia [46 (5)] Disorientation [20 (2.1)] Paresthesia [97 (10.5)] Anosmia [32 (3.4)] Dysgeusia [25 (2.7)] Dizziness [60 (6.5)] Slow walking [37 (4)]	Over 90 days post-discharge, self-reported clinical symptom *via* telephone calls

*Hospitalized.
**Non-hospitalized.
***Mixed (H + NH).

10.3389/fnins.2023.1004957

TABLE 2 Summary of neurological issues reported in patients as late sequelae of SARS-CoV-2 infection.

Neurological issue	Total number studies	Total number of patients studied (*N*)	Number of patients showing symptoms (*n*)	*n*/*N* (%)	
Fatigue	15	6,444	2,581	40.05%	"The most frequent"
Cognitive problems	28	6,873	1,440	20.95%	
Altered smell/taste	21	7,484	1,047	13.93%	"More frequent"
Paresthesia	11	2,844	325	11.43%	
Headache	17	8,427	692	8.21%	"Frequent"
Dizziness	8	5,447	357	6.55%	
Myalgia	4	497	185	37.22%	"Possible significant neurological consequences"
Blurred vision	3	373	71	19.03%	
Vertigo	3	1,040	42	4.04%	"Other rare neurological consequences"
Movement disturbance	5	2,364	90	3.81%	
Muscular debility acquired in ICU	4	983	49	4.98%	
GBS	4	4	4	100.00%	
Seizures/status epilepticus	5	124	5	4.03%	
Sensory disfunction	2	71	12	16.90%	
Cranial nerve disfunction	1	50	5	10.00%	
Dysarthria	1	50	1	2.00%	
Dysphagia	1	50	1	2.00%	
Back/limb pain	2	200	64	32.00%	
Stroke	2	179	3	1.68%	
Bradyphrenia	1	928	46	4.96%	
Restless legs syndrome variant	1	1	1	100.00%	
Transfers myelitis	1	1	1	100.00%	
Functional movement disorders	1	1	1	100.00%	
Extended neuralgic amyotrophy syndrome	1	1	1	100.00%	

months after recovery from COVID-19. Bearing in mind the above data, it is difficult to conclude whether the problem of fatigue has increased during the pandemic. Therefore, further analysis of this issue is necessary.

There is a growing concern about the cognitive aspect of people who have recovered from COVID-19. Hampshire et al., based on cross-sectional cognitive performance data from 81,337 participants, observed that cognitive impairments were most pronounced in people who had been hospitalized but, importantly, were also observed in non-hospitalized patients with no reported breathing difficulties (Hampshire et al., 2021). Therefore, it is easier to understand the cognitive problems in patients hospitalized for COVID-19, who were more likely to have hypoxia, as well as septic complications. Hypoxia is a common cause of neuropsychological changes observed in acute respiratory distress syndrome (Hopkins et al., 2006). Patients who have required ventilation for multiple reasons may need help with daily tasks due to problems with attention, memory, verbal fluency, and information processing speed (Mikkelsen et al., 2012; Sasannejad et al., 2019). Thus, it is well-known that hypoxia, sepsis, and the accompanying immune hyperstimulation contribute to cognitive deficits.

It is less clear that patients with mild COVID-19 course, who have not been hospitalized, may also have objectively measurable Alzheimer's disease (AD)-like cognitive impairment (Albu et al., 2021b; Boesl et al., 2021; Papri et al., 2021). Although the specific mechanisms remain largely unknown, a recent study based on the use of single nuclear RNA sequencing datasets revealed associations between the pathogenic mechanisms of COVID-19 and AD. Researchers have identified significant similarities in neuronal damage, synaptic dysfunction, and neuroinflammation in both diseases. They presented the role of neural cell adhesion molecule 2 (NCAM2) and ICA1L (AD gene marker) in the process leading to cognitive impairment, which may be a potential target for AD intervention (Fu et al., 2022).

There is still too little information in the literature about baseline (before COVID-19 infection) clinical measures of cognitive-affective

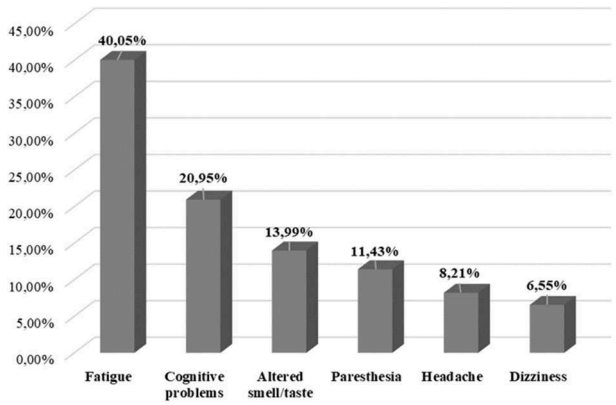


FIGURE 2
Percentage distribution of common neurological symptoms as late sequelae of SARS-CoV-2 infection.

alteration. Therefore, it can only be assumed that the cognitive impairment may be either the result of the direct negative effects of the SARS-CoV-2 virus or the acceleration and aggravation of pre-existing cognitive deficits. Hence, advancing medical scientific knowledge through full case reports with included pre-COVID-19 status seems the most appropriate way. A case report of a young "33-year-old woman" with cognitive deficits 149 days after the first COVID-19 symptoms is a good example, as we can find a comparison for cognitive tests from 12 years ago (Hellmuth et al., 2021).

Cognitive decline is often undiagnosed until it is more advanced, leading to impairment of the ability to perform daily activities. The SARS-CoV-2 infection has spread to all continents, affecting particularly hard older people with comorbidities. This group of people often experiences a diminished quality of life resulting from new impairments with accompanying limitations in activities and restrictions to their participation in life. Therefore, it is necessary to focus on the possible cognitive impact of SARS-CoV-2 infection. When analyzing the topic of cognitive problems after SARS-CoV-2 infection, it is worth paying attention to the promising reports on the reversibility of cognitive disorders. Blazhenets et al. (2021) demonstrated essential reversibility of decreased neocortical glucose metabolism assessed by 18F-FDG PET accompanied by an improvement in cognitive functions in patients with COVID-19 (subjective and objective MoCa examination) from the subacute stage to the chronic stage after SARS-CoV-2 infection.

Future work would benefit from systematic cognitive assessments of ambulatory patients with COVID-19.

A complete or partial loss of smell and taste sensations is the most frequent neurological manifestation of COVID-19. Their occurrence can be explained as the expression of SARS-CoV-2 entry receptors in the olfactory epithelium. Then, SARS-CoV-2 *via* the olfactory nerve can spread to the olfactory bulb (Desforges et al., 2019; Beltrán-Corbellini et al., 2020). Clinicians should be alert regarding olfactory disorders which may mark the onset of some neurodegenerative diseases (Zhu et al., 2021).

Paresthesia is a common non-specific symptom with which patients come to the neurological clinic. There is evidence of changes in nociceptor excitability that COVID-19 could induce through multiple potential mechanisms (McFarland et al., 2021).

Other non-specific symptoms reported in the studies were headaches and dizziness. If they persist for several weeks, it is of concern among symptom-experienced people and physicians. This is often the reason for extended diagnostics procedures. During the COVID-19 pandemic, the incidence of headaches increased 5-fold in the studied region (Lippi et al., 2020). *De novo* headache is common post-COVID-19 and can persist long after infection resolution. Post-COVID-19 headache has often migraineurs features which may reflect an activation of the trigeminovascular system by inflammation or direct involvement of SARS-CoV-2. This hypothesis can be supported by concomitant anosmia (Caronna et al., 2020; Al-Hashel et al., 2021).

Moreover, Al-Hashel et al. (2021) found in the cohort study that a significant number of patients with primary headaches had worsening of their headaches within 3 months after COVID-19 disease. Headache and dizziness were presented very commonly in relevant studies included in our review.

The frequency was above 8 and 6% for headache and dizziness, respectively, reported in 17 different studies for headache and eight for dizziness.

It is worth keeping in mind that the COVID-19 pandemic may contribute to poor mental health manifested by somatization in the form of mental fatigue, cognitive changes, paresthesia, headaches, or dizziness.

Moreover, myalgia and blurred vision were identified as "possible significant neurological consequences". Most cases of myalgia and blurred vision were self-reported and there was no information about the specificity of the symptoms. Rodriguez et al. (2021) considered whether myopathy is a part of long-COVID-19. They presented Multi Voltage Rule Check (MVRC) recordings 3 weeks after the onset of COVID-19 symptoms showed a marked reduction of early supernormality as a sign of muscle membrane depolarization compared to an earlier recording (Rodriguez et al., 2021).

In addition, it is worth referring to rare late neurological consequences of COVID-19 in our reviews, such as GBS and seizures. Most of the cases of GBS described in the literature are para- or directly postinfectious which is beyond the scope of our review. We found four relevant case reports related to GBS within the timeframe of our review where the interval between GBS and SARS-CoV-2 ranged from 1 month to 53 days. Keddie et al. (2021) compared GBS cases reported during the COVID-19 pandemic to GBS cases from 2016 to 2019. This epidemiological and cohort study investigated the UK population. Based on the comparison, the researchers concluded that GBS incidence has fallen during the pandemic. They assumed it might be caused by a lockdown that reduces transmission of GBS-inducing pathogens such as Campylobacter jejuni and respiratory viruses. There were no significant differences in the pattern of weakness, time to nadir, neurophysiology, CSF findings, or outcome between the COVID-19 pandemic group and the control groups (Keddie et al., 2021).

There are many descriptions of seizures during the acute infectious period in patients with COVID-19. Even convulsive and nonconvulsive status epilepticus triggered by SARS-CoV-2 virus infection has also been described (Emami et al., 2020; Somani et al., 2020; Asadi-Pooya et al., 2021). In our review,

we focused on seizures/status epilepticus as a late consequence of COVID-19. Seizures are not a common late manifestation of COVID-19.

Limitations

The main limitations were reliance on self-report measures in many articles. In some cases, there was a lack of clear information about comorbidities, so some symptoms may be due to pre-existing comorbidities. The same situation was about baseline assessment in the analyzed sources, which makes it impossible to reliably estimate the incidence.

Additionally, in some cases, the grouping of symptoms with an overlapping profile was used, which may have contributed to the fact that the frequency of some of the symptoms found in this review may be incorrectly estimated.

Conclusion

According to data from World Health Organization (WHO) by 3 June 2022, the total number of COVID-19 cases worldwide reaches 528,816,317.00.

Assuming that only a minority percentage of patients with COVID-19 will struggle with late neurological issues when calculated on a global scale of patients affected with COVID-19, the prolonged neurological impact has become increasingly recognized and concerning.

We must remember that symptoms such as fatigue, cognitive problems, smell/taste disturbance, paresthesia or headache, and dizziness may accompany patients for many weeks after infection with SARS-CoV-2. Thus, recognition and familiarity with these neurological issues are imperative in managing these patients.

In our review, the long-term neurological consequences of COVID-19 disease have been collected and categorized in a simple and transparent way. Therefore, our study could be an easily accessible source of knowledge for medical professionals.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

KR: conceptualization, project administration, and supervision. AK: data curation, formal analysis, investigation, methodology, and writing—original draft. AK and KR: writing—review and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Aasfara, J., Hajjij, A., Bensouda, H., Ouhabi, H., and Benariba, F. (2021). A unique association of bifacial weakness, paresthesia and vestibulocochlear neuritis as post-COVID-19 manifestation in pregnant women: a case report. *Pan Afr. Med. J.* 38, 30. doi: 10.11604/pamj.2021.38.30.27646

Ahmad, S. A., and Salih, K. H. (2021). Post COVID-19 transverse myelitis; a case report with review of literature. *Ann. Med. Surg.* 69, 102749. doi: 10.1016/j.amsu.2021.102749

Albu, S., Rivas Zozaya, N., and Murillo, N. (2021b). Multidisciplinary outpatient rehabilitation of physical and neurological sequelae and persistent symptoms of covid-19: a prospective, observational cohort study. *Disabil. Rehabil.* 44, 6833–6840. doi: 10.1080/09638288.2021.1977398

Albu, S., Zozaya, N. R., Murillo, N., García-Molina, A., Chacón, C. A. F., Kumru, H., et al. (2021a). What's going on following acute covid-19? Clinical characteristics of patients in an out-patient rehabilitation program. *NeuroRehabilitation* 48, 469–480. doi: 10.3233/NRE-210025

Alemanno, F., Houdayer, E., and Parma, A. (2021). COVID-19 cognitive deficits after respiratory assistance in the subacute phase: A COVID rehabilitation unit experience. *PLoS ONE* 16, 246590. doi: 10.1371/journal.pone.0246590

Al-Hashel, J. Y., Abokalawa, F., Alenzi, M., Alroughani, R., and Ahmed, S. F. J. (2021). Coronavirus disease-19 and headache; impact on pre-existing and characteristics of de novo: a cross-sectional study. *Headache Pain* 22, 97. doi: 10.1186/s10194-021-01314-7

Ali, S. T., Kang, A. K., Patel, T. R., Clark, J. R., Perez-Giraldo, G. S., Orban, Z. S., et al. (2022). Evolution of neurologic symptoms in non-hospitalized COVID-19 "long haulers". *Ann. Clin. Transl. Neurol.* 9, 950–961. doi: 10.1002/acn3.51570

Alvare, A., Amirianfar, E., Mason, M. C., Huang, L., Jose, J., Tiu, T., et al. (2021). Extended neuralgic amyotrophy syndrome in a confirmed COVID-19 patient after intensive care unit and inpatient rehabilitation stay. *Am. J. Phys. Med. Rehabil.* 100, 733–736. doi: 10.1097/PHM.0000000000001795

Anaya, J. M., Rojas, M., Salinas, M. L., Rodríguez, Y., Roa, G., Lozano, M., et al. (2021). Post-COVID syndrome. A case series and comprehensive review. Post-COVID study group. *Autoimmun Rev.* 20, 102947. doi: 10.1101/2021.07.17.21260655

Asadi-Pooya, A. A., Simani, L., Shahisavandi, M., and Barzegar, Z. (2021). COVID-19, *de novo* seizures, and epilepsy: a systematic review. *Neurol. Sci.* 42, 415–431. doi: 10.1007/s10072-020-04932-2

Baig, A. M. (2020). Deleterious outcomes in long-Hauler COVID-19: the effects of SARS-CoV-2 on the CNS in chronic COVID syndrome. *ACS Chem. Neurosci.* 11, 4017–4020. doi: 10.1021/acschemneuro.0c00725

Beltrán-Corbellini, Á., Chico-García, J. L., Martínez-Poles, J., Rodríguez-Jorge, F., Natera-Villalba, E., Gómez-Corral, J., et al. (2020). Acute-onset smell and taste disorders in the context ofCovid-19: a pilot multicenter PCR-based case-control study. *Eur J Neurol.* 27, 1738–1741. doi: 10.1111/ene.14359

Blazhenets, G., Schroeter, N., and Bormann, T. (2021). Slow but evident recovery from neocortical dysfunction and cognitive impairment in a series of chronic COVID-19 patients. *J. Nucl. Med.* 62, 910–915. doi: 10.2967/jnumed.121.262128

Boesl, F., Audebert, H., Endres, M., Prüss, H., and Franke, C. A. (2021). Neurological outpatient clinic for patients with post-COVID-19 syndrome — A report

on the clinical presentations of the first 100 patients. *Front. Neurol.* 12, 738405. doi: 10.3389/fneur.2021.738405

Bozzali, M., Grassini, A., and Morana, G. (2021). Focal seizures with impaired awareness as long-term neurological complication of COVID-19: a case report. *Neurol. Sci.* 42, 2619–2623. doi: 10.1007/s10072-021-05233-y

Bungenberg, J., and Humkamp, K. (2022). Long COVID-19: objectifying most self-reported neurological symptoms. *Ann. Clin. Transl. Neurol.* 9, 141–154. doi: 10.1002/acn3.51496

Carfì, A., Bernabei, R., and Landi, F. (2020). Gemelli against C-P-ACSG. Persistent symptoms in patients after acute COVID-19. *JAMA* 324, 603–605. doi: 10.1001/jama.2020.12603

Caronna, E., Ballvé, A., Llauradó, A., and Gallardo, V. J. (2020). Headache: a striking prodromal and persistent symptom, predictive of COVID-19 clinical evolution *Cephalalgia* 40, 1410–1421. doi: 10.1177/0333102420965157

Carroll, E., Neumann, H., Aguero-Rosenfeld, M. E., Lighter, J., Czeisler, B. M., Melmed, K., et al. (2020). Post-COVID-19 inflammatory syndrome manifesting as refractory status epilepticus. *Epilepsia* 61, 135–139. doi: 10.1111/epi.16683

Carvalho-Schneider, C., Laurent, E., Lemaignen, A., Beaufils, E., Bourbao-Tournois, C., Laribi, S., et al. (2020). Follow-up of adults with noncritical COVID-19 two months after symptom onset. *Clin. Microbiol. Infect.* 27, 258–263. doi: 10.1016/j.cmi.2020.09.052

Collantes, M. E. V., Espiritu, A. I., Sy, M. C. C., Anlacan, V. M. M., and Jamora, R. D. G. (2021). Neurological manifestations in COVID-19 infection: a systematic review and meta-analysis. *Can. J. Neurol. Sci.* 48, 66–76. doi: 10.1017/cjn.2020.146

Datta, S. D., Talwar, A., and Lee, J. T. A. (2020). Proposed framework and timeline of the spectrum of disease due to SARS-CoV-2 infection: illness beyond acute infection and public health implications. *JAMA* 324, 2251–2252. doi: 10.1001/jama.2020.22717

Desforges, M., Le Coupanec, A., Dubeau, P., Bourgouin, A., Lajoie, L., Dubé, M., et al. (2019). Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? *Viruses* 12, 14. doi: 10.3390/v12010014

Divani, A. A., Andalib, S., Biller, J., Di Napoli, M., Moghimi, M., Rubinos, C. A., et al. (2020). Central nervous system manifestations associated with COVID-19. *Curr. Neurol. Neurosci. Rep*. 20:60. doi: 10.1007/s11910-020-01079-7

Emami, A., Fadakar, N., Akbari, A., Lotfi, M., Farazdaghi, M., Javanmardi, F., et al. (2020). Seizure in patients with COVID-19. *Neurol. Sci.* 41, 3057–3061. doi: 10.1007/s10072-020-04731-9

Finsterer, J., and Mahjoub, S. Z. (2014). Fatigue in healthy and diseased individuals. *Am. J. Hosp. Palliat. Med*. 31, 562–575. doi: 10.1177/1049909113494748

Frontera, J. A., Yang, D., and Lewis, A. A. (2021). prospective study of long-term outcomes among hospitalized COVID-19 patients with and without neurological complications. *J. Neurol. Sci*. 426, 117486. doi: 10.1016/j.jns.2021.117486

Fu, Y., Guo, Z., Wang, Y., Zhang, H., Zhang, F., Xu, Z., et al. (2022). Single-nucleus RNA sequencing reveals the shared mechanisms inducing cognitive impairment between COVID-19 and Alzheimer's disease. *Front. Immunol*. 13, 967356. doi: 10.3389/fimmu.2022.967356

Garg, A., Goyal, S., and Comellas, A. P. (2021). Post-acute COVID-19 functional movement disorder. *SAGE Open Med. Case Rep.* 9. doi: 10.1177/2050313X21103 9377

Garrigues, E., Janvier, P., Kherabi, Y., Le Bot, A., Hamon, A., Gouze, H., et al. (2020). Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. *J Infect. Dec.* 81, 4–6. doi: 10.1016/j.jinf.2020.08.029

Graham, E. L., Clark, J. R., Orban, Z. S., Lim, P. H., Szymanski, A. L., Taylor, C., et al. (2021). Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers". *Ann. Clin. Transl. Neurol.* 8, 1073–1085. doi: 10.1002/acn3.51350

Halpin, S. J., McIvor, C., Whyatt, G., Adams, A., Harvey, O., McLean, L., et al. (2021). Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: a cross-sectional evaluation. *J. Med. Virol.* 93, 1013–1022. doi: 10.1002/jmv.26368

Hampshire, A., Trender, W., Chamberlain, S. R., and Jolly, A. E. (2021). Cognitive deficits in people who have recovered from COVID-.19. *EClin. Med*. 39, 101044. doi: 10.1016/j.eclinm.2021.101044

Hellmuth, J., Barnett, T. A., Asken, B. M., Kelly, J. D., Torres, L., Stephens, M. L., et al. (2021). Persistent COVID-19-associated neurocognitive symptoms in non-hospitalized patients. *Neurovirology* 27, 191–195. doi: 10.1007/s13365-021-00954-4

Hopkins, R. O., Gale, S. D., and Weaver, L. K. (2006). Brain atrophy and cognitive impairment in survivors of acute respiratory distress syndrome. *Brain Inj.* 20, 263–271. doi: 10.1080/02699050500488199

Hosseiny, M., Kooraki, S., Gholamrezanezhad, A., Reddy, S., and Myers, L. (2020). Radiology perspective of coronavirus disease 2019 (COVID-19): lessons from severe acute respiratory syndrome and Middle East respiratory syndrome. *Am. J. Roentgenol.* 214, 1078–1082. doi: 10.2214/AJR.20.22969

Huang, C., Huang, L., Wang, Y., Li, X., Ren, L., Gu, X., et al. (2021). 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 397, 220–232. doi: 10.1016/S0140-6736(20)32656-8

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., et al. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 395, 497–506. doi: 10.1016/S0140-6736(20)30183-5

Iqbal, A., Iqbal, K., Arshad, A. S., Azim, D., Farid, E., Baig, M. D., et al. (2021). The COVID-19 sequelae: a cross-sectional evaluation of post-recovery symptoms and the need for rehabilitation of COVID-19 survivors. *Cureus.* 13. doi: 10.7759/cureus.13080

Kanberg, N., Simrén, J., Edén, A., Andersson, L. M., Nilsson, S., Ashton, N. J., et al. (2021). Neurochemical signs of astrocytic and neuronal injury in acute COVID-19 normalizes during long-term follow-up. *Biomedicine* 70, 103512. doi: 10.1016/j.ebiom.2021.103512

Kayaaslan, B., Eser, F., Kalem, A. K., Kaya, G., Kaplan, B., Kacar, D., et al. (2021). Post-COVID syndrome: a single-center questionnaire study on 1007 participants recovered from COVID-19. *Med. Virol.* 93, 6566–6574. doi: 10.1002/jmv.27198

Keddie, S., Pakpoor, J., Mousele, C., Pipis, M., Machado, P. M., Foster, M., et al. (2021). Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barre syndrome. *Brain* 144, 682–693. doi: 10.1093/brain/awaa433

Leth, S., Gunst, J. D., Mathiasen, V., Hansen, K., Søgaard, O., Østergaard, L., et al. (2021). Persistent symptoms in patients recovering from COVID-19 in Denmark. *Open Forum Infect. Dis.* 8. doi: 10.1093/ofid/ofab042

Lippi, G., Mattiuzzi, C., Bovo, C., and Henry, B. M. (2020). Headache is an important symptom in patients with coronavirus disease 2019 (COVID-19) *Diagnosis* 7, 409–411. doi: 10.1515/dx-2020-0048

McFarland, A. J., Yousuf, M. S., Shiers, S., and Price, T. J. (2021). Neurobiology of SARS-CoV-2 interactions with the peripheral nervous system: implications for COVID-19 and pain. *Pain Rep*. 6, 885. doi: 10.1097/PR9.0000000000000885

Mendelson, M., Nel, J., Blumberg, L., Madhi, S. A., Dryden, M., Stevens, W., et al. (2020). Long-COVID: An evolving problem with an extensive impact. *S. Afr. Med. J.* 111, 10–12. doi: 10.7196/SAMJ.2020.v111i11.15433

Mikkelsen, M. E., Christie, J. D., Lanken, P. N., Biester, R. C., Thompson, B. T., Bellamy, S. L., et al. (2012). The adult respiratory distress syndrome cognitive outcomes study. *Am. J. Respir. Crit. Care Med.* 185, 1307–1315. doi: 10.1164/rccm.201111-2025OC

Miskowiak, K. W., Johnsen, S., Sattler, S. M. (2021). Cognitive impairments four months after COVID-19 hospital discharge: Pattern, severity and association with illness variables. *Eur. Neuropsychopharmacol.* 46, 39–48. doi: 10.1016/j.euroneuro.2021.03.019

Misra, S., Kolappa, K., Prasad, M., Radhakrishnan, D., Thakur, K. T., Solomon, T., et al. (2021). Frequency of neurological manifestations in COVID-19: a systematic review and meta-analysis of 350 studies. *medRxiv*. 97, 2269–2281.

Nakamura, I., Itoi, T., and Inoue, T. (2021). Case report of restless anal syndrome as restless legs syndrome variant after COVID-19. *BMC Infect. Dis.* 21, 993. doi: 10.1186/s12879-021-06683-7

Ngai, J. C., Ko, F. W., Ng, S. S., To, K. W., Tong, M., Hui, D. S., et al. (2010). The long-term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. *Respirology* 15, 543–550. doi: 10.1111/j.1440-1843.2010.01720.x

Orr,ù, G., Bertelloni, D., and Diolaiuti, F. (2021). Long-covid syndrome? A study on the persistence of neurological, psychological and physiological symptoms. *Healthcare* 9, 575. doi: 10.3390/healthcare9050575

Palaiodimou, L., Stefanou, M. I., Katsanos, A. H., and Fragkou, P. C. (2021). Prevalence, clinical characteristics and outcomes of Guillain-Barré syndrome spectrum associated with COVID-19: a systematic review and meta-analysis. *Eur. J. Neurol.* 28, 3517–3529. doi: 10.1111/ene.14860

Papri, N., Hayat, S., Mohammed, A., Afsar, M. N. A., Hasan, I., Rahman, A., et al. (2021). Guillain-Barre syndrome associated with SARS-CoV-2 infection: a case report with long term follow up. *Neuroimmunology* 356, 577590. doi: 10.1016/j.jneuroim.2021. 577590

Park, S., Majoka, H., Sheikh, A., and Ali, I. (2021). A presumed case of new-onset focal seizures as a delayed complication of COVID-19 infection. *Epilep. Behav. Rep. Int. Dev. Res. Cent. Can.* 16, 100447. doi: 10.1016/j.ebr.2021.100447

Paterson, R. W., Brown, R. L., Benjamin, L., Nortley, R., Sarah, W., Bharucha, T., et al. (2020). The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. *Brain* 143, 3104–3120. doi: 10.1093/brain/awaa240

Pilotto, A., and Cristillo, V. (2021). Long-term neurological manifestations of COVID-19: prevalence and predictive factors. *Neurol. Sci.* 42, 4903–4907. doi: 10.1007/s10072-021-05586-4

Pistarini, C., Fiabane, E., and Houdayer, E. (2021). Cognitive and emotional disturbances due to COVID-19: an exploratory study in the rehabilitation setting. *Front. Neurol.* 12, 643646. doi: 10.3389/fneur.2021.643646

Poletti, S., Palladini, M., and Mazza, M. G. (2021). Long-term consequences of COVID-19 on cognitive functioning up to 6 months after discharge: role of depression and impact on quality of life. *Eur. Arch. Psychiatry Clin. Neurosci.* 272, 773–782. doi: 10.1007/s00406-021-01346-9

Qin, Y., Wu, J., Chen, T., Li, J., Zhang, G., Wu, D., et al. (2021). Long-term microstructure and cerebral blood flow changes in patients recovered from COVID-19 without neurological manifestations. *J. Clin. Invastig.* 131, 147329. doi: 10.1172/JCI147329

Raahimi, M. M., Kane, A., Moore, C. E., and Alareed, A. W. (2021). Late onset of Guillain-Barre syndrome following SARS-CoV-2 infection: part of 'long COVID-19 syndrome'? *BMJ Case Rep*. (2021) 14:240178. doi: 10.1136/bcr-2020-240178

Rass, V., Beer, R., and Schiefecker, A. J. (2022). Neurological outcomes 1 year after COVID-19 diagnosis: a prospective longitudinal cohort study. *Eur. J. Neurol*. 29, 1685–1696. doi: 10.1111/ene.15307

Rivera-Izquierdo, M., Láinez-Ramos-Bossini, A. J., and Alba de Long, I. G. F. (2022). COVID 12 months after discharge: persistent symptoms in patients hospitalised due to COVID-19 and patients hospitalised due to other causes—a multicentre cohort study. *BMC Med*. 20, 92. doi: 10.1186/s12916-022-02292-6

Rodriguez, B., Nansoz, S., Cameron, D. R., and Z'Graggen, W.J. (2021). Is myopathy part of long-Covid? *Clin. Neurophysiol*. 132, 1241–1242. doi: 10.1016/j.clinph.2021.03.008

Rogers, J. P., Watson, C. J., Badenoch, J., Cross, B., Butler, M., Song, J., et al. (2021). Neurology and neuropsychiatry of COVID-19: a systematic review and meta-analysis of the early literature reveals frequent CNS manifestations and key emerging narratives. *J. Neurol. Neurosurg. Psychiatry* 92, 932–941. doi: 10.1136/jnnp-2021-326405

Romero-Duarte, A., Rivera-Izquierdo, M., and de Alba Guerrero-Fernández, I. (2021). Sequelae, persistent symptomatology and outcomes after COVID-19 hospitalization: the ANCOHVID multicentre 6-month follow-up study. *BMC Med*. 19, 129. doi: 10.1186/s12916-021-02003-7

Romoli, M., Jelcic, I., Bernard-Valnet, R., and Garcia, A. D. (2020). A systematic review of neurological manifestations of SARS-CoV-2 infection: the devil is hidden in the details. *Eur. J. Neurol*. 27, 1712–1726. doi: 10.1111/ene.14382

Roy, D., Ghosh, R., Dubey, S., Dubey, M. J., Benito-León, J., Kanti Ray, B., et al. (2021). Neurological and Neuropsychiatric Impacts of COVID-19 Pandemic. *Can. J. Neurol. Sci*. 48, 9–24. doi: 10.1017/cjn.2020.173

Rudroff, T., Fietsam, A. C., Deters, J. R., Bryant, A. D., and Kamholz, J. (2020). Post-COVID-19 fatigue: potential contributing factors. *Brain Sci*. 10, 1012. doi: 10.3390/brainsci10121012

Santis, L. V. D., Sobrino, B., González, G. E., Ruíz-Mesa, J. D., Plata, A., Márquez-Gómez, I., et al. (2020). Clinical and immunoserological status 12 weeks after infection with COVID-19: prospective observational study. *MedRxiv*. doi: 10.1101/2020.10.06.20206060

Sasannejad, C., Ely, E. W., and Lahiri, S. (2019). Long-term cognitive impairment after acute respiratory distress syndrome: a review of clinical impact and pathophysiological mechanisms. *Crit Care* 23, 352. doi: 10.1186/s13054-019-2626-z

Siow, I., Lee, K. S., Zhang, J. J., Saffari, S. E., and Ng, A. (2021). Encephalitis as a neurological complication of COVID-19: a systematic review and meta-analysis of incidence, outcomes, and predictors. *Eur. J. Neurol*. 28, 3491–3502. doi: 10.1111/ene.14913

Somani, S., Pati, S., Gaston, T., Chitlangia, A., and Agnihotri, S. (2020). *De novo* status epilepticus in patients with COVID-19. *Ann Clin Transl Neurol*. 7, 1240–1244. doi: 10.1002/acn3.51071

Stuby, J., Roth, R., Strecker, N., Teubner, J., and Rudiger, A. (2021). Post-COVID-19 bifacial weakness and paraesthesia: a case report. *Swiss Med. Wkly*. 21, 151. doi: 10.4414/SMW.2021.w30066

Sykes, D. L., Holdsworth, L., Jawad, N., Gunasekera, P., Morice, A. H., and Crooks, M. G. (2021). Post-COVID-19 symptom burden: what is long-COVID and how should we manage it? *Lung* 199, 113–119. doi: 10.1183/13993003.congress-2021.OA4189

Townsend, L., Dyer, A. H., Jones, K., and Dunne, J. (2020). Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. *PLoS ONE* 15, 20164293. doi: 10.1101/2020.07.29.20164293

Vanichkachorn, G., Newcomb, R., and Cowl, C. T. (2021). Post–COVID-19 syndrome (Long Haul Syndrome): description of a multidisciplinary clinic at mayo clinic and characteristics of the initial patient cohort. *Mayo Clin. Proc*. 96, 1782–1791. doi: 10.1016/j.mayocp.2021.04.024

Vogrig, A., Gigli, G. L., Bnà, C., and Morassi, M. (2021). Stroke in patients with COVID-19: clinical and neuroimaging characteristics. *Neurosci. Lett*. 743, 135564. doi: 10.1016/j.neulet.2020.135564

Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X.,' Zhang, J., et al. (2020). Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA* 323, 1061–1069. doi: 10.1001/jama.2020.1585

Wijeratne, T., and Crewther, S. (2020). Post-COVID 19 Neurological Syndrome (PCNS); a novel syndrome with challenges for the global neurology community. *J. Neurol. Sci*. 419, 117179. doi: 10.1016/j.jns.2020.117179

Wong-Chew, R. M., Rodríguez Cabrera, E. X., Rodríguez Valdez, C. A., Tapia-Conyer, R., and Valdez-Vázquez, R. R. (2022). Symptom cluster analysis of long COVID-19 in patients discharged from the temporary COVID-19 hospital in Mexico City, *Ther. Adv. Infect. Dis*. 9 doi: 10.1177/20499361211069264

Woo, M. S., Malsy, J., Pöttgen, J., Seddiq, Z. S., Ufer, F., Hadjilaou, A., et al. (2020). Frequent neurocognitive deficits after recovery from mild COVID-19. *Brain Commun*. 2. doi: 10.1093/braincomms/fcaa205

Yassin, A., Nawaiseh, M., Shaban, A., Alsherbini, K., El-Salem, K., Soudah, O., et al. (2021). Neurological manifestations and complications of coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis. *BMC Neurol*. 21, 138. doi: 10.1186/s12883-021-02161-4

Zhang, X., Wang, F., Shen, Y., Zhang, X., Cen, Y., Wang, B., et al. (2021). Symptoms and health outcomes among survivors of COVID-19 infection 1 year after discharge from hospitals in Wuhan, China.. *JAMA Netw. Open*. 1, 4. doi: 10.1001/jamanetworkopen.2021.27403

Zhu, Y., Cao, M., Zheng, P., and Shen, W. (2021). Residual olfactory dysfunction in coronavirus disease 2019 patients after long term recovery. *J. Clin. Neurosci*. 93, 31–35. doi: 10.1016/j.jocn.2021.07.050

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 24 February 2023
DOI 10.3389/fneur.2023.1092999

OPEN ACCESS

EDITED BY
Sonia Zuñiga,
National Center for Biotechnology (CSIC), Spain

REVIEWED BY
Bekir Kocazeybek,
Istanbul University-Cerrahpasa, Türkiye
Vincenzo Brescia Morra,
University of Naples Federico II, Italy
Marco Iannetta,
University of Rome Tor Vergata, Italy
Klarissa Hanja Stürner,
University of Kiel, Germany

*CORRESPONDENCE
Anastasia Lambrianides
✉ nancyl@cing.ac.cy

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

RECEIVED 08 November 2022
ACCEPTED 07 February 2023
PUBLISHED 24 February 2023

CITATION
Lambrianides A, Deeba E, Hadjiagapiou M, Pantzaris M, Krashias G and Christodoulou C (2023) SARS-CoV-2-specific antibody responses following BNT162b2 vaccination in individuals with multiple sclerosis receiving different disease-modifying treatments. *Front. Neurol.* 14:1092999. doi: 10.3389/fneur.2023.1092999

SARS-CoV-2-specific antibody responses following BNT162b2 vaccination in individuals with multiple sclerosis receiving different disease-modifying treatments

Anastasia Lambrianides[1*†], Elie Deeba[2†], Maria Hadjiagapiou[3], Marios Pantzaris[1], George Krashias[2] and Christina Christodoulou[2]

[1]Department of Neuroimmunology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [2]Department of Molecular Virology, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus, [3]Postgraduate School, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

Introduction: The study aims to evaluate the concentration of IgG antibodies against the receptor-binding domain of the SARS-CoV-2 spike1 protein (S1RBD) in BNT162b2- vaccinated relapsing-remitting multiple sclerosis (RRMS) individuals receiving disease-modifying treatments (DMTs).

Methods: Serum from 126 RRMS volunteers was collected 3 months after the administration of the second dose of the Pfizer-BioNTech BNT162b2 vaccine. Additional samples were analyzed after the administration of the booster dose in fingolimod- treated MS. Anti-S1RBD IgG antibody concentrations were quantified using the ABBOTT SARS-CoV-2 IgG II Quant assay.

Results: Anti-S1RBD IgG antibody concentrations in RRMS individuals receiving natalizumab, interferons, teriflunomide, and dimethyl fumarate showed no significant difference to those in healthy controls. However, fingolimod-treated MS individuals showed a marked inability to produce SARS-CoV-2- specific antibodies ($p < 0.0001$). Furthermore, a booster dose was not able to elicit the production of IgG antibodies in a large portion of matched individuals.

Discussion: A possible explanation for the altered immune response in fingolimod- treated MS individuals could be due to the medication inhibiting the circulation of lymphocytes, and possibly in turn inhibiting antibody production. Overall, patients on DMTs are generally of no disadvantage toward mounting an immune response against the vaccine. Nevertheless, further studies require evaluating non-humoral immunity against SARS-CoV-2 following vaccination, as well as the suitability of such vaccinations on patients treated with fingolimod.

KEYWORDS

SARS-CoV-2, multiple sclerosis, IgG antibodies, vaccines, disease-modifying treatments

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent for Coronavirus disease 2019 (COVID-19), has claimed over 6.5 million lives globally (October, 2022) (1). Vaccines that have received emergency approval for human use by the food and drug administration (FDA) or European medicines agency (EMA) include those from Pfizer-BioNTech, Moderna, AstraZeneca, and Janssen (2, 3). All the above vaccines have gone

through clinical trials where their safety and efficacy were evaluated in previously healthy individuals (4). Of equal importance, there are no diseases, other than history of severe allergic reactions toward vaccinations, that are considered as contraindications for the use of these vaccines in the general population. Nevertheless, it remains to be seen whether the already approved vaccines are effective at inducing an adequate immune response in vaccinated individuals with different chronic neurological diseases, especially those with multiple sclerosis (MS) receiving different disease-modifying treatments (DMTs). Obtaining such information is of primary importance since it would highlight the suitability of the above vaccines for these individuals. This information can be utilized in the clinic by the treating physician for the benefit of the patients.

The Cyprus Institute of Neurology and Genetics (CING), as the reference center for neurological diseases in the Republic of Cyprus, treats patients with a wide range of neurological diseases. Following the guidelines of the WHO, the majority of these patients have been vaccinated against SARS-CoV-2. Interestingly, these patients are also treated with different immunomodulatory or immunosuppressive therapies. The effect of these therapies on the already approved Pfizer-BioNTech's BNT162b2 SARS-CoV-2 vaccines requires exploration to decide whether administration of booster doses would be beneficial.

The current study aims to evaluate for the first time the levels of antibodies against the receptor-binding domain of the SARS-CoV-2 spike1 protein (S1RBD) in BNT162b2-vaccinated MS individuals receiving different DMTs [natalizumab, fingolimod, teriflunomide, dimethyl fumarate, interferon β-1a (IFN β-1a), and interferon β-1b (IFN β-1b)].

2. Materials and methods

2.1. Ethical approval and subject recruitment

This study was approved by the Cyprus National Bioethics Committee (ΕΕΒΚ/ΕΠ/2020/23). All participants completed and signed an informed consent form.

2.2. Study population and sample collection/processing

A total of 126 volunteers with clinically definite relapsing-remitting MS and 52 healthy volunteers (HC) signed up for the study. Blood samples were collected from MS volunteers upon request from the Neuroimmunology department at The Cyprus Institute of Neurology and Genetics. The average number of days from the second dose to the booster dose was 90 days as indicated by the Ministry of Health in Cyprus. Throughout the study, patients that had COVID confirmed with PCR testing were excluded. In more detail, the inclusion criteria were: (1) patients above 18 years of age; (2) patients with clinically definite multiple sclerosis (CDMS) with clear clinical course of relapsing-remitting; (3) patients not experiencing any relapse symptoms during blood collection; (4) availability of a detailed clinical history [age of onset, disease duration calculated as the duration between sample acquisition and age of onset, Expanded Disability Status Scale (EDSS) score obtained on the day of sample acquisition, and treatments received]; and (5) being born in Cyprus and have resided in Cyprus from birth to at least early adult life. Exclusion criteria were: (1) presence of relapse in the 30 days before enrolment in the study; (2) inability or unwillingness to provide informed consent; (3) a history of alcohol or drug abuse; (4) pregnancy; and (5) history of previous SARS-CoV-2 infection. The inclusion and exclusion criteria, that are not solely MS-related, can be similarly extended to the healthy control group, save for the addition of an exclusion criterion that an individual may have any neurodegenerative, autoimmune, or underlying health issues. Table 1 shows the demographic details and clinical characteristics (EDSS, diseases duration, treatment at time of blood collection) of the MS volunteers and HCs. Other relevant data collected included SARS-CoV-2 infection history and lymphocyte counts for MS volunteers receiving fingolimod.

The timing of vaccinations followed the guidelines set by the EMA and the protocol set by the Ministry of Health in Cyprus, where the second dose was administered 3 weeks after the initial dose of BNT162b2 and the booster dose administered 3 months after the second dose. Blood samples were collected from all volunteers 3 months after the second vaccination dose. Reviewing preliminary results warranted additional analysis from a select MS group, as such MS volunteers receiving fingolimod were asked to return for another blood sample at least 2 weeks after receiving the booster dose. Note that due to the volunteering nature of the study, some volunteers were not willing to further donate blood. Additionally, due to volunteers getting infected with SARS-CoV-2 during the time between vaccination doses, a follow-up sample was not suitable for the purpose of the study.

Blood samples were collected in tubes containing clotting activators at the COVID-19 sampling unit of The Cyprus Institute of Neurology and Genetics. Following blood collection, samples were centrifuged for 10 min at 500 × g at 20°C to obtain cell-free serum. Serum was stored at −20°C until analysis.

2.3. Anti-S1RBD IgG quantification analysis

Part of the serum obtained from the two groups of the study was used to quantify the level of Anti-S1RBD IgG antibodies. The quantification was performed using the ABBOTT SARS-CoV-2 IgG II Quant assay (REF# 6S60-22) on an ABBOTT ARCHITECT i1000SR instrument. The assay is an automated, two-step chemiluminescent microparticle immunoassay used for qualitative and quantitative determination of IgG antibodies against S1RBD of the SARS-CoV-2 from human serum and plasma. The SARS-CoV-2 IgG II Quant calibrator package (REF# 6S60-02) and the SARS-CoV-2 IgG II Quant control package (REF# 6S60-12) were run on the instrument prior to sample analysis. According to the manufacturer, the cut-off is set at 50.0 AU/mL, and the analytical measuring interval is set between 21.0 (limit of quantification) and 40,000.0 AU/mL (upper limit of quantification). Additional information on performance characteristics of the assay can be found in the manufacturer's

manual. Based on the recommendations of the National Institute of Biological Standards and Control (NIBSC) and WHO, the concentrations were converted into Binding antibody units per mL (BAU/mL) through multiplying AU/mL by a factor of 0.142. The corresponding cut-off value becomes 7.1 BAU/mL.

2.4. Statistical analysis

The Mann-Whitney *U*-test and Fisher's exact test were used for age- and sex- matching, respectively. The Mann-Whitney *U*-test was used to evaluate significance in the differences between antibody levels in different groups. Simple linear regression and point-biserial correlation were used to analyze the correlation between antibody levels and lymphocyte count. The GraphPad Prism v8·00 for Windows software program was used to perform the statistical analyses (GraphPad Software, La Jolla, California, USA).

3. Results

3.1. Anti-S1RBD IgG antibody concentrations in MS and HC volunteers

Three months after the second vaccination dose, all of the HC group were found positive for anti-S1RBD IgG antibodies at a median (interquartile range) of 415.6 BAU/mL (244.9–686.5). Similarly, MS individuals receiving different medications were found to be positive for anti-S1RBD IgG antibodies, as well as comparable to the HC group, with medians (interquartile range) of 487.3 BAU/mL (197.8–730.6) for $MS_{IFN\beta-1a}$, 495.3 BAU/mL (199.1–999.5) for $MS_{Natalizumab}$, 434.4 BAU/mL (220.9–663.8) for $MS_{Dimethylfumarate}$, 460.4 BAU/mL (119.5–878.5) for $MS_{Teriflunomide}$, and 402.4 BAU/mL (240.6–660.1) for $MS_{IFN\beta-1b}$. On the other hand, around half of the MS individuals receiving fingolimod (18/34; 52.9%) were positive for anti-S1RBD IgG antibodies with a significantly lower concentration [median (interquartile range); 7.5 BAU/mL (1.8–21.6)] compared to the HC group ($p < 0.0001$; Figure 1).

3.2. Anti-S1RBD IgG antibody level vs. lymphocyte count in fingolimod-treated MS individuals

Further analysis focused on the $MS_{Fingolimod}$ group, where lymphocyte count data was collected for 30 individuals and measured independently by their physician around 4 weeks after their second vaccination dose. There was no significant correlation between lymphocyte counts and the concentration of anti-S1RBD IgG antibodies (linear regression; $p = 0.45$, point-biserial correlation; $p = 0.08$; $r = 0.33$; 95% CI = −0.04–0.61) (Figure 2). We note that, although there was no significance, there seems to be a trend showing higher anti-S1RBD IgG antibody concentrations with higher lymphocyte counts (correlation coefficient $r > 0$).

TABLE 1 Demographic and clinical characteristics of MS and healthy volunteers.

Features	MS group ($n = 126$)	HC group ($n = 52$)	*p*-value
Age [mean (SD)]	45.08 ± 9.33	46.67 ± 12.86	0.340
Sex (male/female)	31/95	21/31	**0.046**
Duration of disease in years [median (interquartile range)]	9 (5–16)	N/A	
EDSS [median (interquartile range)]	3 (2–3.5)		
Type of treatment [*n* (%)]		N/A	
IFNβ-1a	42 (33.3%)		
Fingolimod	34 (27%)		
Natalizumab	26 (20.6%)		
Dimethyl fumarate	11 (8.7%)		
Teriflunomide	7 (5.6%)		
IFNβ-1b	6 (4.8%)		

The Mann-Whitney U-test was used for age matching, and the Fisher's exact test was used for sex matching.
MS, Multiple sclerosis; HCs, Healthy volunteers; RR, Relapsing Remitting MS; SP, Secondary Progressive MS; PP, Primary Progressive MS; IFN, Interferon; SD, Standard Deviation; N/A, Not Applicable. Bold value shows statistical significance.

3.3. Change in antibody level following booster dose in fingolimod-treated MS individuals

Based on the low concentrations of anti-S1RBD IgG antibodies measured in $MS_{Fingolimod}$, as well as the recommendations for a SARS-CoV-2 booster dose administration, a follow-up sample was taken from $MS_{Fingolimod}$ volunteers at least 2 weeks after the booster dose (T2) [median (interquartile range); 4.9 weeks (3.4–5.5)]. Anti-S1RBD IgG antibody levels were measured for 26 $MS_{Fingolimod}$, of which 11 were previously found positive 3 months after the second vaccination dose (T1), and 12 were previously found negative at T1. After the booster dose, there was a significant increase in antibody concentration in $MS_{Fingolimod}$ previously found positive at T1 from 20.3 BAU/mL (10.2–90.1) to 96.1 BAU/mL (30.9–236.8) ($p < 0·001$; Figure 3A). Similarly, antibody levels in $MS_{Fingolimod}$ previously found negative at T1 significantly increased at T2 to a median (interquartile range) of 12.1 BAU/mL (3.0–36.9) ($p < 0.001$; Figure 3B), with half of those remaining negative after the booster dose. Analysis comparing antibody levels with lymphocyte count after the booster dose showed that the trend shown above appears to hold true, however without reaching significance (linear regression; $p = 0.64$, point-biserial correlation; $p = 0.46$; $r = 0.15$; 95% CI = −0.26–0.52) (graph not shown).

4. Discussion

With the seemingly unstoppable spread of SARS-CoV-2, and its variants, there was a need to ensure the safety of individuals with underlying comorbidities, specifically immunocompromised

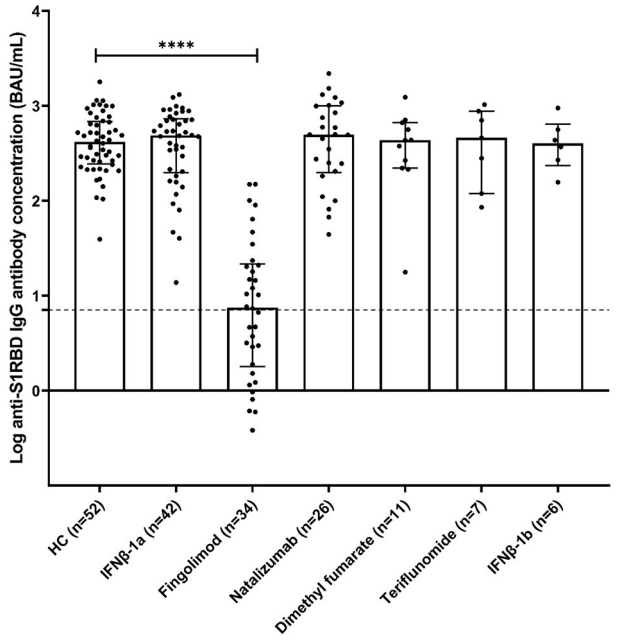


FIGURE 1
Anti-S1RBD IgG antibody levels in healthy volunteers (HC) and MS volunteers receiving various DMTs [interferonβ-1a (IFNβ-1a), interferonβ-1b (IFNβ-1b), natalizumab, fingolimod, dimethyl fumarate, and teriflunomide]. Bars represent median and interquartile ranges. The dotted line represents the cut-off value (7.1 BAU/mL). ****$p < 0.0001$.

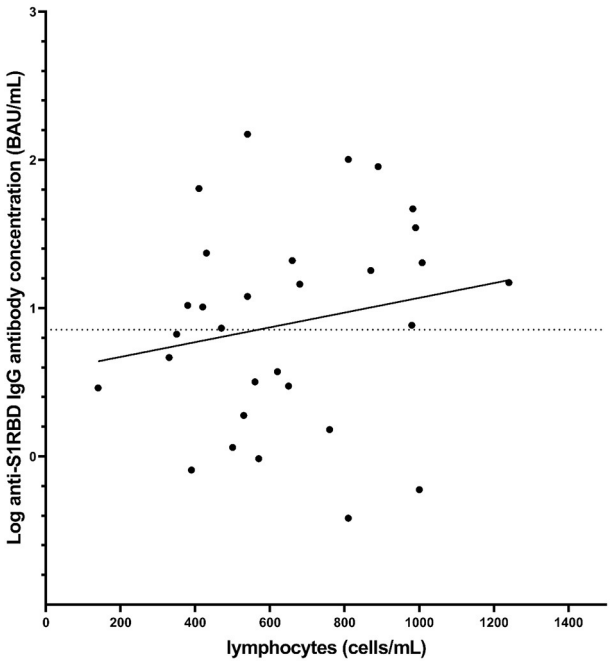


FIGURE 2
Distribution of anti-S1RBD IgG antibody levels measured after the second vaccination dose as a function of lymphocyte count also measured after the second vaccination dose in MS volunteers receiving fingolimod ($n = 30$). The dotted line represents the cut-off value (7.1 BAU/mL). The solid line represents a best fit line based on simple linear regression ($p = 0.45$).

individuals. The neuroimmunology department at CING accepts and oversees the treatment of hundreds of individuals with MS in the Republic of Cyprus. Therefore, we aimed to understand the effect of different DMTs received by MS individuals on the levels of anti-S1RBD IgG antibodies produced after the full vaccination regimen with BNT162b2.

With the exception of one DMT (fingolimod), we did not observe a significant effect of different MS- directed medications on the ability of the immune system to produce anti-S1RBD antibodies against the full course of BNT162b2 vaccination regimen. Other studies have reported similar findings (5–10). However, we point out some discrepancies found between our results and results from Pitzalis et al. (8), whereby their results showed a significantly lower level of antibodies produced in MS treated with teriflunomide and natalizumab compared to the healthy control group. Such a discrepancy could be attributed to our small sample size for the two treatment groups, as well as large range in the antibody levels given the small sample sizes. Hence, we note the importance of unifying global data to further understand the effect of different medications in such niche groups. Our focus then turned to MS volunteers receiving fingolimod where, similar to other reports (5–12), we found significantly lower antibody levels compared to other MS and healthy volunteers. More so, such results were not exclusive to the type of vaccine used but were also observed in MS individuals vaccinated with Oxford-AstraZeneca's ChAdOx1-S (12) and Sinovac's CoronaVac vaccine (13). We can, therefore, further confirm a SARS-CoV-2-specific humoral immune response impairment due to treatment with fingolimod.

Due to the aggressive mode of action of fingolimod, we explored the possible relationship between circulating lymphocyte count and antibody production. Although our results showed a positive trend, i.e., higher lymphocyte counts correlate with higher antibody levels, our analysis did not return significance, possibly due to the low sample size. Nonetheless, this trend was also reported in different studies (7, 10, 14), where both B- and T- cell responses were measured and it was shown that there is a marked immunological impairment in MS individuals treated with fingolimod compared to those treated with natalizumab (7) or IFNβ (10), leading to the limited anti-S antibody production and T-cell activation.

In an effort to continue monitoring the SARS-CoV-2- specific humoral immune response in MS individuals receiving fingolimod, their anti-S1RBD IgG antibody levels were measured again after the administration of the BNT162b2 booster dose. Our results show that the booster shot was able to induce a significant increase in antibody levels. We also note that, of those who had tested negative for antibodies after their second vaccination dose, fifty percent converted to seropositive for anti-S1RBD IgG antibodies. The low number of seroconversions in MS individuals treated with fingolimod following a booster dose has also been observed in other studies as summarized in Table 2. Other studies by König et al. and Idda et al. do not point out changes in seroconversion, but rather report significantly lower concentrations and/or significantly reduced immunity compared to healthy vaccinated individuals

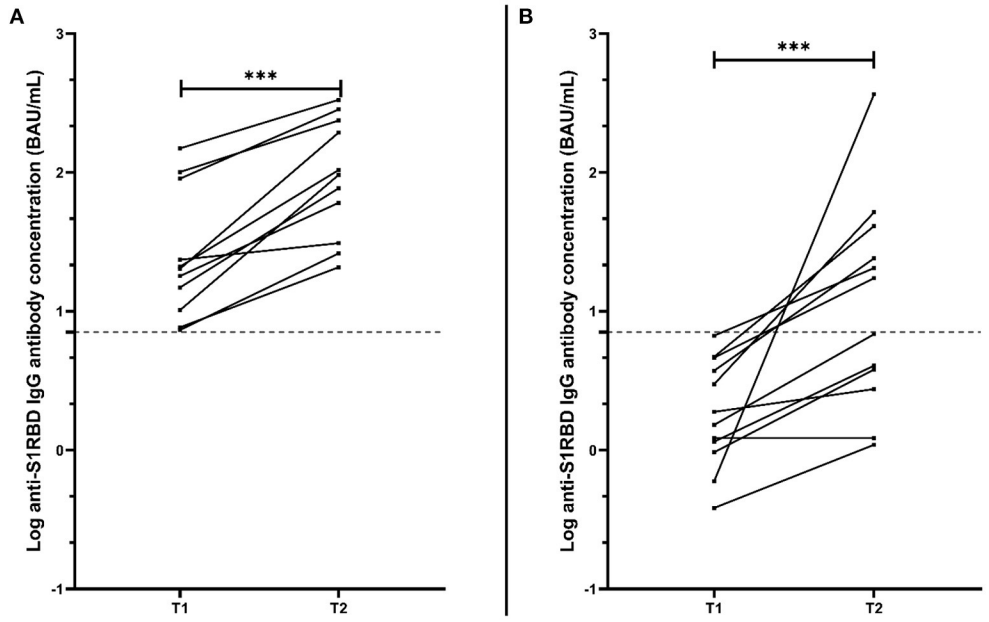


FIGURE 3
Anti-S1RBD IgG antibody level comparison between matched MS volunteers receiving fingolimod at 2 different time points: T1, 3 months after the second vaccination dose; and T2, at least 2 weeks after the booster dose. **(A)** Represents only $MS_{Fingolimod}$ volunteers who were found positive for anti-S1RBD IgG at T1 ($n = 11$), while **(B)** represents those who were found negative for anti-S1RBD IgG at T1 ($n = 12$). The dotted line represents the cut-off value (7.1 BAU/mL). ***$p < 0.001$.

(19, 20). Though these findings do not contradict with our results, they are not directly comparable with our study and therefore could not be included in the table, however we can note that both studies recruit <50 patients treated with fingolimod. Given that these observational studies, similar to this study, recruit a limited sample size, a larger cohort would be needed to confirm and further clarify the effect of DMTs on booster vaccinations, possibly achieved through international collaboration. In terms of the correlation between antibody level and lymphocyte count, our results show a similar trend before and after the booster dose, suggesting that additional vaccine administration might not be as effective if the lymphocyte count is low in fingolimod- treated individuals. Indeed, this conclusion was also inferred in another study that showed discontinuation of fingolimod treatment is significantly correlated with antibody production following booster dose administration (21). We note that natural immunization by SARS-CoV-2 infection, although beneficial for the immunity of the patients, does not interfere with the interpretation of our results, given the aim of the study at analyzing the changes in the levels of antibodies between vaccination doses.

This study has several limitations. Due to low turnout of volunteers, one limitation of the study was sample size, which had restricted the data to a handful of DMTs. Nonetheless, the results and trends shown in this study are consistent with other studies on the topic. Additionally, we were also restricted to the type of vaccine studied, as other types (ChAdOx1-S and mRNA-1273) were not administered in the Republic of Cyprus, in enough numbers to warrant meaningful analysis. The participant dropout after the booster dose led to an even more restricted sample size, which means that such results should be approached with caution and not be considered as wholly representative. Other limitations include the uncertainty of SARS-CoV-2 infection in both the MS and the HC groups. SARS-CoV-2 history was based solely on patient/control declaration and anti-nucleocapsid antibodies have not been checked for asymptomatic events, however, since the purpose of the study was to assess the levels of antibodies against the receptor-binding domain of the SARS-CoV-2 spike1 protein (S1RBD) in BNT162b2-vaccinated MS individuals receiving different DMTs, it is unlikely it would have not affected the comparison. Another limitation is the lack of Indirect information on T cell responses, which would have enabled us to get a more complete picture of a patient's immune status, by using additional tests that measure the presence and function of specific types of immune cells, such as CD4 T lymphocytes and cytokines such as IFN. These tests can indeed provide important information about how the immune system is responding to infection with COVID-19. However, it is important to note that the interpretation of these results can be complex. Future studies could follow the data on a larger longitudinal scale, while also incorporating data on T cell- based responses which might play a larger role in immunity against SARS-CoV-2. Additionally, future studies could focus on understanding the exact mechanism of fingolimod in terms of antibody production, by measuring the relationship between antibody levels and each lymphocyte subset.

5. Conclusions

The current study aimed to evaluate the IgG antibody levels against S1RBD of SARS-CoV-2 in BNT162b2-vaccinated

TABLE 2 Comparison between different studies reporting seroconversion of fingolimod-treated MS patients following SARS-CoV-2 booster dose.

Authors	Vaccine used	Total patients recruited	Antibody-negative patients after second dose	Seroconverted patients following booster dose	References
Achiron et al.	BNT162b2	10	10	2	(14)
Achtnichts et al.	BNT162b2, mRNA-1273	8	8	4	(15)
Maglione et al.	BNT162b2, mRNA-1273	13	11	7	(16)
Meyer-Arndt et al.	BNT162b2, mRNA-1273	29	25	9	(17)
Tallantyre et al.	BNT162b2, mRNA-1273	15	15	7	(18)

Cypriot MS individuals receiving different DMTs. We showed that BNT162b2 was effective at inducing a sufficient humoral response comparable to healthy individuals, regardless of treatments received. However, the vaccine was unable to elicit the same response in fingolimod- treated MS individuals, where antibody levels, if positive, were significantly lower compared to those in MS individuals receiving other DMTs. Even with a booster dose, some MS individuals receiving fingolimod were not able to produce anti-S1RBD IgG antibodies, this could be attributed to the aggressive mode of action of fingolimod which effectively inhibits the immune system's ability to elicit any significant humoral responses toward an infection. Our results may aid the global effort in understanding antibody kinetics across different individuals receiving immunomodulatory medications. This may also help in better informing public health policies regarding vaccine efficacy and humoral immunity in immunocompromised individuals, as well as vaccine considerations against new emerging variants of concern.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Cyprus National Bioethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: CC, GK, and MP. Project administration: CC and MP. Data collection and curation: ED, AL, and MH. Formal analysis and interpretation: ED and AL. Funding acquisition: CC and MP. Methodology: ED and GK. Validation: ED, GK, and CC. Writing—original draft: ED. Writing—review and editing: AL, GK, and CC. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by funds from the Cyprus Institute of Neurology and Genetics intended for research purposes.

Acknowledgments

Special thanks to all study participants for providing blood samples for the successful completion of this study. We would also like to thank Ms. Andria Xyrichi for her help in withdrawing the blood samples from the volunteers. Finally, we would like to extend our gratitude to C&V Kriticos Suppliers LTD for providing the ARCHITECT i1000SR instrument for the purpose of the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. WHO. *Coronavirus WHO. (COVID-19) Dashboard | WHO Coronavirus (COVID-19) Dashboard With Vaccination Data.* (2022). Available online at: https://covid19.who.int/ (accessed October 3, 2022).

2. European Medicines Agency. *COVID-19 Vaccines | European Medicines Agency*. (2022). Available online at: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines (accessed January 21, 2022).

3. FDA. *COVID-19 Vaccines | FDA*. (2022). Available online at: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines#news (accessed January 21, 2022).

4. Knoll MD, Wonodi C. Oxford-AstraZeneca COVID-19 vaccine efficacy. *Lancet.* (2021) 397:72–4. doi: 10.1016/S0140-6736(20)32623-4

5. Achiron A, Dolev M, Menascu S, Zohar DN, Dreyer-Alster S, Miron S, et al. COVID-19 vaccination in patients with multiple sclerosis: what we have learnt by February 2021. *Mult Scler.* (2021) 27:864–70. doi: 10.1177/13524585211003476

6. Bigaut K, Kremer L, Fabacher T, Lanotte L, Fleury MC, Collongues N, et al. Impact of disease-modifying treatments of multiple sclerosis on anti-SARS-CoV-2 antibodies: an observational study. *Neurol Neuroimmunol Neuroinflamm.* (2021) 8:1055. doi: 10.1212/NXI.0000000000001055

7. Iannetta M, Landi D, Cola G, Campogiani L, Malagnino V, Teti E, et al. B- and T-Cell responses after SARS-CoV-2 vaccination in patients with multiple sclerosis receiving disease modifying therapies: immunological patterns and clinical implications. *Front Immunol.* (2022) 12:796482. doi: 10.3389/fimmu.2021.796482

8. Pitzalis M, Idda ML, Lodde V, Loizedda A, Lobina M, Zoledziewska M, et al. Effect of different disease-modifying therapies on humoral response to BNT162b2 vaccine in sardinian multiple sclerosis patients. *Front Immunol.* (2021) 12:e781843. doi: 10.3389/fimmu.2021.781843

9. Sormani MP, Schiavetti I, Carmisciano L, Inglese M, Laroni A, Lapucci C, et al. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. *EBioMedicine.* (2021) 72:103581. doi: 10.1016/j.ebiom.2021.103581

10. Tortorella C, Aiello A, Gasperini C, Agrati C, Castilletti C, Ruggieri S, et al. Humoral- and T-cell-specific immune responses to SARS-CoV-2 mRNA vaccination in patients with MS using different disease-modifying therapies. *Neurology.* (2022) 98:e541–54. doi: 10.1212/WNL.0000000000013108

11. Capuano R, Bisecco A, Conte M, Donnarumma G, Altieri M, Grimaldi E, et al. Six-month humoral response to mRNA SARS-CoV-2 vaccination in patients with multiple sclerosis treated with ocrelizumab and fingolimod. *Mult Scler Relat Disord.* (2022) 60:e541–e554. doi: 10.1016/j.msard.2022.103724

12. Guerrieri S, Lazzarin S, Zanetta C, Nozzolillo A, Filippi M, Moiola L, et al. Serological response to SARS-CoV-2 vaccination in multiple sclerosis patients treated with fingolimod or ocrelizumab: an initial real-life experience. *J Neurol.* (2022) 269:39–43. doi: 10.1007/s00415-021-10663-x

13. Türkoglu R, Baliç N, Kizilay T, Erol R, Akbayir E, Yilmaz V, et al. Fingolimod impairs inactivated vaccine (CoronaVac)-induced antibody response to SARS-CoV-2 spike protein in persons with multiple sclerosis. *Mult Scler Relat Disord.* (2022) 58:103524. doi: 10.1016/j.msard.2022.103524

14. Achiron A, Mandel M, Dreyer-Alster S, Harari G, Magalashvili D, Sonis P, et al. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. *Ther Adv Neurol Disord.* (2021) 14:835. doi: 10.1177/17562864211012835

15. Achtnichts L, Ovchinnikov A, Jakopp B, Oberle M, Nedeltchev K, Fux CA, et al. SARS-CoV-2 mRNA vaccination in people with multiple sclerosis treated with Fingolimod: protective humoral immune responses may develop after the preferred third shot. *Vaccines.* (2022) 10:341. doi: 10.3390/vaccines10020341

16. Maglione A, Morra M, Meroni R, Matta M, Clerico M, Rolla S, et al. Humoral response after the booster dose of anti-SARS-CoV-2 vaccine in multiple sclerosis patients treated with high-efficacy therapies. *Mult Scler Relat Disord.* (2022) 61:103776. doi: 10.1016/j.msard.2022.103776

17. Meyer-Arndt L, Braun J, Fauchere F, Vanshylla K, Loyal L, Henze L, et al. SARS-CoV-2 mRNA vaccinations fail to elicit humoral and cellular immune responses in patients with multiple sclerosis receiving fingolimod. *J Neurol Neurosurg Psychiatry.* (2022) 93:960–71. doi: 10.1136/jnnp-2022-329395

18. Tallantyre EC, Scurr MJ, Vickaryous N, Richards A, Anderson V, Baker D, et al. Response to COVID-19 booster vaccinations in seronegative people with multiple sclerosis. *Mult Scler Relat Disord.* (2022) 64:103937. doi: 10.1016/j.msard.2022.103937

19. Idda ML, Pitzalis M, Lodde V, Loizedda A, Frau J, Lobina M, et al. Cross-sectional analysis of the humoral response after SARS-CoV-2 vaccination in Sardinian multiple sclerosis patients, a follow-up study. *Front Immunol.* (2022) 13:e946356. doi: 10.3389/fimmu.2022.946356

20. König M, Torgauten HM, Tran TT, Holmøy T, Vaage JT, Lund-Johansen F, et al. Immunogenicity and safety of a third SARS-CoV-2 vaccine dose in patients with multiple sclerosis and weak immune response after COVID-19 vaccination. *JAMA Neurol.* (2022) 79:307–9. doi: 10.1001/jamaneurol.2021.5109

21. Achiron A, Mandel M, Gurevich M, Dreyer-Alster S, Magalashvili D, Sonis P, et al. Immune response to the third COVID-19 vaccine dose is related to lymphocyte count in multiple sclerosis patients treated with fingolimod. *J Neurol.* (2022) 269:2286–92. doi: 10.1007/s00415-022-11030-0

frontiers | Frontiers in Neuroscience

TYPE Mini Review
PUBLISHED 03 March 2023
DOI 10.3389/fnins.2023.1128157

OPEN ACCESS

EDITED BY
Gonzalo Emiliano Aranda-Abreu,
Universidad Veracruzana, Mexico

REVIEWED BY
Pawel Grieb,
Mossakowski Medical Research Institute, Polish Academy of Sciences, Poland
Omar El Hiba,
Université Chouaib Doukkali, Morocco

*CORRESPONDENCE
Roger F. Butterworth
✉ rb@enceph.com

SPECIALTY SECTION
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

RECEIVED 20 December 2022
ACCEPTED 14 February 2023
PUBLISHED 03 March 2023

CITATION
Butterworth RF (2023) Adamantanes for the treatment of neurodegenerative diseases in the presence of SARS-CoV-2. *Front. Neurosci.* 17:1128157. doi: 10.3389/fnins.2023.1128157

Adamantanes for the treatment of neurodegenerative diseases in the presence of SARS-CoV-2

Roger F. Butterworth*

Department of Medicine, University of Montreal, Montreal, QC, Canada

Advent of the acute respiratory coronavirus SARS-CoV-2 has resulted in the search for novel antiviral agents and in the repurposing of existing agents with demonstrated efficacy against other known coronaviruses in the search for an agent with antiviral activity for use during the COVID-19 pandemic. Adamantanes including amantadine, rimantadine, and memantine have well-established benefit in the treatment of neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD) and fatigue related to Multiple sclerosis (MS) all of which are known comorbidities related to COVID-19 Moreover, results of basic pharmacological studies both *in vitro* and *in vivo* reveal that amantadine has the potential to inhibit SARS-CoV-2 *via* down-regulation of host-cell proteases resulting in impaired viral genome release into the host cell and *via* amantadine's property as an NMDA receptor antagonist resulting in the prevention of the acute lung injury and respiratory distress that is characteristic of COVID-19. Cases suggestive of COVID-19 prophylaxis have been reported in patients with PD or MS or severe cognitive impairment treated in all cases for several months with an adamantane [amantadine or memantine] who were subsequently infected with SARS-CoV-2 confirmed by RT-PCR, and, in all cases, no signs of infectious disease were encountered. Amantadine is effective for the treatment of fatigue in MS and for the neurological complications of Traumatic Brain Injury (TBI).

KEYWORDS

neurodegenerative diseases, Parkinson's disease, Alzheimer's disease, Multiple sclerosis, traumatic brain injury, amantadine, memantine

Introduction

Neurodegenerative diseases including Parkinson's disease [PD], Alzheimer's disease [AD], and Multiple sclerosis [MS] are increasingly considered to represent comorbidities in patients infected with SARS-CoV-2, the coronavirus responsible for the 2019 pandemic currently known as COVID-19. The presence of these disorders has the potential to impact negatively on the severity of symptoms of the infection as well as the efficacy of treatment strategies and on patient survival.

Members of the adamantane family of agents have established beneficial effects on neurodegenerative diseases that include amantadine [for PD and for the treatment of fatigue in MS], memantine [for AD] and amantadine for the treatment of the decreased levels of consciousness and cognitive/behavioral sequelae of traumatic brain injury [TBI]. Evidence supports the notion that SARS-CoV-2-infection of a patient with AD results in worsening of both conditions. On the other hand, treatment with amantadine has the

potential to benefit both situations *via* distinct neurophysiologic and antiviral mechanisms. The present article reviews these issues in an evidence-based manner from basic mechanisms to results of systematic reviews and meta-analyses in support of therapeutic efficacy in these neurodegenerative diseases during the COVID-19 pandemic.

Parkinson's disease

Parkinson's disease [PD] is an age-related neurodegenerative disease characterized by the progressive selective deterioration and ultimate death of dopaminergic neurons situated in substantia nigra of the basal ganglia. PD shares several common features with COVID-19 including age-dependency and co-morbidities that include cardiovascular disorders, obesity, and diabetes with the capacity to the impact of COVID-10 on Strategies implicated in PD patient care and, conversely on the effects of PD on immune status resulting in possible increases in severity of COVID-19 (Prasad et al., 2020). Other common features of COVID-19 such as fever, stress, and anxiety may have deleterious effects on tremor, gait, and dyskinesias in PD in addition to compromise of the efficacy of L-Dopa (Butterworth, 2020a). Moreover, enhancement of antibody responses to coronaviruses have been described in cerebrospinal fluid [CSF] samples of PD patients and substantia nigra is a brain structure that is susceptible to viral infections including the MHV-A59 coronavirus (Takahashi et al., 1995).

The functional pathophysiologic links between PD, viral infection and adamantanes became evident following the publication of the serendipitous observation in a 68 year-old female patient with moderate-severe PD who, upon taking the adamantane compound amantadine for the treatment of influenza, noted a marked remission of her rigidity and tremor both of which reappeared upon cessation of amantadine. The molecular structures of amantadine and related adamantanes known to be effective for the treatment of neurodegenerative disease are shown in **Figure 1**.

Beneficial effects were confirmed in a subsequent clinical trial in 163 PD patients (Schwab et al., 1969) and is currently widely employed for the motor symptoms characteristic of PD and particularly for the treatment of L-Dopa-induced dyskinesias as demonstrated by meta-analysis of the results of several randomized clinical trials (Kong et al., 2017).

Enhancement of antibody responses to a range of coronaviruses have been reported in CSF samples from patients with PD and other evidence suggests that Parkinsonism is a common feature a range of viral encephalitides with associated regional neuropathology reminiscent of PD. For example, substantia nigral damage has been reported in association with the H1-N1 influenza virus and MHV-A59 coronaviral infection shows selective affinity for basal ganglia structures with accompanying postural and locomotor deficits resulting from neuronal cell death and gliosis in substantia nigra (Fishman et al., 1985) and a first case of meningitis/encephalitis associated with SARS-CoV-2 the virus responsible for the COVID-19 pandemic was reported in 2020 (Moriguchi et al., 2020).

Investigations into the potential beneficial effects of adamantanes against coronaviruses including SARS-CoV-2 continue at pace resulting in the discovery of novel mechanisms responsible for their neurotropic and neuroinvasive properties as well as those implicated in the protective effects of adamantanes (Butterworth, 2020b, 2021). Other examples include studies of the human respiratory coronavirus HCoV-OC43, a strain known to activate neuroinflammatory and neurodegenerative processes in human neural cell populations cells leading to motor dysfunction and paralytic disease in virus-infected mice (Brison et al., 2014). Treatment with the adamantane analog memantine [structure shown in **Figure 1**) resulted in the reduction of viral replication rates together with improvements in survival times in a dose-dependent manner. Both amantadine and memantine are potent non-competitive antagonists of the N-Methyl-D-Aspartate [NMDA] subclass of receptor for glutamate, the principal excitatory amino acid neurotransmitter of mammalian brain (**Figure 2**). Over-activation of NMDA receptors has the potential to cause release of excess Ca++ and neuronal cell death. Similar mechanisms have been proposed to explain the pathogenesis of neuronal cell death in PD.

There is preliminary evidence from a series of published case reports that is suggestive of a protective effect of amantadine against infection by SARS-CoV-2. The study involved five PD patients all taking amantadine and L-Dopa for several weeks for treatment of motor symptoms of PD who tested positive for the virus by RT-PCR. None of the five patients went on to manifest symptoms of viral infection and improvements in motor function were maintained in all cases (Rejdak and Grieb, 2020). Confirmation of these interesting findings is now required under randomized controlled clinical trial conditions.

Alzheimer's disease

Alzheimer's disease [AD] is a neurodegenerative disease characterized by acquired progressive memory loss with prevalence estimated of 25–30% in the population of Europe aged between the ages of 80 and 85 years in 1990. From a neurochemical pathologic standpoint, AD is characterized by significant neuronal cell loss from the nucleus basalis of Meynert resulting in a central cholinergic deficit. These conclusions led to intensive supplementary investigations and clinical trials of novel agents with the potential to restore the cholinergic deficit. One such group of compounds, the cholinesterase inhibitors having the appropriate structure/activity profile were initially shown to be beneficial from a symptomatic cognitive standpoint but were unfortunately shown to provide little by way of evidence in support of their use for the prevention of the neuronal cell damage and death characteristic of AD. On the other hand, results of a systematic review with meta-analysis of the results of 30 RCTs involving 7,567 patients demonstrated that the adamantane analog, memantine (**Figure 1**) was effective for the improvement of cognitive function in patients with AD compared to placebo, a finding that was highly significant either with or without the addition of cholinesterase inhibitors (Kishi et al., 2017). The case for the use of memantine progressed to gain FDA approval for the treatment of moderate-to-severe AD in the same year.

In recent years, several mechanisms have been proposed to account for the efficacy of memantine for the treatment of AD. Like amantadine, memantine is a potent NMDA receptor

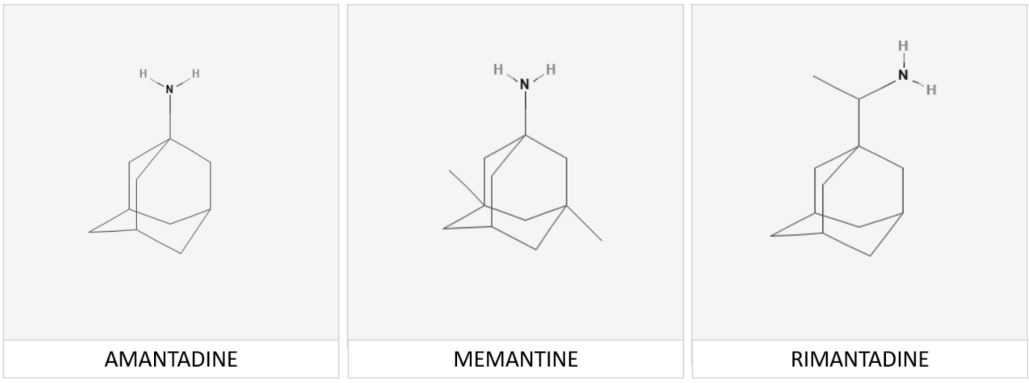


FIGURE 1
Memantine [adamantan-1-amine] is a member of the adamantane family of agents. The adamantane molecule is composed of three condensed cyclohexane ring structures fused together in an armchair configuration with a functional group substituted at one of the four methyne positions that determines the specificity of each individual compound. Chemical structures of memantine and its analogs rimantadine and amantadine are shown above.

antagonist with the potential to inhibit the excess release of Ca^{++} following receptor activation as described above. Alternatively [or additionally], memantine is an established anti-inflammatory agent acting by attenuation of microglial activation known to be of key importance in the pathogenesis of neuronal cell death in AD (Wu et al., 2009).

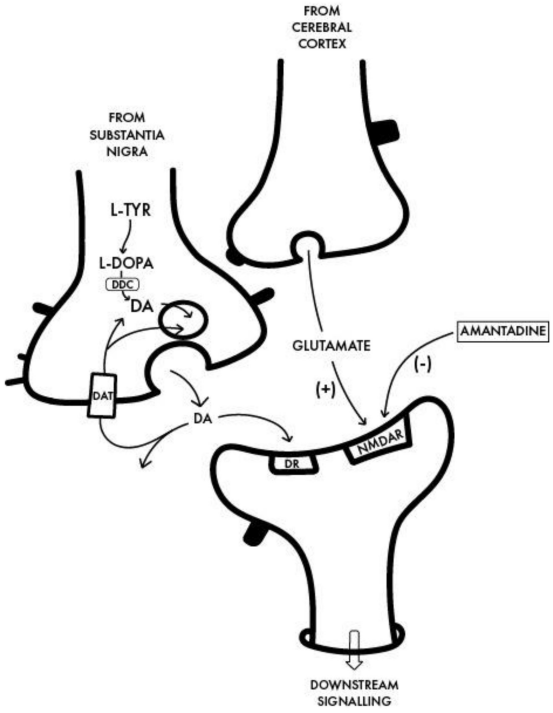


FIGURE 2
Interface between a dopaminergic nigrostriatal nerve terminal in which DA is synthesized from L-Tyrosine [L-TYR] *via* L-DOPA to dopamine with a glutamatergic terminal of the cortico-striatal tract and the postsynaptic neuron. The benefit of amantadine for the treatment of the motor disturbances in PD is attributed to its non-competitive antagonist action on the post-synaptic NMDA receptor [NMDAR] resulting in the restoration of the balance between nigrostriatal and corticostriatal inputs in favor of increased net production of DA. DDC, Dopa decarboxylase; DAT, dopamine transporter; DR, post-synaptic DA receptor.

Memantine, in common with other adamantanes, is effective against numerous viral infections including the human coronavirus HCoV-OC43 and an ongoing thesis proposes that the Herpes Simplex Virus Type 1 [HSV-1] rather than p-tau is responsible for the inter-neuronal trans-synaptic pathological cascade proposed for the inter-cerebral propagation of AD (Ball et al., 2013).

Evidence of functional links between AD and COVID-19 continues to accumulate. In common with other neurodegenerative diseases, AD is considered a co-morbidity for COVID-19 and the presence of one of the conditions frequently results in worsening of the other (Xia et al., 2021). Each condition results in neurocognitive impairment and neurodegeneration that is linked to the accumulation of amyloid precursor protein [APP] as well as to NMDA receptor activation and, since they share proinflammatory signaling cascades, neuronal cell dysfunction and loss has been attributed to microglial-mediated responses in both conditions (Butterworth, 2022). In relation to these mechanistic considerations, it is interesting to note that amyloid-beta oligomers are known to transit into the plasma membrane leading to the formation of pores that favor the passage of Ca^{++} following activation of NMDA receptors. It is interesting in this regard that both AD and COVID-19 appear to derive therapeutic benefit from treatment with the potent NMDA receptor antagonist memantine. Memantine exerts dose-dependent antiviral and neuroprotective against the human respiratory neuroinvasive coronavirus HCoV-OC43, a relative of SARS-CoV-2 where the beneficial effects were attributed to the reduction of microglial activation. Moreover, in relation to inflammatory responses, AD-related neuroinflammation coupled with that resulting from SARS-CoV-2 infection has the potential to result in a "cytokine storm" leading to extremely poor clinical outcomes in both situations.

Studies of the effects of adamantanes on the SARS-CoV-2 virus *per se* continue apace. In one such investigation, the antiviral actions of memantine, amantadine and rimantadine were compared in Vero E6 cells; results are shown in **Figure 3**. While all three analogs were effective, rimantadine was the most potent showing the highest selectivity index (Zhou et al., 2021).

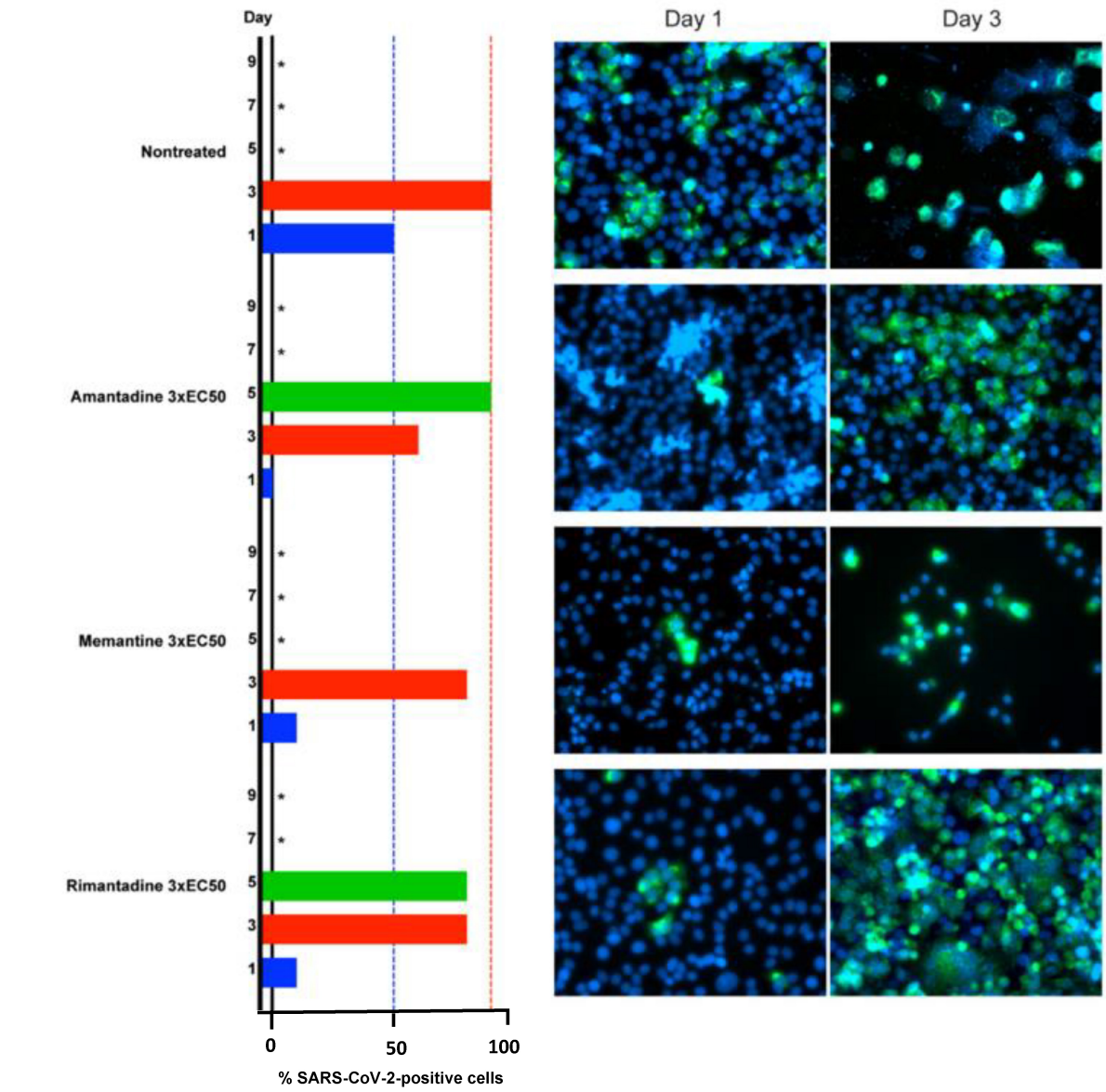


FIGURE 3
Barrier to SARS-CoV-2 escape by memantine, amantadine and rimantadine in their roles as ion channel inhibitors in Vero E6 cells at concentrations of 3 x EC50 on days 1,3,5,7,9 post-infection. Infected cells identified by immunostaining for SARS-CoV-2 spike protein (green) relative to counterstaining of cell nuclei with Hoechst dye (blue). *Not recorded/cell death.

Mechanisms responsible for the antiviral properties of these agents include blocking of the viroporin channel of the E protein of SARS-CoV-2 leading to prevention of release of the viral nucleus into the host cell cytoplasm (Singh Tomar and Arkin, 2020) as well as down-regulation in expression of host cell proteases such as Cathexin L (Smieszek et al., 2020) and targeting ion channels encoded by the virus (Toft-Bertelsen et al., 2021).

Multiple sclerosis

Central fatigue in Multiple sclerosis [MS] has a significant negative impact on disability scores and health-related quality of life [HRQOL] that occurs in patients with MS with increased severity and frequency in those with primary or secondary progressive disease compared to those with a relapsing-remitting presentation. Modern neuroimaging and spectroscopic techniques continue to support the thesis that predominantly centrally mediated mechanisms underpin the pathogenesis of fatigue in MS. Both the burden of Magnetic Resonance Imaging [MRI] lesions and abnormalities of motor-evoked potentials [MEPs] correlate in an independent manner with fatigue severity in MS patients consistent with its central origin (Colombo et al., 2000). In addition, region-selective cerebral metabolic dysfunction was confirmed using 18-fluorodeoxyglucose Positron Emission Tomography [PET] (Roelcke et al., 1997) and functional 1-H-Magnetic Resonance Spectroscopy [MRS] (DeLuca et al., 2008). Brain regions implicated included basal ganglia and frontal cortex

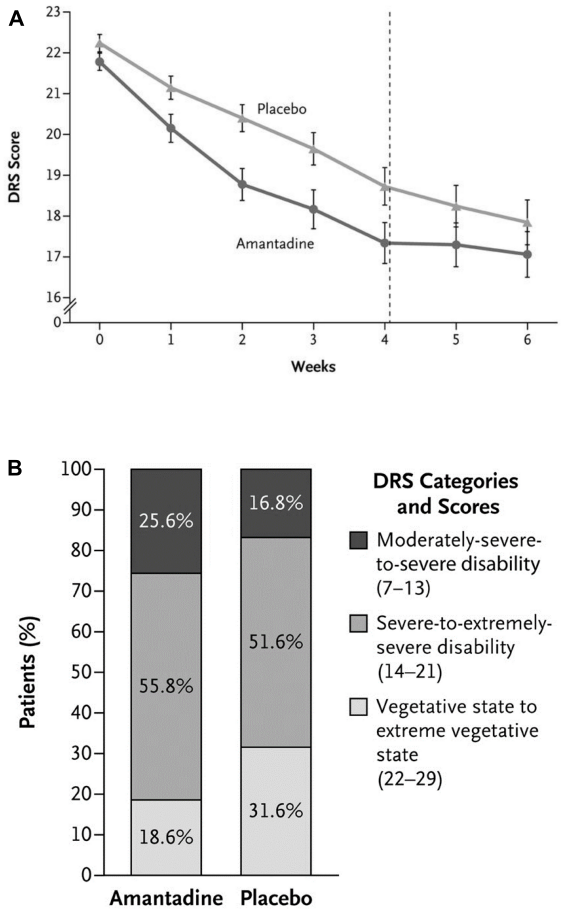


FIGURE 4
(A) Rate of functional recovery (DRS score) as a function of duration of treatment with amantadine compared to placebo in patients with severe TBI. DRS scores were improved significantly more rapidly following amantadine during the 4 week treatment period compared to placebo. On weeks 5 and 6 (washout interval), recovery rates in the amantadine group were significantly slower. Error bars indicate mean values ± SE. **(B)** Effects of amantadine treatment compared to placebo as a function of the category of functional disability (DRS score). After 4 weeks of treatment, the proportion of patients in a vegetative-to-extreme vegetative state was significantly lower in the amantadine group by post-hoc analysis.

giving credence to the notion that functional modifications of the striatal-thalamic-frontal cortical network play a key role in MS-related fatigue (Genova et al., 2013). Possible central mediators proposed include the neurotransmitter dopamine and the pro-inflammatory cytokine tumor necrosis factor alpha [TNFα].

Medications currently employed for the treatment of fatigue in MS. Amantadine is one such agent where results of 11 RCTs some comparing its efficacy to placebo, others comparing amantadine to that of other agents that included modafinil, pemoline, L-carnitine, ondansetron or methylphenidate were published resulting in six systematic reviews with two associated meta-analyses. The majority of cases confirmed that amantadine provided significant degrees of relief from fatigue in patients with either chronic persistent MS or in relapsing-remitting forms of the disorder. The consistency of these results contributed to the recommendations from the clinical practice guidelines published by The Royal College of Physicians: Multiple Sclerosis [NICE, UK) and by The German Society of Multiple Sclerosis recommending that amantadine be employed for the effective treatment of fatigue in MS (Pilling and Butterworth, 2021).

Traumatic brain injury

Traumatic Brain Injury [TBI] and its associated neurological disabilities [decreased levels of consciousness, cognitive impairment] although not classically included as neurodegenerative diseases, share certain similarities with the latter that include favorable responses to amantadine treatment *via* well-established mechanisms of action. Furthermore, certain neurobehavioral sequelae of TBI such as hyperexcitability, disinhibition and agitation may also manifest improvements (**Figure 4**) that include metabolic activity in sagittal, coronal and axial planes following amantadine treatment (Giacino et al., 2018) accompanied by faster improvements in Disability Rating Score [DRS] values (Butterworth, 2020c). Improvements of executive cognitive ability concomitant with improved prefrontal cortical function determined by PET has also been reported in amantadine-treated MS patients (Kraus et al., 2005).

Adverse events reported during use of adamantanes for treatment of PD, AD, MS, TBI are relatively minor in severity and most commonly include confusion, light headedness, swelling of hands, legs, sleep disturbances [rarely].

Conclusion

Close inspection of the published articles cited in this review reveals important links between neurodegenerative diseases and COVID-19 with respect to both basic pathophysiology and treatment with members of the adamantane family of agents. Both PD and AD are important age-related co-morbidities with the potential to impact on the severity of COVID-19 and, conversely, the symptoms of COVID-19 [fever, stress, fatigue] are known to aggravate gait abnormalities, tremor and effectiveness of L-Dopa in PD patients. Furthermore, enhanced antibody responses to coronaviruses have been demonstrated in CSF samples from patients with PD.

Links that are distinct from those encountered in PD are known to occur in AD where the presence of one of the two conditions may result in worsening of the other (Xia et al., 2021). Again, as for PD, severity of symptoms of both AD and COVID-19 are age-related and, interestingly, both are pathologically to mechanistic factors including the deposition of APP and to the activation of putative cell-death mechanisms that include excitotoxicity due to NMDA-receptor activated uptake of Ca^{++} in addition to microglial-mediated proinflammatory responses shared by COVID-19 and AD. The notion of a viral etiology in AD remains popular one version of which proposes that herpes simplex virus type 1 [HSV-1] rather than tau may cause the inter-neuronal trans-synaptic pathological cascade involved in the inter-cerebral progression of AD. It should also be noted that additional mechanisms have also been proposed; for example, some years ago, amantadine was found to improve resolution of the dysfunction of peripheral airways

in influenza (Little et al., 1976) similar mechanisms could be implicated in the case of COVID-19.

Adamantanes [particularly amantadine and memantine] have evidence-based support from a series of randomized controlled trials [RCTs], some with associated meta-analysis for the treatment of PD and of L-Dopa-related dyskinesias in PD [amantadine] and for cognitive dysfunction in mild-to-moderate AD with US-FDA approval granted in both cases.

Accumulating evidence is now available in support of direct antiviral actions of adamantanes including [particularly amantadine and memantine] against coronaviruses including SARS-CoV-2. Results of initial clinical studies based upon limited patient numbers support the use of amantadine for the treatment of COVID-19 in patients even in the presence of pre-existing PD where benefit for both conditions was reported (Rejdak and Grieb, 2020). Similar reports indicate beneficial effects of memantine in COVID-19-infected patients with cognitive impairment (Rejdak and Grieb, 2020). Amantadine is also effective for the treatment of traumatic brain injury and its CNS complications and for the relief of fatigue in patients with MS (Giacino et al., 2018; Butterworth, 2020c).

The present review touches on other issues related for example, to the effects of prolonged exposure to SARS-CoV-2 on the efficacy and durability of amantadine and/or memantine commonly employed for the treatment of neurodegenerative disorders while bearing in mind that many of these disorders are themselves considered as co-morbidities for COVID-19. In this latter regard, given the recent mechanistic and therapeutic advances of significant antiviral action of amantadine, the possibility emerges whereby treatment of the neurological symptoms characteristic of the degenerative disorder as well as the co-morbidity associated with severe COVID-19 infection could be envisaged in a simultaneous manner. Indeed, the present review cites evidence from case reports and pilot studies that is consistent with such a possibility. It will be important to now confirm and extend these interesting findings in appropriate randomized clinical trials in the near future.

With regards to this latter eventuality, it is important to note that there are a number of clinical trials planned or currently ongoing registered in ClinicalTrials.gov relating directly to studies of the efficacy of amantadine for the treatment of COVID-19 summarized in Table 2.

Author contributions

RB contributed to the design and the manuscript production, includes editing as following: Literature search of adamantanes for treatment of PD, AD, MS, and TBI, writing of the manuscript, reviewing of the manuscript, formatting of the manuscript, creation of figures, references list, compliance with author's guidelines, and online submission.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Ball, M. J., Lukiw, W. J., Kammerman, E. M., and Hill, J. M. (2013). Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology. *Alzheimers Dement.* 9, 169–175. doi: 10.1016/j.jalz.2012.07.005

Brison, E., Jacomy, H., Desforges, M., and Talbot, P. J. (2014). Novel treatment with neuroprotective and antiviral properties against a neuroinvasive human respiratory virus. *J. Virol.* 88, 1548–1563. doi: 10.1128/JVI.02972-13

Butterworth, R. F. (2020a). Amantadine for the treatment of Parkinson's disease and its associated dyskinesias. *J. Parkinsons Dis. Alzheimer Dis.* 7:7.

Butterworth, R. F. (2020b). Amantadine, Parkinson's disease and COVID-19. *Covid Perspect. Res. Rev.* 01, 1–6.

Butterworth, R. F. (2020c). Amantadine for the treatment of traumatic brain injury and its associated cognitive and neurobehavioural complications. *Pharmacol. Pharm. Res.* 3, 1–5. doi: 10.1089/neu.2018.5738

Butterworth, R. F. (2021). Potential for the Repurposing of Adamantane Antivirals for COVID-19. *Drugs R D.* 21, 267–272. doi: 10.1007/s40268-021-00351-6

Butterworth, R. F. (2022). Memantine for the treatment of Alzheimer's disease: novel mechanisms and future opportunities. *Neurol. Neurorehabil.* 4, 17–20. doi: 10.37532/22.4.2.17-20

Colombo, B., Martinelli Boneschi, F., Rossi, P., Rovaris, M., Maderna, L., Filippi, M., et al. (2000). MRI and motor evoked potential findings in nondisabled multiple sclerosis patients with and without symptoms of fatigue. *J. Neurol.* 247, 506–509. doi: 10.1007/s004150070148

DeLuca, J., Genova, H. M., Hillary, F. G., and Wylie, G. (2008). Neural correlates of cognitive fatigue in multiple sclerosis using functional MRI. *J. Neurol. Sci.* 270, 28–39. doi: 10.1016/j.jns.2008.01.018

Fishman, P. S., Gass, J. S., Swoveland, P. T., Lavi, E., Highkin, M. K., and Weiss, S. R. (1985). Infection of the basal ganglia by a murine coronavirus. *Science* 229, 877–879. doi: 10.1126/science.2992088

Genova, H. M., Rajagopalan, V., Deluca, J., Das, A., Binder, A., Arjunan, A., et al. (2013). Examination of cognitive fatigue in multiple sclerosis using functional magnetic resonance imaging and diffusion tensor imaging. *PLoS One* 8:e78811. doi: 10.1371/journal.pone.0078811

Giacino, J. T., Katz, D. I., Schiff, N. D., Whyte, J., Ashman, E. J., Ashwal, S., et al. (2018). Practice guideline update recommendations summary: Disorders of consciousness: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology; the American Congress of Rehabilitation Medicine; and the National Institute on Disability, Independent Living, and Rehabilitation Research. *Neurology* 91, 450–460. doi: 10.1212/WNL.0000000000005926

Kishi, T., Matsunaga, S., Oya, K., Nomura, I., Ikuta, T., and Iwata, N. (2017). Memantine for Alzheimer's disease: an updated systematic review and meta-analysis. *J. Alzheimers Dis.* 60, 401–425. doi: 10.3233/JAD-170424

Kong, M., Ba, M., Ren, C., Yu, L., Dong, S., Yu, G., et al. (2017). An updated meta-analysis of amantadine for treating dyskinesia in Parkinson's disease. *Oncotarget* 8, 57316–57326. doi: 10.18632/oncotarget.17622

Kraus, M. F., Smith, G. S., Butters, M., Donnell, A. J., Dixon, E., Yilong, C., et al. (2005). Effects of the dopaminergic agent and NMDA receptor antagonist amantadine on cognitive function, cerebral glucose metabolism and D2 receptor availability in chronic traumatic brain injury: a study using positron emission tomography (PET). *Brain Inj.* 9, 471–479. doi: 10.1080/02699050400025059

Little, J. W., Hall, W. J., Gordon Douglas, R., Hyde, R. W., and Speers, D. M. (1976). Amantadine effect on peripheral airways abnormalities in influenza. *Ann. Int. Med.* 85, 177–182. doi: 10.7326/0003-4819-85-2-177

Moriguchi, T., Harii, N., Goto, J., Harada, D., Sugawara, H., Takamino, J., et al. (2020). A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int. J. Infect. Dis.* 94, 55–58. doi: 10.1016/j.ijid.2020.03.062

Pilling, K., and Butterworth, R. F. (2021). Amantadine for the treatment of fatigue in multiple sclerosis: systematic review and summary of the evidence base. *J. Mult. Sclerosis* 8:272.

Prasad, S., Holla, V. V., Neeraja, K., Surisetti, B. K., Kamble, N., Yadav, R., et al. (2020). Parkinson's disease and COVID-19: Perceptions and implications in patients and caregivers. *Mov. Disord.* 35, 912–914. doi: 10.1002/mds.28088

Rejdak, K., and Grieb, P. (2020). Adamantanes might be protective from COVID-19 in patients with neurological diseases: multiple sclerosis, parkinsonism and cognitive impairment. *Mult. Scler. Relat. Disord.* 42:102163. doi: 10.1016/j.msard.2020.102163

Roelcke, U., Kappos, L., Lechner-Scott, J., Brunnschweiler, H., Huber, S., Ammann, W., et al. (1997). Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. *Neurology* 48, 1566–1571. doi: 10.1212/wnl.48.6.1566

Schwab, R. S., England, A. C. Jr., Poskanzer, D. C., and Young, R. R. (1969). Amantadine in the treatment of Parkinson's disease. *JAMA* 208, 1168–1170.

Singh Tomar, P. P., and Arkin, I. T. (2020). SARS-CoV-2 E protein is a potential ion channel that can be inhibited by Gliclazide and Memantine. *Biochem. Biophys. Res. Commun.* 530, 10–14. doi: 10.1016/j.bbrc.2020.05.206

Smieszek, S. P., Przychodzen, B. P., and Polymeropoulos, M. H. (2020). Amantadine disrupts lysosomal gene expression: A hypothesis for COVID19 treatment. *Int. J. Antimicrob. Agents* 55:106004. doi: 10.1016/j.ijantimicag.2020.106004

Takahashi, M., Yamada, T., Nakajima, S., Nakajima, K., Yamamoto, T., and Okada, H. (1995). The substantia nigra is a major target for neurovirulent influenza A virus. *J. Exp. Med.* 181, 2161–2169. doi: 10.1084/jem.181.6.2161

Toft-Bertelsen, T. L., Jeppesen, M. G., Tzortzini, E., Xue, K., Giller, K., Becker, S., et al. (2021). Amantadine has potential for the treatment of COVID-19 because it inhibits known and novel ion channels encoded by SARS-CoV-2. *Commun. Biol.* 4:1347. doi: 10.1038/s42003-021-02866-9

Wu, H. M., Tzeng, N. S., Qian, L., Wei, S. J., Hu, X., Chen, S. H., et al. (2009). Novel neuroprotective mechanisms of memantine: increase in neurotrophic factor release from astroglia and anti-inflammation by preventing microglial activation. *Neuropsychopharmacology* 34, 2344–2357. doi: 10.1038/npp.2009.64

Xia, X., Wang, Y., and Zheng, J. (2021). COVID-19 and Alzheimer's disease: how one crisis worsens the other. *Transl. Neurodegener.* 10:15. doi: 10.1186/s40035-021-00237-2

Zhou, Y., Gammeltoft, K. A., Galli, A., Offersgaard, A., Fahnøe, U., Ramirez, S., et al. (2021). Efficacy of ion-channel inhibitors amantadine, memantine and rimantadine for the treatment of SARS-CoV-2 in vitro. *Viruses* 13:2082. doi: 10.3390/v13102082

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 19 June 2023
DOI 10.3389/fimmu.2023.1205879

OPEN ACCESS

EDITED BY
Sonia Zuñiga,
Spanish National Research Council (CSIC), Spain

REVIEWED BY
Matteo Lucchini,
Catholic University of the Sacred Heart, Italy
Domizia Vecchio,
University of Eastern Piedmont, Italy

*CORRESPONDENCE
Simona Rolla
simona.rolla@unito.it

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share senior authorship

RECEIVED 14 April 2023
ACCEPTED 30 May 2023
PUBLISHED 19 June 2023

CITATION
Maglione A, Francese R, Arduino I, Rosso R, Matta M, Rolla S, Lembo D and Clerico M (2023) Long-lasting neutralizing antibodies and T cell response after the third dose of mRNA anti-SARS-CoV-2 vaccine in multiple sclerosis.
Front. Immunol. 14:1205879.
doi: 10.3389/fimmu.2023.1205879

Long-lasting neutralizing antibodies and T cell response after the third dose of mRNA anti-SARS-CoV-2 vaccine in multiple sclerosis

Alessandro Maglione[1†], Rachele Francese[2†], Irene Arduino[2], Rachele Rosso[1], Manuela Matta[3], Simona Rolla[1*], David Lembo[2‡] and Marinella Clerico[1,3‡]

[1]Laboratory of Neuroimmunology, Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy, [2]Laboratory of Molecular Virology and Antiviral Research, Department of Clinical and Biological Sciences, University of Turin, Orbassano, Italy, [3]San Luigi Gonzaga University Hospital, Orbassano, Italy

Background and objectives: Long lasting immune response to anti-SARS-CoV-2 vaccination in people with Multiple Sclerosis (pwMS) is still largely unexplored. Our study aimed at evaluating the persistence of the elicited amount of neutralizing antibodies (Ab), their activity and T cell response after three doses of anti-SARS-CoV-2 vaccine in pwMS.

Methods: We performed a prospective observational study in pwMS undergoing SARS-CoV-2 mRNA vaccinations. Anti-Region Binding Domain (anti-RBD) of the spike (S) protein immunoglobulin G (IgG) titers were measured by ELISA. The neutralization efficacy of collected sera was measured by SARS-CoV-2 pseudovirion-based neutralization assay. The frequency of Spike-specific IFNγ-producing CD4+ and CD8+ T cells was measured by stimulating Peripheral Blood Mononuclear Cells (PBMCs) with a pool of peptides covering the complete protein coding sequence of the SARS-CoV-2 S.

Results: Blood samples from 70 pwMS (11 untreated pwMS, 11 under dimethyl fumarate, 9 under interferon-γ, 6 under alemtuzumab, 8 under cladribine, 12 under fingolimod and 13 under ocrelizumab) and 24 healthy donors were collected before and up to six months after three vaccine doses. Overall, anti-SARS-CoV-2 mRNA vaccine elicited comparable levels of anti-RBD IgGs, neutralizing activity and anti-S T cell response both in untreated, treated pwMS and HD that last six months after vaccination. An exception was represented by ocrelizumab-treated pwMS that showed reduced levels of IgGs ($p<0.0001$) and a neutralizing activity under the limit of detection ($p<0.001$) compared to untreated pwMS. Considering the occurrence of a SARS-CoV-2 infection after vaccination, the Ab neutralizing efficacy ($p=0.04$), as well as CD4+ ($p=0.016$) and CD8+ ($p=0.04$) S-specific T cells, increased in treated COVID+ pwMS compared to uninfected treated pwMS at 6 months after vaccination.

Discussion: Our follow-up provides a detailed evaluation of Ab, especially in terms of neutralizing activity, and T cell responses after anti-SARS-CoV-2 vaccination in MS context, over time, considering a wide number of therapies, and eventually breakthrough infection. Altogether, our observations highlight the vaccine response data to current protocols in pwMS and underline the necessity to carefully follow-up anti-CD20- treated patients for higher risk of breakthrough infections. Our study may provide useful information to refine future vaccination strategies in pwMS.

KEYWORDS

anti-SARS-CoV-2 vaccination, multiple sclerosis, COVID-19, neutralizing antibodies, T-cell response, disease modifying therapies

Introduction

The mRNA vaccines rapidly became the most used to counteract severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread (1) especially in frail subjects such as people with Multiple Sclerosis (pwMS). Whereas vaccination showed an adequate safety profile (2) and high efficacy in preventing COVID-19 transmission and severe disease outcomes in immunocompetent people (3, 4), pwMS are usually treated to prevent or block inflammation with disease-modifying therapies (DMTs) that modulate the immune system and, consequently, may lead to a suboptimal response to vaccination and increased probability of infection/re-infection (5–7). Several studies have shown that high-efficacy DMTs induced a weak immune response to anti-SARS-CoV-2 vaccination in pwMS: after two doses of mRNA vaccines, pwMS treated with ocrelizumab (anti-CD20 therapy) and fingolimod (sphingosine-1-phosphate receptor modulator) showed reduced levels of anti-SARS-CoV-2 spike IgG compared to healthy individuals and pwMS under other treatments (8–12). Due to humoral response decrease over 6 months following the second vaccine dose, authorities suggested the booster dose (10). Despite an increase in seroconversion after the booster (or third) dose, anti-SARS-CoV-2 spike IgG levels are still reduced in pwMS under anti-CD20 or sphingosine-1-phosphate receptor modulators (2, 13–15).

Antibodies (Ab) directed toward the Receptor Binding Domain (RBD) of the SARS-CoV-2 Spike (S) protein are widely considered to be a good representation of the Ab neutralizing activity as they positively correlate with SARS-CoV-2 neutralizing Ab measured in neutralization assays (16, 17). ELISA-based tests present advantages such as low cost, speed, and safety, but only Ab that block the RBD/ACE2 interaction are detected, thus both the actual neutralizing activity and the presence of non-RBD binding Ab, which may also be neutralizing, are missing (18–21). The most direct methods to evaluate the neutralizing Ab induced by SARS-CoV-2 vaccination and predict their function and efficacy are the live virus-based or alternatively the pseudovirion-based infection inhibition tests. As opposed to the use of live virus, neutralization tests with pseudovirions can be easily carried out in BSL-2 conditions and the presence of a reporter gene enables an objective, rapid and quantitative detection (21, 22). To the best of our knowledge, only one report investigated the Ab neutralizing activity with the above-mentioned methods in the context of pwMS (23). Therefore, the actual Ab neutralizing response in pwMS still remains an open question.

The longevity of elicited immunity after the third dose of anti-SARS-CoV-2 vaccination is currently under investigation. A study on healthcare workers showed that reduction in Ab levels 5 months after the third vaccine dose was slower than after the second (24), while a mid/long-term follow-up of the immune response after booster vaccination dose in pwMS is missing to date. Moreover, data indicate that immunity induced by anti-SARS-CoV-2 vaccination is mediated both by neutralizing Ab that block infection by preventing viral entry into host cells, and cellular immunity that rapidly activates once the infection has occurred, hence protecting from severe disease (25). Actually, low neutralizing Ab levels are a relevant risk factor for breakthrough infection risk in pwMS (6) while SARS-CoV-2 antigen-specific T cell response seems to be preserved in the majority of pwMS (15, 26–28).

Here, 70 pwMS and 24 healthy donors (HD) were followed up for 6 months after three vaccination doses to evaluate long-term Ab neutralizing activity and T cells response. Humoral response was evaluated by both anti-RBD IgG titration and neutralization assay using SARS-CoV-2 pseudovirions. Antigen-specific T cell response was quantified by *in vitro* restimulation of Peripheral Blood Mononuclear Cells (PBMCs) with S peptides. Our findings provide additional information to refine future vaccination strategies in MS patients.

Materials and methods

Subjects

PwMS and HD, belonging to this prospective single-center study, were recruited at the AOU San Luigi Gonzaga, Orbassano

(TO, Italy) according to the following inclusion/exclusion criteria. A diagnosis of MS, according to the most recently revised McDonald criteria (29), and eligibility for anti-SARS-CoV-2 vaccination were considered as inclusion criteria. Any medical condition that does not allow the signing of informed consent and a prior history of symptomatic SARS-CoV-2 infection or breakthrough infection before the third dose were considered as exclusion criteria. All the subjects in the study were vaccinated with two doses of Comirnaty (ex mRNA BNT162b2) mRNA vaccine (Pfizer/BioNTech Inc, BioNTech Manufacturing GmbH) and then with the third dose (booster) of Comirnaty or Spikevax (ex mRNA-1273) vaccine (Moderna, Moderna Biotech Spain S.L.). COVID-19 disease was not reported from any of the subjects before vaccination. COVID-19 infection after vaccination was determined by self-reported positive COVID-19 test during the follow-up and/or presence of nucleocapsid Ab (Anti-N) in collected serum samples.

Blood and sera collection

Blood and sera were collected immediately before the first dose of Comirnaty vaccine (Pfizer/BioNTech Inc, BioNTech Manufacturing GmbH) (P0), 4 weeks (± 15 days) (P1) and 6 months (± 15 days) (P6) after the booster vaccination. Sera were immediately frozen for further analysis. PBMCs were isolated by density gradient centrifugation using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA) from heparinized venous blood.

Anti-SARS-Cov2 ELISA

Anti-RBD IgG titers were measured with the SARS-CoV-2 RBD IgG ELISA (EIA-6150, DRG Diagnostics, Marburg, Germany, EU; lot number 142K061) following manufacturer instructions. Results are expressed in IU/ml (log10), and the cut-off threshold corresponded to 1.4 IU/ml (log10), according to manufacturer indications. Anti-N IgG were measured with the SARS-CoV-2 (COVID-19) IgG ELISA test (NOVATEC Immunodiagnostica GmBH, Dietzenbach, Germany, EU; lot number COVG-053) according to manufacturer method.

Cell lines

The human embryonic kidney cells (293T, ATCC, CRL-3216), the baby hamster kidney cells (BHK21, ATCC, C-13) and the hepatocyte derived cellular carcinoma cell line (Huh7) (ECACC, Cat num: 01042712) were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich St. Louis, MO, USA), supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) (Sigma-Aldrich) and a 1% (v/v) antibiotic solution (penicillin–streptomycin, Sigma-Aldrich) at 37°C in 5% CO2 atmosphere.

SARS-CoV-2 pseudovirion production, titration and characterization

SARS-CoV-2 pseudotyped viruses were produced and titrated according to Nie et al. (21) and were analyzed by means of Western Blot analysis to verify the presence of the SARS-CoV-2 S protein on the VSV envelope (30). The detailed protocols are reported in the Supplementary Materials.

SARS-Cov-2 pseudovirion-based neutralization assay

The neutralization assay was performed according to Almahboub et al. (31) and Nie et al. (21). Huh7 cells were pre-seeded in a 96 well/plate at a density of $1.2x10^4$ cell/well. The following day, serum samples were heat inactivated at 56°C for 30 min in a water bath. Then, a fixed amount of SARS-CoV-2 pseudovirions (650 $TCID_{50}$/well) (21) was incubated with serial dilutions of serum samples (from 1:20 to 1:14580 in duplicate) for 1h at 37°C in continuous oscillation. As controls, six wells were incubated with only culture medium (CC wells) and six wells were infected but not treated (VC wells). After 1h incubation, the pre-treated virus was inoculated on Huh7 cells for 24h at 37°C to evaluate the residual viral infectivity. The detection was performed by adding the Britelite plus reporter gene assay system (PerkinElmer) to cells in a 1:1 ratio with the culture medium, for 2 min in the darkness at RT. 150µl of each well were then transferred to a corresponding 96-well chemiluminescence detection plate and the RLU were read in the Infinite F200 luminescence reader (TECAN). Inhibition (%) of luciferase activity from each serum dilution was calculated as follows: *100 - [(mean RLU of each sample - mean RLU of CC)/(mean RLU of VC - mean RLU of CC) x 100]*. Inhibition (%) were then plotted against each dilution using four-parameter logistic (4PL) curve, and 50% inhibitory dilution (ID_{50}) values for each sample were calculated using GraphPad Prism software, version 8.0 (GraphPad Software, San Diego, CA, USA). As recommended by the World Health Organization (WHO) (29), the neutralization assay was calibrated and validated with the Working Standard Reagent for anti-SARS-CoV-2 immunoglobulin (National Institute for Biological Standards and Controls –NIBSC-, code: 21/234), that was also employed as positive control at each run of the experiment. As negative control, a serum sample from an uninfected and unvaccinated person was used.

Evaluation of T cells response

PBMCs were cultured at 10^7/mL in RPMI-1640 Medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% FBS (Corning, New York, NY, USA) and stimulated or not with PepTivator® SARS-CoV-2 Prot_S Complete (Miltenyi Biotec, Bergisch Gladbach, Germany, EU) at a final concentration of

0.6 nmol of each peptide/mL. PepTivator SARS-CoV-2 Prot_S Complete is a pool of lyophilized peptides, covering the complete protein coding sequence (aa 5–1273) of the surface or S glycoprotein of SARS-CoV-2 (GenBank MN908947.3, Protein QHD43416.1). Cells were incubated at 37°C for two hours and then 5µg/mL of Brefeldin A (Sigma-Aldrich, St. Louis, MO, USA) was added to cells to allow intracellular cytokine staining. PBMCs were incubated for further 16 hours and then prepared for staining. To detect anti-S specific CD4 and CD8 T cells, stimulated cells were stained for the surface antigen CD4 and CD8 (BioLegend, San Diego, CA, USA); fixed with Cyto-Fast Fix/Perm Buffer Set (BioLegend) and intracellular stained with anti-IFN-γ mAb (BioLegend). Stained PBMCs were acquired on CELESTA FACS (BD Biosciences, San Jose, CA, USA) and analyzed with FlowJo software (Ashland, OR, USA) Version 10. 50.000 CD4+ events were acquired and analyzed. The frequency of Spike-specific IFNγ-CD4+ and CD8+ T cells was obtained by subtracting cytokine background obtained from unstimulated cells.

Standard protocol approvals, registrations, and patient consents

This study obtained ethics approval from the ethics committee of AOU San Luigi Gonzaga, Orbassano (TO), Italy; Ref. number #117-2021). All the subjects included in the study consented to participate in the study.

Data availability

Data sets used during this study are available from the corresponding author on reasonable request.

Statistics

Anti-RBD IgGs titers and ID_{50} values of the inhibition curves were calculated by a regression analysis using GraphPad Prism software, version 8.0 (GraphPad Software, San Diego, CA, USA) by fitting a quadratic curve and a variable slope-sigmoidal dose-response curve and statistically compared with the F-test, respectively. Ab levels were transformed on a log10 scale, to normalize their distribution and according to previous literature (12, 14).

Statistical analysis was performed using ANOVA Analysis of variance followed by Bonferroni post-test or t-test as reported in the legends to the Figures. Multivariable analysis was performed using a linear regression model computed by R version 3.6.3 (2020-02-29). Model was used to compare log-transformed P1 Anti-RBD IgG titer across patients treated with different DMTs, after adjusting for age, sex, EDSS score, MS type: relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), MS disease duration, booster type (Comirnaty/Spikevax/COVID-19), Ab levels in the P0 samples. P1 Ab titers were included in the model to compare log-transformed P6 Anti-RBD IgG titers across subjects.

Results

Anti-RBD IgGs titers persist up to six months after SARS-CoV-2 vaccination in pwMS

11 untreated pwMS, 59 pwMS under different DMTs and 24 HD were recruited and prospectively followed-up from their first shot of anti-COVID-19 mRNA vaccine (Pfizer) to 6 months after the third dose (Pfizer/Moderna). Demographic and clinical characteristics are reported in Table 1.

The titer of anti-RBD IgGs induced by the full cycle of anti-SARS-CoV-2 vaccination (three doses) was evaluated in serum samples collected immediately before vaccination (P0), one (P1) and six months (P6) after booster. Moreover, the anti-N Ab titration was performed to evaluate a response to the natural infection occurred after vaccination.

Treated (T) pwMS (2.4 ± 1.2; p = 0.001) showed a significant lower level of anti-RBD IgGs compared to HD (3.6 ± 0.2) at P1 while not treated (NT) pwMS (3.3 ± 0.3) showed comparable levels with HD (Figure 1A). At P6, no significant difference was observed comparing anti-RBD IgG levels in HD (3.5 ± 0.2) with NT pwMS (3.4 ± 0.5) and T pwMS (2.7 ± 1) (Figure 1A). Subsequently, pwMS were divided according to anti-N positivity and their anti-RBD IgGs level were compared (Figure 1B). No statistical difference was observed, suggesting that anti-RBD IgGs is not related to a possible natural infection after vaccination. PwMS under interferon were excluded from this analysis because none of these subjects experienced natural COVID-19 infection after vaccination.

To investigate the effect of therapies on anti-RBD IgGs, pwMS were then divided according to DMTs (Figure 1C). All T pwMS showed comparable levels of anti-RBD IgGs with exception of T pwMS under ocrelizumab (1.3 ± 1; p<0.0001) and fingolimod (1.6 ± 1.3; p=0.0009) that showed significant lower levels of Ab respect to NT pwMS (3.3 ± 0.3). This difference is maintained at P6 for pwMS under ocrelizumab (1.1 ± 0.7, p=<0.0001) compared to NT pwMS at P6 (3.4 ± 0.45). Interestingly, a significant difference in anti-RBD IgG titers was not observed between P1 and P6 within each group suggesting a long-lasting durability of anti-RBD IgGs.

Finally, the association of factors included in Table 1 to anti-RBD IgG levels at P1 and P6 was explored by a multivariable regression analysis. Results of this analysis are reported in Table 2. Ab titers at P6 were significantly associated with P1 Ab level (p = 0.0099) and ocrelizumab therapy (p = 0.0012). We confirmed that anti-RBD IgG titers at P1 were associated with treatment with ocrelizumab (p < 0.00005) and fingolimod (p = 0.0005) which both showed significantly reduced anti-RBD Ab levels compared to NT pwMS. Moreover, anti-RBD IgG titers at P1 were significantly increased in subjects that had Spikevax booster with respect to Comirnaty (p = 0.0294). No association with any other considered factor was found.

10.3389/fimmu.2023.1205879

TABLE 1 Clinical characteristics.

Therapy	HD (n=24)	NT MS (n=11)	T MS (n= 59)					
			DMF (n=11)	IFN (n=9)	ALEM (n=6)	CLAD (n=8)	FING (n=12)	OCR (n=13)
Sex (F/M)	20/6	11/0	5/6	4/5	4/2	5/2	10/2	10/3
Age	43.5 (32.3 – 50.3)	51 (47.5 – 63.5)	39 (38 – 48.5)	47 (43 – 55)	37 (36.3 – 47.5)	46.5 (42.8 – 51.8)	52 (46.3 – 59.3)	57 (53 – 64)
MS disease duration (years)	NA	8 (3 –17.5)	6 (5 – 0)	14 (10 – 19)	13.5 (9.5 – 2)	10 (1.8 – 13.5)	18.5 (7 – 24)	12 (9 – 22)
EDSS	NA	2 (1 – 2)	1 (0.5 – 1.25)	1 (1 – 1.5)	3 (1.6 – 5.5)	2.8 (2 – 3.9)	4 (2 – 6.5)	5.5 (3.5 – 6.5)
MS type								
RRMS	–	8	11	9	4	7	7	9
SPMS	–	1	–	–	2	1	5	3
PPMS	–	2	–	–	–	–	–	1
Booster type								
Spikevax	8	5	3	8	–	3	4	4
Comirnaty	16	6	8	1	6	5	8	9
COVID-19 infection between P1 and P6								
COVID-19 -	15	7	10	9	3	4	8	9
COVID-19 +	9	4	1	–	3	4	4	4
Relapses								
No relapses	NA	10	11	–	3	7	11	12
After two doses	NA	1	–	–	2	1	–	1
After three doses	NA	–	–	–	1	–	1	–
Time between last infusion of depletive agents and vaccination (months)	NA	NA	NA	NA	45 (40-45)	6.4 (6-10)	NA	4.4 (3-7.6)

Results are expressed as Median and Inter-quartile range (IQR). HD, healthy donors; NT, not treated; DMF, dimethyl fumarate; IFN, Interferon; ALEM, Alemtuzumab; CLAD, Cladribine; FING, Fingolimod; OCR, Ocrelizumab.
NA = not applicable.

An efficient neutralizing response is present in the majority of pwMS over-time and is increased by natural infection in treated patients

SARS-CoV-2 pseudovirions were produced according to a previously reported protocol published on Nature Protocols by Nie et al. (21), which is briefly described in the Material and methods section. A viral stock with a titer corresponding to $1.5x10^5$ $TCID_{50}$/ml was produced and used throughout the study. As reported by Figures 2A, B, a moderate to extensive cytopathic effect was observed in flasks transfected with pcDNA3.1.S2 plasmid and infected with the VSV-G pseudotyped virus after 48h or 72h, respectively. In order to verify the incorporation of SARS-CoV-2 spike glycoprotein on the VSV particles, pseudovirion production was characterized by means of western blot analysis. As reported in Figure 2C, the S protein was efficiently expressed on pseudovirion envelop: specific bands were detected in the lane of SARS-CoV-2 pseudovirions, whilst no specific band was found in the VSV-G pseudovirions (generated with the same procedure as SARS-CoV-2 pseudovirions) in the corresponding position. The monomer of the S protein (S1 + S2) was observed at a position of about 190 kDa and the S2 domain was detected at 90kDa. The SARS-CoV-2 pseudovirions, together with G*ΔG-VSV, were tested against a VSV-M specific Ab, showing a common band at 26kDa. Altogether, our results indicated that we generated a well-defined SARS-CoV-2 pseudovirion production suitable for the neutralization assays.

The serum samples collected at P1 and P6 were subsequently tested by means of the neutralization assay, in order to directly evaluate the Ab function and efficacy against SARS-CoV-2 pseudovirions. Focusing on sera collected at P1, we compared the neutralizing activity of samples from pwMS with that of HD (Figure 3A, grey dots). As reported, no statistical difference in the neutralizing activity was observed between HD (2.7 ± 0.4) and NT pwMS (2.5 ± 0.6) or pwMS under different therapies, with exception

10.3389/fimmu.2023.1205879

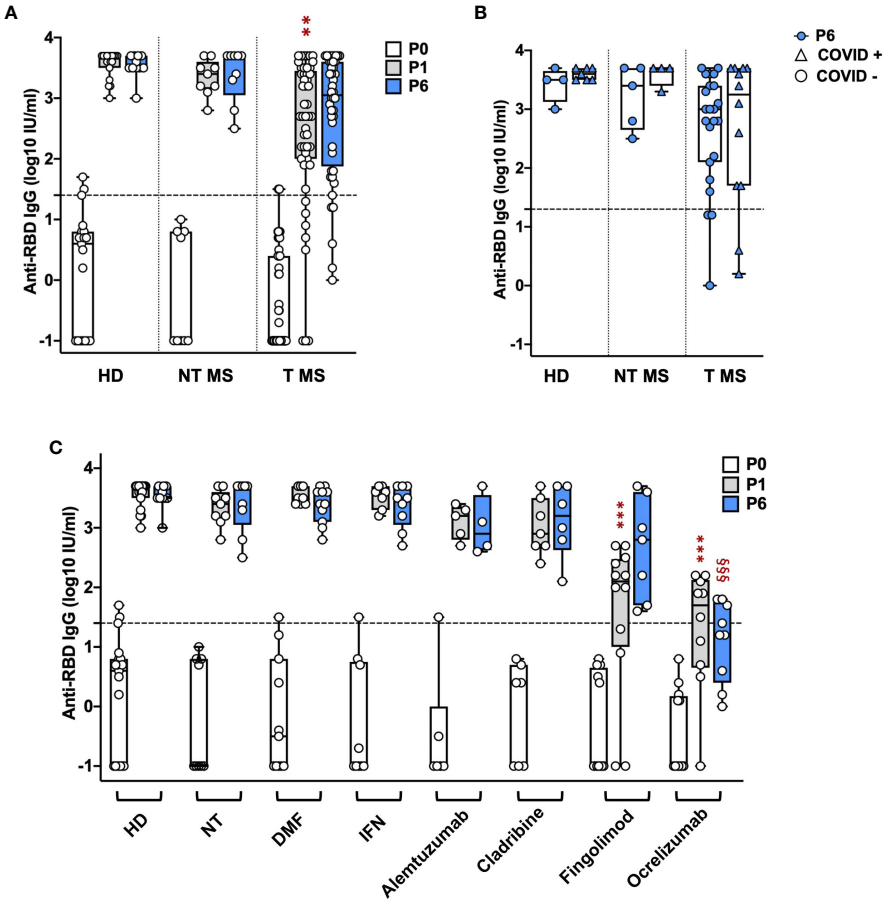


FIGURE 1
Kinetics of anti-RBD IgG levels in pwMS and HD. IgG titers have been compared between HD and untreated and treated pwMS **(A)**; then stratified by the occurrence of a natural infection after three doses of vaccine **(B)** or by specific therapy **(C)**. Anti-RBD IgGs have been quantified at three time points: immediately before vaccination (P0), one (P1) and six months (P6) after the third dose of vaccine. Dotted line corresponds to the cut-off threshold of 1.4 IU/ml (log10). **(A)** Statistic was performed by one-way ANOVA with Bonferroni correction for multiple comparisons. Asterisks correspond to p-value thresholds of one-way ANOVA with Bonferroni correction for multiple comparisons: * indicates HD at P1 vs. each other group at P1 (**p<0,002) **(B)** Subjects under interferon have been excluded from the analysis because no subjects under interferon experienced natural infection after vaccination. Statistic was assessed by the two-tailed unpaired t-test. **(C)** Statistic was performed by one-way ANOVA with Bonferroni correction for multiple comparisons. Different symbols have been used for different comparisons within each group: * indicates NT at P1 vs. each other group at P1 (***p<0.001); § indicates NT at P6 vs. each other group at P6(§§§p<0.001). HD, healthy donors; NT, not treated; T, treated, DMF, dimethyl fumarate; IFN, Interferon; COVID +, anti-N positive subjects; COVID -, anti-N negative subjects.

of pwMS under ocrelizumab. As expected, considering the mechanism of action of ocrelizumab and the previously reported low levels of anti-RBD Ab, a significant reduction of the neutralizing activity was observed in pwMS under anti-CD20 therapy (0.8 ± 0.4; p=0.0001) compared to NT pwMS (2.5 ± 0.6). In particular, despite a small production of anti-RBD Ab, the neutralizing activity of sera from ocrelizumab-treated MS patients was always under the limit of detection (i.e NT_{50} <1.3), indicating an absence of neutralization capacity.

Similar results were observed focusing on sera collected at P6 (Figure 3A, blue dots). No statistical difference in the neutralizing activity at P6 was observed between HD (3.0 ± 0.4) and untreated pwMS (3.2 ± 0.9) or pwMS under different therapies, with the exception of pwMS treated with ocrelizumab (0.5 ± 0.5; p< 0.001) showing a neutralizing activity always under the limit of detection. Additionally, the potential reduction of the neutralizing ability after several months from the three doses was evaluated. As reported in Figure 3A, we didn't observe significant differences comparing the neutralizing titers at P1 and P6 within each group, suggesting that, where present, the neutralization activity against SARS-CoV-2 is maintained over time. A difference in neutralizing efficacy is visible at P6 comparing T pwMS in which a natural SARS-CoV-2 infection occurred after vaccination (3 ± 0.6, p = 0.04) with uninfected T pwMS (2.4 ± 0.5) (Figure 3B), suggesting that natural infection may increase neutralizing response in these subjects. However, the same difference is not visible in HD and NT pwMS. Similarly to what was previously done for anti-RBD quantification, we excluded pwMS under interferon from this analysis because none of these subjects got COVID-19 after vaccination.

Overall, we observed a robust correlation between the previously reported anti-RBD Ab levels and the Ab neutralizing efficacy in HD (Pearson correlation; R=0.78, p= 6.9e-06) and in pwMS (Pearson correlation; R=0.85, p< 2.2e-16) (Figure 4).

10.3389/fimmu.2023.1205879

TABLE 2 Multivariable analysis assessing factors associated with anti-RBD levels at P1 and P6.

Multivariable analysis P1					Multivariable analysis P6				
Variable	Beta coef.	Robust SE	p		Variable	Beta coef.	Robust SE	p	
					Anti-RBD IgGs at P0	2.67E-03	0.01725	0.8779	
Anti-RBD IgGs at P0	-1.73E-04	6.55E-04	0.7928		**Anti-RBD IgGs at P1**	5.19E-04	1.89E-04	0.0099	***
Sex (Male vs. Female)	-0.03953	0.666	0.9529		**Sex (Male vs. Female)**	-0.6582	0.6412	0.3128	
Age (Years)	8.11E-03	0.02606	0.7571		**Age (Years)**	-0.04094	0.02651	0.133	
EDSS score	9.13E-03	0.1716	0.9578		**EDSS score**	0.2179	0.1619	0.1885	
MS disease duration (years)	0.01051	0.03697	0.7775		**MS disease duration (years)**	-4.62E-03	0.03245	0.8878	
MS type					MS type				
RRMS	Ref.				RRMS	Ref.			
PPMS	1.297	1.036	0.2177		PPMS	0.5475	0.9328	0.5616	
SPMS	-0.6854	0.8333	0.4154		SPMS	-1.163	0.9888	0.2486	
Booster type					Booster type				
Comirnaty	Ref.				Comirnaty	Ref.			
Spikevax	1.161	0.5154	0.0294	*	Spikevax	0.3137	0.4456	0.4869	
Therapy					Therapy				
Not treated	Ref.				Not treated	Ref.			
Dimethyl fumarate	0.7545	0.6146	0.2262		Dimethyl fumarate	-0.1137	0.537	0.8337	
Interferon	0.03858	0.6325	0.9516		Interferon	-0.2345	0.6195	0.7078	
Alemtuzumab	0.3403	0.8722	0.6984		Alemtuzumab	-0.6895	0.9647	0.4803	
Cladribine	-0.5561	0.6361	0.3869		Cladribine	0.0202	0.9358	0.9829	
Fingolimod	-3.637	0.9721	0.0005	***	Fingolimod	-0.09006	1.409	0.9494	
Ocrelizumab	-4.446	0.936	<0.00005	***	Ocrelizumab	-3.544	0.9935	0.0012	**

p-values indicate a statistically significant relationship with the response variable in the model. Asterisks correspond to significance thresholds (***p<0.001; **p <0.02; *p<0.03)."Not treated" was chosen as the reference class (Ref.) for therapy, "Pfizer" was chosen as reference class for booster type and "RRMS" was chosen as reference class for MS type. EDSS, Expanded Disability Status Scale; RRMS, Relapsing Remitting Multiple Sclerosis; PPMS, Primary Progressive Multiple Sclerosis; SPMS, Secondary progressive Multiple Sclerosis.

PwMS display a good spike-specific CD4+ and CD8+ T immune response that is increased by COVID-19 and is independent of DMTs

To determine the levels of S-specific T-cell activity, the number of CD4+ and CD8+ cells releasing IFNγ was assessed by cytofluorimetry after exposure of PBMCs to a 15-mer peptide pool covering the S protein of Wuhan wild-type SARS-CoV-2 (Supplementary Figure 1). One and six months after vaccination, all groups of pwMS showed a similar frequency of S-specific IFNγ producing- CD4+ and CD8+ T cells comparable to that of HD (Figures 5A, C). Notably, T pwMS in which occurred a natural COVID-19 infection after vaccination display a higher frequency of both CD4 (0.24% ± 0.15; p=0.016) and CD8+(0.19% ± 0.18; p= 0.04) S-specific T cells response compared to T pwMS who remains protected from the infection (0.09% ± 0.03 and 0.05% ± 0.02 respectively, Figures 5B, D). These results suggest that COVID-19 disease may increase the S-specific T cells repertoire, more than the vaccination alone.

Discussion

Here we report the results of our observational, monocentric, prospective cohort study on SARS-CoV-2 vaccinated pwMS and HD followed up to 6 months after the third dose, in terms of elicited humoral and T cell responses, with a special focus on the neutralizing activity of Abs. This cohort is extremely peculiar as only subjects receiving the three doses of anti-SARS-CoV-2 vaccine without prior or breakthrough SARS-CoV-2 infection were included, allowing us to characterize the specific immune response to a well-defined new antigen in pwMS and to address several unmet clinical questions on the immune response to eventual natural infections in pwMS under DMTs.

Regarding humoral immunity, our main result indicated that after three doses of anti-SARS-CoV-2 mRNA vaccine, pwMS develop a significant Ab response. This result is concordant with previous observations (8, 32–34), albeit obtained with a different methodology of Ab quantification as CLIA (32–34), or for a different target as recombinant S1 subunit (8) or trimeric S (34)

10.3389/fimmu.2023.1205879

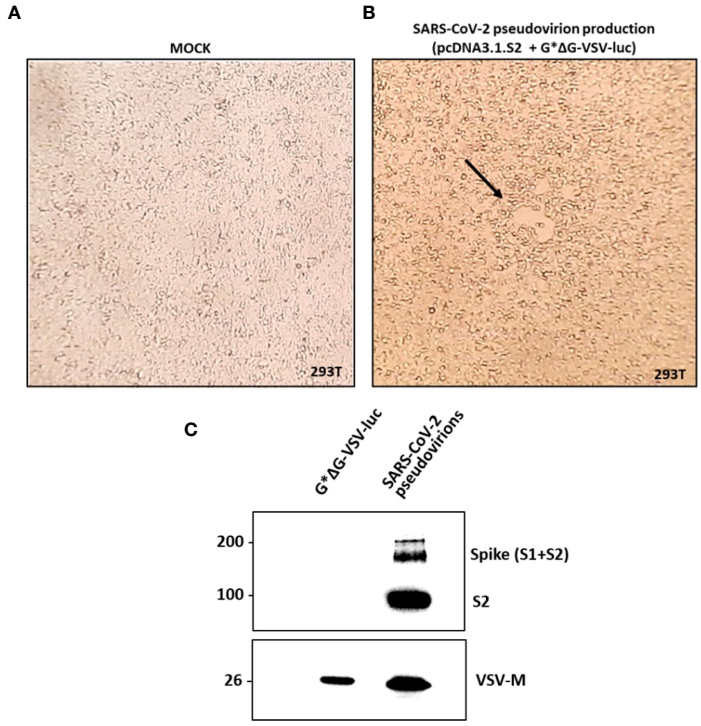


FIGURE 2
SARS-CoV-2 pseudovirion production and characterization. In **(A, B)** the SARS-CoV-2 pseudovirion production is reported. **(A)** Uninfected and untreated 293T cells (mock control). **(B)** 293T cells transfected with pcDNA3.1.S2 and infected with G*ΔG-VSV-luc observed under inverted microscope at 48h. The arrow highlights the observed syncytia. In **(C)** SARS-CoV-2 pseudovirion characterization by means of WB analysis is reported, showing the incorporation of the SARS-CoV-2 spike on the VSV virions.

instead of RBD region. In line with other studies (2, 13–15), we observed a weak anti-RBD IgG production still after the third dose in pwMS under ocrelizumab and fingolimod, even if the booster dose was able to induce seroconversion in several patients (2, 13–15).

A key observation was the maintenance after six-months of high anti-RBD IgG levels after three doses not only in our cohort of HD, similarly to what was observed in a study on healthcare workers (24), but also in the majority of pwMS. The increasing trend between P1 and P6 in the fingolimod group could be due to

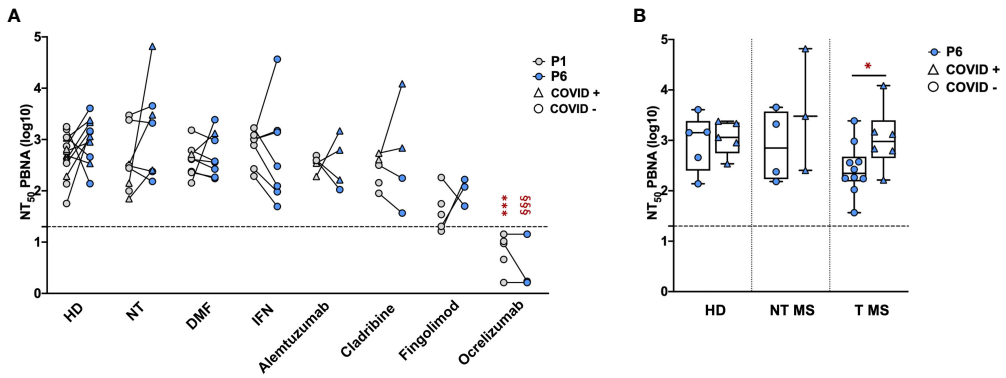


FIGURE 3
Neutralizing activity of serum samples from pwMS and HD. Neutralization titer has been compared between HD and pwMS stratified by therapy **(A)** or by the occurrence of a natural infection after three doses of vaccine **(B)**. Neutralization assay were performed at two time points: one (P1) and six months (P6) after the third vaccination dose. Dotted line corresponds to the cut-off threshold of 1.3 NT_{50} (log10). **(A)** Statistical significance was assessed by one-way ANOVA with Bonferroni correction for multiple comparisons. Different symbols were used for different comparisons within each group: * indicates NT at P1 vs. each other group at P1 (***p<0,001); § indicates NT at P6 vs. each other group at P6 (§§§ p<0.001). **(B)** Statistical significance was assessed by two-tailed unpaired t-test within each group (* p<0.03). Subjects under Interferon were excluded from the analysis because no subjects under interferon experienced natural infection after vaccination. All the results are presented as the mean values of two independent experiments. NT_{50} PBNA, neutralizing titer 50 calculated with pseudovirion based neutralization assay; HD, healthy donors; NT, not treated; T, treated; DMF, dimethyl fumarate; IFN, Interferon; COVID +, anti-N positive subjects; COVID -, anti-N negative subjects.

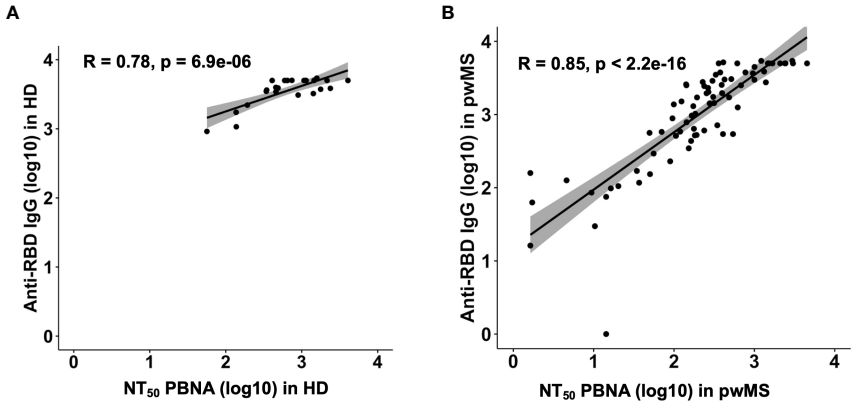


FIGURE 4
Correlation plots between anti-RDB IgG levels and neutralizing titer 50 in HD **(A)** and pwMS **(B)**. Plots have been generated using values from all groups. Pearson correlation coefficients were computed to assess the linear relationship between Anti-RDB IgG levels and NT_{50}.

intercurrent SARS-CoV-2 infection as the mean growth of Ab titers between P1 and P6 was ten folder higher in pwMS COVID-19+ compared to COVID-19-. PwMS under ocrelizumab showed the lowest levels of Ab at 6 months after the third dose compared to differently treated pwMS, suggesting that these subjects are more at risk of a breakthrough COVID-19 infection (6). Indeed, low neutralizing Ab levels are a relevant risk factor for breakthrough infections in pwMS, since neutralizing Ab prevent viral entry into the host cell (6). On the other hand, cellular immunity protects from severe disease (25). Our results on cellular immunity showed comparable levels of spike-specific IFNγ-producing CD4+ and CD8+ T cell among pwMS, confirming that antigen-specific T cell response seems to be preserved in pwMS under anti-CD20 treatment, as reported by previous studies (26, 27). We did not observe a reduced S-specific T cell response in pwMS under fingolimod, as reported by other studies (15, 28, 35), but this is

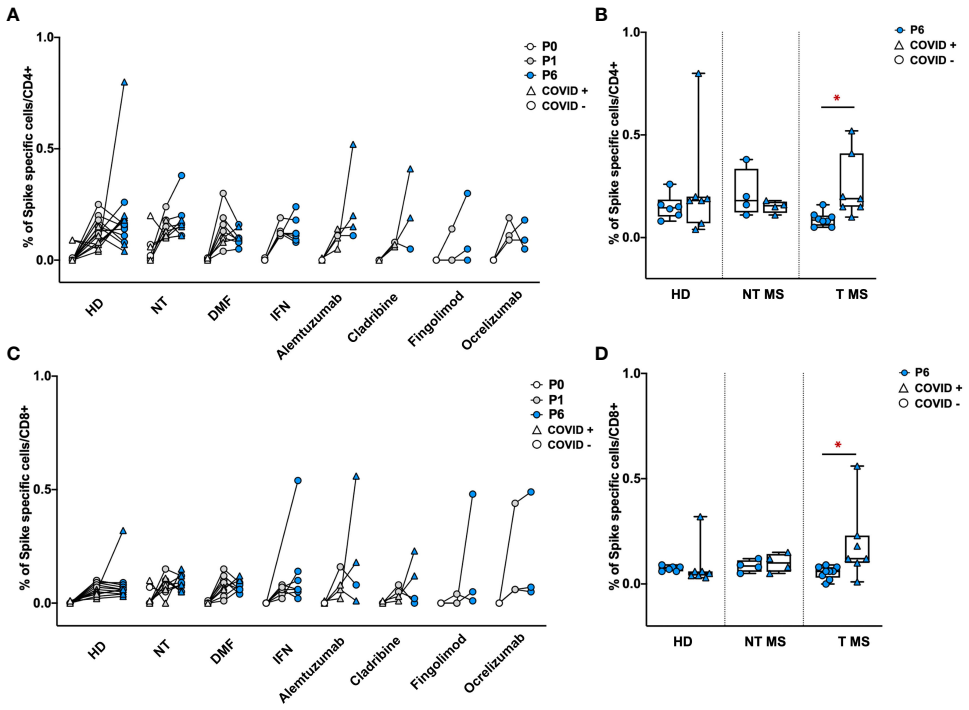


FIGURE 5
Frequency of Spike specific CD4+ and CD8+ T cells in PBMCs of pwMS and HD. Percentage of Spike specific CD4+ and CD8+ T cells has been obtained by *in-vitro* restimulation of PBMCs with Spike peptides, followed by intracellular staining for IFNγ. The percentage of Spike specific CD4+ **(A, B)** or CD8+ T **(C, D)** cells was obtained by subtracting values of unstimulated cells. Obtained percentage were compared between HD and pwMS stratified by therapy **(A, C)** or by the occurrence of a natural infection after three doses of vaccine **(B, D)**. Statistics were assessed by two-tailed unpaired t-test (*p<0.03). HD, healthy donors; NT, not treated; T, treated; DMF, dimethyl fumarate; IFN, Interferon; COVID +, anti-N positive subjects; COVID -, anti-N negative subjects.

not surprising as other studies on influenza vaccination showed that pwMS under fingolimod are able to elicit a T cell response similar to HD (36).

With the aim of investigating deeply the humoral response, we evaluated not only the anti-RBD IgG levels, which are considered a good representation of the Ab neutralizing activity (16, 17), but also the neutralizing Ab (nAb) function and efficacy by means of a pseudovirion-based neutralization assay. The use in a MS context of a novel, sensitive and high-throughput pseudovirion-based assay, which allows the direct evaluation of the nAb function and that directly correlates with a live virus neutralization assay, is one of the strengths of our work (21, 37). So far, a small number of studies have investigated the Ab neutralizing activity through this method (23), whereas the majority of studies employed the analysis of the anti-RBD IgGs levels as surrogate of the direct evaluation of the Ab neutralizing activity (8, 9, 12). Herein, we found a good correlation between the neutralizing activity and the anti-RBD levels both in HD (as expected) (17) and in pwMS group, showing R-values of 0.78 and 0.85 respectively. The results obtained with the evaluation of the anti-RBD IgG levels were confirmed with the pseudovirion-based assay: no statistical difference in the neutralizing activity was observed between HD and pwMS under all the considered therapies, with the exception of ocrelizumab, and the protective capacity was maintained over time (six-month observation). Nevertheless, the direct analysis of Ab neutralization allowed us to highlight novel aspects of the vaccination response in a MS context. Differently from what we observed from the analysis of the anti-RBD levels, we did not observe a statistically reduced neutralizing response in fingolimod-treated patients, thus indicating that despite a reduced number of Ab, a partial ability in neutralizing the virus is maintained. Consistently with our findings, Gyang et al., through a neutralization assay based on SARS-CoV-2 pseudotyped lentivirus, demonstrated that pwMS under B-cell depleting therapies (rituximab and ocrelizumab) have a reduced neutralizing response compared to other pwMS, which correlated with the time from the last anti-CD20 infusion. Additionally, the authors showed that prior COVID-19 illness, DMT category, and pyramidal function were significant predictors of vaccine responsiveness, and that circulating absolute lymphocyte count (ALC) and IgG levels correlated with neutralizing Ab levels (23).

We additionally investigated how the occurrence of SARS-CoV-2 natural infection after the third vaccination dose affected the immune response in pwMS and HD. We found no differences in anti-RBD IgG amount between P1 and P6 suggesting that anti-RBD IgG might not be significantly increased by a natural infection. Despite this result, natural infection acquired between P1 and P6 determined an increased neutralizing activity in MS-treated group. A possible explanation of this finding could be that, beside the Ab targeting the RDB domain, other Ab with a different specificity can contribute to the overall neutralizing activity. Indeed, anti-N-terminal domain (NTD) and anti-C terminal domain of S1 subunit were found to be nAbs in convalescent and vaccinated patients respectively, even if less prevalent than those targeting RBD (38–40). Along with neutralization, also S-specific CD4+ and CD8+ T cell response is increased in MS-treated group, suggesting that COVID-19 infection may increase both humoral and cellular immune response in these subjects. This phenomenon could relay first to the mechanism of actions of DMTs: the majority of patients showing this peculiar pattern were under the immunoreconstitution therapies alemtuzumab and cladribine, in which both T and B cells were depleted and then reconstitute toward a less inflammatory phenotype. Furthermore, the increase in IFNγ production can contribute to Ab affinity maturation, therefore augmenting Abs neutralizing efficacy (39).

Notably none of the pwMS under treatment with interferons experienced natural COVID-19 infection. Indeed, IFN-β administration has been related to a reduced viral load and a faster clearance of the mucosa, reducing the risk of severe disease (41–44).

The current study has several strengths. First, the usage of a pseudovirion-based neutralization assay to determine the real activity of elicited Ab. Secondly, the design of a prospective study allowed us to get a complete and detailed evaluation of humoral and T cell responses over time (up to 6 months after the third vaccine dose), in relation to specific DMTs taking into account the effects of likely confounding factors such as breakthrough infections.

A limitation of our work could be the size of each group resulting from the stratification of patients by therapy; however, as a monocentric longitudinal study, this cohort well represents the general MS population and the distribution of therapies used in clinical practice. Furthermore, we did not include analysis of B cell activation and phenotype; however, S-specific B-cell response was investigated in previous studies (45) showing reduced levels of B cell activity in pwMS under S1P modulators and anti-CD20 that is also influenced by post-vaccine anti-CD20 infusions.

Altogether, our observations combined with recent literature on the topic (2, 6, 8, 13–15, 26, 27, 32–35, 45) highlight the vaccine response data to current protocols applied in pwMS. The majority of pwMS under DMTs develop an efficient and long-term immune response comparable to HD. Collectively, fingolimod and ocrelizumab therapies show the lowest levels of protective immunity, underlying the necessity to carefully follow-up these subjects for the risk of a breakthrough SARS-CoV-2 infection and to have up-to-date vaccination coverage before starting these DMTs. Finally, we underline the necessity to rapidly generate a test combining Ab titers and neutralizing activity to determine which is the threshold required for protection to infection and/or severe COVID-19 disease.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics committee of AOU San Luigi Gonzaga, Orbassano (TO), Italy (Ref. number #117-2021). The patients/

participants provided their written informed consent to participate in this study.

Author contributions

AM and RF: experimental design, interpreted the data; drafted the manuscript for intellectual content; IA and RR: major role in the acquisition and analysis of data; MM and MC: patient enrolment and follow-up: SR, MC and DL: designed and conceptualized the study; revised the manuscript for intellectual content. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the University of Turin (grant LEMD_RILO_20_01 to DL; grant CLEM_RILO_21_01 to MC), by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT, to DL) and by donations of Silvana Legnani to DL and Merck Serono to MC. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We acknowledge all the subjects enrolled in this work for their precious contribution and Raffaella Meroni for her support in the organization of subject follow-up.

Conflict of interest

MM received personal compensations for advisory boards and travel grants from Novartis, Sanofi-Genzyme; SR received personal compensations for public speaking and travel grants from Sanofi-Genzyme and Merck Serono; MC received personal compensations for advisory boards, public speaking, editorial commitments or travel grants from Biogen Idec, Merck Serono, Fondazione Serono, Novartis, Pomona, Sanofi-Genzyme and Teva.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1205879/full#supplementary-material

References

1. Wu Q, Dudley MZ, Chen X, Bai X, Dong K, Zhuang T, et al. Evaluation of the safety profile of COVID-19 vaccines: a rapid review. *BMC Med* (2021) 19:173. doi: 10.1186/s12916-021-02059-5

2. Dreyer-Alster S, Menascu S, Mandel M, Shirbint E, Magalashvili D, Dolev M, et al. COVID-19 vaccination in patients with multiple sclerosis: safety and humoral efficacy of the third booster dose. *J Neurol Sci* (2022) 434:120155. doi: 10.1016/j.jns.2022.120155

3. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. *N Engl J Med* (2020) 383:2603–15. doi: 10.1056/NEJMoa2034577

4. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* (2021) 384:403–16. doi: 10.1056/NEJMoa2035389

5. Sormani MP, De Rossi N, Schiavetti I, Carmisciano L, Cordioli C, Moiola L, et al. Disease-modifying therapies and coronavirus disease 2019 severity in multiple sclerosis. *Ann Neurol* (2021) 89:780–9. doi: 10.1002/ana.26028

6. Sormani MP, Schiavetti I, Inglese M, Carmisciano L, Laroni A, Lapucci C, et al. Breakthrough SARS-CoV-2 infections after COVID-19 mRNA vaccination in MS patients on disease modifying therapies during the delta and the omicron waves in Italy. *EBioMedicine* (2022) 80:104042. doi: 10.1016/j.ebiom.2022.104042

7. Madelon N, Heikkilä N, Sabater Royo I, Fontannaz P, Breville G, Lauper K, et al. Omicron-specific cytotoxic T-cell responses after a third dose of mRNA COVID-19 vaccine among patients with multiple sclerosis treated with ocrelizumab. *JAMA Neurol* (2022) 79:399–404. doi: 10.1001/jamaneurol.2022.0245

8. Achiron A, Mandel M, Dreyer-Alster S, Harari G, Magalashvili D, Sonis P, et al. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. *Ther Adv Neurol Disord* (2021) 14:17562864211012836. doi: 10.1177/17562864211012835

9. Achiron A, Mandel M, Dreyer-Alster S, Harari G, Dolev M, Menascu S, et al. Humoral immune response in multiple sclerosis patients following PfizerBNT162b2 COVID19 vaccination: up to 6 months cross-sectional study. *J Neuroimmunol* (2021) 361:577746. doi: 10.1016/j.jneuroim.2021.577746

10. Bigaut K, Kremer L, Fabacher T, Lanotte L, Fleury M-C, Collongues N, et al. Impact of disease-modifying treatments of multiple sclerosis on anti–SARS-CoV-2 antibodies: an observational study. *Neurol - Neuroimmunol Neuroinflamm* (2021) 8(5): e1055. doi: 10.1212/NXI.0000000000001055

11. Drulovic J, Ivanovic J, Martinovic V, Tamas O, Veselinovic N, Cujic D, et al. Humoral response to SARS-CoV-2 COVID-19 vaccines in patients with multiple sclerosis treated with immune reconstitution therapies. *Multiple Sclerosis Related Disord* (2021) 54:103150. doi: 10.1016/j.msard.2021.103150

12. Sormani MP, Schiavetti I, Carmisciano L, Sormani MP, Inglese M, Laroni A, et al. Effect of SARS-CoV-2 mRNA vaccination in MS patients treated with disease modifying therapies. *EBioMedicine* (2021) 72:103581. doi: 10.1016/j.ebiom.2021.103581

13. Capuano R, Altieri M, Conte M, Bisecco A, d'Ambrosio A, Donnarumma G, et al. Humoral response and safety of the third booster dose of BNT162b2 mRNA COVID-19 vaccine in patients with multiple sclerosis treated with ocrelizumab or fingolimod. *J Neurol* (2022) 269:6185–92. doi: 10.1007/s00415-022-11296-4

14. Maglione A, Morra M, Meroni R, Matta M, Clerico M, Rolla S. Humoral response after the booster dose of anti-SARS-CoV-2 vaccine in multiple sclerosis patients treated with high-efficacy therapies. *Multiple Sclerosis Related Disord* (2022) 61:103776. doi: 10.1016/j.msard.2022.103776

15. Tallantyre EC, Scurr MJ, Vickaryous N, Richards A, Anderson V, Baker D, et al. Response to COVID-19 booster vaccinations in seronegative people with multiple sclerosis. *Multiple Sclerosis Related Disord* (2022) 64:103937. doi: 10.1016/j.msard.2022.103937

16. Kohmer N, Westhaus S, Rühl C, Ciesek S, Rabenau HF. Brief clinical evaluation of six high-throughput SARS-CoV-2 IgG Ab assays. *J Clin Virol* (2020) 129:104480. doi: 10.1016/j.jcv.2020.104480

17. Müller L, Ostermann PN, Walker A, Wienemann T, Mertens A, Adams O, et al. Sensitivity of anti-SARS-CoV-2 serological assays in a high-prevalence setting. *Eur J Clin Microbiol Infect Dis* (2021) 40:1063–71. doi: 10.1007/s10096-021-04169-7

18. Suryadevara N, Shrihari S, Gilchuk P, VanBlargan LA, Binshtein E, Zost SJ, et al. Neutralizing and protective human monoclonal antibodies recognizing the n-terminal domain of the SARS-CoV-2 spike protein. *Cell* (2021) 184:2316–2331.e15. doi: 10.1016/j.cell.2021.03.029

19. Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* (2020) 584:450–6. doi: 10.1038/s41586-020-2571-7

20. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, et al. A neutralizing human Ab binds to the n-terminal domain of the spike protein of SARS-CoV-2. *Science* (2020) 369:650–5. doi: 10.1126/science.abc6952

21. Nie J, Li Q, Wu J, Zhao C, Hao H, Liu H, et al. Quantification of SARS-CoV-2 neutralizing Ab by a pseudotyped virus-based assay. *Nat Protoc* (2020) 15:3699–715. doi: 10.1038/s41596-020-0394-5

22. Chmielewska AM, Czarnota A, Bieńkowska-Szewczyk K, Grzyb K. Immune response against SARS-CoV-2 variants: the role of neutralization assays. *NPJ Vaccines* (2021) 6:142. doi: 10.1038/s41541-021-00404-6

23. Gyang TV, Evans JP, Miller JS, Alcorn K, Peng J, Bell EH, et al. Neutralizing Ab responses against SARS-CoV-2 in vaccinated people with multiple sclerosis. *Mult Scler J Exp Transl Clin* (2022) 8:20552173221087356. doi: 10.1177/20552173221087357

24. Gilboa M, Regev-Yochay G, Mandelboim M, Indenbaum V, Asraf K, Fluss R, et al. Durability of immune response after COVID-19 booster vaccination and association with COVID-19 omicron infection. *JAMA Net Open* (2022) 5:e2231778. doi: 10.1001/jamanetworkopen.2022.31778

25. Wherry EJ, Barouch DH. T Cell immunity to COVID-19 vaccines. *Science* (2022) 377:821–2. doi: 10.1126/science.add2897

26. Brill L, Rechtman A, Zveik O, Haham N, Oiknine-Djian E, Wolf DG, et al. Humoral and T-cell response to SARS-CoV-2 vaccination in patients with multiple sclerosis treated with ocrelizumab. *JAMA Neurol* (2021) 78:1510–4. doi: 10.1001/jamaneurol.2021.3599

27. Apostolidis SA, Kakara M, Painter MM, Goel RR, Mathew D, Lenzi K, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat Med* (2021) 27:1990–2001. doi: 10.1038/s41591-021-01507-2

28. Tortorella C, Aiello A, Gasperini C, Agrati C, Castilletti C, Ruggieri S, et al. Humoral- and T-Cell-Specific immune responses to SARS-CoV-2 mRNA vaccination in patients with MS using different disease-modifying therapies. *Neurology* (2022) 98:e541–54. doi: 10.1212/WNL.0000000000013108

29. Mattiuzzo G, Bentley EM, Hassall M, Routley S, Bernasconi V, Kristiansen P, et al. *Establishment of the WHO international standard and reference panel for anti-SARS-CoV-2 Ab*. (2020). (Geneva:World Health Organization).

30. Whitt MA. Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J Virol Methods* (2010) 169:365–74. doi: 10.1016/j.jviromet.2010.08.006

31. Almahboub SA, Algaissi A, Alfaleh MA, ElAssouli M-Z, Hashem AM. Evaluation of neutralizing antibodies against highly pathogenic coronaviruses: a detailed protocol for a rapid evaluation of neutralizing antibodies using vesicular stomatitis virus pseudovirus-based assay. *Front Microbiol* (2020) 11. doi: 10.3389/fmicb.2020.02020

32. Milo R, Staun-Ram E, Karussis D, Karni A, Hellmann MA, Bar-Haim E, et al. Humoral and cellular immune responses to SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis: an Israeli multi-center experience following 3 vaccine doses. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.868915

33. Sabatino JJ, Mittl K, Rowles WM, McPolin K, Rajan JV, Laurie MT, et al. Multiple sclerosis therapies differentially affect SARS-CoV-2 vaccine–induced Ab and T cell immunity and function. *JCI Insight* (2022) 7(4):e156978. doi: 10.1172/jci.insight.156978

34. Dominelli F, Zingaropoli MA, Tartaglia M, Tortellini E, Guardiani M, Perri V, et al. Multiple sclerosis-disease modifying therapies affect humoral and T-cell response to mRNA COVID-19 vaccine. *Front Immunol* (2022) 13:1050183. doi: 10.3389/fimmu.2022.1050183

35. Palomares Cabeza V, Kummer LYL, Wieske L, Hagen RR, Duurland M, Konijn VAL, et al. Longitudinal T-cell responses after a third SARS-CoV-2 vaccination in patients with multiple sclerosis on ocrelizumab or fingolimod. *Neurol Neuroimmunol Neuroinflamm* (2022) 9:e1178. doi: 10.1212/NXI.0000000000001178

36. Mehling M, Hilbert P, Fritz S, Durovic B, Eichin D, Gasser O, et al. Antigen-specific adaptive immune responses in fingolimod-treated multiple sclerosis patients. *Ann Neurol* (2011) 69:408–13. doi: 10.1002/ana.22352

37. Xiong H-L, Wu Y-T, Cao J-L, Yang R, Liu Y-X, Ma J, et al. Robust neutralization assay based on SARS-CoV-2 s-protein-bearing vesicular stomatitis virus (VSV) pseudovirus and ACE2-overexpressing BHK21 cells. *Emerg Microbes Infect* (2020) 9:2105–13. doi: 10.1080/22221751.2020.1815589

38. Brouwer PJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* (2020) 369:643–50. doi: 10.1126/science.abc5902

39. Phakaratsakul S, Manopwisedjaroen S, Boonarkart C, Kupatawintu P, Chaiwanichsiri D, Roytrakul T, et al. Dynamics of neutralizing antibodies and binding antibodies to domains of SARS-CoV-2 spike protein in COVID-19 survivors. *Viral Immunol* (2022) 35:545–52. doi: 10.1089/vim.2022.0059

40. Garrett ME, Galloway JG, Wolf C, Logue JK, Franko N, Chu HY, et al. Comprehensive characterization of the Ab responses to SARS-CoV-2 spike protein finds additional vaccine-induced epitopes beyond those for mild infection. *Elife* (2022) 11:e73490. doi: 10.7554/eLife.73490

41. Jhuti D, Rawat A, Guo CM, Wilson LA, Mills EJ, Forrest JI. Interferon treatments for SARS-CoV-2: challenges and opportunities. *Infect Dis Ther* (2022) 11:953–72. doi: 10.1007/s40121-022-00633-9

42. Chen L-F, Yang C-D, Cheng X-B. Anti-interferon autoantibodies in adult-onset immunodeficiency syndrome and severe COVID-19 infection. *Front Immunol* (2021) 12:788368. doi: 10.3389/fimmu.2021.788368

43. Eskandarian Boroujeni M, Sekrecka A, Antonczyk A, Hassani S, Sekrecki M, Nowicka H, et al. Dysregulated interferon response and immune hyperactivation in severe COVID-19: targeting STATs as a novel therapeutic strategy. *Front Immunol* (2022) 13. doi: 10.3389/fimmu.2022.888897

44. Galbraith MD, Kinning KT, Sullivan KD, Araya P, Smith KP, Granrath RE, et al. Specialized interferon action in COVID-19. *Proc Natl Acad Sci U.S.A.* (2022) 119:e2116730119. doi: 10.1073/pnas.2116730119

45. Disanto G, Galante A, Cantu' M, Sacco R, Mele F, Eisler JJ, et al. Longitudinal postvaccine SARS-CoV-2 immunoglobulin G titers, memory b-cell responses, and risk of COVID-19 in multiple sclerosis over 1 year. *Neurol Neuroimmunol Neuroinflamm* (2023) 10:e200043. doi: 10.1212/NXI.0000000000200043

frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 28 June 2023
DOI 10.3389/fnins.2023.1198219

OPEN ACCESS

EDITED BY
Sonia Zuñiga,
Spanish National Research Council (CSIC), Spain

REVIEWED BY
Kun Leng,
University of California, San Francisco, United States
Helena Therese Hogberg,
National Institute of Environmental Health Sciences (NIH), United States

*CORRESPONDENCE
Osama Al-Dalahmah
✉ oa2298@cumc.columbia.edu

†These authors have contributed equally to this work

RECEIVED 31 March 2023
ACCEPTED 05 June 2023
PUBLISHED 28 June 2023

CITATION
Madden N, Mei YZJ, Jakubiak K, Li J, Hargus G, Goldman JE and Al-Dalahmah O (2023) The link between SARS-CoV-2 related microglial reactivity and astrocyte pathology in the inferior olivary nucleus.
Front. Neurosci. 17:1198219.
doi: 10.3389/fnins.2023.1198219

The link between SARS-CoV-2 related microglial reactivity and astrocyte pathology in the inferior olivary nucleus

Nacoya Madden†, Ying Zi Jessy Mei†, Kelly Jakubiak†, Juncheng Li, Gunnar Hargus, James E. Goldman and Osama Al-Dalahmah*

Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York Presbyterian Hospital, New York, NY, United States

The pathological involvement of the central nervous system in SARS-CoV2 (COVID-19) patients is established. The burden of pathology is most pronounced in the brain stem including the medulla oblongata. Hypoxic/ischemic damage is the most frequent neuropathologic abnormality. Other neuropathologic features include neuronophagia, microglial nodules, and hallmarks of neurodegenerative diseases: astrogliosis and microglial reactivity. It is still unknown if these pathologies are secondary to hypoxia versus a combination of inflammatory response combined with hypoxia. It is also unknown how astrocytes react to neuroinflammation in COVID-19, especially considering evidence supporting the neurotoxicity of certain astrocytic phenotypes. This study aims to define the link between astrocytic and microglial pathology in COVID-19 victims in the inferior olivary nucleus, which is one of the most severely affected brain regions in COVID-19, and establish whether COVID-19 pathology is driven by hypoxic damage. Here, we conducted neuropathologic assessments and multiplex-immunofluorescence studies on the medulla oblongata of 18 COVID-19, 10 pre-pandemic patients who died of acute respiratory distress syndrome (ARDS), and 7–8 control patients with no ARDS or COVID-19. The comparison of ARDS and COVID-19 allows us to identify whether the pathology in COVID-19 can be explained by hypoxia alone, which is common to both conditions. Our results showed increased olivary astrogliosis in ARDS and COVID-19. However, microglial density and microglial reactivity were increased only in COVID-19, in a region-specific manner. Also, olivary hilar astrocytes increased YKL-40 (CHI3L1) in COVID-19, but to a lesser extent than ARDS astrocytes. COVID-19 astrocytes also showed lower levels of Aquaporin-4 (AQP4), and Metallothionein-3 in subsets of COVID-19 brain regions. Cluster analysis on immunohistochemical attributes of astrocytes and microglia identified ARDS and COVID-19 clusters with correlations to clinical history and disease course. Our results indicate that olivary glial pathology and neuroinflammation in the COVID-19 cannot be explained solely by hypoxia and suggest that failure of astrocytes to upregulate the anti-inflammatory YKL-40 may contribute to the neuroinflammation. Notwithstanding the limitations of retrospective studies in establishing causality, our experimental design cannot adequately control for factors external to our design. Perturbative studies are needed to confirm the role of the above-described astrocytic phenotypes in neuroinflammation.

KEYWORDS

astrocyte, microglia, inferior olivary nucleus, COVID-19, hypoxia

Introduction

COVID-19, an infection caused by the coronavirus SARS-CoV-2, can lead to an acute severe respiratory syndrome that has caused millions of deaths in recent years Patients with COVID-19 exhibit respiratory symptoms severe enough to cause acute respiratory distress syndrome (ARDS) requiring hospitalization and usually mechanical ventilation (Aiyegbusi et al., 2021; Swenson and Swenson, 2021). The pathologic counterpart of ARDS is known as diffuse alveolar damage (DAD) (Konopka et al., 2020), a condition that leads to alveolar damage and failure of gas exchange, culminating in hypoxemia (Swenson and Swenson, 2021). The brain is particularly vulnerable to hypoxemia and COVID-19 patients are known to exhibit acute and chronic neurologic symptoms and sequelae (Mao et al., 2020; von Weyhern et al., 2020). We and several other groups have conducted neuropathologic studies to determine the neuropathologic features of COVID-19 in the brain (al-Dalahmah et al., 2020a; Deigendesch et al., 2020; Matschke et al., 2020; Solomon et al., 2020; Colombo et al., 2021; Fabbri et al., 2021; Thakur et al., 2021; Wierzba-Bobrowicz et al., 2021; Agrawal et al., 2022). The main neuropathologic findings across multiple datasets point to ischemia, hemorrhage, astrogliosis and microgliosis as the primary neuropathologic insults, with very little evidence to support direct invasion of the brain by the virus (Maiese et al., 2021). Given that these neuropathologic insults are non-specific, and can be seen in, and therefore explained by, brain hypoxia, we designed this study to directly address this question: Can the neuropathologic findings in COVID-19 be explained by hypoxic injury alone?

Astrogliosis and microgliosis are salient to neurodegeneration and neuroinflammation (Kwon and Koh, 2020; Muzio et al., 2021; Vandenbark et al., 2021). In COVID-19 brains, astrogliosis and microgliosis are common (Maiese et al., 2021). Astrogliosis is characterized by morphologic and functional alterations secondary to pathologic tissue damage and can lead to a combination of changes in homeostatic, neuroprotective, and/or neurotoxic functions (Escartin et al., 2021). Usually, reactive astrocytes exhibit increased GFAP levels associated with hypertrophy and/or proliferation (Sofroniew and Vinters, 2010). Likewise, microgliosis or microglial reactivity is associated with morphologic and functional alterations secondary to pathologic insults and tissue damage. This is usually associated with morphologic changes including loss of the ramified appearance and retraction of cell processes, and increased expression of activation molecules like MHCII proteins and CD68 (Woodburn et al., 2021). Astrogliosis and microgliosis may be secondary to tissue damage, but can also adopt central roles in neurodegeneration. For instance, mutations that impair microglial function such as those involving TREM2 are associated with increased risk of Alzheimer's disease (Hansen et al., 2018). Importantly, the cross-talk between microglia and astrocytes is an actively researched topic in glial biology and neurodegeneration (Matejuk and Ransohoff, 2020). Microglia can drive astrogliosis (Liddelow et al., 2017), and astrocytes can regulate microglial reactivity (Cekanaviciute and Buckwalter, 2016; Chhatbar et al., 2018).

We are interested in the phenotypes of astrocytes in COVID-19 brains. We chose to study reactive astrocytes by performing detailed immunohistochemical analyses of astrocyte protein expression in the inferior olivary nucleus (ION), which is one of the most commonly and severely affected regions in COVID-19 brains (al-Dalahmah et al., 2020a; Thakur et al., 2021). The inferior olivary nuclei are located bilaterally within the rostral part of the medulla oblongata and participate in motor learning and coordination. ION neurons project via the hilum to contralateral cerebellar Purkinje cells (Schweighofer et al., 2013). In this study, we used post-mortem human tissue from control subjects who died with no neuropathologic abnormalities ($n = 7–8$), patients who died with ARDS before the COVID-19 pandemic ($n = 10$), and subjects who died from COVID-19 ($n = 19$). We performed immunohistochemistry and multiplex immunofluorescence studies for markers and microglia. We first established that ARDS and COVID-19 patients exhibited increased astrogliosis compared to controls. Because both ARDS and COVID- 19 patients had similar clinical courses, with profound hypoxia, in most cases requiring intubation, and the main difference between the two groups is the presence or absence of COVID-19 infection, we focused on these groups to investigate microglial reactivity and astrocyte protein expression. The ventral, lateral and dorsal regions of the ION along with the hilum were analyzed to examine the differences in microglial reactivity and astrocyte protein expression between ARDS and COVID-19 patients. We further employed principal component analysis and clustering methods to correlate astrocyte protein expression to the clinicopathologic attributes of the patients. Our findings represent one of the first attempts to address the question of whether neuropathology in COVID-19 is due to hypoxia alone vs. other factors, and link astrocyte protein expression to an exaggerated microglial response in the ION.

Materials and methods

Human brain samples

This study is in compliance with the Declaration of Helsinki. Consent for autopsy was obtained from the patient's next of kin through standardized consenting procedures. No IRB approval was required given that the autopsy material used herein is considered to be non-human subjects. Pre-COVID autopsy material was obtained from donors who died between January 2018–2019, or during 2020–2021 and were negative for COVID-19. COVID-19 cases are previously thoroughly described (al-Dalahmah et al., 2020a; Thakur et al., 2021). Only the medulla oblongata tissue was analyzed in this study. The demographic information and relevant information regarding hospital course, including whether patients had histologic evidence of Diffuse Alveolar Damage (DAD) in the case when a full autopsy had been conducted, are provided in Supplementary Table S1. For some of our control cases, the clinical history is not available. This is because these were brain-only autopsies of patients who died elsewhere (not in our hospital). For these cases, we ensured that no hypoxic changes were neuropathologically detected (i.e., red neurons, nuclear pyknosis, and neuronal shrinkage) so as to use them as non-hypoxic controls. Autopsy brains, fixed in 10% formalin for 10–14 days after removal, were sectioned coronally and samples from representative areas of the CNS were removed and embedded in paraffin blocks, cut at 7 μm thickness, and mounted on charged glass slides. All studies reported herein are from the medulla at the level of the inferior olivary nuclei and hypoglossal nuclei.

Immunohistochemistry

All immunostains were conducted on a Leica© Bond RXm automated stainer. For chromogenic 3,3′-Diaminobenzidine (DAB) stains, a generic immunohistochemistry protocol was employed as per manufacturer protocols. For multiplexing immunostains using antibodies raised in non-overlapping hosts, we used a generic immunofluorescence protocol. Briefly, slides were baked in a 65°C oven for a minimum of 2 h. The following protocol was then used: After a dewaxing step, incubation in BOND Epitope Retrieval Solution 2 (cat# AR9640) for 20 min was used for heat-induced epitope retrieval. Next, the slides were washed in 1X PBS before washing twice in Bond Wash Solution (Ref#AR9590)—10 min/wash. Next, they were incubated in a 10% donkey serum blocking buffer for 60 min followed by the primary antibody diluted in blocking buffer for 60 min. After three washes, the slides were incubated in the secondary antibody containing buffer for 60 min. After three washes, A DAPI containing mounting solution (Everbright TrueBlack Hardset Mounting Medium with DAPI Cat#23018) was used to label nuclei and quench autofluorescence prior to coverslipping. One hundred fifty microliters/slide was the volume we used for all steps. All steps were conducted at ambient temperature—excluding the antigen retrieval step.

For multiplexing immunostains using primary antibodies raised in overlapping hosts (ALDH1L1, MT3 and AQP4 and ALDH1L1, YKL-40 and C3), the Opal 4-color Automation IHC kit Ref#220126024 from Akoya© Biosciences was used in accordance with the manufacturer protocol. Briefly, two wash steps were followed by incubation in PKI Blocking buffer for 5 min before incubation in the first primary antibody for 30 min. After 3 wash steps, the slides were incubated in Opal Polymer HRP for 10 min followed by 6 wash steps prior to incubation in Opal 520 reagent for 10 min. This was followed by 4 additional wash steps. Next, the slides were incubated in Bond ER 1 solution for 20 min at 95° to elute the antibody complexes before 3 more wash steps. This procedure was repeated twice, once with the second primary antibody and Opal 570 reagent and once with the third primary antibody and Opal 690 reagent. Following the 3 wash steps at the end of the third round, the slides were incubated with Spectral DAPI for 5 min before the final 3 wash steps.

The following primary antibodies and dilutions were used: Rabbit ALDH1L1 (1:100, EnCor, Cat#RPCA-ALDH1L1), Rabbit YKL-40 (1:250, Abcam, Cat#ab255297), Rabbit C3 (1:200, Abcam, Cat#ab200999), Chicken GFAP (1:1000, Abcam, Cat#4674), Goat Clusterin (1:200, Thermo fisher, PA5-46931), Rabbit CD44 (1:100, Abcam, Cat#ab101531), Rabbit MT3 (1:100, millipore, Cat#HPA004011), Rabbit, AQP4 (1:2000, Millipore, Cat# ABN910), Goat IBA1 (1,500, Abcam, Cat#ab5076), Rabbit Trem2 (1,100, Cell Signaling, Cat#91068). Secondary antibodies conjugated to fluorophores: anti-mouse Alexa Fluor 488, 568, and 633, anti-rabbit Alexa Fluor 488, 594, anti-chicken Alexa Fluor 488 and 647, and anti-goat Alexa Fluor 488, 568, 633; all from goat or donkey (1:500, ThermoFisher Scientific, Eugene, OR).

Imaging

All brightfield images were taken using a Leica Aperio LSM™ slide scanner under 20X objective. All immunofluorescent images were taken on the Leica Thunder imager DMi8. Images were acquired at 20x using a Leica K5 camera. Leica biosystems LAS X software was used for image capture. Tiles covering the entire ION were taken and stitched. Leica Thunder instant computational clearing was used to remove out of focus light. The images were exported as tiff files for downstream analysis.

Image analysis

All image analysis was done in QuPath 0.30 (Bankhead et al., 2017). Annotations delineating the ventral, lateral and dorsal ION Parenchyma as well as the hilum were manually drawn. To detect cells, we used the "cell detection" function under the analysis menu. The DAPI Channel was selected for the Detection Channel. We modified the background threshold for each image to eliminate non-specific detections. Next, we trained an object classifier to classify the detections for the different channels. Training data were created from each image to delineate cells that are positive for the specific antigens in question. One classifier per channel was trained by calling the "train object classifier" function under classify with the following parameters: type = Random Trees, measurements = Cell: measurements = Cell: Channel X standard deviation, mean, max, and min measurements for the channel in question. To increase the accuracy of the classifier, additional training annotations were created on the image in question until the classification results matched the impression of the observer. Once a classifier was trained for each channel, "create composite classifier" was called to create a classifier consisting of multiple individual classifiers, one for each channel on the image. Classifiers were trained for each image separately. For CD44 and AQP4 analysis, we created a pixel classifier to classify positive and negative pixels. Training annotations were created for each image for positive and negative pixels. "Train pixel classifier" function was then called with the classifier type set to random trees, with a resolution of 2.60 µm/pixel, and selected all the features from only the channel in question.

To measure the minimal distance between microglial and olivary neurons, we first detected microglia using positive cell detection to identify IBA1+ cells. Next, QuPath pixel classifier was used to classify IBA1− ION neurons, which have characteristic large cytoplasm and eccentric nuclei. Using more than 20 manually annotated neurons as the training set, the pixel classifier accurately detected all neurons. We next converted the pixel classifications into annotations which we used to measure the distance against by calling analyze > spatial analysis > distance to annotations 2D measurements function between microglia (as positive cells) and neurons (as annotations). The measurements were exported as .csv files for downstream analysis in R. After −1*log10(1 + value) normalization, and binning into 100 bins, the kernel density distribution of the counts of cells that fall within each bin was used to calculate the modes for each condition using the multimode package in R by calling the locmodes function with the following options (lowsup = 0.00001, uppsup = 6, mod0 = 2, display = T). The supports were chosen to fit the data empirically—the upper support was ≤ to the maximum value in the data. We assumed two modes for the distribution (mod0 = 2). The Gaussian kernel density estimator is employed in the package. The distributions were compared using the ks test (two-sided) in R.

To classify microglia by activation state, an object classifier was used on objects detected by setting the detection channel to the IBA1 channel. This allowed the full tracing of microglial processes. The training images for microglial reactivity were compiled from examples

taken from all images included in the analysis. Training objects were assigned by setting the class of microglia as quiescent vs. activated cells. The key characteristics used to identify a quiescent microglial cell were lightly-stained processes and small somata, while the activated microglia were marked by darker stains, larger soma, and thickened and retracted processes.

PCA and cluster analysis

PCA analysis was done in FactoMineR R package (Lê et al., 2008). A total of 28 donor brains (10 ARDS and 18 COVID) were analyzed in four brain regions (dorsal, lateral, and ventral ION parenchyma (OP), and hilum), for a total of 107 data points representing the results of the image quantification after outlier removal. Metadata was included in the analysis as supplementary variables. Numerical values (age and length of hospitalization) were categorized into three bins. Other qualitative data included presence or absence of diffuse alveolar damage (DAD), intubation, and sepsis, as well as sex, condition, and brain region. −1*Log10 (value+1) normalization was performed on all immunohistochemical data measured as number of positive cells per area; no normalization was performed for data measured by percentage of area covered (AQP4 and CD44). Outliers, denoted in Supplementary Table S1, were identified in both using the Grubb's method (see Section Statistical Analysis section below) and in cluster analysis. Outliers formed small 1–2 sample clusters. The few missing values, such as those resulting from low quality images, were imputed using the imputePCA function of the MissMDA package in R. Principal component analysis (PCA) was performed on the IHC data alongside supplementary qualitative variables, comprised of the metadata variables (Supplementary Figure S5A). The dimdesc function, part of the FactoMineR package, provided further details of factor analysis of samples (Supplementary Table S2). These results were then used for hierarchical clustering analysis, using the FactoMineR package's HCPC function with the distance metric set to 'Manhattan', to provide four hierarchical clusters of the data (Figure 5B). Proportions of qualitative variables comprising each hierarchical cluster were then calculated using Dplyr functions (Supplementary Figure S5C). Heatmaps were generated using the pheatmap R package.

Statistical analysis

All statistical analyses were conducted in GraphPad® Prism 9 or R v4.03. For all data sets, outliers were identified using the Grubbs' method with an Alpha = 0.2. All statistical tests and graphs were done using the outlier-free data. For analyzing two groups we used two-tailed and unpaired *t*-tests. For analyzing more than two groups, we used one way Brown-Forsythe and Welch ANOVA correcting for multiple comparisons using Original FDR method of Benjiamini and Hochberg. All data sets that were analyzed using one-way ANOVA were tested for normality using Shapiro–Wilk test and transformed using Y = −1*Log(Y + 1) if they did not pass the normality test. *p* values reported are those of the transformed data where transformation was done. To further validate our ANOVA test results, a beta regression model was also used as implemented in the betareg package in R. The independent variables used were condition (either ARDS, COVID with microglial nodules (MN), and COVID with no MN, or Control, ARDS, and COVID) and region (dorsal, lateral, and ventral OP), with the counts (microglia per area, proportion of activated microglia, GFAP per area, or percent MT3) as the dependent variable. The results of this analysis are provided in Supplementary Table S4.

Results

Increased microglial activation in the ION of COVID patients

Microglial reactivity is a common feature of COVID-19 pathology. We first set out to replicate this finding in our cohort, focusing on the ION. We included control patients who died without COVID-19 or ARDS, patients who died with ARDS but not COVID-19, and patients who died of COVID-19. This allows us to answer the following question: is microglial reactivity in COVID-19 due to hypoxia? Thus, we quantified the number of IBA1+ cells per unit area in different regions of the ION: the lateral, dorsal, and ventral sectors (Figure 1A). ANOVA analysis of IBA1+ cells/area was significant in all three regions of the ION, and there was a significant increase in the number of microglia per unit area in COVID-19 cases compared to the non-hypoxic controls in the lateral ION, but there was no difference between the non-hypoxic controls and the ARDS cohort (Figure 1B). This indicates that factors in COVID-19, in addition to hypoxia, were necessary to drive the increase in microglia in the ION. Beta regression analysis for microglia per area returned, for the COVID condition, a coefficient of 0.472 and *p* value 2.89E-05 (Supplementary Table S4), suggesting that COVID-19 condition can explain the increased microglial numbers in the COVID-19 cases in our cohort. Because ARDS and controls were not significantly different in the density of ION microglia, and ARDS cases can be considered matching controls for hypoxia, we compared microglial reactivity between the ARDS and COVID-19. We used morphologic attributes of microglia to train a machine learning algorithm to classify microglia into quiescent versus activated (see Section Materials and methods). We wanted a simple way to classify microglia based on morphology, knowing that microglial reactivity falls on a spectrum of states, and that activated microglia generally have retracted thick processes compared to quiescent cells (Davis et al., 2017; Leyh et al., 2021). We opted for a simple binary classification of quiescent vs. activated microglia; we show examples of these classifications in Figure 1C. We also chose to split our COVID-19 group into two groups: a group with high abundance of microglial nodules (MN), and another group with relatively few/no microglial nodules (No-MN). These designations were based on previously reported neuropathologic assessments (Thakur et al., 2021). Comparing the proportion of activated microglia across the ventral, dorsal, and lateral ION in these three groups (ARDS, COVID-19 no-MN, COVID-19 MN) revealed that in the ventral ION, the COVID-19 MN group had a significantly larger proportion of activated microglia (Figure 1D). Subsequent beta regression testing of proportion of activated microglia returned a coefficient of 0.509 with *p* value 0.000553 for the COVID-MN condition, but the coefficients were not significant for ION regions, suggesting that

10.3389/fnins.2023.1198219

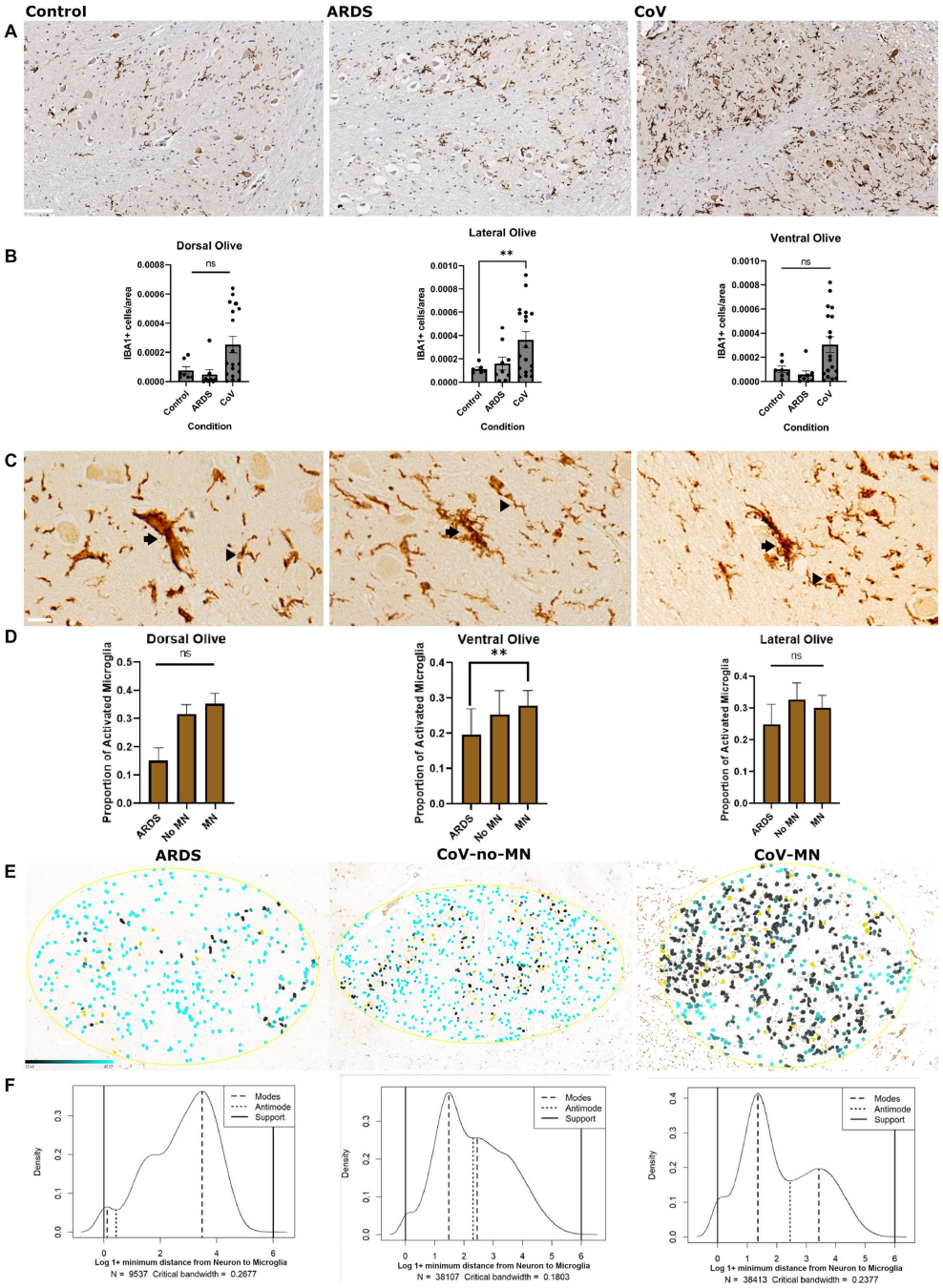


FIGURE 1
Microglial reactivity in COVID-19. **(A)** Immunohistochemical stain for IBA1 to label microglia in the ventral ION. Scale bar is 100 μm. **(B)** Quantification of the number of IBA1+ microglia per unit area in the dorsal, ventral and lateral ION. *N*= 18 for COVID-19, 10 for ARDS, and 7 for Control. The data was transformed using Y=-1*Log(Y) prior to calculating *P* values. ANOVA p-value= dorsal:0.0098, lateral: 0.0416, and ventral: 0.0076 based on transformed data. The graphs of transformed data are provided in table S4. *P* values of multiple comparisons: Control vs. CoV: dorsal: 0.1547, lateral: 0.0089 and ventral: 0.1372.Control vs ARDS: dorsal: 0.0582, lateral: 0.6543, and ventral: 0.0858.**(C)** Examples of different microglia classified as activated (arrow) versus quiescent (arrowhead). Scale bar is 20 μm. **(D)** Quantification of the percentage of total microglia that were classified as activated in each anatomic region (dorsal, ventral, and lateral). *N*= 10 for COVID-19 MN, 8 for COVID-19 No-MN, and 10 for ARDS. Normality was confirmed using a Shapiro-Wilk test. ANOVA *P*-values= dorsal: 0.84236, lateral: 0.1995, and ventral: 0.0174. Multiple comparisons *P* values = Control vs no-MN: dorsal:,0.0853, lateral: 0.2590 and ventral: 0.1973. Control vs CoV-MN: dorsal: 0.5654, lateral: 0.1551 and ventral: 0.0050. **(E)** Distance maps depicting the distance between neurons (masked in Yellow) and microglia in the Ventral Olive. The distance is shown as a color gradient (Black: close, cyan: far). The gradient is shown in the bottom left part of the left panel. **(F)** Probability density plots showing the probability distributions of the proportion of microglia that fall within a specified distance from the closest neuron, binned into 100 bins after log normalization. The modes (peaks) and anti-modes (troughs) are indicated. The supports indicate the upper and lower bounds of the distributions. The condition depicted in each graph is as in panel **E**. ARDS: Acute respiratory distress syndrome. MN: COVID-19 with microglial nodules. No-MN: COVID-19 without microglial nodules. **B** One way Brown-Forsythe and Welch ANOVA. Comparisons are against Control. **D** One way Brown-Forsythe and Welch ANOVA. Comparisons are against ARDS. Data is shown as mean +/- SEM.

condition rather than ION region drives microglial activation (Supplementary Table S4). Next, we asked if the minimum distance between any microglial cell and the closest neuron to it is different between the groups. This in effect is a way to quantify the proximity of microglia to neurons. We reasoned that we would see more microglia close to neurons if there is more neuronophagia or microglial nodules. We measured the distance between microglia and neurons in the ION (Figure 1E) and found that the distribution of minimal distance between microglia and neurons is quite different between the groups (Figure 1F—Asymptotic two-sample Kolmogorov–Smirnov test—a non-parametric test to compare distributions). The results are as follows: D values = 0.28, 0.21, 0.12, for MN vs. ARDS, No-MN vs. ARDS, and MN vs. No-MN, respectively, for all comparisons the p-value is less than 2.2e-16. Comparing the distribution across different anatomic sectors of the ION revealed similar results (data not shown). The distribution was truly bimodal in the COVID-19 MN group, and the two modes were 1.36 (higher probability mode) and 3.45 (lower probability mode). Conversely, the modes for the ARDS group were 3.47 (highest probability mode) and 0.11 (lower probability mode) and for the No-MN group were 1.48 (highest probability mode) and 2.45 (lower probability mode). The fact that the mode with the highest probability (density) in the MN was lower than that in the ARDS group can be seen as an indirect measure of the presence of microglial nodules in the MN group—which is previously established. Altogether, we found that microglia are more activated and closer to neurons in the ION in COVID-19, especially the MN group.

We also asked if microglia in COVID-19 brains expressed more TREM2 compared to microglia in ARDS in the ION. TREM2 labels phagocytic microglia (Takahashi et al., 2005). Although we could detect TREM2 in microglia in the white matter surrounding the ION (for example—in the pyramids Supplementary Figure S1A), there was no significant specific labeling of microglia in the ION in COVID-19 or in ARDS (Supplementary Figure S1B).

Increased astrogliosis in ARDS and COVID-19 patients

Given that astrogliosis is a prominent feature of COVID-19 neuropathology, we asked if we could recapitulate this finding in the ION. To address this question, we conducted a series of immunohistochemical and multiplex immunofluorescence studies to quantify the expression of proteins related to reactive astrogliosis or alterations in astrocyte function. First, we quantified the number of Glial fibrillary acidic protein (GFAP) positive astrocytes in the ION in controls, ARDS, and COVID-19 (Figure 2A). Interestingly, we found that compared to control, both ARDS and COVID-19 exhibited increased numbers of GFAP+ astrocytes, defined as GFAP+ somata, per unit area in all ION regions (Figure 2B). Notably, while control samples showed many GFAP+ astrocytic processes, few astrocytic cell bodies were labeled. Additionally, beta regression testing of GFAP per area data returned coefficient −0.0828 and p value 2.1E-08 for the control condition, consistent with our ANOVA results (Supplementary Table S4).

A caveat is worth mentioning here: detecting increased GFAP+ cells does not necessarily suggest that there were more astrocytes in one group vs. the other. Some astrocytes may exhibit lower levels of GFAP below the sensitivity of the assay, and can upregulate GFAP in pathologic contexts allowing its detection. Either way, the downstream interpretation of this phenomenon supports that in hypoxia (ARDS and COVID-19), there are elevated levels of astrogliosis.

Ventral ION astrocytes in COVID-19 show decreased Aquaporin-4 compared with ARDS

Reactive astrocytes upregulate the expression of Aquaporin-4 (AQP4) (Tomas-Camardiel et al., 2004; Tourdias et al., 2011), and redistribute its expression to the cell soma from the astrocytic end-feet, where it is normally localized (Eid et al., 2005; Steiner et al., 2012). Moreover, AQP4 has important implications in hypoxic–ischemic conditions (Shi et al., 2012), and studies have shown that loss of AQP4 protects against early cytotoxic edema associated with stroke (Papadopoulos and Verkman, 2008). Also, AQP4 expression in astrocytes has important implications in neuroinflammation secondary to ischemia, and AQP4 knockout mice exhibit exaggerated post-stroke microglial reactivity (Shi et al., 2012). Thus, we asked if AQP4 levels were altered in COVID-19 vs. ARDS. We measured the area covered by AQP4 in ION (Figure 3A). In patients who had died of COVID-19, there was decreased expression of AQP4 in the ventral ION compared to the ARDS patients (Figure 3B). Together, these findings link lower AQP4 levels to increase neuroinflammation in COVID-19.

Hilar astrocytes of COVID-19 donors exhibit reduced levels of YKL-40 compared with ARDS

Encoded by the *CHI3L1* gene, Chitinase-3-like protein (YKL-40) is a secreted glycoprotein primarily expressed in astrocytes in the brain that is a common marker of neurodegeneration (Querol-Vilaseca et al., 2017; Lananna et al., 2020; Zhao et al., 2020; Ferrari-Souza et al., 2022). Astrocytes increase the expression of YKL-40 in several neurodegenerative diseases including AD, tauopathies, and prion disease (Bonneh-Barkay et al., 2010; Llorens et al., 2017; Querol-Vilaseca et al., 2017). *In vitro* studies showed that YKL-40 could be induced in astrocytes by macrophages (Bonneh-Barkay et al., 2012). A recent study showed that YKL-40 knockout mice exhibit reduced amyloid plaques and increased expression of CD68 in microglia in an AD model, suggesting that YKL-40 suppresses microglial reactivity (Lananna et al., 2020). Thus, we examined the expression of YKL-40 in astrocytes in our cohort (Figure 4A). We were interested in knowing whether hypoxia in general can increase YKL-40, so for this analysis, we included the non-ARDS controls. We quantified the proportion of ALDH1L1 positive astrocytes that were also positive for YKL-40 and found that there were significantly more YKL-40 positive astrocytes in the hilum of ARDS and COVID-19 brains compared to non-ARDS controls (Figure 4B). This was not the case in the ION parenchyma (data not shown). However, there were fewer YKL-40 positive astrocytes in the ION hilum of the COVID-19 cohort compared to ARDS (Figure 4B). We examined astrocytic protein expression in all the comparisons we conducted,

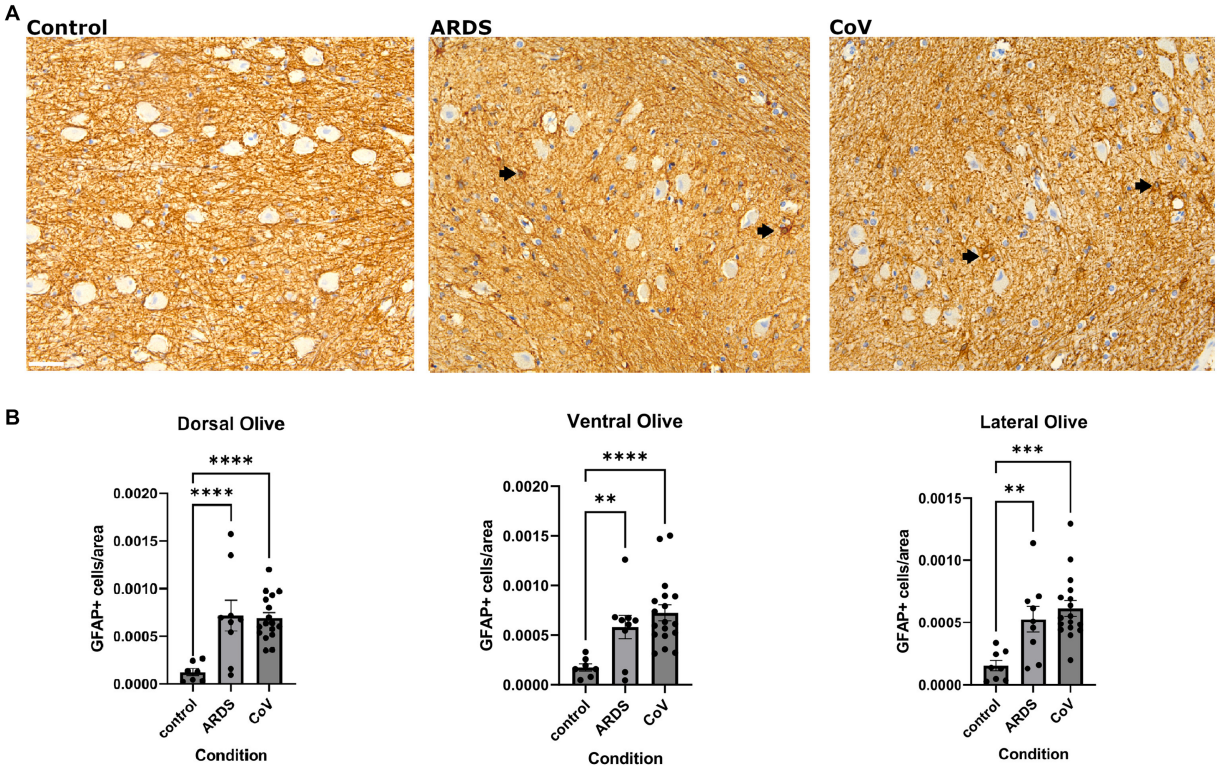


FIGURE 2
Astrogliosis in COVID-19. **(A)** Immunohistochemical stain for GFAP in the ventral ION. Black arrows point to GFAP positive cells. Scale bar indicates 50 μm. **(B)** Quantification of GFAP positive cells per unit area in the non-hypoxic controls, ARDS and COVID-19 samples across the dorsal, lateral, and ventral regions of the ION. One way Brown-Forsythe and Welch ANOVA correcting for multiple comparisons using Original FDR method of Benjiamini and Hochberg. Comparisons are against for COVID-19 and ARDS are both against Control. *N*= 18 for COVID-19, 10 for ARDS, and 7 for Control. Data is shown as mean +/- SEM. The data was transformed using Y=-1*log10(Y) before calculating p-values. ANOVA *P* value= dorsal: <.0001, lateral: <0.0001 and ventral: 0.0002. Multiple comparison *P*=values:<0.0001 for ARDS and CoV in dorsal, 0.0002 for ARDS and <0.0001 for CoV in the lateral and 0.0049 for ARDS and <0.0001 for CoV in the ventral.

and YKL-40 is the only protein that we found dysregulated in the hilum. This is interesting given that it is a secreted protein (Zhao et al., 2020). Together, these results indicate that ION astrocytes behave differently under hypoxia in the setting of COVID-19 systemic infection; they fail to upregulate YKL-40 to the same extent as in ARDS. A caveat is that YKL-40 is a secreted protein, and that changes in YKL-40 levels between COVID-19 and ARDS may reflect changes in secretion patterns.

Other markers of astrogliosis

To further characterize astrogliosis in COVID-19 ION astrocytes, we performed multiplex immunofluorescence for other protein markers associated with reactive astrogliosis. We first quantified the expression of metallothionein-3 (MT3), a zinc-binding protein that has been shown to be upregulated in reactive astrocytes in Huntington disease (al-Dalahmah et al., 2020b). Metallothioneins are thought to be neuroprotective (Stankovic et al., 2007). Quantification of MT3 in different sectors of the ION of ARDS and COVID-19 showed no significant difference in the proportion of astrocytes that label with MT-3 (unpaired *t*-test, ARDS and COVID-19 mean ± SEM = 8.901 ± 2.731 and 14.27 ± 4.462, respectively, *p* value = 0.2262), however, when we stratified COVID-19 by the presence or absence of microglial nodules, we detected significantly lower proportions of lateral ION astrocytes in the COVID-19 with microglial nodules compared with ARDS patients (Supplementary Figures S2A,B). In accordance with this result, beta regression testing showed that COVID-MN condition had coefficient of—0.6191 and *p* value 0.0016, and that ION regions also had significant coefficients (Supplementary Table S4). We next asked if ION astrocytes in COVID-19 increase the expression of complement factor 3 (C3), which is a gene that is upregulated in and therefore a marker of putative neurotoxic "A1" astrocytes (Liddelow et al., 2017). We found no significant increase in the proportion of C3+ astrocytes in COVID-19 vs. ARDS (Supplementary Figures S3A,B). Finally, we examined the expression of CD44, an astrocyte protein expressed in white matter astrocytes [see our preprint (Al Dalahmah et al., 2023)], astrocytes around large vessels, interlaminar astrocytes, and a subset of cortical astrocytes (Sosunov et al., 2014), as well as Clusterin (CLU), which is increased in neurodegenerative astrocytes in AD (Wojtas et al., 2020; Chen et al., 2021). We quantified the area covered by CD44 and again found no significant increase in CD44 labeling in the COVID-19 ION (Supplementary Figures S4A,B). Likewise, we found no significant differences in the proportion of ION astrocytes that

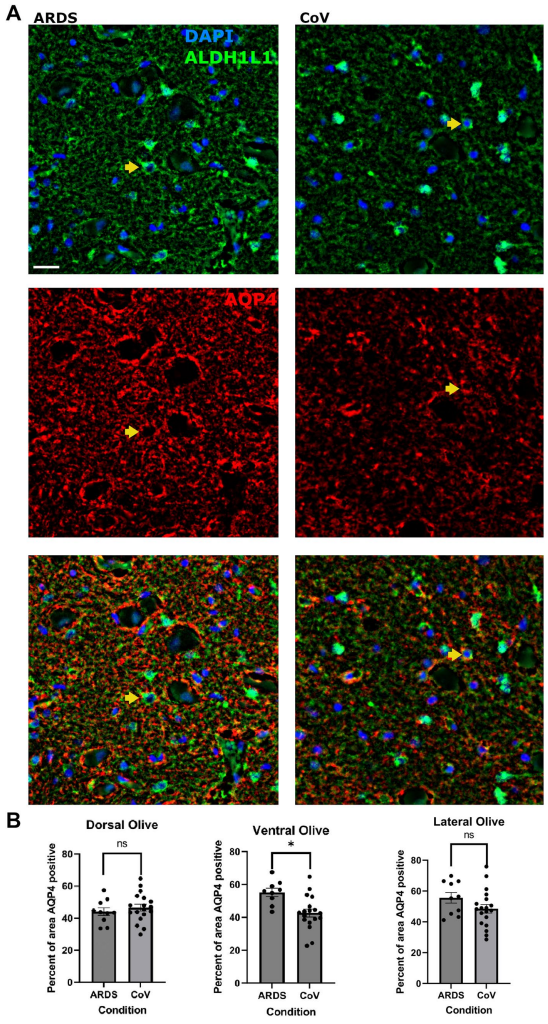


FIGURE 3
Lower AQP4 levels in the ventral ION in COVID-19 compared to ARDS. **(A)** Multiplex immunofluorescence showing ARDS (left) and COVID-19 (right) in the ventral ION labeled for nuclei (DAPI - blue) and ALDH1L1 (green - top panel), AQP4 (red – middle panel), and merged panels (lower panels). Arrows indicate cells positive for DAPI, ALDH1L1 and AQP4. Scale bar = 20 μm. **(B)** Quantification of the percent area positive for AQP4 per ION region. Unpaired two-tailed t-test. N= 18 for COVID-19, 10 for ARDS. *P* value = 0.0035. Data is shown as mean +/- SEM.

were CLU-positive between COVID-19 and ARDS (Supplementary Figures S4A,C).

Astrocyte IHC profiles and microglial reactivity drive cohort clustering

In our design, we tried to control for relevant demographic and clinical variables (metadata), however this is not always possible. To determine the correlation between metadata variables and biological results, we performed principal component analysis (PCA) on all the immunohistochemical data from images from different regions in ION, using the metadata as supplementary variables, allowing us to predict their PCA coordinates from the IHC data. The input to the PCA analysis is provided in Supplementary Table S2. First, we plotted the brain donors in PCA space and found that ARDS and COVID-19 donors were relatively well separated (Figure 5A). This highlights the biological differences between the two groups. A closer look at the PCA results showed that a number of quantitative IHC variables were responsible for the greatest amount of variation in dimensions 1 and 2 (Supplementary Figure S5; Supplementary Table S3). In PC1, YKL40, C3, and a combination of the two (YKL40.C3) had correlation values of 0.898, 0.839, and 0.910, and *p* values of 3.27E-39, 1.77E-29, and 4.68E-42, respectively. Interestingly, the proportion of activated microglia was only weakly correlated with PC1. Metadata variables length of hospital stay, condition, sex, and DAD had low correlation (R2 values of 0.206, 0.069, 0.039, and 0.038, respectively—Supplementary Table S3) with PC1, suggesting that our case-control matching is not perfect, but sufficient. CD44 proportion and CLU per area were the most significant IHC variables associated with PC2, with correlation values of 0.620 and 0.508, and *p* values of 1.09E-12 and 2.35E-08, respectively. Astrocytes per unit area and proportion of MT3 positive astrocytes were significantly and strongly negatively correlated with PC2. The most relevant qualitative variable for PC2 was age, with a relatively low R2 value of 0.080 (Supplementary Table S3). This again shows high significance but low correlation of qualitative variables with the variance shown in the dimension, suggesting that our ARDS- COVID-19 matching was relatively effective. Supplementary Figure S5A shows the correlation circle depicting the IHC variables and their correlation to PC1 and PC2. Supplementary Figure S5B shows the correlation between the metadata variables and the first 5 PCs.

We next asked if clustering the samples (IHC images) based on the PCA dimensions would give us clusters that reflect condition, and/or other relevant variables like anatomic region for example. To achieve that, we clustered the data on the first 5 PC's using hierarchical clustering on the Euclidian distance matrix derived from the PC1-5 coordinates for each sample. We identified four clusters as shown in Figure 5B. Examination of the hierarchical clustering results show that clusters 1 and 4 were relatively deplete of samples derived from the ION hilum compared to clusters 2 and 3. This is expected because the hilum is composed of white matter harboring axons and glia, unlike the ION parenchyma, which harbors neurons, too. To highlight any relationships between clusters and condition (ARDS vs. COVID-19), we plotted the proportion of images that fell under each cluster against condition in a heatmap (Figure 5C). The results show that ARDS samples were mainly enriched in clusters 1 and 3, while COVID-19 samples were distributed between clusters 2, 3, and 4. Together, these findings demonstrate that our samples cluster based on the major factors that our analysis set out to investigate, biological condition and anatomic locale.

A closer look at the distribution of metadata variables shows cluster 1 appears to be most enriched with old age (13), and short hospital stay (16) samples, and cluster 2 with COVID (20), short hospital stay (22), male (19), DAD (20), and non-septic (23) samples. Cluster 3 is most enriched with median-age (20), ARDS (15), short hospital stay (22), DAD (22), and non-septic (26) samples, and cluster 4 with COVID (18) and female (16) samples (Supplementary Figure S2C). The cos2 value of each variable, a good metric of variable correlation with the circumference of the correlation circle, also shows the same patterns we described by looking at the R2 above. Briefly, correlations in PC1 with length of hospital stay, sex, DAD, disease condition including the presence of microglial nodules in COVID-19, in PC2 with region

10.3389/fnins.2023.1198219

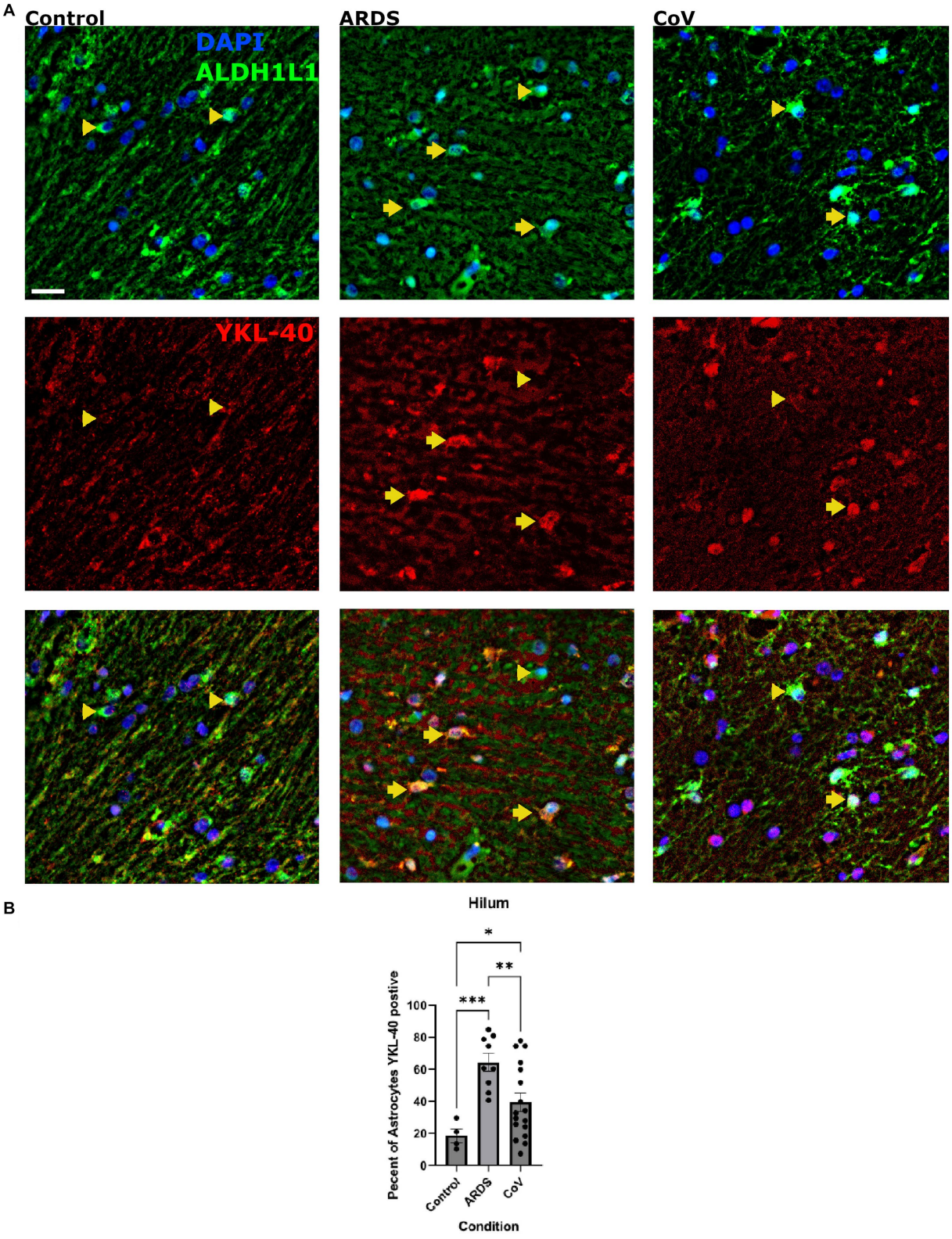


FIGURE 4
YKL-40 expression in the hila of the Controls, COVID-19 and ARDS. **(A)** Cells in the hilum stained for DAPI (blue) to detect nuclei of all cells and ALDH1L1 (green) to detect astrocytes. Scale bar = 20 μm. The next row shows YKL-40 (red) alone with the last figure being the merge of all three. **(B)** Quantification of the proportion of astrocytes positive for YKL-40 positive astrocytes per unit area in the hilum of non-hypoxic control, ARDS and COVID-19 cases per ION region. One way Brown-Forsythe and Welch ANOVA correcting for multiple comparisons using Original FDR method of Benjiamini and Hochberg. *N*= 17 for COVID-19, 10 for ARDS, and 4 for controls. ANOVA *P* value = >0.0001. *P* value =<0.0001 for ARDS and 0.0106 for COVID-19. *P*-value of CoV in comparison to ARDS is 0.0091. Data is shown as mean +/- SEM.

10.3389/fnins.2023.1198219

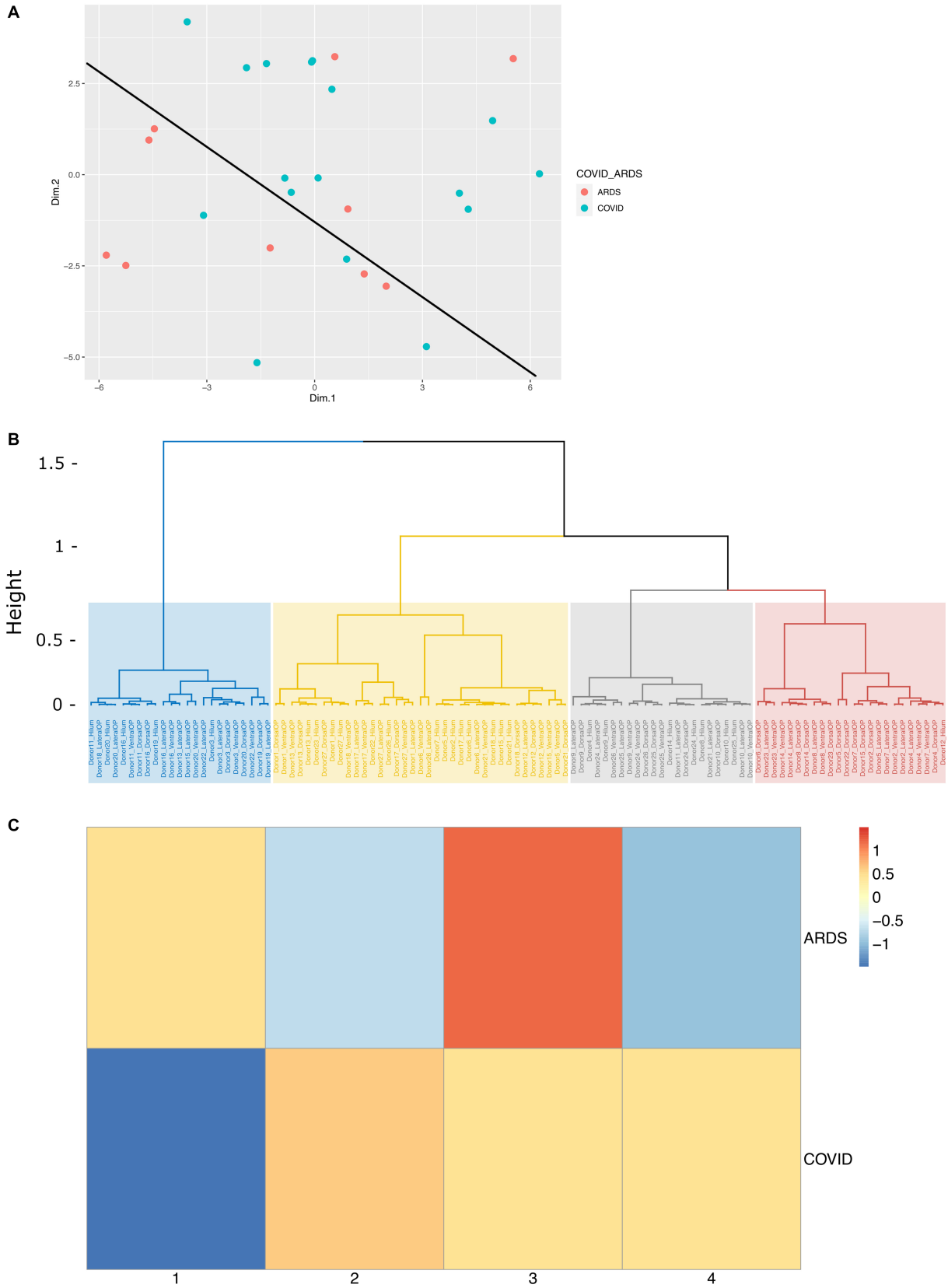


FIGURE 5
Cluster analysis of immunohistochemical data on COVID and ARDS cases. **(A)** PCA scatterplot of donors along PC1 and PC2, colored by condition (ARDS and COVID), which a line depicting the separation of COVID patients and ARDS patients in PCA space. **(B)** Dendrogram depicting the distribution of each sample into four hierarchical clusters. **(C)** Heatmap showing the proportion of all COVID samples and ARDS samples represented in each hierarchical cluster, scaled by row. Columns represent each of the four hierarchical clusters.

and age, and in PC3 with region and length of hospital stay, respectively (Supplementary Figure S2B). All together, these data suggest that in addition to condition and anatomic locale, sex, concomitant DAD, and length of hospital stay were variables that correlated with IHC features and contributed to clustering. However, their overall correlation with the PC's that explain the variance was low, suggesting they had a modest influence on the reactivity of astrocytes and microglia in the ION.

Discussion

This study investigated the effects of the SARS-CoV-2 (COVID-19) virus on astrocytes and microglia in the ION. We designed this study to control for hypoxemia by including controls with ARDS and no COVID-19 infection, allowing us to determine if systemic infection with SARS-CoV-2, independently drives glial pathology in the ION—one of the most severely involved brain regions in COVID-19 neuropathology (Thakur et al., 2021). We confirmed that our non-hypoxia controls had no neuropathologic evidence of hypoxia compared with the ARDS cases, which exhibited widespread hypoxic changes. We found that the ION in COVID-19 and ARDS exhibits significant astrogliosis, and in COVID-19 alone displays significant microgliosis. We found that COVID-19 microglia are closer to ION neurons compared with non-COVID-19 counterparts. We also found morphologic evidence for increased microglial reactivity in the ventral region of the ION. In parallel, we quantified astrocytic protein expression and found that in both COVID-19 and ARDS, YKL40 levels were increased in the hilum, however, the proportion of YKL-40+ hilar astrocytes was lower in COVID-19. Finally, ventral and lateral ION astrocytes in COVID-19 showed lower levels of AQP4 and MT3, respectively. Overall, our findings indicate that the pathology in COVID-19 cannot be explained by hypoxia alone, and that astrocytic pathology in COVID-19 may contribute to the prominent neuroinflammatory response in the brainstem.

Astrocytes play important roles in mediating the tissue response to hypoxia-ischemia (Vella et al., 2015), which is the most common neuropathologic abnormality in COVID-19 (Maiese et al., 2021). Astrocytes are primary drivers of cytotoxic edema in the acute phase of ischemia (Choi and Rothman, 1990; Pantoni et al., 1996; Nielsen et al., 1997), and vasogenic edema if the blood brain barrier breaks down (Badaut et al., 2002). AQP4 levels are increased in reactive conditions, and AQP4 can redistribute to the astrocytic somata during ischemia (Tourdias et al., 2011). Loss of AQP4 protects against early cytotoxic edema associated with stroke (Papadopoulos and Verkman, 2008). Therefore, it is possible that the reduction of AQP4 in the ventral ION in COVID-19 might be a protective response against ischemia. On the flip side, AQP4 knockout mice exhibit exaggerated post-stroke microglial reactivity (Shi et al., 2012), and this may explain the heightened microglial reactivity we see in COVID-19 ION. Perhaps this picture becomes more compelling when combined with the other phenotypic alterations we see in astrocytes, namely, the relative reduction of YKL-40, which is a secreted cytokine (Zhao et al., 2020) thought to suppress microglial reactivity (Lananna et al., 2020), and the relative failure of upregulation of the putative neuroprotective MT3. These findings along with those reported in this paper demonstrate the need for further mechanistic studies to investigate the functional roles of MT3, AQP4, and YKL-40 in astrocytes in animal or cell-based models. We did not find a gain of C3, which is a marker of putative neurotoxic "A1" astrocytes (Liddelow et al., 2017). Thus, it appears that COVID-19 astrocytes exhibit phenotypic alterations that may result in failure to check the immune response in the ION. Given that our controls were matched for hypoxemia, the alteration in astrocytic protein expression cannot be solely attributed to hypoxia. We can only conclude that some other factor, such as systemic infection with SARS-CoV-2, may underlie this astrocytic phenotype.

In considering potential causes for the astrocytic protein expression changes in the COVID-19 brains, we have to consider the role of comorbidities such as sepsis. We tried to control for this factor by patient matching, however, this is not always possible. Our PCA analysis indicates that sepsis is not significantly correlated with the first 2 PC's, supporting that our patient matching approach was relatively effective at controlling for sepsis in this cohort. It has been shown that astrocytes in an animal model of lipopolysaccharide-induced sepsis increased expression of C3 (Zamanian et al., 2012; Liddelow et al., 2017). Had sepsis been the underlying reason behind astrocytic phenotypic changes, we would have detected changes in C3 expression, which was not the case. Another explanation for ION COVID-19 astrocyte phenotypes could be the increase in systemic levels of cytokines in COVID-19 (Chen et al., 2020). Unfortunately, we do not have data on the cytokine profiles from our cohort, and it would be impossible to retrieve that retrospectively from a postmortem dataset. Moreover, it is possible that systemic inflammation, as seen in COVID-19, may lead to alterations in the blood brain barrier (Varatharaj and Galea, 2017) which may then lead to changes in astrocyte phenotypes. Finally, we considered that astrocytes may be infected by SARS-CoV-2 directly leading to their phenotypic changes. To date, there is no convincing evidence that this happens in human tissue (al-Dalahmah et al., 2020a; Deigendesch et al., 2020; Matschke et al., 2020; Solomon et al., 2020; Colombo et al., 2021; Fabbri et al., 2021; Maiese et al., 2021; Thakur et al., 2021; Wierzba-Bobrowicz et al., 2021; Agrawal et al., 2022). Although we cannot rule it out completely, we conclude that direct infection of astrocytes by the virus given the available evidence is unlikely. We contend that systemic infection with SARS-CoV-2 indirectly alters astrocytic protein expression, and further studies are needed to examine this hypothesis.

There are notable limitations of this study. For starters, we examined astrocytic and microglial reactivity in only one region of the brainstem, the ION. We used this region as representative of the most severely affected brain regions acknowledging that there are other brain nuclei, like the dentate nucleus and the pontine nuclei, which also exhibit significant pathology in COVID-19 (al-Dalahmah et al., 2020a; Thakur et al., 2021). Future studies will examine these brain regions including others, to elaborate on the heterogeneous glial responses to injury in COVID-19. Another limitation is the incompleteness of the clinical data. It would have been optimal if the clinical records were complete so as to allow us to conduct more comprehensive analyses of the impact of several clinical variables on glial reactivity. We only included a limited number of variables for which we had data on most cases. We had to exclude our non-hypoxic controls from the analysis because our clinical records on these patients are lacking. These brain donors died elsewhere, outside the NY Presbyterian hospital, so we have no way of getting the relevant clinical information. Finally, our experimental design matched

COVID-19 with ARDS patients for prolonged hypoxia, however, we cannot adequately control for other unmeasured factors that are beyond hypoxia and viral infection.

In conclusion, our data is the first to perform controlled immunophenotypic astrocytes in COVID-19 brains to determine whether the observed glial pathology can be explained by hypoxia. We found that hypoxia alone cannot explain glial pathology in COVID-19 in ION—one of the most severely affected regions in the brain. Future studies are needed to extend this approach to other brain regions that are severely affected vs. relatively preserved, to expand our understanding of the disease pathology. An unanswered question remains as to the regional heterogeneity of astrocytic and microglial reactivity in the ION, and further studies are needed to understand this phenomenon.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

OA-D conceived and designed the study. NM, YM, KJ, and JL performed the immunostains. NM, YM, KJ, JL, GH, JG, and OA-D analyzed the data. NM, KJ, JG, and OA-D wrote the paper. All authors contributed to the article and approved the submitted version.

Funding

OA-D was supported by the American Brain tumor Association—fully supported by the Uncle Kory Foundation and NIA ADRC REC program (Grant Number P30AG066462).

Acknowledgments

The authors thank Dr. Phyllis Faust for helping us with control cases.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1198219/full#supplementary-material

SUPPLEMENTARY TABLE 1
Cohort demographic and clinical data.

SUPPLEMENTARY DATA SHEET 1
Data file containing the original set of immunohistochemical and meta data, following log10 normalization, outlier removal, and missing value imputation, used as input for all downstream analysis.

SUPPLEMENTARY DATA SHEET 2
PCA description of dimensions: Results from PCA analysis, giving details of the contribution of variables to dimensions 1 and 2, including only those variables with the greatest bearing on explanation of variance in those dimensions.

SUPPLEMENTARY DATA SHEET 3
Results from beta regression on proportions and percentage data implicated in ANOVA testing. Plots of transformed data where appropriate.

SUPPLEMENTARY FIGURE 1
TREM2 expression in ION microglia. **(A)** Immunohistochemical stains for TREM2 in a COVID-19 brain in the pyramid. Note the labeling of microglia. **(B)** Immunohistochemical stains for TREM2 in a COVID-19 brain in the ION. Note the absence of strong labeling of microglial cell bodies. Scale bars = 50 μm.

SUPPLEMENTARY FIGURE 2
C3 expression in ION astrocytes. **(A)** Immunofluorescent images of the ION labeled for nuclei (DAPI - blue) and ALDH1L1 (green) to detect astrocytes (upper row), and C3 (white – middle row). Merged panels are show on the bottom row. Arrow indicates a ALDH1L1, DAPI and C3 positive cells and arrowheads indicate C3 negative astrocyte. Scale bar = 10μm. The Condition is shown by column. **(B)** Quantification of the proportion of MT3 positive astrocytes in the different ION regions. N= 17 for COVID-19 MN and 10 for ARDS controls. Data is shown as mean +/- SEM. P value= dorsal: 0.8465, lateral: 0.7734 and ventral: 0.4666.

SUPPLEMENTARY FIGURE 3
MT3 expression in ION astrocytes. **(A)** Immunofluorescent images of the ION labeled for nuclei (DAPI - blue) and ALDH1L1 (green) to detect astrocytes (upper row), and MT3 (white – middle row). Merged panels are show on the bottom row. Arrow indicates a ALDH1L1, DAPI and MT3 positive cells and arrowheads indicate MT3 negative astrocyte. Scale bar = 10μm. **(B)** Quantification of the proportion of MT3 positive astrocytes in the different ION regions. One way BrownForsythe and Welch ANOVA correcting for multiple comparisons using Original FDR method of Benjiamini and Hochberg. Comparisons are against ARDS. *N*= 10 for COVID19-MN, 8 for COVID-19 No-MN, 10 for ARDS. *P* values =0.0120 for COVID-19 MN 0.5410 for No-MN in the lateral ION. Data is shown as mean +/- SEM. MN: COVID19 with microglial nodules. No-MN: COVID-19 with no microglial nodules.

SUPPLEMENTARY FIGURE 4
CD44 and CLU expression in ION astrocytes. **(A)** Immunofluorescent images of the ION labeled for nuclei (DAPI - blue) and GFAP (green) to detect astrocytes (upper row), and CD44 (white – second row), and CLU (third row – red). Merged CLU GFAP panels are show on the bottom row. Scale bar = 20 μm. **(B)** Quantification of CD44 positive area in the different ION regions. **(C)** Quantification of CLU positive astrocytes in the different ION regions. Unpaired two-tailed t-test. *N*=

10 for COVID-19, 18 for ARDS. *P* values = dorsal: 0.0790, lateral: 0.4304 and ventral: 0.2036. Data is shown as mean +/- SEM.

SUPPLEMENTARY FIGURE 5
Contributions of data points to principal components and hierarchical clusters. **(A)** PCA plot depicting the extent to which each immunohistochemical variable is responsible for variation in dimensions 1 and 2. **(B)** Correlation plot with meta data variables along the y-axes and dimensions from PCA analysis on the x-axis. Size and color of each dot represent the extent to which each variable's cos2 value from PCA is represented in each dimension. **(C)** Heatmap with the numbers of samples (images) versus metadata variables in each hierarchical cluster. Rows represent each of the four hierarchical clusters.

References

Agrawal, S., Farfel, J. M., Arfanakis, K., al-Harthi, L., Shull, T., Teppen, T. L., et al. (2022). Brain autopsies of critically ill COVID-19 patients demonstrate heterogeneous profile of acute vascular injury, inflammation and age-linked chronic brain diseases. *Acta Neuropathol. Commun.* 10:186. doi: 10.1186/s40478-022-01493-7

Aiyegbusi, O. L., Hughes, S. E., Turner, G., Rivera, S. C., McMullan, C., Chandan, J. S., et al. (2021). Symptoms, complications and management of long COVID: a review. *J. R. Soc. Med.* 114, 428–442. doi: 10.1177/01410768211032850

Al Dalahmah, O., Sosunov, A. A., Sun, Y., Liu, Y., Akpan, N., Madden, N., et al. (2023). The matrix receptor CD44 is present in astrocytes throughout the human CNS and accumulates in hypoxia and seizures. *Preprints.org*:2023030444. doi: 10.20944/preprints202303.0444.v1

al-Dalahmah, O., Thakur, K. T., Nordvig, A. S., Prust, M. L., Roth, W., Lignelli, A., et al. (2020a). Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. *Acta Neuropathol. Commun.* 8:147. doi: 10.1186/s40478-020-01024-2

al-Dalahmah, O., Sosunov, A. A., Shaik, A., Ofori, K., Liu, Y., Vonsattel, J. P., et al. (2020b). Single-nucleus RNA-seq identifies Huntington disease astrocyte states. *Acta Neuropathol. Commun.* 8:19. doi: 10.1186/s40478-020-0880-6

Badaut, J., Lasbennes, F., Magistretti, P. J., and Regli, L. (2002). Aquaporins in brain: distribution, physiology, and pathophysiology. *J. Cereb. Blood Flow Metab.* 22, 367–378. doi: 10.1097/00004647-200204000-00001

Bankhead, P., Loughrey, M. B., Fernández, J. A., Dombrowski, Y., McArt, D. G., Dunne, P. D., et al. (2017). QuPath: open source software for digital pathology image analysis. *Sci. Rep.* 7:16878. doi: 10.1038/s41598-017-17204-5

Bonneh-Barkay, D., Bissel, S. J., Kofler, J., Starkey, A., Wang, G., and Wiley, C. A. (2012). Astrocyte and macrophage regulation of YKL-40 expression and cellular response in neuroinflammation. *Brain Pathol.* 22, 530–546. doi: 10.1111/j.1750-3639.2011.00550.x

Bonneh-Barkay, D., Wang, G., Starkey, A., Hamilton, R. L., and Wiley, C. A. (2010). *In vivo* CHI3L1 (YKL-40) expression in astrocytes in acute and chronic neurological diseases. *J. Neuroinflammation* 7:34. doi: 10.1186/1742-2094-7-34

Cekanaviciute, E., and Buckwalter, M. S. (2016). Astrocytes: integrative regulators of Neuroinflammation in stroke and other neurological diseases. *Neurotherapeutics* 13, 685–701. doi: 10.1007/s13311-016-0477-8

Chen, F., Swartzlander, D. B., Ghosh, A., Fryer, J. D., Wang, B., and Zheng, H. (2021). Clusterin secreted from astrocyte promotes excitatory synaptic transmission and ameliorates Alzheimer's disease neuropathology. *Mol. Neurodegener.* 16:5. doi: 10.1186/s13024-021-00426-7

Chen, X., Yin, Y., Gao, H., Guo, Y., Dong, Z., Wang, X., et al. (2020). Clinical utility of in-house metagenomic next-generation sequencing for the diagnosis of lower respiratory tract infections and analysis of the host immune response. *Clin. Infect. Dis.* 71, S416–S426. doi: 10.1093/cid/ciaa1516

Chhatbar, C., Detje, C. N., Grabski, E., Borst, K., Spanier, J., Ghita, L., et al. (2018). Type I interferon receptor Signaling of neurons and astrocytes regulates microglia activation during viral encephalitis. *Cell Rep.* 25, 118–129.e4. doi: 10.1016/j.celrep.2018.09.003

Choi, D. W., and Rothman, S. M. (1990). The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* 13, 171–182. doi: 10.1146/annurev.ne.13.030190.001131

Colombo, D., Falasca, L., Marchioni, L., Tammaro, A., Adebanjo, G. A. R., Ippolito, G., et al. (2021). Neuropathology and inflammatory cell characterization in 10 Autoptic COVID-19 brains. *Cells* 10:2262. doi: 10.3390/cells10092262

Davis, B. M., Salinas-Navarro, M., Cordeiro, M. F., Moons, L., and De Groef, L. (2017). Characterizing microglia activation: a spatial statistics approach to maximize information extraction. *Sci. Rep.* 7:1576. doi: 10.1111/j.1755-3768.2017.03623

Deigendesch, N., Sironi, L., Kutza, M., Wischnewski, S., Fuchs, V., Hench, J., et al. (2020). Correlates of critical illness-related encephalopathy predominate postmortem COVID-19 neuropathology. *Acta Neuropathol.* 140, 583–586. doi: 10.1007/s00401-020-02213-y

Eid, T., Lee, T. S. W., Thomas, M. J., Amiry-Moghaddam, M., Bjørnsen, L. P., Spencer, D. D., et al. (2005). Loss of perivascular aquaporin 4 may underlie deficient water and K+ homeostasis in the human epileptogenic hippocampus. *Proc. Natl. Acad. Sci. U. S. A.* 102, 1193–1198. doi: 10.1073/pnas.0409308102

Escartin, C., Galea, E., Lakatos, A., O'Callaghan, J. P., Petzold, G. C., Serrano-Pozo, A., et al. (2021). Reactive astrocyte nomenclature, definitions, and future directions. *Nat. Neurosci.* 24, 312–325. doi: 10.1038/s41593-020-00783-4

Fabbri, V. P., Foschini, M. P., Lazzarotto, T., Gabrielli, L., Cenacchi, G., Gallo, C., et al. (2021). Brain ischemic injury in COVID-19-infected patients: a series of 10 post-mortem cases. *Brain Pathol.* 31, 205–210. doi: 10.1111/bpa.12901

Ferrari-Souza, J. P., Ferreira, P. C. L., Bellaver, B., Tissot, C., Wang, Y. T., Leffa, D. T., et al. (2022). Astrocyte biomarker signatures of amyloid-β and tau pathologies in Alzheimer's disease. *Mol. Psychiatry* 27, 4781–4789. doi: 10.1038/s41380-022-01716-2

Hansen, D. V., Hanson, J. E., and Sheng, M. (2018). Microglia in Alzheimer's disease. *J. Cell Biol.* 217, 459–472. doi: 10.1083/jcb.201709069

Konopka, K. E., Nguyen, T., Jentzen, J. M., Rayes, O., Schmidt, C. J., Wilson, A. M., et al. (2020). Diffuse alveolar damage (DAD) resulting from coronavirus disease 2019 infection is morphologically indistinguishable from other causes of DAD. *Histopathology* 77, 570–578. doi: 10.1111/his.14180

Kwon, H. S., and Koh, S. H. (2020). Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener* 9:42. doi: 10.1186/s40035-020-00221-2

Lananna, B. V., McKee, C. A., King, M. W., del-Aguila, J. L., Dimitry, J. M., Farias, F. H. G., et al. (2020). Chi3l1/YKL-40 is controlled by the astrocyte circadian clock and regulates neuroinflammation and Alzheimer's disease pathogenesis. *Sci. Transl. Med.* 12:eaax3519. doi: 10.1126/scitranslmed.aax3519

Lê, S., Josse, J., and Husson, F. (2008). FactoMineR: a package for multivariate analysis. *J. Stat. Softw.* 25, 1–18.

Leyh, J., Paeschke, S., Mages, B., Michalski, D., Nowicki, M., Bechmann, I., et al. (2021). Classification of microglial morphological phenotypes using machine learning. *Front. Cell. Neurosci.* 15:701673. doi: 10.3389/fncel.2021.701673

Liddelow, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L., et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* 541, 481–487. doi: 10.1038/nature21029

Llorens, F., Thüne, K., Tahir, W., Kanata, E., Diaz-Lucena, D., Xanthopoulos, K., et al. (2017). YKL-40 in the brain and cerebrospinal fluid of neurodegenerative dementias. *Mol. Neurodegener.* 12:83. doi: 10.1186/s13024-017-0226-4

Maiese, A., Manetti, A. C., Bosetti, C., del Duca, F., la Russa, R., Frati, P., et al. (2021). SARS-CoV-2 and the brain: a review of the current knowledge on neuropathology in COVID-19. *Brain Pathol.* 31:e13013. doi: 10.1111/bpa.13013

Mao, L., Jin, H., Wang, M., Hu, Y., Chen, S., He, Q., et al. (2020). Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol* 77, 683–690. doi: 10.1001/jamaneurol.2020.1127

Matejuk, A., and Ransohoff, R. M. (2020). Crosstalk between astrocytes and microglia: an overview. *Front. Immunol.* 11:1416. doi: 10.3389/fimmu.2020.01416

Matschke, J., Lütgehetmann, M., Hagel, C., Sperhake, J. P., Schröder, A. S., Edler, C., et al. (2020). Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* 19, 919–929. doi: 10.1016/S1474-4422(20)30308-2

Muzio, L., Viotti, A., and Martino, G. (2021). Microglia in Neuroinflammation and neurodegeneration: from understanding to therapy. *Front. Neurosci.* 15:742065. doi: 10.3389/fnins.2021.742065

Nielsen, S., Arnulf Nagelhus, E., Amiry-Moghaddam, M., Bourque, C., Agre, P., and Petter Ottersen, O. (1997). Specialized membrane domains for water transport in glial cells: high-resolution Immunogold Cytochemistry of Aquaporin-4 in rat brain. *J. Neurosci.* 17, 171–180. doi: 10.1523/JNEUROSCI.17-01-00171.1997

Pantoni, L., Garcia, J. H., and Gutierrez, J. A. Cerebral white matter is highly vulnerable to ischemia. *Stroke* 27, 1641–1646. discussion 1647 (1996), doi: 10.1161/01.STR.27.9.1641

Papadopoulos, M. C., and Verkman, A. S. (2008). Potential utility of aquaporin modulators for therapy of brain disorders. *Prog. Brain Res.* 170, 589–601. doi: 10.1016/S0079-6123(08)00446-9

Querol-Vilaseca, M., Colom-Cadena, M., Pegueroles, J., San Martín-Paniello, C., Clarimon, J., Belbin, O., et al. (2017). YKL-40 (Chitinase 3-like I) is expressed in a subset of astrocytes in Alzheimer's disease and other tauopathies. *J. Neuroinflammation* 14:118. doi: 10.1186/s12974-017-0893-7

Schweighofer, N., Lang, E. J., and Kawato, M. (2013). Role of the olivo-cerebellar complex in motor learning and control. *Front Neural Circuits* 7:94. doi: 10.3389/fncir.2013.00094

Shi, W. Z., Zhao, C. Z., Zhao, B., Shi, Q. J., Zhang, L. H., Wang, Y. F., et al. (2012). Aggravated inflammation and increased expression of cysteinyl leukotriene receptors in the brain after focal cerebral ischemia in AQP4-deficient mice. *Neurosci. Bull.* 28, 680–692. doi: 10.1007/s12264-012-1281-z

Sofroniew, M. V., and Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathol.* 119, 7–35. doi: 10.1007/s00401-009-0619-8

Solomon, I. H., Normandin, E., Bhattacharyya, S., Mukerji, S. S., Keller, K., Ali, A. S., et al. (2020). Neuropathological features of Covid-19. *N. Engl. J. Med.* 383, 989–992. doi: 10.1056/NEJMc2019373

Sosunov, A. A., Wu, X., Tsankova, N. M., Guilfoyle, E., McKhann, G. M. II, and Goldman, J. E. (2014). Phenotypic heterogeneity and plasticity of isocortical and hippocampal astrocytes in the human brain. *J. Neurosci.* 34, 2285–2298. doi: 10.1523/JNEUROSCI.4037-13.2014

Stankovic, R. K., Chung, R. S., and Penkowa, M. (2007). Metallothioneins I and II: neuroprotective significance during CNS pathology. *Int. J. Biochem. Cell Biol.* 39, 484–489. doi: 10.1016/j.biocel.2006.09.010

Steiner, E., Enzmann, G. U., Lin, S., Ghavampour, S., Hannocks, M. J., Zuber, B., et al. (2012). Loss of astrocyte polarization upon transient focal brain ischemia as a possible mechanism to counteract early edema formation. *Glia* 60, 1646–1659. doi: 10.1002/glia.22383

Swenson, K. E., and Swenson, E. R. (2021). Pathophysiology of acute respiratory distress syndrome and COVID-19 lung injury. *Crit. Care Clin.* 37, 749–776. doi: 10.1016/j.ccc.2021.05.003

Takahashi, K., Rochford, C. D., and Neumann, H. (2005). Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. *J. Exp. Med.* 201, 647–657. doi: 10.1084/jem.20041611

Thakur, K. T., Miller, E. H., Glendinning, M. D., al-Dalahmah, O., Banu, M. A., Boehme, A. K., et al. (2021). COVID-19 neuropathology at Columbia University Irving medical Center/New York Presbyterian hospital. *Brain* 144, 2696–2708. doi: 10.1093/brain/awab148

Tomas-Camardiel, M., Venero, J. L., de Pablos, R. M., Rite, I., Machado, A., and Cano, J. (2004). *In vivo* expression of aquaporin-4 by reactive microglia. *J. Neurochem.* 91, 891–899. doi: 10.1111/j.1471-4159.2004.02759.x

Tourdias, T., Mori, N., Dragonu, I., Cassagno, N., Boiziau, C., Aussudre, J., et al. (2011). Differential aquaporin 4 expression during edema build-up and resolution phases of brain inflammation. *J. Neuroinflammation* 8:143. doi: 10.1186/1742-2094-8-143

Vandenbark, A. A., Offner, H., Matejuk, S., and Matejuk, A. (2021). Microglia and astrocyte involvement in neurodegeneration and brain cancer. *J. Neuroinflammation* 18:298. doi: 10.1186/s12974-021-02355-0

Varatharaj, A., and Galea, I. (2017). The blood-brain barrier in systemic inflammation. *Brain Behav. Immun.* 60, 1–12. doi: 10.1016/j.bbi.2016.03.010

Vella, J., Zammit, C., Di Giovanni, G., Muscat, R., and Valentino, M. (2015). The central role of aquaporins in the pathophysiology of ischemic stroke. *Front. Cell. Neurosci.* 9:108. doi: 10.3389/fncel.2015.00108

von Weyhern, C. H., Kaufmann, I., Neff, F., and Kremer, M. (2020). Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet* 395:e109. doi: 10.1016/S0140-6736(20)31282-4

Wierzba-Bobrowicz, T., Krajewski, P., Tarka, S., Acewicz, A., Felczak, P., Stępień, T., et al. (2021). Neuropathological analysis of the brains of fifty-two patients with COVID-19. *Folia Neuropathol.* 59, 219–231. doi: 10.5114/fn.2021.108829

Wojtas, A. M., Sens, J. P., Kang, S. S., Baker, K. E., Berry, T. J., Kurti, A., et al. (2020). Astrocyte-derived clusterin suppresses amyloid formation *in vivo*. *Mol. Neurodegener.* 15:71. doi: 10.1186/s13024-020-00416-1

Woodburn, S. C., Bollinger, J. L., and Wohleb, E. S. (2021). The semantics of microglia activation: neuroinflammation, homeostasis, and stress. *J. Neuroinflammation* 18:258. doi: 10.1186/s12974-021-02309-6

Zamanian, J. L., Xu, L., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., et al. (2012). Genomic analysis of reactive astrogliosis. *J. Neurosci.* 32, 6391–6410. doi: 10.1523/JNEUROSCI.6221-11.2012

Zhao, T., Su, Z., Li, Y., Zhang, X., and You, Q. (2020). Chitinase-3 like-protein-1 function and its role in diseases. *Signal Transduct. Target. Ther.* 5:201. doi: 10.1038/s41392-020-00303-7

frontiers | Frontiers in Neuroscience

TYPE Review
PUBLISHED 30 August 2023
DOI 10.3389/fnins.2023.1198327

OPEN ACCESS

EDITED BY
Gonzalo Emiliano Aranda-Abreu,
Universidad Veracruzana, Mexico

REVIEWED BY
Sergiu Chirila,
Ovidius University, Romania
Georgios E. Manousakis,
University of Minnesota, United States

*CORRESPONDENCE
Ashir Mehra
✉ ashir.mehra@health.missouri.edu

RECEIVED 01 April 2023
ACCEPTED 31 July 2023
PUBLISHED 30 August 2023

CITATION
Gomez F, Mehra A, Ensrud E, Diedrich D and Laudanski K (2023) COVID-19: a modern trigger for Guillain-Barre syndrome, myasthenia gravis, and small fiber neuropathy.
Front. Neurosci. 17:1198327.
doi: 10.3389/fnins.2023.1198327

COVID-19: a modern trigger for Guillain-Barre syndrome, myasthenia gravis, and small fiber neuropathy

Francisco Gomez[1], Ashir Mehra[1]*, Erik Ensrud[1], Daniel Diedrich[2] and Krzysztof Laudanski[2]

[1]Department of Neurology, University of Missouri, Columbia, MO, United States, [2]Department of Anesthesiology and Perioperative Care, Mayo Clinic, Rochester, MN, United States

COVID-19 infection has had a profound impact on society. During the initial phase of the pandemic, there were several suggestions that COVID-19 may lead to acute and protracted neurologic sequelae. For example, peripheral neuropathies exhibited distinctive features as compared to those observed in critical care illness. The peripheral nervous system, lacking the protection afforded by the blood–brain barrier, has been a particular site of sequelae and complications subsequent to COVID-19 infection, including Guillain-Barre syndrome, myasthenia gravis, and small fiber neuropathy. We will discuss these disorders in terms of their clinical manifestations, diagnosis, and treatment as well as the pathophysiology in relation to COVID-19.

KEYWORDS

COVID-19, SARS-CoV-2, peripheral neuropathy, Guillain-Barre, GBS, myasthenia gravis, small fiber neuropathy

Introduction

Severe manifestations of COVID-19 may be partly accounted for by an autoimmune reaction mediated by a dysregulated network of circulating proinflammatory cytokines and inflammatory markers, including IL-1β, IL-6, IL-2, IL-8, IL-17, TNF-α, C-reactive protein, D-dimer, and antibodies (da Silva et al., 2021; Qin et al., 2022). It has been postulated that the resulting hyperinflammatory state causes endothelial dysfunction with increased vascular permeability, and hypercoagulability. These may progress to more severe complications such as acute respiratory distress syndrome and multi-organ failure (Ginikopoulou, 2022; Qin et al., 2022). Additionally, the inflammatory state may incite damage to the unprotected nerve fibers and prolonged resolution may result in ongoing exposure to non-specific inflammatory reactions. The emergence of autoimmunity can occur via numerous mechanisms; (a) if there is failure to suppress autoreactive clones (breakdown of immune tolerance measures) (b) if viral proteins that share an anatomical resemblance to innate proteins trigger an immune response (molecular mimicry) (c) if progressive infection leads to epitope diversification and thereby provoking an autoimmune response (Jovanova-Nesic and Shoenfeld, 2006; Morsy, 2020; Jacob et al., 2022). Interestingly, other evidence suggested autoreactive molecules resembling severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) like NCAM-1 were elevated (Laudanski et al., 2021). The present manuscript describes the pathogenesis, clinical presentation, and management of three neurological disorders in the setting of recent SARS-CoV-2; namely these include Guillain-Barre

Syndrome (GBS), Myasthenia Gravis (MG), and Small Fiber Neuropathy (SFN).

Guillain-Barre Syndrome (GBS) and Myasthenia Gravis (MG) are recognized autoimmune illnesses. Likewise, because some cases of SFN are immune mediated, they can be triggered by COVID-19 as well (Zhou, 2019). Thus, these disorders of the peripheral nervous system may be caused or worsened by the dysregulated systemic immune response to COVID-19 infection or its aftermath. On the other side, if dysregulated response underlies post-COVID-19 peripheral neuropathies, immunomodulating strategies commonly employed in the treatment of neurological autoimmune diseases would ameliorate post-COVID-19 neurological sequelae.

Methods

Search strategy and selection criteria: PubMed and Google Scholar searches were employed utilizing the following keywords: "COVID-19" "Sars-COVID-19" in combinations with "Peripheral Neuropathy," "GBS," "Guillain-Barre," "MG," and "SFN" was conducted for the years 2018–2022. Additional review articles explaining previously established pathophysiology for said diseases was included, dated prior to 2018.

Review articles and meta-analyses were included on rare occasions to provide readers with further details and references. Articles were evaluated for relevancy related to concomitant establishment of the above described neurologic and COVID diagnose by EE, AM, and FG. FG also served as the final arbiter for inclusion. Relevant references from these publications that focused on COVID-19 pathophysiology were also included.

Discussion

COVID-19 and Guillain Barre-syndrome

Definition

Guillain-Barré syndrome (GBS) comprises a gamut of autoimmune polyneuropathies varying in pathophysiology and symptoms (Fokke et al., 2013; Guidon and Amato, 2020). The hallmark clinical findings in these disorders are flaccid weakness and hyporeflexia (Shahrizaila et al., 2021). GBS can be broadly divided into demyelinating and axonal variants depending on the peripheral nerve site of autoimmune response (Shang et al., 2021).

Epidemiology

The incidence of pre-covid GBS is estimated at 100,000 new cases per year worldwide with regional variability. The incidence increases with age and is higher in men (Shahrizaila et al., 2021). A precipitating infection within 4 weeks often precedes GBS. Known associated viruses including Influenza A, Epstein–Barr, hepatitis E, and Zika have all been reported and well described (Shahrizaila et al., 2021; Shang et al., 2021). GBS associated COVID-19 cases have followed a similar epidemiological pattern, with older men, averaging 61 years old, being affected more frequently than women, at a nearly 2:1 ratio in case series (Pimentel et al., 2023). Similarly, a lag between the COVID-19 infection and GBS symptoms onset averages 14–19 days which is similar to previously described precipitating infections (Shahrizaila et al., 2021; Aladawi et al., 2022; Pimentel et al., 2023). These similarities indicate that the pathophysiology of GBS in the setting of recent COVID-19 is similar to GBS triggered by other infectious agents (Aladawi et al., 2022).

Reports have varied on the association between GBS and COVID-19, with no early conclusive evidence of an increased risk for GBS (Suh and Amato, 2021). Rather, a cohort study conducted in Britain found a decrease in GBS incidence during the pandemic, which the authors attributed to a generalized decrease in the incidence of precipitating infections due to the adopted lockdown measures (Keddie et al., 2021). Additionally, it is possible that GBS cases were under-reported during said period. Notwithstanding, the sheer number of reported cases of GBS in association with prior COVID-19 infection does suggest an association to the authors. However, most reports detailing the association between COVID-19 and GBS arose early in the epidemic (Abu-Rumeileh et al., 2020; Caress et al., 2020; Paterson et al., 2020; Toscano et al., 2020). Further assays have shown a possible slight increase, wherein a multi-center study involving 61 emergency departments in Spain found a slight increase in relative frequency of GBS among COVID (0.15‰) vs. non-COVID (0.02‰) patients (odds ratio [OR] = 6.30, 95% confidence interval [CI] = 3.18–12.5) The authors concluded that GBS is not often a debuting presentation for COVID infection (Fragiel et al., 2020).

Considering that GBS is an autoimmune disease secondary to a trigger that activates the immune system, it is unsurprising COVID-19 infection is linked to an increased risk of GBS (Kanou et al., 2022). Unfortunately, several of said reports were confounded by the concomitant use of experimental therapies for COVID-19 including steroids, antiviral medications as well as other sequelae of COVID-19 such as critical care illness neuropathy (FINSTERER et al., 2021). Furthermore, establishing a link is further complicated by the concomitant administration of vaccines which may trigger non-specific immune reactions with potential to impact the nervous system. In any case, the frequency of post-COVID-19 vaccine related GBS is lower than GBS provoked by COVID19 infection (Patone et al., 2021).

Pathogenesis

Traditionally, GBS is thought to arise from molecular mimicry between offending (infectious, vaccine, drugs) agents and peripheral neuron gangliosides leading to the generation of anti-ganglioside antibodies (Guidon and Amato, 2020; Suh and Amato, 2021). It is important to note that various forms of GBS have unique pathogenic mechanisms. The most common form of GBS, Acute Inflammatory Demyelinating Polyneuropathy (AIDP) occurs because of T-cell mediated cytokine storm and does not routinely have detectable antibodies (Shang et al., 2021). Conversely, the axonal variants of GBS, namely Acute Motor Axonal Neuropathy (AMAN) and Acute Motor and Sensory Axonal Neuropathy (AMSAN) are associated with the traditional anti-ganglioside antibodies (Shang et al., 2021; Figure 1).

Known GBS specific auto-antibodies have been found in COVID-19-related GBS. Sporadic cases with positive auto-antibodies such as anti-GM1, GM2, GD1a, GD1b, GD3, GM1, GT1b or contactin have been found only rarely (Suh and Amato, 2021; Taga and Lauria, 2022). Further meta-analysis reported antiganglioside antibodies in merely 2% of cases, the most common

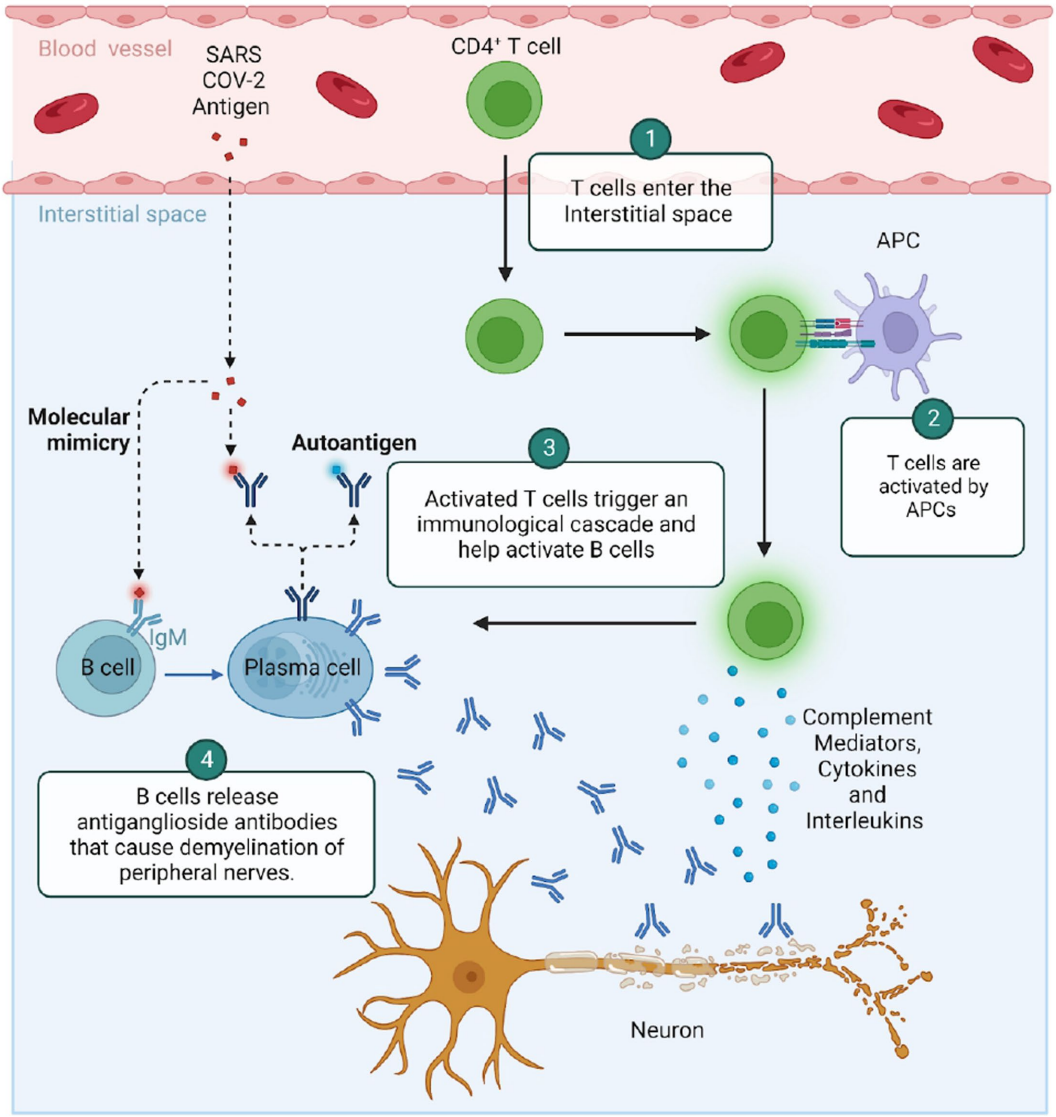


FIGURE 1
Suspected pathogenesis of Guillain-Barré syndrome in the setting of COVID-19.

being anti-GD1b IgG (Pimentel et al., 2023). *In vitro* assays have identified molecular similarities between COVID-19-encoded protein and host neuronal proteins raising the potential of autoimmune mimicry (da Silva et al., 2021), similarly, cross reactivity between COVID-19 neutralizing and neuronal epitopes has been reported (Kreye et al., 2020). *Per contra*, in-silico peptidome studies showed conflicting results (da Silva et al., 2021). One analysis identified molecular structural similarities at the molecular level between COVID-19 peptide sequences and adhesion molecules expressed by neurons and Schwann cells Another noted potential for molecular mimicry between COVID-19 and heat shock proteins-60 and -90 (Hsp) (Lucchese and Flöel, 2020; Shang et al., 2021). Both Hsp were linked to the emergence of GBS. An example of potential mimicry is a single viral open reading frame (ORF1) protein, a part being coded by SARS-CoV-2 genome that shares a sequence with human mono-ADP-ribosyltransferase (PARP14), with a 32% match suggesting structural mimicry (Keddie et al., 2021). These suggestions are not universal as another study found no homologies between viral membrane, spike or nucleocapsid COVID-19 encoded peptides and those in human neural tissue. Thus, the matter of whether a specific COVID-19 encoded protein is generally causative of GBS remains to be fully ascertained. It has been suggested that given para-infectious or a post-infectious symptomatic debut, the trigger for GBS associated with COVID may be overactivation of the systemic inflammatory response rather than specific epitope per se causative of molecular mimicry. These authors cite the abundance of circulating IL-6 and other inflammatory cytokines to be a more likely suspect (Ahmad et al., 2022). Another proposed etiopathogenesis suggests it is feasible that virus neurotropism for olfactory bulb cells with resulting inflammation and demyelination leads not only to described anosmia and dysgeusia but expression of hitherto unexposed epitopes, including GD1b (Fragiel et al., 2020).

There is also the potential that non-specific mimicry is induced via antibody activation. *Campylobacter jejuni*, is the most studied model in the axonal form of GBS with ample evidence supportive of molecular mimicry (Suh and Amato, 2021). In this model, B and T

cells are activated by antigen presenting cells by processing the offending pathogen and selecting reactive T and B cells to produce antibodies via hypermutation mechanism. However, the fault in the system can cause B cells to produce antibodies that are avid for ganglioside antigens. These immunoglobulins bind proteins on Schwann cell Ranvier nodes triggering complement and attracting acquired immunity components. Subsequently, neuronal axolemma is damaged resulting in primary neuropathy.

Via independent and complementary mechanisms, co-activated T cells produce proinflammatory cytokines and chemokines that facilitate entry of macrophages into the neural tissue (Shang et al., 2021). Prior histologic examinations in AIDP have demonstrated neural T-cell and macrophage infiltration, as well as complement deposition in Schwann cells while acute motor axonal neuropathy (AMAN) variants exhibit primary macrophage-mediated axonal injury with scarce demyelination or T-cell infiltration (Shahrizaila et al., 2021). In COVID-19-related AIDP, one small histological series demonstrated no viral invasion but primarily $CD68^{+++}$ histiocytes, often accompanied by cytotoxic $CD8^{++}$ T-cells and less frequently helper $CD4^{+}$ T cells. This process is often fueled by interferons (Suh et al., 2021). This leukocyte composition demonstrates an activated immune system with little control over its response. The outcome is a damage to Schwann cells with subsequent deterioration of the peripheral nerve function. In an interesting observation, perivascular inflammation was demonstrated in 67% of samples, with endoneurial infiltrates in only 11%. This may suggest a potential link between endothelial inflammation and peripheral nerve function but more definite studies are needed.

Clinical presentation

Classical Guillain-Barré syndrome comprises flaccid ascending limb weakness with hyporeflexia. Miller-Fisher Syndrome is a common variant consisting of hyporreflexia, accompanied by bilateral ophthalmoplegia, and ataxia. Other less common presentations include facial diplegia or pharyngeal-cervical-brachial paresis (Shahrizaila et al., 2021). Thus, GBS should be suspected in patients with rapidly progressive bilateral leg or arm paresis in the absence of CNS involvement. Concomitant distal paraesthesias or hypoesthesia are common in the sensorimotor variant (AIDP) (Leonhard et al., 2019). Concurrent respiratory paresis in GBS and COVID-19 infection necessitates early recognition given its rapidly progressive nature and potential tractability (Sriwastava et al., 2021).

COVID-19 related GBS infection most commonly presents with the classic sensorimotor variant, often accompanied by facial paresis. Electrophysiological testing showed a preponderance of demyelinating patterns (Aladawi et al., 2022). One single center study comparing 20 patients with COVID + GBS and GBS alone, those patients with concomitant COVID presented with statistically significant higher disability upon admission, higher incidence of cranial neuropathies and lower lymphocyte count (Ahmad et al., 2022). Rare variants such as the Pharyngo-cervico-brachial variant of GBS have been reported (Table 1; Randhawa et al., 2021).

Respiratory failure in GBS can be caused by a combination of respiratory muscle paresis, airway compromise or an inability to control secretions (Shang et al., 2021). COVID-19 related GBS cases have a similar presentation and are expectedly at risk for respiratory failure (Aladawi et al., 2022). In one meta-analysis involving 436 patients, respiratory muscle paresis was described in 18% of the study population wherein 10% progressed to frank respiratory failure necessitating endotracheal intubation and mechanical ventilation (Pimentel et al., 2023). Although this may make it appear that the incidence of respiratory failure in COVID-19 related GBS necessitating mechanical ventilation would appear slightly lower than the 30% as reported in pre-COVID-19 literature (Shang et al., 2021), it must be noted that authors Pimental et al. specifically note that an additional 54 of the reviewed 436 patients were admitted to ICU for unspecified reasons and may have had respiratory failure (Pimentel et al., 2023).

TABLE 1 Reported symptomatology in COVID-19-related AIDP.

Symptoms	Aladawi, $n = 99$
Hyporreflexia	93% ($n = 93$)
Paraparesis	82% ($n = 81$)
Sensory symptoms	41% ($n = 41$)
Quadriparesis	65% ($n = 64$)
Facial Palsy	42% ($n = 42$)
Dysphagia	18% ($n = 18$)
Bulbar paresis	12% ($n = 12$)
Dysarthria	11% ($n = 11$)
Diplopia	11% ($n = 11$)
Ophthalmoplegia	11% ($n = 11$)
Ataxia	18% ($n = 18$)

Symptoms encountered in COVID-19-Related GBS (Aladawi et al., 2022). Of note 84/99 patients included by Aladawi reportedly had Brighton criteria for level 1–3 certainty.

Autonomic failure is another severe complication of GBS, associated with increased mortality and length of ICU stay previously described in 3–38% of GBS patients (Chakraborty et al., 2019; Leonhard et al., 2019). One meta-analysis described dysautonomia in COVID-19 related GBS in addition to the following (with frequency); hypotension (6.9%), arrhythmias (6%), urinary retention or incontinence (5%), hypertension (4%), fecal incontinence or diarrhea (3%) (Pimentel et al., 2023).

In general, the mortality from GBS is estimated at 5% and complications from the disease are common, with up to 20% of patients unable to walk independently at 1 year (Shahrizaila et al., 2021). A recent meta analysis showed COVID-19-related GBS patients fared worse with 9% mortality and 22% showing residual paresis (Pimentel et al., 2023).

Diagnostics

The diagnosis of GBS by biomarkers alone remains difficult with numerous antibodies being described. Negative antibody testing does not rule out GBS (Leonhard et al., 2019). That being said, antibodies can be useful in distinguishing between variants such as Miller-Fisher Syndrome in which AntiGQ1b are positive in 90% of cases (Leonhard et al., 2019). Other variants have less specific associations, AIDP is associated with anti-LM1 and Gal-C, while AMAN is associated with Anti-GM1, GM2, GD1b, GT1b, GM3, GD1a, and GalNac-GD1a (Shang et al., 2021). Again, antiganglioside Ab have been found very rarely in COVID-19 related GBS cases (Pimentel et al., 2023). A systematic review conducted by Aladawi et al. (2022) demonstrated that only 14% of COVID-19 associated GBS had demonstratable antiganglioside antibodies. Magnetic Resonance Imaging (MRI) which demonstrates lumbar radicular enhancement with 83%

sensitivity in the acute phase (Shahrizaila et al., 2021). In a small case series, Berciano et al., 2017 employed ultrasound and described C5–C7 cervical radicular enlargement. Improvements in those parameters may correlate with the clinical course (Berciano et al., 2017).

Therefore, diagnosis of GBS relies largely on clinical manifestations. The Brighton Criteria remain the most widely adopted, wherein cases are divided into levels of certainty 1 through 4. Level 1 confers the highest degree of certainty but necessitates positive Cerebrospinal fluid (CSF) or electrodiagnostic findings consistent with the disease.

Cerebrospinal fluid testing

Cerebrospinal fluid findings consistent with the disease include <50/μl cells and elevated protein levels, termed cyto-albumin dissociation (Fokke et al., 2013). However, CSF results may not be diagnostic in the early course of the disease and up to 50% of patients may exhibit normal findings in the first week, and 30% in the second (Leonhard et al., 2019).

Electrodiagnostic testing

Electrodiagnostic tests can be helpful in differentiating GBS variants, but can be falsely negative within the first week of symptoms (Leonhard et al., 2019) hence studies can be performed (Rajabally et al., 2014). Furthermore, EMG can distinguish different types of GBS. AMAN demonstrates reversible conduction failure and may occasionally show reduced compound muscle action potentials (Shang et al., 2021). AIDP exhibits slowed sensory motor nerve conductions, with early F wave abnormalities and later an increased distal response latency (Rajabally et al., 2014; Shang et al., 2021). A preponderance of demyelinating AIDP patterns was encountered in 77% of patients, followed by motor sensory axonal variants in 13%, and motor axonal variants in 10% (Aladawi et al., 2022).

This variant distribution is quite similar to that previously described in the literature; wherein AIDP reported 72%; of cases and AMAN 14–18% (Rajabally et al., 2014).

Treatment

The mainstay of GBS treatment is immunomodulation via primarily immunoglobulin removal by plasma exchange (PLEX) or increased degradation with intravenous immunoglobulins (IVIG). Both have shown nearly equal effectiveness (Leonhard et al., 2019), improving the speed of recovery but not necessarily disease progression (Shahrizaila et al., 2021).

Plasma exchange

Plasma exchange is an extracorporeal therapeutic technique where plasma is removed from whole blood via membrane filtration, centrifugation, or a combination of both (Gwathmey et al., 2014; Fernández-Zarzoso et al., 2019; Bauer et al., 2022). The patient then receives replacement fluid and cellular blood components. In general, PLEX allows for the removal of various pathogenic substances or molecules including autoantibodies, immune complexes, and toxins (Fernández-Zarzoso et al., 2019). It is believed that predominant benefit of PLEX in GBS is related to diminished titer of the autoantibodies and removal of a causative agent. Unfortunately, large fluid shifts during implementation of PLEX may cause hemodynamic instability. In GBS patients with dysautonomia, PLEX is more problematic as the autonomic system has an impaired ability to compensate for large fluid shifts and the therapy may lead to an increase in hypotensive events (Shahrizaila et al., 2021).

Plasma exchange is a mainstay of GBS treatment, as a Level I recommendation with grade A evidence (Fernández-Zarzoso et al., 2019; Bauer et al., 2022). Dosing recommendations vary between four and seven sessions dosed at 50 mL/kg every other day (Fernández-Zarzoso et al., 2019; Shahrizaila et al., 2021). This is theorized to be a consequence of the accumulation of newly synthesized antibodies (Melzer et al., 2016). Elevated necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, levels have been reported in severe COVID-19 (Lu et al., 2021). PLEX may exert benefits via direct removal of proinflammatory cytokines (Ginikopoulou, 2022; Qin et al., 2022). One early study found a significantly decreased D-dimer, ferritin, CRP, IL-6 and procalcitonin in COVID-19 patients who underwent PLEX (Gucyetmez et al., 2020). Furthermore, it has been suggested that convalescent plasma used as the replacement solution could possibly enhance derived benefits (Ginikopoulou, 2022).

Multiple studies have found PLEX to be beneficial, or at minimum safe in SARS-Cov-2 infections (Khamis et al., 2020; Faqihi et al., 2021; Kamran et al., 2021; Cegolon et al., 2022). Thus, it would be reasonable to recommend PLEX in the setting of COVID-19-related GBS.

Intravenous immunoglobulins

Intravenous immunoglobulins is a blood product consisting of pooled healthy donor immunoglobulins with pleiotropic immunomodulating and anti-inflammatory effects (Shang et al., 2021). While IVIG's mechanism of action remains to be fully elucidated, several hypotheses have been described or proposed. These include increased antibody catabolism, blockade of autoantibody Fc tail region, complement protein scavenging and inhibition, and macrophage and mononuclear phagocyte inhibition (Norris et al., 2020). Antiganglioside antibody dimerization leading to decreased serum immunogenicity has been posited as an additional mechanism in GBS patients (Shang et al., 2021).

For GBS, daily administration at 2 g/kg over 5 days has shown efficacy (Leonhard et al., 2019; Shahrizaila et al., 2021). IVIG may be preferable to PLEX in patients with dysautonomia (Shahrizaila et al., 2021). Side effects of IVIG include anaphylaxis in patients with pre-existing IgA deficiency, aseptic meningitis, headache hypertension, pulmonary edema and dermatitis (Melzer et al., 2016). Hepatic dysfunction and thrombosis are less commonly encountered (Shahrizaila et al., 2021).

Numerous meta-analyses and case series have demonstrated that IVIG is safe to administer in COVID-19 patients (Cao et al., 2020; Xiang et al., 2021; Marcec et al., 2022).

COVID-19 Vaccination and Guillain Barre-Syndrome

Since 1976, vaccinations against viral infections have been linked to the development of GBS where an increase in cases was observed after a widespread vaccination program was undertaken in the US (Schonberger et al., 1979). Further studies suggested an increased incidence of one additional GBS case per 1 million influenza vaccinations (Leonhard et al., 2019). Thus far, one multicenter case series reported 9 cases of GBS following COVID-19 vaccination but the denominator is unclear (Karimi et al., 2021). A meta-analysis, including data from 17 countries, has reported a total

of 88 cases of GBS. Of note, 63% of these patients were male, and neurological symptoms appeared 14 days post-vaccination in keeping with previously reported GBS epidemiology (Abolmaali et al., 2022). The Center for Disease Control did report an increased risk of GBS among adults who received the J&J/Janssen COVID-19 vaccination but not after Pfizer-BioNTech or Moderna COVID-19 vaccination (Centers for Disease Control and Prevention, 2019; Hanson et al., 2022). Specifically, Hanson et al. reported that the risk of developing GBS within 21 days of Ad.26.COV2.S (Janssen) vaccine was 32.4 per 100,000 person-years. Patients that received mRNA vaccines though showed a much lower rate of 1.3 per 100,000 person-years that was similar to the background (Hanson et al., 2022).

This data is not surprising given vaccines are by design immunogenic, a pathophysiological predisposition to GBS development is plausible and clinicians should be alert to developing symptoms in patients following recent COVID-19 vaccinations. The most important message is that the benefits of vaccine administration continue to outweigh risks in terms of overall mortality and GBS incidence (Abolmaali et al., 2022). Recent meta-analysis including 48 publications including 2,110,441,600 participants revealed COVID vaccine related GBS at a rate of 3.09 per 1 million people within 6 weeks of vaccination, higher to that of the influenza vaccine (Finsterer et al., 2022).

COVID-19 and myasthenia gravis

Definition

Myasthenia gravis is an autoimmune disorder caused by antibodies targeting components of the neuromuscular junction, most commonly postsynaptic acetylcholine receptors leading to paresis (Farmakidis et al., 2018).

Epidemiology

Myasthenia gravis is the most prevalent neuromuscular junction disorder (Gilhus and Verschuuren, 2015; Farmakidis et al., 2018; Bubuioc et al., 2021; Punga et al., 2022). MG has been increasing with an annual incidence in adults estimated to be 10–29/1,000,000 with a prevalence ranging between 100 and 350/1,000,000. Between the ages of 15–64, it is more common in women at a 2:1 ratio, whereas late-onset myasthenia after age 64 has a higher incidence in men (Gilhus and Verschuuren, 2015). A genetic predisposition has been described, wherein siblings or first-degree relatives exhibit a 4.5% increase in risk for developing MG (Melzer et al., 2016). Additionally, chronic immunosuppression or treatment with multiple drugs of this type has been described as a risk factor for the development of COVID-19 or a more severe course (Sanders et al., 2016; Guidon and Amato, 2020).

Pathogenesis

Myasthenia gravis is caused by antibodies binding the neuromuscular junction (NMJ) epitopes within the postsynaptic membrane (Bubuioc et al., 2021). The acetylcholine receptor (AChR) is the most commonly targeted (Gilhus and Verschuuren, 2015). NMJ physiopathology in these cases has been well documented; synapses are impaired via receptor blockage, increased internalization hence decreased receptor availability, and complement deposition leading to distortion of the endplate thus widening of the synaptic cleft. AChR antibody levels correlate with disease severity (Melzer et al., 2016). Other recognized causative antibodies include muscle-specific kinase (MUSK), lipoprotein-related protein 4 (LRP4), agrin, titin, and ryanodine (Bubuioc et al., 2021). The prevalence of said antibodies in one review has been reported as AChR in 80% of patients, MUSK in 4% and LRP4 in 2%, the remaining 5% remaining seronegative (Gilhus and Verschuuren, 2015). Geographic variations have been reported (Punga et al., 2022).

A thymoma is associated with myasthenia gravis in 10–15% of cases (Melzer et al., 2016) wherein AChR auto reactive T-cells escape physiological surveillance and are released, subsequently activating B-cells. Thus, mediastinal imaging is recommended in all patients with this disease (Gilhus and Verschuuren, 2015; Punga et al., 2022). AChR expression by thymic epithelial cells may be incited by a viral infection via cytokine and receptor signaling (Gilhus and Verschuuren, 2015). MG subsequent to a viral infection has been previously reported following Epstein–Barr and Varicella-Zoster infections (Shah et al., 2022). Thus, unsurprisingly, new onset MG after SARS-Cov-2 infection has been reported with patients commonly testing positive for AChR Ab in the setting of ocular and bulbar symptoms (Huber et al., 2020; Restivo et al., 2020; Sriwastava et al., 2020; Assini et al., 2021; Essajee et al., 2021; Karimi et al., 2021). And although cases with positive MUSK antibodies have been documented as well, these appear to be less common (Figure 2) (Assini et al., 2021).

Clinical presentation

Generalized myasthenia

The most common symptoms in MG include fluctuating paresis, and muscle fatigability which is often progressive throughout the day (Gilhus and Verschuuren, 2015; Melzer et al., 2016; Bubuioc et al., 2021). Up to 60% of patients present with ptosis or diplopia, or a combination thereof (Gilhus and Verschuuren, 2015). Generalized myasthenia is described as paresis affecting any muscle groups beyond the ocular muscles. Weakness is most commonly found within bulbar or proximal limb muscle groups (Melzer et al., 2016). Bulbar weakness comprises dysphagia, dysphonia, difficulty chewing and dysphagia (Punga et al., 2022).

Ocular myasthenia

Ocular myasthenia is defined as paresis limited to the extraocular muscles leading to diplopia or ptosis and comprises 10–20% of cases (Melzer et al., 2016; Sanders et al., 2016). Ocular myasthenia is more often seen in patients with AChR antibodies, MUSK+ cases have been described, but are much rarer (Gilhus and Verschuuren, 2015).

Myasthenic crisis

A myasthenic crisis is defined as a rapid life-threatening exacerbation leading to respiratory failure (Sanders et al., 2016; Nelke et al., 2022) Respiratory failure can occur from a loss of airway protection or an inability to clear secretions both of which can occur from bulbar weakness. Respiratory failure can also occur from diaphragmatic paralysis and may affect up to 15% of MG patients (Punga et al., 2022). Infections are a common trigger for myasthenic crisis (Anand et al., 2020; Tugasworo et al., 2022) and associated with worse outcomes (Nelke et al., 2022). Previously used therapies

that were used inappropriately to treat COVID-19, without solid evidence of efficacy, including HCQ and Azithromycin may actually worsen NMJ transmission (Qin et al., 2022). A few publications have reported MG following a SARS-CoV-2 infection and include a small retrospective case series involving 8 patients where MG exacerbation was attributed to a SARS-CoV-2 (Rodrigues et al., 2022). One particular case series reported myasthenic crisis necessitating rescue therapy in 36/91 (40%) of MG patients following COVID-19 (Muppidi et al., 2020). Another case series found more disturbing results with a high mortality (80%) in MG patients whom contracted COVID-19 (Lupica et al., 2022). These results could be attributed to the combined and synergistic effect of critical care illness and COVID-19 pathology.

Diagnostics

AChR antibodies, specifically the binding and modulating varieties, are highly specific for myasthenia gravis, and a positive assay in patients with muscle weakness is considered pathognomonic to the point of obviating electrodiagnostic tests (Gilhus and Verschuuren, 2015). Other detectable antibodies are described under the pathology section but are less common or reliable.

Electrodiagnostic

Electrodiagnostic tests continue to be of value, especially in seronegative patients. Single fiber EMG is the most sensitive test, while low-frequency repetitive nerve stimulation is often considered the first line procedure in patients with synaptic transmission failure (Gilhus and Verschuuren, 2015; Punga et al., 2022). Low-frequency repetitive nerve stimulation (3 Hz) is considered positive when there is a response amplitude decrease at a minimum of 6–10% between the first and fourth elicited compound motor action potentials (Punga et al., 2022). Single fiber electromyography measures muscle jitter, defined as the time interval variation between action potentials, which is increased in MG (Melzer et al., 2016; Punga et al., 2022).

Treatment

Maintenance treatment

Myasthenia gravis management involves enhancement of acetylcholine availability within the NMJ via inhibition of cholinesterase enzymes, or immunosuppression/immunomodulation (Farmakidis et al., 2018). Pyridostigmine, an acetylcholinesterase inhibitor (AChEI) is considered the first line treatment that also improves electrodiagnostic measures (Melzer et al., 2016). In an early randomized trial where the treatment arm (94/188) received pyridostigmine vs. placebo, the initial results demonstrated a tendency towards improved survival of 11.7%. Unfortunately, this trial was halted early due to lack of recruitment (Fragoso-Saavedra et al., 2022). Acetylcholinesterase inhibitors along with corticosteroids or azathioprine are considered first-line treatments (Melzer et al., 2016; Sanders et al., 2016). A variety of immunosuppressive therapies are employed as second line, or steroid sparing agents (Sanders et al., 2016). Notably, patients with ocular myasthenia exhibit a reduced rate of progression to the generalized form with the management strategy (Melzer et al., 2016). MUSK+ patients tend to respond less to acetylcholinesterase inhibitors and IVIG (Melzer et al., 2016; Sanders et al., 2016). However, commonly utilized immunosuppressants may influence COVID-19 outcomes (Rodrigues et al., 2022) (Table 2).

Some immunosuppressive therapies utilized for the management of myasthenia have shown possible dual benefits. Tocilizumab, an IL-6 inhibiting monoclonal antibody indicated for treatment of severe COVID-19 has shown safety and efficacy in two previously refractory MG patients, and safety in a third patient in a small case series (Anand et al., 2020). A meta-analysis including 3,924 patients of which 433 received tocilizumab showed promising results. The treatment arm exhibited a lower adjusted mortality risk of 27.5% vs. 37.1% (95% CI, 21.2–33.8 and 95% CI, 35.5–38.7%, respectively) (Gupta et al., 2021). That being said, Tocilizumab is not yet approved for use in MG and it is still considered an experimental therapy.

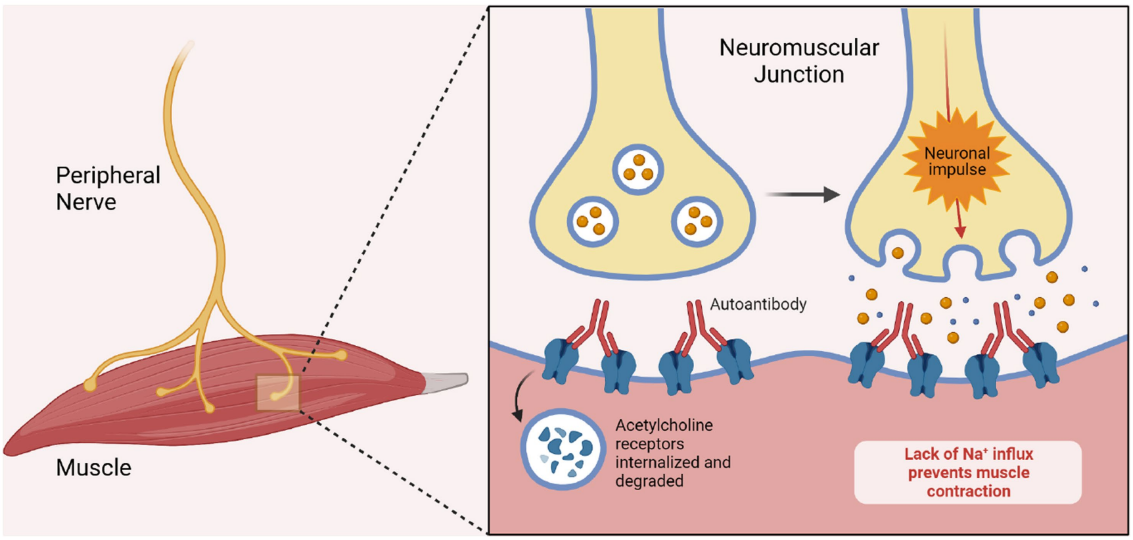


FIGURE 2
Pathogenesis of myasthenia gravis. The image demonstrates the pathogenesis of myasthenia gravis in the setting of ACh Receptor blocking antibodies. The antibodies bind to the post-synaptic acetylcholine receptors and thereby prevent depolarization of the muscular membrane. Biorender.com software.

TABLE 2 Effects of immunosuppressants on COVID-19 mortality, note that some of this data was derived from Rheumatology patients and not exclusive to neuromuscular complications.

Treatment	Mechanism of action	Effect on COVID-19
Corticosteroids (Melzer et al., 2016; Farmakidis et al., 2018; Anand et al., 2020; Korsukewitz et al., 2020; Rodrigues et al., 2022)	Inhibit cytokine response, leukocyte recruitment. T-cell activation and differentiation suppressors	Increased mortality at higher doses
Azathioprine (Melzer et al., 2016; Farmakidis et al., 2018; Anand et al., 2020; Korsukewitz et al., 2020; Strangfeld et al., 2021; Rodrigues et al., 2022)	Purine synthesis suppressant. Inhibits cellular replication, and lymphocyte function	Possibly increased mortality
Cyclophosphamide (Melzer et al., 2016; Farmakidis et al., 2018; Korsukewitz et al., 2020; Strangfeld et al., 2021)	Cytotoxic guanine alkylating agent. Inhibits cell replicating by forming DNA-cross bonds. Marrow suppressant, inhibits B- and T-cells	Increased mortality
Cyclosporine (Farmakidis et al., 2018; Strangfeld et al., 2021; Rodrigues et al., 2022)	Calcineurin activation inhibitor, suppresses IL-2 and IFN-γ, Inhibits T-helper cell activation.	Did not affect outcomes
Eculizumab (Melzer et al., 2016; Farmakidis et al., 2018; Diurno et al., 2020; Korsukewitz et al., 2020; Mimori et al., 2022)	Monoclonal C5 complement inhibitor	Potentially beneficial
Tocilizumab (Anand et al., 2020; Korsukewitz et al., 2020; Rodrigues et al., 2022)	Monoclonal IL-6 inhibitor	Potentially beneficial*
Intravenous Immunoglobulins IVIG (Melzer et al., 2016; Farmakidis et al., 2018; Anand et al., 2020; Korsukewitz et al., 2020; Xiang et al., 2021; Rodrigues et al., 2022)	Inhibits macrophage Fc receptor expression, cytokine synthesis antibody production and complement activation.	Potentially beneficial
Mycophenolate (Melzer et al., 2016; Farmakidis et al., 2018; Anand et al., 2020; Korsukewitz et al., 2020; Strangfeld et al., 2021; Rodrigues et al., 2022)	Guanosine (purine) synthesis inhibitor	Possibly increased mortality
Methotrexate (Melzer et al., 2016; Farmakidis et al., 2018; Korsukewitz et al., 2020; Strangfeld et al., 2021; Rodrigues et al., 2022)	Dihydrofolate reductase inhibitor, thus inhibiting nucleotide synthesis.	No effect
Rituximab (Melzer et al., 2016; Farmakidis et al., 2018; Korsukewitz et al., 2020; Strangfeld et al., 2021; Rodrigues et al., 2022)	Recombinant antibody targeting CD-20+ B-cells.	Increased mortality
Plasma Exchange (Melzer et al., 2016; Farmakidis et al., 2018; Korsukewitz et al., 2020; Rodrigues et al., 2022)	Removal of autoimmune antibodies and cytokines	Potentially beneficial
Tacrolimus (Melzer et al., 2016; Strangfeld et al., 2021)	Calcineurin inhibitor, decreasing antigen-specific lymphocyte activation	Increased mortality

*Very limited data for tocilizumab as it is considered to be an experimental therapy for MG (Melzer et al., 2016; Farmakidis et al., 2018; Anand et al., 2020; Diurno et al., 2020; Korsukewitz et al., 2020; Strangfeld et al., 2021; Xiang et al., 2021; Mimori et al., 2022; Rodrigues et al., 2022).

Eculizumab, a monoclonal antibody directed at the complement attack complex, has demonstrated a benefit in the treatment of refractory MG in early trials (Melzer et al., 2016). Similarly, one small cohort study which included 10 patients in the eculizumab arm found the treatment to be safe and well tolerated in severe COVID-19 patients preventing them from being treated with advanced respiratory support, as well as noted improvement in respiratory distress and inflammatory markers. Moreover, the authors concluded the treatment arm tended towards decreased in-hospital mortality or respiratory sequelae (Ruggenenti et al., 2021).

Crisis treatment

Plasma exchange and IVIg are the mainstay of rescue management in myasthenic crisis (Melzer et al., 2016; Sanders et al., 2016). There is some data to suggest PLEX may exhibit quicker effect onset (Županić, 2021), but the guidelines do not strongly recommend one treatment over the other in the general MG population. Safety profile and effects for these treatments in the treatment of COVID-19 is as aforementioned.

COVID-19 vaccination and myasthenia gravis

Vaccinations are generally recommended for MG patients, including COVID-19. Non-live formulations may be preferable given common concurrent immunosuppressive treatments. Prior trials regarding seasonal influenza vaccines showed safety in MG (Županić, 2021). One retrospective case series evaluated 22 MG patients receiving inactivated or recombinant vaccines, 77% were on chronic immunomodulators. In total, two patients reported mild worsening symptoms, treated successfully with pyridostigmine (Ruan et al., 2021). Another study which included 53 MG patients receiving vaccinations showed similar results wherein the measured myasthenia gravis activities of daily living score was unaffected in 58.5%, improved in 15% and demonstrated worsening symptoms in 28.3%, independent of vaccine formulation, prior antibody titers, or MG variant (Lupica et al., 2022). Yet another case series found MG symptomatic decline after COVID-19 vaccination in 7.7% of 104 included cases, mostly mild (Farina et al., 2022).

In one multinational retrospective study involving COVID vaccinations and immune mediated disease, a total of 2 *de novo* myasthenia gravis cases occurred, both after the second dose of BNT162b2 vaccine, with one case described as severe (Watad et al., 2021). At the time of publication, a mere 6 cases of COVID-19 vaccine related MG have been reported (Lee et al., 2022; Sansone and Bonifati, 2022) with one of these patients presenting with myasthenic crisis (Sansone and Bonifati, 2022). Given the relative infrequency and mild symptomatology of adverse reactions following COVID-19 vaccine in MG patients, and the lack of robust information to infer association, vaccination is recommended in this

patient population (Shah et al., 2022). Cases of wherein varying the administered vaccine type and immunomodulatory therapy resulted in a satisfactory rise in titers in patients with known MG (Sansone and Bonifati, 2022).

COVID-19 and small fiber neuropathy

Definition

Small fiber neuropathy is an umbrella term comprising a varied group of disorders involving the peripheral thinly myelinated Aδ fibers and unmyelinated C nerve fibers (Zhou, 2019; Devigili et al., 2020). The pathophysiology of this disorder is unclear and appears to have multiple etiologies (Zhou, 2019). The symptom common to all is neuropathic pain (Strangfeld et al., 2021) and autonomic symptoms are a common finding (Sène, 2018; Devigili et al., 2020).

Epidemiology

Given protean symptoms and various causes, varying reports on epidemiological data are unsurprising. One study in Olmsted county, Minnesota United States reported an incidence of 1.3/100,000 which increased during the study period (Johnson et al., 2021). Reports on prevalence have ranged between 13 and 53 per 100,000 in the Netherlands and United States, respectively with conflicting data on predilection for men or women (Peters et al., 2013; Johnson et al., 2021). SFN is likely underdiagnosed leading to an underestimation of the true incidence and prevalence (Farhad, 2019). Exacerbations or, more importantly, *de novo* cases of SNF manifesting as COVID-19 sequelae have been reported and described as "not uncommon" (Abrams et al., 2021; Shouman et al., 2021).

Pathogenesis

The term "Small Fiber" refers to small somatosensory fibers, which mediate pinprick and thermal sensations, and autonomic C fibers, which innervate the smooth muscles of blood vessels, gastrointestinal track and genitourinary tract (Zhou, 2019). Thus, symptomatology comprises primarily dysesthesias or dysautonomia, respectively. SFN can be classified by pattern of involvement; length-dependent debuting commonly with distal sensory symptoms, non-length-dependent neuropathy with patchy involvement, or neuropathy multiplex or monoplex (Devigili et al., 2020). The most common variant is length dependent neuropathy (Zhou, 2019) as seen in Diabetes Mellitus, and is thought to account for 4.5–31% of cases (Sène, 2018; Farhad, 2019).

Small Fiber Neuropathy has been broadly organized into etiological categories which include: metabolic, inflammatory, toxic, infectious, genetic or idiopathic (Sène, 2018; Johnson et al., 2021). Specific diseases associated with autoimmune SFN include systemic lupus erythematosus, Sjogren's syndrome, sarcoidosis or paraneoplastic syndromes (Shoenfeld et al., 2020). SFN and fibromyalgia have been linked to autoimmune processes (Oaklander and Nolano, 2019). Given an observed delayed symptomatic debut measured in weeks, some authors have postulated a postinfectious autoimmune injury mechanism for SFN subsequent to COVID-19 cases (Burakgazi, 2022). Yet another case series found 13 patients debuting with new onset paresthesias after COVID infection, wherein 6 had SFN confirmed via skin biopsy. Authors concluded SFN may underlie the paresthesias associated with so called "long-haul" COVID (Abrams et al., 2021), which these authors consider a neurologic sequelae.

Clinical presentation

A majority of patients do not self-report SFN symptoms as disabling, but quality of life can be severely decreased (Johnson et al., 2021). Dysautonomia results from autonomic C-fiber dysfunction and can affect several organ systems (Zhou, 2019). Gastrointestinal involvement may lead to chronic diarrhea or constipation, gastroparesis, pseudo-obstruction or fecal incontinence. Genitourinary involvement may manifest as dysuria, incontinence or impotence. Exocrine dysfunction of the sweat, salivary and lacrimal glands may also be encountered. Ocular manifestations may manifest as impaired accommodation, or photosensitivity (Sène, 2018).

Sensory symptoms can be described as negative or positive, the latter more commonly encountered (Devigili et al., 2020). Negative symptoms comprise decreased perception of stimuli while positive symptoms comprise perceived sensation disproportionate to or in the absence of stimuli. Sensory symptoms are most common in length-dependent SFN. Patients often present with sharp pain in the affected area, characterized as burning, lancinating or akin to an electrical discharge. Hyperalgesia and allodynia have been reported as well leading to discomfort with footwear or sheets (Zhou, 2019; Devigili et al., 2020). A squeezing sensation, coldness, or pruritus within the affected areas have been reported as well (Zhou, 2019). Positive symptoms may worsen at night time (Farhad, 2019; Devigili et al., 2020). Negative symptoms include hypoesthesia, as well as thermal perception and nociception (Devigili et al., 2020). Muscle strength would be preserved, as these functions are exerted by large nerve fibers (Zhou, 2019).

Cardiovagal dysfunction may be seen in up to 64% of SFN patients (Blackmore and Siddiqi, 2017). Signs and symptoms of cardiovascular dysautonomia include blood pressure lability including orthostatic hypotension, arrhythmias and sinus bradycardia or tachycardia (Sène, 2018). SFN patients may be at higher risk of myocardial infarctions with study finding an incidence of 46% vs. 27% in controls ($p < 0.0001$) (Johnson et al., 2021).

Neurological symptoms consistent with SFN following severe SARS-CoV-2 infection have been reported (Abrams et al., 2021). Furthermore, there is limited literature that has linked small fiber neuropathy to chronic fatigue syndrome (Shoenfeld et al., 2020). In view of previously described autoimmune etiologies and the fact that numerous SFN patients report a prior viral infection (Farhad, 2019), an autoimmune etiology to COVID-19-related SFN and a link between SFN and reported sequelae is possible.

An initial case report described a 64-year-old woman who developed a new painful SFN with concomitant fatigue, orthostatic dizziness, and urinary incontinence 2 weeks after COVID-19. The clinical condition improved with empiric IVIG (Novak, 2020) and the authors suggested a link with an autoimmune cause. Further case reports found 2 cases of length dependent neuropathy responding to pregabalin and duloxetine, respectively (Burakgazi, 2022).

Another single center's retrospective review identified 27 patients with autonomic dysfunction subsequent to SARS-CoV-2 infection. Reported symptoms included lightheadedness (93%), orthostatic headache (22%), syncope (11%), hyperhidrosis (11%), and burning pain (11%). An abnormal sweat test was found in 36%, and cardiovagal dysfunction in 27% (Shouman et al., 2021).

Another case series included 13 patients with new onset symptoms after COVID-19. The authors took efforts to exclude confounding causes by testing HbA1c, antinuclear antibodies, vitamin B12, thyroid stimulating hormone and free T4, and performed serum immunofixation testing. Furthermore, none exhibited large fiber involvement in nerve conduction studies or electromyography. Biopsy confirmed SFN in 46% of cases. Painful paresthesias followed a length dependent distribution in 54% and a multifocal patchy distribution in the remaining 46% while orthostasis was also noted in 46% of the study population. The authors noted that although the study was likely underpowered, an association could be inferred (Abrams et al., 2021). A third case series involving 17 patients presenting after COVID-19 with no identified systemic or immune risk factors, SFN was confirmed in 6 via skin biopsy (Oaklander et al., 2022). While the data is limited, there exists a possible autoimmune etiology to COVID-19-related SFN and a link between SFN and reported sequelae.

Diagnostics

Small fiber neuropathy has for a long time been a clinical diagnosis based on the symptoms previously described. Allodynia with pinprick testing may be present evaluation (Blackmore and Siddiqi, 2017). Since deep tendon reflexes are mediated by large muscle fibers, hyporeflexia would not be expected (Zhou, 2019). Electrodiagnostic testing via nerve conduction studies is normal given this test does not measure the function of small fibers (Abrams et al., 2021). It should be noted that altered nerve conduction studies do not rule out SFN, but rule in further large fiber neuropathy as both pathologies can coexist (Sopacua et al., 2019). Thus, skin biopsy to evaluate nerve fiber density is considered by some authors to be the gold standard (Zhou, 2019). That being said, it must be noted that skin biopsy findings must be interpreted within the right clinical context and often in conjunction with already-established clinical criteria.

Diagnostic criteria for small fiber neuropathy have been proposed previously (Tesfaye et al., 2010; Blackmore and Siddiqi, 2017) However, established criteria may be biased towards the detection of length-dependent SFN as opposed to non-length dependent forms of the SFN. For example, the criteria proposed by Blackmore et al. include length dependent dysesthesias and abnormal pinprick sensation, altered pain or heat perception in addition to dysautonomia as tallied via quantitative sudomotor reflexes or abnormal heart rate variability testing (Blackmore and Siddiqi, 2017).

Treatment

The mainstay of SFN treatment comprises the identification and abatement of potential underlying causes. However, heterogeneity of the potential causes makes this an aspirational target. Symptom management includes gabapentin or pregabalin as well as antidepressants of the tricyclic and serotonin/norepinephrine uptake inhibitor variety as first line (Zhou, 2019). Varying success has been reported with duloxetine, amitriptyline, gabapentin and pregabalin in case series data (Abrams et al., 2021). In general, patients with normal skin biopsy tend to have better outcomes as compared to those with abnormal skin biopsy findings (Abrams et al., 2021). IVIG has also demonstrated considerable success (Novak, 2020; McAlpine et al., 2022). In a case series of patients with SFN in the setting of COVID-19, all three out of four patients that agreed to proceed with IVIG demonstrated significant improvement of their symptoms with one patient having complete clinical resolution (McAlpine et al., 2022). Corticosteroids are also known to be effective, especially in young patients with rapid onset SFN (Dabby et al., 2006).

COVID-19 vaccination and small fiber neuropathy

One case reported SNF onset 1 week post COVID-19 vaccination. Symptoms were described as subacute intense burning dysesthesias in an apparent length dependent distribution, debuting at the feet and subsequently hands. SFN was confirmed via skin biopsy (Waheed et al., 2021). There is little data at this time to support any link between COVID-19 Vaccination and SFN.

Conclusion

In summary, peripheral neuropathies including GBS, MG and SFN can be caused or worsened by COVID-19. The incidence of severe cases has abated, in part due to a decreasing prevalence of SARS-CoV-2 infections worldwide. That being said, increased survival rates, emerging variants and the fact that vaccines are by design immunogenic (da Silva et al., 2021) signifies that a large population remains vulnerable to autoimmune mediated neurological complications of COVID-19. Thus, clinicians treating acutely ill or convalescent patients must be alert to this.

Given there is a lag between COVID-19 and symptomatic debut (Shahrizaila et al., 2021; Lee et al., 2022; Sansone and Bonifati, 2022), little can be said of treatment for concurrent COVID-19 and GBS, SFN or MG. PLEX appeared to offer the most benefit for these diseases with concomitant severe COVID-19 patients. Yet this is not standard of care and further studies are needed. Furthermore it is possible said immunomodulatory therapies will be superseded by more targeted therapies. It is possible emerging treatments for COVID-19-mediated hyperimmune cytokine response can be parlayed into novel therapies for peripheral neuropathies in the future, as appears to be the case for tocilizumab (Anand et al., 2020).

Author contributions

EE, AM, and FG evaluated the relevancy articles. DD and KL aided in reviewing the manuscript for accuracy and editing. FG served as the final arbiter for inclusion and is cited when appropriate. FG additionally reviewed the relevant references from these publications that focused on COVID-19 pathophysiology. All authors contributed to the article and approved the submitted version.

Acknowledgments

FG would like to thank Nizar Souayah, who was his first mentor in the field of Neuromuscular Neurology. All figures in this manuscript were made using Biorender (Biorender.com).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Abolmaali, M., Rezania, F., Behnagh, A. K., Hamidabad, N. M., Gorji, A., and Mirzaasgari, Z. (2022). Guillain-Barré syndrome in association with COVID-19 vaccination: a systematic review. *Immunol. Res.* 70, 752–764. doi: 10.1007/s12026-022-09316-6

Abrams, R. M. C., Simpson, D. M., Navis, A., Jette, N., Zhou, L., and Shin, S. C. (2021). Small fiber neuropathy associated with SARS-CoV -2 infection. *Muscle Nerve* 65, 440–443. doi: 10.1002/mus.27458

Abu-Rumeileh, S., Abdelhak, A., Foschi, M., Tumani, H., and Otto, M. (2020). Guillain-Barré syndrome spectrum associated with COVID-19: an up-to-date systematic review of 73 cases. *J. Neurol.* 268, 1133–1170. doi: 10.1007/s00415-020-10124-x

Ahmad, L., Businaro, P., Regalbuto, S., Gastaldi, M., Zardini, E., Panzeri, M., et al. (2022). COVID-19 and Guillain-Barré syndrome: a single-center prospective case series with a 1-year follow-up. *Medicine* 101:e29704. doi: 10.1097/MD.0000000000029704

Aladawi, M., Elfil, M., Abu-Esheh, B., Abu Jazar, D., Armouti, A., Bayoumi, A., et al. (2022). Guillain Barre syndrome as a complication of COVID-19: a systematic review. *Can. J. Neurol. Sci.* 49, 38–48. doi: 10.1017/cjn.2021.102

Anand, P., Slama, M. C. C., Kaku, M., Ong, C., Cervantes-Arslanian, A. M., Zhou, L., et al. (2020). COVID-19 in patients with myasthenia gravis. *Muscle Nerve* 62, 254–258. doi: 10.1002/mus.26918

Assini, A., Gandoglia, I., Damato, V., Rikani, K., Evoli, A., and Del Sette, M. (2021). Myasthenia gravis associated with anti-MuSK antibodies developed after SARS-CoV-2 infection. *Eur. J. Neurol.* 28, 3537–3539. doi: 10.1111/ene.14721

Bauer, P. R., Ostermann, M., Russell, L., Robba, C., David, S., Ferreyro, B. L., et al. (2022). Plasma exchange in the intensive care unit: a narrative review. *Intensive Care Med.* 48, 1382–1396. doi: 10.1007/s00134-022-06793-z

Berciano, J., Sedano, M. J., Pelayo-Negro, A. L., García, A., Orizaola, P., Gallardo, E., et al. (2017). Proximal nerve lesions in early Guillain-Barré syndrome: implications for pathogenesis and disease classification. *J. Neurol.* 264, 221–236. doi: 10.1007/s00415-016-8204-2

Blackmore, D., and Siddiqi, Z. A. (2017). Diagnostic criteria for small Fiber neuropathy. *J. Clin. Neuromuscul. Dis.* 18, 125–131. doi: 10.1097/CND.0000000000000154

Bubuioc, A. M., Kudebayeva, A., Turuspekova, S., Lisnic, V., and Leone, M. A. (2021). The epidemiology of myasthenia gravis. *J. Med. Life* 14, 7–16. doi: 10.25122/jml-2020-0145

Burakgazi, A. Z. (2022). Small-Fiber neuropathy possibly associated with COVID-19. *Case Rep. Neurol.* 14, 208–212. doi: 10.1159/000524205

Cao, W., Liu, X., Bai, T., Fan, H., Hong, K., Song, H., et al. (2020). High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with coronavirus disease 2019. *Open Forum Infect. Dis.* 7:ofaa102. doi: 10.1093/ofid/ofaa102

Caress, J. B., Castoro, R. J., Simmons, Z., Scelsa, S. N., Lewis, R. A., Ahlawat, A., et al. (2020). COVID-19–associated Guillain-Barré syndrome: the early pandemic experience. *Muscle Nerve* 62, 485–491. doi: 10.1002/mus.27024

Cegolon, L., Einollahi, B., Panahi, Y., Imanizadeh, S., Rezapour, M., Javanbakht, M., et al. (2022). On therapeutic plasma exchange against severe COVID-19-associated pneumonia: an observational clinical study. *Front. Nutr.* 9:9. doi: 10.3389/fnut.2022.809823

Chakraborty, T., Kramer, C. L., Wijdicks, E. F. M., and Rabinstein, A. A. (2019). Dysautonomia in Guillain–Barré syndrome: prevalence, clinical Spectrum, and outcomes. *Neurocrit. Care* 32, 113–120. doi: 10.1007/s12028-019-00781-w

da Silva, S. J. R., de Lima, S. C., da Silva, R. C., Kohl, A., and Pena, L. (2021). Viral load in COVID-19 patients: implications for prognosis and vaccine efficacy in the context of emerging SARS-CoV-2 variants. *Front. Med.* 8:836826. doi: 10.3389/fmed.2021.836826

Dabby, R., Gilad, R., Sadeh, M., Lampl, Y., and Watemberg, N. (2006). Acute steroid responsive small-fiber sensory neuropathy: a new entity? *J. Peripher. Nerv. Syst.* 11, 47–52. doi: 10.1111/j.1085-9489.2006.00062.x

Devigili, G., Cazzato, D., and Lauria, G. (2020). Clinical diagnosis and management of small fiber neuropathy: an update on best practice. *Expert. Rev. Neurother.* 20, 967–980. doi: 10.1080/14737175.2020.1794825

Diurno, F., Numis, F. G., Porta, G., Cirillo, F., Maddaluno, S., Ragozzino, A., et al. (2020). Eculizumab treatment in patients with COVID-19: preliminary results from real life ASL Napoli 2 Nord experience. *Eur. Rev. Med. Pharmacol. Sci.* 24, 4040–4047. doi: 10.26355/eurrev_202004_20875

Essajee, F., Lishman, J., Solomons, R., Abraham, D. R., Goussard, P., and Van Toorn, R. (2021). Transient acetylcholine receptor-related myasthenia gravis, post multisystem inflammatory syndrome in children (MIS-C) temporally associated with COVID-19 infection. *BMJ Case Rep.* 14:e244102. doi: 10.1136/bcr-2021-244102

Faqihi, F., Alharthy, A., Abdulaziz, S., Balhamar, A., Alomari, A., AlAseri, Z., et al. (2021). Therapeutic plasma exchange in patients with life-threatening COVID-19: a randomised controlled clinical trial. *Int. J. Antimicrob. Agents* 57:106334. doi: 10.1016/j.ijantimicag.2021.106334

Farhad, K. (2019). Current diagnosis and treatment of painful small Fiber neuropathy. *Curr. Neurol. Neurosci. Rep.* 19:103. doi: 10.1007/s11910-019-1020-1

Farina, A., Falso, S., Cornacchini, S., Spagni, G., Monte, G., Mariottini, A., et al. (2022). Safety and tolerability of SARS-Cov-2 vaccination in patients with myasthenia gravis: a multicenter experience. *Eur. J. Neurol.* 29, 2505–2510. doi: 10.1111/ene.15348

Farmakidis, C., Pasnoor, M., Dimachkie, M. M., and Barohn, R. J. (2018). Treatment of myasthenia gravis. *Neurol. clini.* 36, 311–337. doi: 10.1016/j.ncl.2018.01.011

Fernández-Zarzoso, M., Gómez-Seguí, I., and de la Rubia, J. (2019). Therapeutic plasma exchange: review of current indications. *Transfus. Apher. Sci.* 58, 247–253. doi: 10.1016/j.transci.2019.04.007

Finsterer, J., Matovu, D., and Scorza, F. A. (2022). SARS-CoV-2 vaccinations reduce the prevalence of post-COVID Guillain-Barre syndrome. *Clinics* 77:100064. doi: 10.1016/j.clinsp.2022.100064

FINSTERER, J., SCORZA, F. A., SCORZA, C. A., and FIORINI, A. C. (2021). Peripheral neuropathy in COVID-19 is due to immune-mechanisms, pre-existing risk factors, anti-viral drugs, or bedding in the intensive care unit. *Arq. Neuropsiquiatr.* 79, 924–928. doi: 10.1590/0004-282X-ANP-2021-0030

Fokke, C., van den Berg, B., Drenthen, J., Walgaard, C., van Doorn, P. A., and Jacobs, B. C. (2013). Diagnosis of Guillain-Barre syndrome and validation of Brighton criteria. *Brain* 137, 33–43. doi: 10.1093/brain/awt285

Fragiel, M., Miró, Ò., Llorens, P., Jiménez, S., Piñera, P., Burillo, G., et al. (2020). Incidence, clinical, risk factors and outcomes of Guillain-Barré in Covid-19. *Ann. Neurol.* 89, 598–603. doi: 10.1002/ana.25987

Fragoso-Saavedra, S., Núñez, I., Audelo-Cruz, B. M., Arias-Martínez, S., Manzur-Sandoval, D., Quintero-Villegas, A., et al. (2022). Pyridostigmine reduces mortality of patients with severe SARS-CoV-2 infection: a phase 2/3 randomized controlled trial. *Mol. Med.* 28:131. doi: 10.1186/s10020-022-00553-x

Gilhus, N. E., and Verschuuren, J. J. (2015). Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol.* 14, 1023–1036. doi: 10.1016/S1474-4422(15)00145-3

Ginikopoulou, E. (2022). Plasma exchange and COVID 19. *Transfus. Apher. Sci.* 61:103598. doi: 10.1016/j.transci.2022.103598

Gucyetmez, B., Atalan, H. K., Sertdemir, I., Cakir, U., and Telci, L. (2020). Therapeutic plasma exchange in patients with COVID-19 pneumonia in intensive care unit: a retrospective study. *Crit. Care* 24:492. doi: 10.1186/s13054-020-03215-8

Guidon, A. C., and Amato, A. A. (2020). COVID-19 and neuromuscular disorders. *Neurology* 94, 959–969. doi: 10.1212/WNL.0000000000009566

Centers for Disease Control and Prevention. *Guillain-Barré Syndrome Concerns [Internet]*. (2019). Available at: https://www.cdc.gov/vaccinesafety/concerns/guillain-barre-syndrome.html

Gupta, S., Wang, W., Hayek, S. S., Chan, L., Mathews, K. S., Melamed, M. L., et al. (2021). Association between early treatment with tocilizumab and mortality among critically ill patients with COVID-19. *JAMA Intern. Med.* 181, 41–51. doi: 10.1001/jamainternmed.2020.6252

Gwathmey, K., Balogun, R. A., and Burns, T. (2014). Neurologic indications for therapeutic plasma exchange: 2013 update. *J. Clin. Apher.* 29, 211–219. doi: 10.1002/jca.21331

Hanson, K. E., Goddard, K., Lewis, N., Fireman, B., Myers, T. R., Bakshi, N., et al. (2022). Incidence of Guillain-Barré syndrome after COVID-19 vaccination in the vaccine safety datalink. *JAMA Netw. Open* 5:e228879. doi: 10.1001/jamanetworkopen.2022.8879

Huber, M., Rogozinski, S., Puppe, W., Framme, C., Höglinger, G., Hufendiek, K., et al. (2020). Postinfectious onset of myasthenia gravis in a COVID-19 patient. *Front. Neurol.* 11:11. doi: 10.3389/fneur.2020.576153

Jacob, S., Kapadia, R., Soule, T., Luo, H., Schellenberg, K. L., Douville, R. N., et al. (2022). Neuromuscular complications of SARS-CoV-2 and other viral infections. *Front. Neurol.* 13:914411. doi: 10.3389/fneur.2022.914411

Johnson, S. A., Shouman, K., Shelly, S., Sandroni, P., Berini, S. E., Dyck, P. J. B., et al. (2021). Small fiber neuropathy incidence, prevalence, longitudinal impairments, and disability. *Neurology* 97, e2236–e2247. doi: 10.1212/WNL.0000000000012894

Jovanova-Nesic, K., and Shoenfeld, Y. (2006). MMP-2, VCAM-1 and NCAM-1 expression in the brain of rats with experimental autoimmune encephalomyelitis as a trigger mechanism for synaptic plasticity and pathology. *J. Neuroimmunol.* 181, 112–121. doi: 10.1016/j.jneuroim.2006.08.013

Kamran, S. M., Mirza, Z. H., Naseem, A., Liaqat, J., Fazal, I., Alamgir, W., et al. (2021). Therapeutic plasma exchange for coronavirus disease-2019 triggered cytokine release syndrome; a retrospective propensity matched control study. *PLoS One* 16:e0244853. doi: 10.1371/journal.pone.0244853

Kanou, S., Wardeh, L., Govindarajan, S., and Macnay, K. (2022). Guillain-Barre syndrome (GBS) associated with COVID-19 infection that resolved without treatment in a child. *BMJ Case Rep.* 15:e245455. doi: 10.1136/bcr-2021-245455

Karimi, N., Boostani, R., Fatehi, F., Panahi, A., Okhovat, A. A., Ziaadini, B., et al. (2021). Guillain-Barre syndrome and COVID-19 vaccine: a report of nine patients. *Basic Clin. Neurosci.* 12, 703–710. doi: 10.32598/bcn.2021.3565.1

Karimi, N., Okhovat, A. A., Ziaadini, B., Haghi Ashtiani, B., Nafissi, S., and Fatehi, F. (2021). Myasthenia gravis associated with novel coronavirus 2019 infection: a report of three cases. *Clin. Neurol. Neurosurg.* 208:106834. doi: 10.1016/j.clineuro.2021.106834

Keddie, S., Pakpoor, J., Mousele, C., Pipis, M., Machado, P. M., Foster, M., et al. (2021). Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barré syndrome. *Brain* 144, 682–693. doi: 10.1093/brain/awaa433

Khamis, F., Al-Zakwani, I., Al Hashmi, S., Al Dowaiki, S., Al Bahrani, M., Pandak, N., et al. (2020). Therapeutic plasma exchange in adults with severe COVID-19 infection. *Int. J. Infect. Dis.* 99, 214–218. doi: 10.1016/j.ijid.2020.06.064

Korsukewitz, C., Reddel, S. W., Bar-Or, A., and Wiendl, H. (2020). Neurological immunotherapy in the era of COVID-19 — looking for consensus in the literature. *Nat. Rev. Neurol.* 16, 493–505. doi: 10.1038/s41582-020-0385-8

Kreye, J., Reincke, S. M., and Prüss, H. (2020). Do cross-reactive antibodies cause neuropathology in COVID-19? *Nat. Rev. Immunol.* 20, 645–646. doi: 10.1038/s41577-020-00458-y

Laudanski, K., Yakhkind, A., Restrepo, M., Draham, L., and Lang, A. E. (2021). Guillain–Barré syndrome in COVID-19—the potential role of NCAM-1 and immunotherapy. *Bio. Med.* 1, 80–92. doi: 10.3390/biomed1010006

Lee, M. A., Lee, C., Park, J. H., and Lee, J. H. (2022). Early-onset myasthenia gravis following COVID-19 vaccination. *J. Korean Med. Sci.* 37:50. doi: 10.3346/jkms.2022.37.e50

Leonhard, S. E., Mandarakas, M. R., Gondim, F. A. A., Bateman, K., Ferreira, M. L. B., Cornblath, D. R., et al. (2019). Diagnosis and management of Guillain–Barré syndrome in ten steps. *Nat. Rev. Neurol.* 15, 671–683. doi: 10.1038/s41582-019-0250-9

Lu, W., Kelley, W., Fang, D. C., Joshi, S., Kim, Y., Paroder, M., et al. (2021). The use of therapeutic plasma exchange as adjunctive therapy in the treatment of coronavirus disease 2019: a critical appraisal of the current evidence. *J. Clin. Apher.* 36, 483–491. doi: 10.1002/jca.21883

Lucchese, G., and Flöel, A. (2020). SARS-CoV-2 and Guillain-Barré syndrome: molecular mimicry with human heat shock proteins as potential pathogenic mechanism. *Cell Stress Chaperones.* 25, 731–735. doi: 10.1007/s12192-020-01145-6

Lupica, A., Di Stefano, V., Iacono, S., Pignolo, A., Quartana, M., Gagliardo, A., et al. (2022). Impact of COVID-19 in AChR myasthenia gravis and the safety of vaccines: data from an Italian cohort. *Neurol. Int.* 14, 406–416. doi: 10.3390/neurolint14020033

Marcec, R., Dodig, V. M., Radanovic, I., and Likic, R. (2022). Intravenous immunoglobulin (IVIg) therapy in hospitalised adult COVID-19 patients: a systematic review and meta-analysis. *Rev. Med. Virol.* 32:e2397. doi: 10.1002/rmv.2397

McAlpine, L., Zubair, A., and Spudich, S. (2022). Case series: post-COVID small fiber neuropathy. *Neurol. Int.* 98, 9–9.002.

Melzer, N., Ruck, T., Fuhr, P., Gold, R., Hohlfeld, R., Marx, A., et al. (2016). Clinical features, pathogenesis, and treatment of myasthenia gravis: a supplement to the guidelines of the German neurological society. *J. Neurol.* 263, 1473–1494. doi: 10.1007/s00415-016-8045-z

Mimori, M., Komatsu, T., Maku, T., Mitsumura, H., and Iguchi, Y. (2022). Generalized myasthenia gravis patients infected with COVID-19 should continue eculizumab. *Neurol. Sci.* 43, 4081–4083. doi: 10.1007/s10072-022-05922-2

Morsy, S. (2020). NCAM protein and SARS-COV-2 surface proteins: in-silico hypothetical evidence for the immunopathogenesis of Guillain-Barré syndrome. *Med. Hypotheses* 145:110342. doi: 10.1016/j.mehy.2020.110342

Muppidi, S., Guptill, J. T., Jacob, S., Li, Y., Farrugia, M. E., Guidon, A. C., et al. (2020). COVID-19-associated risks and effects in myasthenia gravis (CARE-MG). *Lancet Neurol.* 19, 970–971. doi: 10.1016/S1474-4422(20)30413-0

Nelke, C., Stascheit, F., Eckert, C., Pawlitzki, M., Schroeter, C. B., Huntemann, N., et al. (2022). Independent risk factors for myasthenic crisis and disease exacerbation in a retrospective cohort of myasthenia gravis patients. *J. Neuroinflammation* 19:89. doi: 10.1186/s12974-022-02448-4

Norris, P. A. A., Kaur, G., and Lazarus, A. H. (2020). New insights into IVIg mechanisms and alternatives in autoimmune and inflammatory diseases. *Curr. Opin. Hematol.* 27, 392–398. doi: 10.1097/MOH.0000000000000609

Novak, P. (2020). Post COVID-19 syndrome associated with orthostatic cerebral hypoperfusion syndrome, small fiber neuropathy and benefit of immunotherapy: a case report. *eNeurologicalSci* 21:100276. doi: 10.1016/j.ensci.2020.100276

Oaklander, A. L., Mills, A. J., Kelley, M., Toran, L. S., Smith, B., Dalakas, M. C., et al. (2022). Peripheral neuropathy evaluations of patients with prolonged long COVID. *Neurol. Neuroimmunol. Neuroinflamm.* 9:e1146. doi: 10.1212/NXI.0000000000001146

Oaklander, A. L., and Nolano, M. (2019). Scientific advances in and clinical approaches to small-fiber polyneuropathy: a review. *JAMA Neurol.* 76, 1240–1251. doi: 10.1001/jamaneurol.2019.2917

Paterson, R. W., Brown, R. L., Benjamin, L., Nortley, R., Wiethoff, S., Bharucha, T., et al. (2020). The emerging spectrum of COVID-19 neurology: clinical, radiological and laboratory findings. *Brain* 143, 3104–3120. doi: 10.1093/brain/awaa240

Patone, M., Handunnetthi, L., Saatci, D., Pan, J., Katikireddi, S. V., Razvi, S., et al. (2021). Neurological complications after first dose of COVID-19 vaccines and SARS-CoV-2 infection. *Nat. Med.* 27, 1–10. doi: 10.1038/s41591-021-01556-7

Peters, M. J. H., Bakkers, M., Merkies, I. S. J., Hoeijmakers, J. G. J., van Raak, E. P. M., and Faber, C. G. (2013). Incidence and prevalence of small-fiber neuropathy: a survey in the Netherlands. *Neurol. Int.* 81, 1356–1360. doi: 10.1212/WNL.0b013e3182a8236e

Pimentel, V., Luchsinger, V. W., Carvalho, G. L., Alcará, A. M., Esper, N. B., Marinowic, D., et al. (2023). Guillain–Barré syndrome associated with COVID-19: a systematic review. *Brain Behav. Immun. Health* 28:100578. doi: 10.1016/j.bbih.2022.100578

Punga, A. R., Maddison, P., Heckmann, J. M., Guptill, J. T., and Evoli, A. (2022). Epidemiology, diagnostics, and biomarkers of autoimmune neuromuscular junction disorders. *Lancet Neurol.* 21, 176–188. doi: 10.1016/S1474-4422(21)00297-0

Qin, J., Wang, G., and Han, D. (2022). Benefits of plasma exchange on mortality in patients with COVID-19: a systematic review and meta-analysis. *Int. J. Infect. Dis.* 122, 332–336. doi: 10.1016/j.ijid.2022.06.014

Rajabally, Y. A., Durand, M. C., Mitchell, J., Orlikowski, D., and Nicolas, G. (2014). Electrophysiological diagnosis of Guillain-Barré syndrome subtype: could a single study suffice? *J. Neurol. Neurosurg. Psychiatry* 86, 115–119. doi: 10.1136/jnnp-2014-307815

Randhawa, J., Randhawa, H. S., and Toor, P. (2021). Pharyngeal-cervical-brachial variant of Guillain-Barré syndrome in a patient of COVID-19 infection. *Cureus* 13:e17945. doi: 10.7759/cureus.17945

Restivo, D. A., Centonze, D., Alesina, A., and Marchese-Ragona, R. (2020). Myasthenia gravis associated with SARS-CoV-2 infection. *Ann. Intern. Med.* 173, 1027–1028. doi: 10.7326/L20-0845

Rodrigues, C. L., de Freitas, H. C., Lima, P. R. O., de Oliveira Junior, P. H., Fernandes, J. M. A., D'Almeida, J. A. C., et al. (2022). Myasthenia gravis exacerbation and myasthenic crisis associated with COVID-19: case series and literature review. *Neurol. Sci.* 43, 2271–2276. doi: 10.1007/s10072-021-05823-w

Ruan, Z., Tang, Y., Li, C., Sun, C., Zhu, Y., Li, Z., et al. (2021). COVID-19 vaccination in patients with myasthenia gravis: a single-center case series. *Vaccines* 9:1112. doi: 10.3390/vaccines9101112

Ruggenenti, P., Di Marco, F., Cortinovis, M., Lorini, L., Sala, S., Novelli, L., et al. (2021). Eculizumab in patients with severe coronavirus disease 2019 (COVID-19) requiring continuous positive airway pressure ventilator support: retrospective cohort study. *PLoS One* 16:e0261113. doi: 10.1371/journal.pone.0261113

Sanders, D. B., Wolfe, G. I., Benatar, M., Evoli, A., Gilhus, N. E., Illa, I., et al. (2016). International consensus guidance for management of myasthenia gravis. *Neurol. Int.* 87, 419–425. doi: 10.1212/WNL.0000000000011124

Sansone, G., and Bonifati, D. M. (2022). Vaccines and myasthenia gravis: a comprehensive review and retrospective study of SARS-CoV-2 vaccination in a large cohort of myasthenic patients. *J. Neurol.* 269, 3965–3981. doi: 10.1007/s00415-022-11140-9

Schonberger, L. B., Bregman, D. J., Sullivan-Bolyai, J. Z., Keenlyside, R. A., Ziegler, D. W., Retailliau, H. F., et al. (1979). Guillain-Barre syndrome following vaccination in the national influenza immunization program, United States, 1976–19771. *Am. J. Epidemiol.* 110, 105–123. doi: 10.1093/oxfordjournals.aje.a112795

Sène, D. (2018). Small fiber neuropathy: diagnosis, causes, and treatment. *Joint Bone Spine* 85, 553–559. doi: 10.1016/j.jbspin.2017.11.002

Shah, S. M. I., Yasmin, F., Memon, R. S., Jatoi, N. N., Savul, I. S., Kazmi, S., et al. (2022). COVID-19 and myasthenia gravis: a review of neurological implications of the SARS-COV-2. *Brain Behav.* 12:e2789. doi: 10.1002/brb3.2789

Shahrizaila, N., Lehmann, H. C., and Kuwabara, S. (2021). Guillain-Barré syndrome. *Lancet* 397, 1214–1228. doi: 10.1016/S0140-6736(21)00517-1

Shang, P., Feng, J., Wu, W., and Zhang, H. L. (2021). Intensive care and treatment of severe Guillain–Barré syndrome. *Front. Pharmacol.* 12:608130. doi: 10.3389/fphar.2021.608130

Shang, P., Zhu, M., Wang, Y., Zheng, X., Wu, X., Zhu, J., et al. (2021). Axonal variants of Guillain-Barré syndrome: an update. *J. Neurol.* 268, 2402–2419. doi: 10.1007/s00415-020-09742-2

Shoenfeld, Y., Ryabkova, V. A., Scheibenbogen, C., Brinth, L., Martinez-Lavin, M., Ikeda, S., et al. (2020). Complex syndromes of chronic pain, fatigue and cognitive impairment linked to autoimmune dysautonomia and small fiber neuropathy. *Clini. Immunol.* 214:108384. doi: 10.1016/j.clim.2020.108384

Shouman, K., Vanichkachorn, G., Cheshire, W. P., Suarez, M. D., Shelly, S., Lamotte, G. J., et al. (2021). Autonomic dysfunction following COVID-19 infection: an early experience. *Clin. Auton. Res.* 31, 385–394. doi: 10.1007/s10286-021-00803-8

Sopacua, M., Hoeijmakers, J. G. J., Merkies, I. S. J., Lauria, G., Waxman, S. G., and Faber, C. G. (2019). Small-fiber neuropathy: expanding the clinical pain universe. *J. Peripher. Nerv. Syst.* 24, 19–33. doi: 10.1111/jns.12298

Sriwastava, S., Kataria, S., Tandon, M., Patel, J., Patel, R., Jowkar, A., et al. (2021). Guillain Barré syndrome and its variants as a manifestation of COVID-19: a systematic review of case reports and case series. *J. Neurol. Sci.* 420:117263. doi: 10.1016/j.jns.2020.117263

Sriwastava, S., Tandon, M., Kataria, S., Daimee, M., and Sultan, S. (2020). New onset of ocular myasthenia gravis in a patient with COVID-19: a novel case report and literature review. *J. Neurol.* 268, 2690–2696. doi: 10.1007/s00415-020-10263-1

Strangfeld, A., Schäfer, M., Gianfrancesco, M. A., Lawson-Tovey, S., Liew, J. W., Ljung, L., et al. (2021). Factors associated with COVID-19-related death in people with rheumatic diseases: results from the COVID-19 global rheumatology Alliance physician-reported registry. *Ann. Rheum. Dis.* 80, 930–942. doi: 10.1136/annrheumdis-2020-219498

Suh, J., and Amato, A. A. (2021). Neuromuscular complications of coronavirus disease-19. *Curr. Opin. Neurol.* 34, 669–674. doi: 10.1097/WCO.0000000000000970

Suh, J., Mukerji, S. S., Collens, S. I., Padera, R. F., Pinkus, G. S., Amato, A. A., et al. (2021). Skeletal muscle and peripheral nerve histopathology in COVID-19. *Neurol. Int.* 97, e849–e858. doi: 10.1212/WNL.0000000000012344

Taga, A., and Lauria, G. (2022). COVID-19 and the peripheral nervous system. A 2-year review from the pandemic to the vaccine era. *J. Peripher. Nerv. Syst.* 27, 4–30. doi: 10.1111/jns.12482

Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., et al. (2010). Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 33, 2285–2293. doi: 10.2337/dc10-1303

Toscano, G., Palmerini, F., Ravaglia, S., Ruiz, L., Invernizzi, P., Cuzzoni, M. G., et al. (2020). Guillain–Barré syndrome associated with SARS-CoV-2. *N. Engl. J. Med.* 382, 2574–2576. doi: 10.1056/NEJMc2009191

Tugasworo, D., Kurnianto, A., Retnaningsih, A. Y., Ardhini, R., and Budiman, J. (2022). The relationship between myasthenia gravis and COVID-19: a systematic review. *Egypt. J. Neurol. Psychiatr. Neurosurg.* 58:83. doi: 10.1186/s41983-022-00516-3

Waheed, W., Carey, M. E., Tandan, S. R., and Tandan, R. (2021). Post COVID-19 vaccine small fiber neuropathy. *Muscle Nerve* 64, E1–E2. doi: 10.1002/mus.27251

Watad, A., De Marco, G., Mahajna, H., Druyan, A., Eltity, M., Hijazi, N., et al. (2021). Immune-mediated disease flares or new-onset disease in 27 subjects following mRNA/DNA SARS-CoV-2 vaccination. *Vaccine* 9:435. doi: 10.3390/vaccines9050435

Xiang, H., Cheng, X., Li, Y., Luo, W., Zhang, Q., and Peng, W. (2021). Efficacy of IVIG (intravenous immunoglobulin) for corona virus disease 2019 (COVID-19): a meta-analysis. *Int. Immunopharmacol.* 96:107732. doi: 10.1016/j.intimp.2021.107732

Zhou, L. (2019). Small Fiber neuropathy. *Semin. Neurol.* 39, 570–577. doi: 10.1055/s-0039-1688977

Županić, S. (2021). Treatment of myasthenia gravis patients with COVID-19: review of the literature. *Acta Clin. Croat.* 60, 496–509. doi: 10.20471/acc.2021.60.03.21

frontiers | Frontiers in Neuroscience

TYPE Original Research
PUBLISHED 08 January 2024
DOI 10.3389/fnins.2023.1249282

OPEN ACCESS

EDITED BY
Gonzalo Emiliano Aranda-Abreu,
Universidad Veracruzana, Mexico

REVIEWED BY
Ulises Gomez-Pinedo,
Health Research Institute of Hospital Clínico San Carlos, Spain
Ela Isabel Olivares,
Autonomous University of Madrid, Spain

*CORRESPONDENCE
Fuleah A. Razzaq
✉ fuleah@neuroinformatics-collaboratory.org
Roberto Rodriguez-Labrada
✉ roberto.rodriguez@neuroinformatics-collaboratory.org
Maria L. Bringas-Vega
✉ maria.bringas@neuroinformatics-collaboratory.org
Pedro A. Valdes-Sosa
✉ pedro.valdes@neuroinformatics-collaboratory.org

†These authors have contributed equally to this work

RECEIVED 28 June 2023
ACCEPTED 11 December 2023
PUBLISHED 08 January 2024

CITATION
Yasir S, Jin Y, Razzaq FA, Caballero-Moreno A, Galán-García L, Ren P, Valdes-Sosa M, Rodriguez-Labrada R, Bringas-Vega ML and Valdes-Sosa PA (2024) The determinants of COVID-induced brain dysfunctions after SARS-CoV-2 infection in hospitalized patients.
Front. Neurosci. 17:1249282.
doi: 10.3389/fnins.2023.1249282

The determinants of COVID-induced brain dysfunctions after SARS-CoV-2 infection in hospitalized patients

Shahwar Yasir[1†], Yu Jin[1†], Fuleah A. Razzaq[1*], Antonio Caballero-Moreno[2], Lidice Galán-García[3], Peng Ren[1], Mitchell Valdes-Sosa[3], Roberto Rodriguez-Labrada[3*], Maria L. Bringas-Vega[1,3*] and Pedro A. Valdes-Sosa[1,3*]

[1]Joint China-Cuba Laboratory for Neurotechnology and Bioengineering (JCCLNB), The Clinical Hospital of Chengdu Brain Science Institute, School of Life Sciences and Technology, University of Electronic Science and Technology of China, Chengdu, China, [2]Servicio de Psiquiatria Galigarcia, Hospital Nacional, La Habana, Cuba, [3]The Cuban Neuroscience Center, La Habana, Cuba

The severity of the pandemic and its consequences on health and social care systems were quite diverse and devastating. COVID-19 was associated with an increased risk of neurological and neuropsychiatric disorders after SARS-CoV-2 infection. We did a cross-sectional study of 3 months post-COVID consequences of 178 Cuban subjects. Our study has a unique CUBAN COVID-19 cohort of hospitalized COVID-19 patients and healthy subjects. We constructed a latent variable for pre-health conditions (PHC) through Item Response Theory (IRT) and for post-COVID neuropsychiatric symptoms (Post-COVID-NPS) through Factor Analysis (FA). There seems to be a potential causal relationship between determinants of CIBD and post-COVID-NPS in hospitalized COVID-19 patients. The causal relationships accessed by Structural Equation Modeling (SEM) revealed that PHC ($p < 0.001$) and pre-COVID cognitive impairments ($p < 0.001$) affect the severity of COVID-19 patients. The severity of COVID-19 eventually results in enhanced post-COVID-NPS ($p < 0.001$), even after adjusting for confounders (age, sex, and pre-COVID-NPS). The highest loadings in PHC were for cardiovascular diseases, immunological disorders, high blood pressure, and diabetes. On the other hand, sex ($p < 0.001$) and pre-COVID-NPS including neuroticism ($p < 0.001$), psychosis ($p = 0.005$), cognition ($p = 0.036$), and addiction ($p < 0.001$) were significantly associated with post-COVID-NPS. The most common neuropsychiatric symptom with the highest loadings includes pain, fatigue syndrome, autonomic dysfunctionalities, cardiovascular disorders, and neurological symptoms. Compared to healthy people, COVID-19 patients with pre-health comorbidities or pre-neuropsychiatric conditions will have a high risk of getting severe COVID-19 and long-term post-COVID

neuropsychiatric consequences. Our study provides substantial evidence to highlight the need for a complete neuropsychiatric follow-up on COVID-19 patients (with severe illness) and survivors (asymptomatic patients who recovered).

KEYWORDS

SARS-CoV-2, neuropsychology, COVID-19, post-COVID neuropsychiatric symptoms/disorders, long-COVID

1 Introduction

COVID-19 patients most frequently experience respiratory impairments. However, the disease's high susceptibility and morbidity indicate the effect of pre-existing health conditions in COVID-19 patients. The degrees of symptomatology in COVID-19 are caused by host-viral interactions and immunological responses, leading to severe infection and mortality. We briefly summarized some of the literature that shows that patients with a history of diabetes, hypertension, or respiratory diseases have much worse immune function, which affects vascular and respiratory impairment (Huang et al., 2022; Sharun et al., 2022). Recent Research into COVID-19 revealed that SARS-CoV-2 affects the brain, which results in neurological and neuropsychiatric consequences. Indeed, after recovering from COVID-19, some patients still suffer from post-COVID symptoms, which include depression, anxiety, headache, sleep disturbances, cognitive decline, or other health comorbidities that can lead to long-COVID (Fahriani et al., 2021; Bigdelou et al., 2022, 2022; Efstathiou et al., 2022).

The risk of anxiety or trauma-related disorders is highest for people with pre-health comorbidities such as complicated diabetes, obesity, and cardiovascular diseases (Treskova-Schwarzbach et al., 2021). According to US administrative database data, 14% of individuals with SARS-CoV-2 infection in 6 months developed new clinical disorders affecting multi-organ systems, including cardiovascular, respiratory, or brain disorders (Daugherty et al., 2021). Another study of 4,899,447 hospitalized patients examined underlying medical conditions related to SARS-CoV-2 infection (Kompaniyets et al., 2021). Careful evaluation and management of underlying health conditions in COVID-19 patients can aid in risk stratification for severe infection (Calixto-Calderón et al., 2021). However, much is yet to be discovered about the detailed mechanisms, treatment, efficacy, and long-term outcomes of post-COVID symptoms (Adab et al., 2022; Choutka et al., 2022). Significant evidence shows that chronic conditions can develop from acute COVID-19, where hospitalization and pre-existing conditions are generally linked to higher risk and severity of COVID-19 (Cunningham et al., 2021).

There is increasing scientific evidence of an association between SARS-CoV-2 infection and the subsequent occurrence of new-onset post-COVID neuropsychiatric symptoms, especially among patients with increased disease severity (Charlton et al., 2021; Busatto et al., 2022; De Erausquin et al., 2022; Efstathiou et al., 2022). It is mandatory to understand the impact of the neurotropic nature of SARS-CoV-2 and its effects on the long-term consequences of cognitive decline and other neuropsychiatric sequelae in COVID-19 patients (Rogers et al., 2020; De Erausquin et al., 2022; Taquet et al., 2022). Of particular importance is that pre-existing neuropsychiatric disorders induce a higher risk of contracting COVID-19 infection (Douaud et al., 2022; Taquet et al., 2022). So, it is essential to understand the attributes of post-COVID or long-COVID symptoms in patients (Augustin et al., 2021; Fernández-de-Las-Peñas et al., 2021; Magnusson et al., 2022; Nalbandian et al., 2023).

Despite the numerous clinical manifestations of post-COVID sequelae, studies employing statistical approaches to analyze patterns of symptom co-occurrence and their biological connections explicitly are sparse. We leveraged a unique cohort obtained in Cuba to describe the long-term post-COVID consequences. It is a 3-month follow-up study on hospitalized COVID-19 patients and survivors after recovery. We have studied the potential causal relations between determinants of COVID-Induced Brain Dysfunctions, analyzing age, sex, pre-health conditions (PHC), pre-COVID neuropsychiatric conditions (Pre-COVID-NPS), COVID-19 severity, and long-term post-COVID neuropsychiatric symptoms (post-COVID-NPS) in hospitalized COVID-19 patients and healthy controls. The COVID-19-positive and COVID-19-negative patients were hospitalized and shared the same psychological stress. The risk/incidence for neuropsychiatric sequelae may increase with pre-existing health conditions, pre-neuropsychiatric conditions, and admission to the hospital for SARS-CoV-2 infection. This study identified the potential risk factors, including COVID-19 severity, the effect of pre-health comorbidities, and pre-neuropsychiatric conditions in patients. The uniqueness of this sample is that both PCR + and PCR- participants were hospitalized and were subject to some degree of isolation and stress.

The main goal is to check whether the patients with prior health comorbidities or pre-neuropsychiatric conditions have a high risk of a more severe COVID-19 infection, eventually leading to long-term post-COVID neuropsychiatric symptoms. So, we studied the effect of pre-health conditions (PHC) on COVID-19 severity. PHC includes chronic obstructive pulmonary disease, immunological disorders, renal disorders, high blood pressure, cardiovascular symptoms, and diabetes. Post-COVID-NPS analysis includes somatomorphic symptomatology and autonomic dysfunctionalities. The somatomorphic symptomatology includes pain, gastrointestinal symptoms, cardiovascular disorders, urogenital and neurological symptoms. Autonomic dysfunctionalities include fatigue syndrome, panic symptoms, symptoms of generalized anxiety, depression, sleep disorders,

sexual dysfunctions, and trauma. Pre-health conditions were categorized and analyzed using Item Response Theory (IRT) by generating a latent variable. Symptom co-occurrence for post-COVID-NPS was investigated by Factory Analysis (FA). After adjusting for confounders (age, sex, and pre-COVID-NPS), we further explored the associations of latent variables with the long-term neuropsychiatric consequences in COVID-19 patients via Structural Equation Modeling (SEM).

2 Materials and methods

2.1 Study design

This study is a prospective follow-up study of convalescent COVID-19 subjects after epidemiological discharge who were recruited from community-based policlinics in three municipalities in Havana City. The assessment was conducted for 8 months, from July 2020 to March 2021. It included baseline assessments and a follow-up visit 18–24 months later. For this study, 178 participants were recruited. All Participants were asked to visit the Cuban Centre for Neurosciences for the health assessments.

2.2 Participants

2.2.1 Inclusion and exclusion criteria

For this study, the SARS-CoV-2 infection cohort was selected retrospectively based on the following inclusion criteria: (i) age 18-80 years, (ii) at least primary school education, (iii) diagnosis of COVID-19 by Polymerase Chain Reaction (PCR) test; and (iv) after discharge period of 3–6 months. The exclusion criteria were (i) diagnosis of neurologic or psychiatric disorders before the COVID-19 infection, (ii) diagnosis of severe organ-specific disease (e.g., cancer, hepatopathy, cardiomyopathy, advanced renal disease), and (iii) alcohol or drug abuse.

Most of the patients analyzed in this study were infected between March and December 2020. They were evaluated between June and December 2020. The molecular epidemiological study conducted in Cuba identified that the common SARS-CoV-2 variant in Cuba during this time was D614G (Guzmán et al., 2022). However, a few COVID-19 cases were taken in early 2021 as well, which were infected with another variant of SARS-CoV-2. It is impossible to distinguish the variant of those few cases; however, the dominant variant of SARS-CoV-2 at that time was D614G.

2.2.2 Patients

A total of 91 participants were confirmed convalescent COVID-19 patients who had contracted the D614G variant of the SARS-COV-2 virus and had a positive reverse transcription polymerase chain reaction (RT-PCR) result.

2.2.3 Controls

To ensure the robustness of the study, we selected an RT-PCR negative control group (n = 87) comprising individuals of age, sex, and education-matched individuals. These controls were in close contact with COVID-19-positive cases. They were isolated and evaluated in healthcare facilities according to the "Cuban Protocol for the Management and Care of COVID-19 in 2020" until their PCR test results were available. Those who tested negative were discharged, while those who tested positive were shifted to a hospital for COVID-19 treatment. This control group was included to mitigate the potential impact of psychological determinants, such as anxiety and depression, on intergroup differences. The unique aspect of this study design allowed us to isolate the virus's effects from other confounding factors. Including this control group is a noteworthy feature of the study, as it enhances the reliability and validity of the findings.

2.3 Measures/determinants of COVID-induced brain dysfunctions (CIBD)

2.3.1 COVID-19 severity

The degree of severity for all the participants was classified into four categories. Non-COVID patients were coded as "0." On the other hand, COVID patients (PCR positive) had distinct degrees of severity for SARS-CoV-2 infection. There were 44 asymptomatic patients coded as "1," 37 mild symptomatic patients coded as "2," and 10 severe symptomatic patients coded as "3."

2.3.2 Pre-health conditions (PHC)

We have analyzed seven different health categories to study the prior-health comorbidities in COVID-19 patients, which are as follows:

i **Cardiovascular diseases** include ischemia, stroke, hemorrhage, insufficient blood circulatory system issues, and arrhythmia.
ii **Immunological disorders** include immunodepression, immunosuppression, HIV, and rheumatoid arthritis.
iii **Renal disorders** include urinary sepsis, renal colic disorder, urinary incontinence, and kidney stones.
iv **Neurological diseases** include epilepsy, peripheral manifestations, aneurysm, migraine, multiple sclerosis, trigeminal neuralgia, craniofacial syndrome, neuropathy, and meningoencephalitis.
v **Chronic obstructive pulmonary disease (COPD)** includes lungs-related injury/disorder and fibrosis.
vi **High blood pressure (HBP).**
vii **Diabetes.**

2.3.3 Confounders/confounding factors

2.3.3.1 Age

Our study sample includes participants with an age range from 20 to 85 years. The mean age was 48.25 for the COVID-19 patients and 44.82 for the Control group. We treated age as a confounding factor to check its effect on other confounders (sex and pre-COVID-NPS) and COVID-19-related variables (PHC and post-COVID-NPS).

2.3.3.2 Sex

We analyzed 36 males and 55 females in the COVID-19 group and 39 males and 48 females in the control group. It is to study

whether sex (male or female) is associated with COVID-19 severity and post-COVID-NPS.

2.3.3.3 Pre-COVID neuropsychiatric conditions (pre-COVID-NPS)

The psychiatrist's team consisted of 4–6 psychiatrists from different hospitals in Cuba, who were in charge of interviewing the patients. The psychiatrists employed a few categories from Section 0 of the SCAN 2.1 (Schedules for Clinical Assessment in Neuropsychiatry), which includes sociodemographic items such as age, date of birth, sex, marital status, educational level, years of education, address, and skin color for a detailed analysis. Furthermore, they diagnosed the previous psychiatric conditions (Personal Pathological and Psychiatric Antecedents) after the interview, using different standardized screening questionnaires, and finally concluded their remarks to make a diagnosis.

The following are the categories of pre-COVID-NPS in COVID-19 patients.

The Neurocognitive symptoms category was based on three categories of syndromes: delirium, mild neurocognitive disorder, and major neurocognitive disorder (dementia). **The Psychotic spectrum** category classified all subjects referred to as having any psychotic symptom or episode. They are characterized by an impaired relationship with reality, usually associated with behavioral changes, such as hearing voices, visual hallucinations, or delusions. There are several psychotic disorders with different diagnostic criteria, as mentioned in the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-5) 5th edition. **The Neuroticism spectrum** reflects a person's level of emotional stability. It is usually defined as a personality trait with negative emotions, poor self-regulation (an inability to manage urges), trouble dealing with stress, and the tendency to complain. Non-psychotic symptoms, such as depression, panic, or anxiety episodes, were also classified. Lastly, **Addiction** includes those subjects who are referred to have abuse of substances. They have substance use disorder criteria that involve using more substance than usual, urges to use the substance often, neglecting responsibilities at home, work, or school because of intense cravings to use the substance, giving up social and extracurricular activities, and continual use of the substance despite causing problems in physical and mental health, etc.

Notably, Taquet et al. (2021) reported that among 236,379 patients diagnosed with COVID-19, the most common incidences at 6 months post-COVID consequences were 1.40% for psychotic disorders, 6.58% for substance use disorder, 5.42% for insomnia, and 0.67% for dementia.

2.3.3.4 Post-COVID neuropsychiatric symptoms (post-COVID-NPS)

Neuropsychiatric data of COVID-19 patients was taken from Schedules for Clinical Assessment in Neuropsychiatry (SCAN), version 2.1 (Schützwohl et al., 2007). It is a semi-structured interview developed by the World Health Organization to assess psychiatric disorders that consists of 1,872 items distributed in 28 sections. For the present study, only sections 0 (sociodemographic items); II (Physical health, somatoform, and dissociative disorders), IV (Panic, anxiety, and phobias); VI (Depressed mood and ideation); VII (Thinking, concentration, energy, interest); VIII (Bodily functions), and XIII (Interference and attributions for part one) were analyzed. However, during the interview, the psychiatrists gathered additional information about the duration of symptoms, the initial and final date of symptoms, and interference of the symptoms, which helped make the diagnosis.

We have used Somatomorphic symptomatology items categorized by SCAN 2.1 (Schützwohl et al., 2007), which are defined as follows:

i **Pain** includes headache, pain in the back, arms, or legs, muscle pain, pain in the joints, pain in the chest, pain while urinating, abdominal pain, menstrual pain, pain during sexual intercourse, pain in the ass, pain in other parts of the body and erratic pain.

ii **Gastrointestinal** include nausea, vomiting, a sensation of fullness, bloating, diarrhea, constipation, anal fluids, bad sensation in the mouth, burning sensation in the chest or esophagus, aerophagia, hiccups, intolerance to some food, and other gastric symptoms.

iii **Cardiovascular** includes palpitation, precordial discomfort, hyperventilation, shortness of breath (without physical exercise), dyspnea during exercise, and other cardiovascular complaints.

iv **Urinary/Urogenital** include frequent urination, urinary retention, uncomfortable sensation around the urethra or genitalia, irregular menstruation, fluid in the vagina, excessive bleeding during menstruation, decrement of menstruation, vomiting during pregnancy, lack of sexual interest, erectile dysfunction, other complaints in the urinary and genital area.

v **Neurological** include lack of balance or equilibrium, a sensation of paresis or focal muscle weakness, swallowing problems, knot in the throat, aphonia, twinkling, painful anesthesia, diplopia, blindness, deafness, fainting, fading, loss of consciousness, amnesia, other neurological manifestations.

Of note, the neurological symptomatology included in our analysis is based on the participant's answers asked during psychiatric assessments and is not actually from the neurological manifestations category defined by a neurologist. That is why, in our study, we have mainly focused on neuropsychiatric symptoms instead of neurological consequences. The complete list of the items included in this study can be found in **Supplementary material 1**.

3 Statistical analysis

This section describes the details of the statistical analysis for our data. **Figure 1** shows an overview of the proposed methodology in this article.

3.1 Data pre-processing and cleaning

We pre-processed and cleaned the data and stored it in "tidyverse" format using the "tidyverse" package (Wickham et al., 2019) in R (version 4.3.0). To understand the data distribution, we have plotted individual data points in a Scatter plot between COVID-19 severity and post-COVID-NPS, available in **Supplementary material 2** of the article. Furthermore, checking for

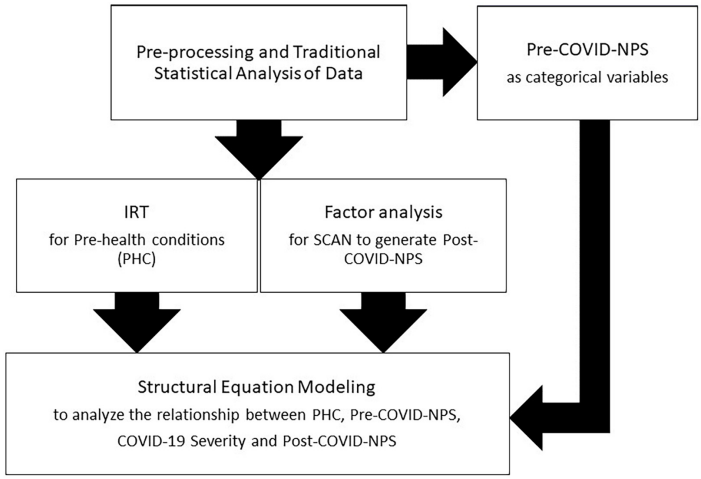


FIGURE 1
Overview of methodology (statistical analysis) for our CUBAN COVID-19 dataset.

linearity, additivity, normality, and homoscedasticity assumptions is important for any causal or inference-based analysis to determine the authenticity of the results. The violation of assumptions may degrade the outcome of the analysis (Williams et al., 2013; Ernst and Albers, 2017).

Initially, we check for data missingness, followed by multivariate outlier detection. A multivariate outlier is any subject whose combination of values for all variables differs from most participants (Tabachnick et al., 2007). We used Mahalanobis distance to assign a score to each subject based on the distance between the subject and the center of a distribution. We detected this using the "Mahalanobis" function in R with a *p*-value of 0.001 as a distance-cut off (Tabachnick et al., 2007). We examined normality, where all errors are generally distributed around zero using the standardized regression residuals. A histogram was plotted using the "moments" package (Komsta and Novomestky, 2022) to check the skewness and kurtosis in the data. Additivity was checked by looking into correlation for measured variables. The correlations were checked and plotted with the "corrplot" package (Wei and Simko, 2021). The homoscedasticity assumption is fulfilled when residuals have the same variance for all independent variable values. To check for homoscedasticity, we employed a scatter plot of standardized residuals vs. fitted values (Tabachnick et al., 2007). Power analysis was done to check the power of our sample by using "semTools" (Jorgensen et al., 2022) and "semPower" (Moshagen and Bader, 2023) packages in R.

3.2 Item response theory (IRT) for PHC

We obtained a latent variable for pre-health conditions (PHC) by Item response theory (IRT) as they are binary variables with YES/NO questions. IRT consists of mathematical tools that explain the relationship between latent variables and their observable values (true/false or multiple-choice questions). The goal of IRT in our analysis is to quantify the likelihood or tendency of a given response based on the relationship between trends of pre-health conditions in participants and the ability to distinguish characteristics of these items. Therefore, a dominant factor explaining most of the variance scores is expected.

Item Response Theory implies three assumptions: A unidimensional trait denoted by theta, the local independence of items, and a response to an item can be modeled by a mathematical item response function (IRF). In this study, the models predict the likelihood or probability of a correct response. One way to choose the best-fitted model is to assess its relative fit through its Information criteria. AIC estimates are compared, and the model with the lower AIC is chosen. It also helps to identify their optimal linear combination obtained by non-linear factor analysis to produce an inferred overall score, also called a latent variable. We used "MIRT," a Multidimensional item response theory (Chalmers, 2012) package in R, to identify latent variables. The latent variables are independent of the evaluator and are more robust against fluctuations in score recording.

3.3 Factor analysis for post-COVID-NPS

Factor analysis (FA) is a data reduction tool that helps to reduce a large number of variables into a smaller number of components (factor) or latent variables. It removes any redundancy from a set of correlated variables in the data. As post-COVID-NPS is a continuous score from SCAN 2.1, we generated a latent variable for post-COVID-NPS with the help of factor analysis. This technique combines the highest common variances and projects them onto a common score. We use this score for further analysis to summarize all constituent variables.

3.4 Structural equation modeling (SEM) for statistical analysis

Structural equation modeling (SEM) is a comprehensive set of multivariate statistical techniques. This method combines component and multiple regression analyses to explore the

structural relationship between measured and latent variables for testing different hypotheses (Beran and Violato, 2010). We used SEM to analyze the relationship between COVID-19-related variables and their effect on COVID-19 severity and post-COVID-NPS. SEM examines linear causal relationships among variables while accounting for measurement error. After getting a post-COVID-NPS latent variable from factor analysis, we analyzed the effect of age, sex, pre-health conditions, and pre-COVID-NPS on COVID-19 severity and post-COVID-NPS.

Structural Equation Modeling was performed with the help of "Lavaan," defined as a latent variable analysis. Thus, SEM with Lavaan helps test path models with latent variables. Our model in SEM analyzed the direct effect of PHC on COVID-19 Severity. And how COVID-19 Severity enhances post-COVID-NPS chances even after adjusting for confounders. Statistical fit indices provide a way to quantify how well our model fits the data. The SEM model's Comparative Fit Index (CFI) is the discrepancy function for any sample size. CFI ranges from 0 to 1, with a larger value indicating a better model fit. Acceptable model fit is indicated by a CFI value of 0.90 or greater. The Root Mean Square Error of Approximation (RMSEA) describes the residual in the model. RMSEA values range from 0 to 1, with a smaller RMSEA value indicating a better model fit. It is highly significant if $p < 0.05$ between the two nodes in the path model.

4 Results

4.1 Demographics

Table 1 shows the analyses of demographic characteristics of our CUBAN COVID-19 dataset with significant differences in age, sex, and year of education between the two groups. It was tabulated using the "table one" package (Yoshida and Bartel, 2022).

4.2 Data pre-processing and assumptions check

Our CUBAN COVID-19 dataset has no missingness. We implemented outlier detection based on Mahalanobis distance and excluded three subjects from the analysis based on the distance cut-off value. After outlier elimination, we have $n = 175$ participants to carry out the rest of the analysis with more authenticity, as shown in Table 2.

All the assumptions were checked and analyzed carefully by residual plots, histograms, and Quantile-Quantile (Q-Q) plots. Linearity was checked through a Q-Q plot where the x-axis has theoretical quantiles, showing the residuals in standard normal distribution if the variance is derived from a normal distribution. On the other hand, the y-axis has the sample quantiles for each data point. So, if the data have a normal distribution, it will be represented around a straight line. The recommended range is from −2 to + 2 for a linear relationship (Tabachnick et al., 2007). There is a minimal deviation in the tail, but overall, the Q-Q plot for standardized regression residuals shows a linear trend, as shown in Figure 2. Normality was checked and plotted as a Standardized Histogram to test whether residuals are normally distributed visually. The histogram for standardized residuals shows a normal data distribution (symmetrical in a bell shape), as shown in Figure 3, so no corrective measures were needed.

Furthermore, homogeneity/homoscedasticity was checked, depicting the residuals' constant variance. We have plotted z-scores-fitted values vs. standardized residuals in a scatter plot. The data points have a constant spread along the regression line, as shown in Figure 4. The results are homogenous as the spread across both axes [−2, 2] is the same. The solid line in the plot marks zero for both axes. The additivity assumption was checked using "corrplot" to study the correlation between two independent variables (Wei and Simko, 2021). It shows that all the variables fall under the correlation value of less than 0.9, depicting a weak correlation/strength among variables, as shown in Figure 5. So, the analysis will not be biased, and the results will be accurate. The dots in Figures 2, 4 show the regression residuals as we are predicting the outcome of a random variable, so the errors should be randomly distributed (centered around zero). Each dot represents a standardized residual plotted against the theoretical residual for that area of the standardized distribution. By standardizing the errors, the results can be interpreted easily. In short, Figures 2–4 summarizes the results for linearity, normality, additivity, and data homogeneity using standardized results from regression.

4.3 IRT scores for pre-health conditions (PHC)

We have analyzed pre-health comorbidities to get a latent variable for PHC by Item Response Theory, as IRT models estimate respondents' responses to the items based on their position or trend on the latent trait spectrum. Each item's response indicates a certain degree of loadings on the latent traits. Simply put, the latent variable (θ) influences and distinguishes the likelihood of reporting positive on the items in pre-health conditions. The Item Characteristic Curve is a graphical representation of this relationship. The curve is S-shaped (Sigmoid/Ogive), as indicated in Figure 6.

Furthermore, the higher the latent variable estimated, the higher the probability or likelihood of giving a positive response in terms of loadings. It is to be noted that theoretically, this latent variable (θ) ranges from $-\infty$ to $+\infty$. However, practically, it usually ranges between −3 and + 3. This analysis was conducted with all 178 subjects, taking advantage of the flexibility of the IRT approach for handling missing values. We got specific loadings for pre-health conditions of COVID-19 patients through item response theory (IRT). The curves in Figure 6 show the probability of a patient being positive for values of an underlying variable, and the theta represents the outcome in the form of the disease state (COVID-19 severity and post-COVID-NPS). All items, specifically cardiovascular diseases, immunological disorders, and high blood pressure, loaded well onto the latent factors produced by IRT analysis with 47% of the variance, as listed in Table 3. It states that IRT classifies the participants suffering from any of these pre-health comorbidities to have a higher chance of getting a severe COVID-19 infection and post-COVID neuropsychiatric symptoms.

TABLE 1 The demographic characteristics of our CUBAN COVID-19 dataset.

	Description	COVID-19 group (Positive = 0)	Control group (Negative = 1)	*P*-value
N = Number of patients	178	91	87	–
Age [mean (SD)]	20–85 years	48.25 (12.68)	44.82 (12.83)	0.074
Sex [*n* (%)]	Male = 0	36 (39.6)	39 (44.8)	0.576
	Female = 1	55 (60.4)	48 (55.2)	
Education [mean (SD)]	Years of study/grade	14.10 (3.60)	14.79 (2.88)	0.158
Marital status [*n* (%)]	Divorced	8 (9.1)	7 (8.9)	0.943
	Married	24 (27.3)	21 (26.6)	
	Single	18 (20.5)	20 (25.3)	
	Union consensual	36 (40.9)	30 (38.0)	
	Widow	2 (2.3)	1 (1.3)	
Degree of severity [*n* (%)]	No COVID = 0	0 (0.0)	87 (100.0)	<0.001
	Asymptomatic = 1	44 (48.4)	0 (0.0)	
	Mild symptomatic = 2	37 (40.7)	0 (0.0)	
	Severe symptomatic = 3	10 (11.0)	0 (0.0)	

TABLE 2 Outlier detection via Mahalanobis distance in R.

Mahal < cutoff		
Mode	TRUE	FALSE
Logical	175	3

4.4 Post-COVID neuropsychiatric symptoms (post-COVID-NPS) scores from FA

We did factor analysis for post-COVID-NPS and obtained factor loadings to describe the relationship between the factors and the observed variables. The loadings can evaluate the relationship strength between each variable and the factor. Additionally, we can identify the observed variables corresponding to a specific factor and interpret loadings as correlation coefficients. Values usually range from −1 to + 1. However, the sign indicates the direction of the relations, either positive or negative, while the absolute value indicates the strength. Stronger relationships in the factor analysis have factor loadings closer to −1 and + 1, which shows that the factors explain maximum variance in the observed variable. We have set the threshold to be >0.3 to check the significant values. For post-COVID-NPS, the highest loadings observed are for pain, fatigue syndrome, autonomic, cardiovascular, and neurological symptoms, with a threshold range from 0.82 to 0.74, explaining 47% of the variance, as indicated in Table 4.

4.5 Structural equation modeling (SEM) output

Structural Equation Modeling is a multivariate, hypothesis-driven technique based on a structural model. It represents a hypothesis about the causal relations among several variables. Path models are diagrams in SEM used to visually display the hypotheses and help to examine variable relationships. The relationship between constructs (variables that are not directly measurable) and their assigned indicators is depicted by arrows. Single-headed arrows have predictive relationships and can be interpreted as causal relationships, as shown in Figure 7.

The psychiatrists analyzed pre-neuropsychiatric conditions (Pre-COVID-NPS). We treated them as categorical variables as they belong to four different categories to study the effect of pre-COVID-NPS on post-COVID-NPS. The pre-COVID-NPS items were directly put in the Structural equation modeling formula along with other regression coefficients and confounding factors. SEM analysis helps to study the effect of these COVID-19 determinants of COVID-Induced Brain Disorders on post-COVID neuropsychiatric symptoms in COVID-19 patients. Significant and non-significant pathways (*p*-values) were obtained for latent variables and confounding factors to study the strength of their relationship, as shown in Table 5. The specific linear regressions tested are expressed using the Wilkinson-Rogers notation, which gives a compact and intuitive notation for liner models (Wilkinson and Rogers, 1973) as shown in Equations (1, 2). Wilkinson notations describe regression and repeated measure models without mentioning coefficient values. It also specifies and identifies the response variable as well as which predictor variables should be included or excluded from the model. So, it allows the inclusion of only the interaction terms of interest in the main model, be it squared, higher order terms, or grouping variables.

$$\text{Severity} \sim \text{PHC} + \text{Age} + \text{Sex} + \text{Neuroticism} + \text{Cognitive} + \text{Psychosis} + \text{Addiction} \quad (1)$$

$$\text{NPS} \sim \text{COVID} - 19\ \text{Severity} + \text{Age} + \text{Sex} + \text{Neuroticism} + \text{Cognitive} + \text{Psychosis} + \text{Addiction} \quad (2)$$

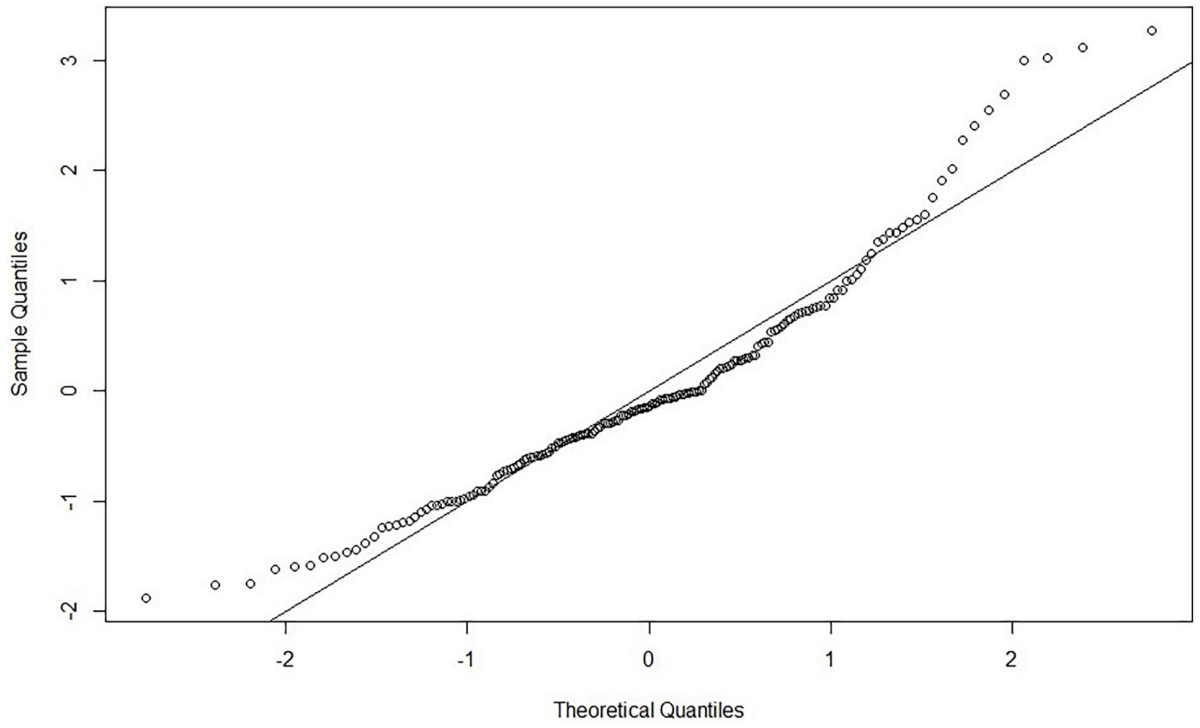


FIGURE 2
A Quantile-Quantile (Q–Q) plot for the standardized residual vs. a theoretical normal distribution to ensure a linear trend.

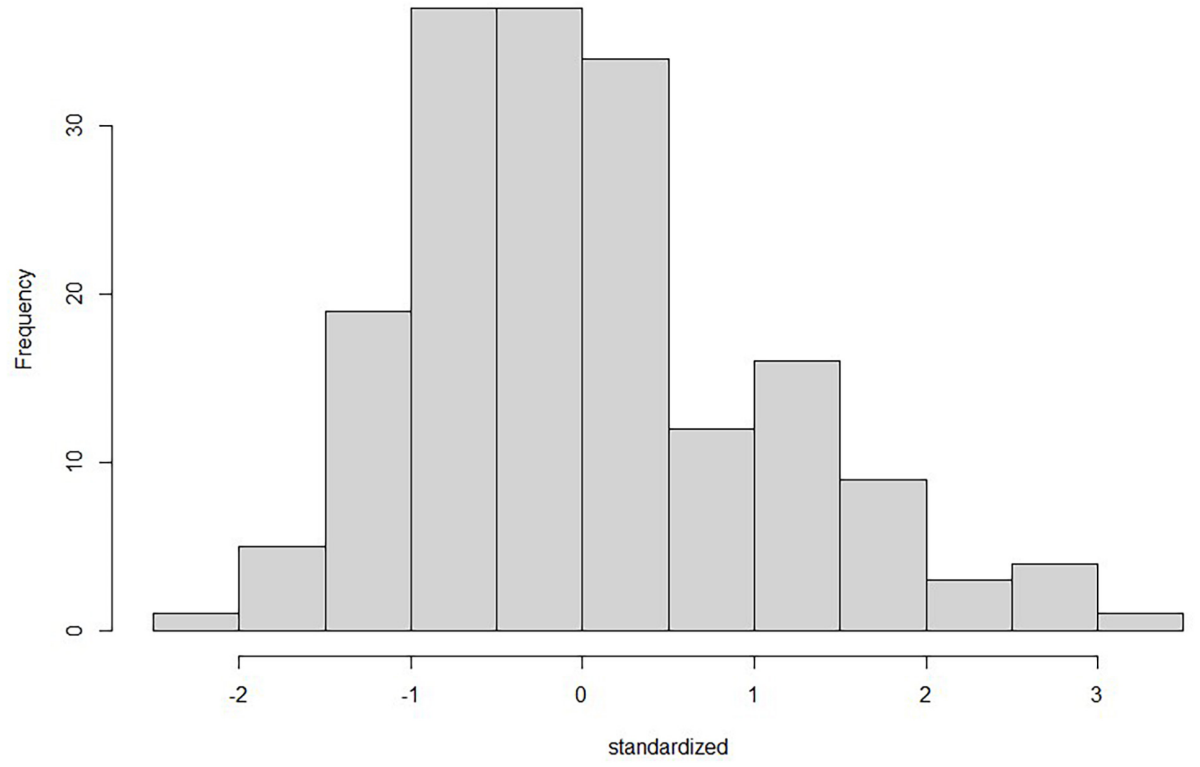


FIGURE 3
Histogram for standardized regression residuals to check for normality using the "moments" package in R. It shows that the data has a symmetrical distribution and is not skewed.

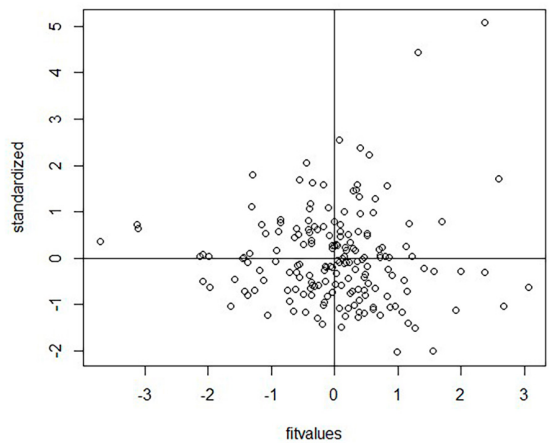


FIGURE 4
A homoscedasticity graph (scatter plot) between standardized regression residuals and z-scored fitted value where the data spread is homogeneous.

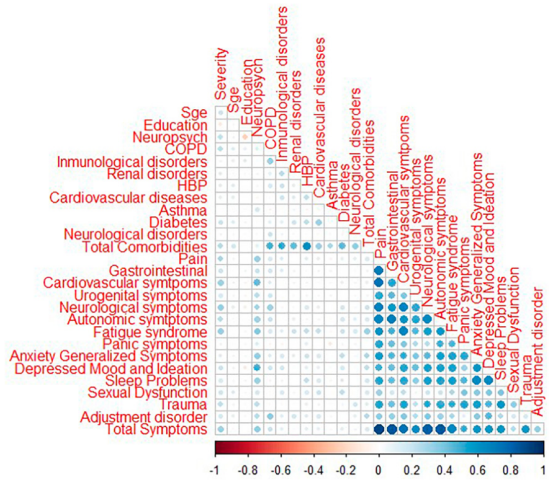


FIGURE 5
Correlation plot for measured variables using the "corrplot" package in R. The blue color shows a positive correlation; the dark color depicts a value of 0.96.

$$\text{NPS} = \text{Post} - \text{COVID} - \text{NPS}$$

$$\text{Neuroticism} + \text{Cognitive} + \text{Psychosis} + \text{Addiction} = \textit{Pre} - \textit{COVID} - \textit{NPS}$$

The results summarize the factors affecting COVID-19 severity and post-COVID-NPS tested via Structural Equation Modeling (SEM). The output of the SEM model has significant fit indices. The comparative fit index, or CFI, is a popular fit index, which is 0.927 for our model. On the other hand, SRMR is 0.022, and RMSEA = 0.186, which shows that our model is a good fit with maximum loadings for latent variables. We obtained significant paths and *p*-values for sex, COVID-19 severity, PHC, pre-COVID-NPS, and post-COVID-NPS. For PHC and severity $p = 0.005$, for severity and NPS $p < 0.001$, for sex and post-COVID-NPS $p < 0.001$, and for pre-COVID-NPS (neuroticism, cognitive, psychosis, addiction) to post-COVID-NPS $p < 0.05$ as shown in Table 5.

The present study aims to assess the true extent of post-COVID brain dysfunctions in the Cuban population and its role in the progression or *de novo* diagnosis of neurodegenerative diseases. It depicts that COVID-19 is associated with a certain degree of severity and post-COVID neuropsychiatric symptoms (Post-COVID-NPS) in patients. Patients with pre-health comorbidities, like chronic obstructive pulmonary diseases, diabetes, immunological disorders, renal disorders, or cardiovascular symptoms, have a high risk of getting more severe COVID-19 and, eventually, more long-term neuropsychiatric symptoms. The most common neuropsychiatric symptoms included pain, cardiovascular, trauma, fatigue syndrome, anxiety, depression, and neurological symptoms. A study by Tauqet et al. has also shown that pre-COVID-NPS conditions enhance the chances of getting post-COVID-NPS comorbidities in COVID-19 patients.(Taquet et al., 2022).

5 Discussion

In this study, we used statistical methods to document patterns of co-occurrence of several post-COVID symptoms in a relatively moderate sample of patients with mild or severe COVID-19 infection. The participants were evaluated 3 months after hospitalization to determine how such a symptom co-occurrence was related to COVID-19 severity, followed by signs of post-COVID neuropsychiatric symptoms in patients and survivors (both hospitalized). Evidence from COVID-19 infection suggests a potential development and risk of long-term neuropsychiatric disorders (Kumar et al., 2021). We analyzed the pre-health conditions of COVID-19 patients and controls by Item Response Theory (IRT) and got PHC scores in the form of loadings. Another study illustrates and emphasizes the features of IRT to refine and increase the validity and reliability of the analysis (Zanon et al., 2016). In our study, the highest loadings (cardiovascular, immunological disorders, and high blood pressure) imply that the patients with such prior health comorbidities will have a high level of COVID-19 severity. However, neurological diseases in PHC loaded extremely low as our sample has few patients who had neurological disorders before getting SARS-CoV-2 infection. Similar other studies state that pre-existing health comorbidities may cause disease severity or post-COVID-related consequences (Sanyaolu et al., 2020; Calixto-Calderón et al., 2021; Edison, 2021a; Richardson et al., 2021; Treskova-Schwarzbach et al., 2021; Russell et al., 2023). Several underlying diseases were linked to severe COVID-19 infection. The most common condition was high blood pressure, but the significant risk factors for severe COVID-19 were obesity, diabetes with other comorbidities, and psychological disorders (Kompaniyets et al., 2021).

On the other hand, pre-COVID-19 neuropsychiatric conditions directly impact the severity of the infection and post-COVID neuropsychiatric symptoms. Patients with cognitive impairments prior to infection are more likely to get severe COVID-19 (Kumar et al., 2021). However, prior neuropsychiatric conditions like neuroticism, psychosis, addiction, and cognitive

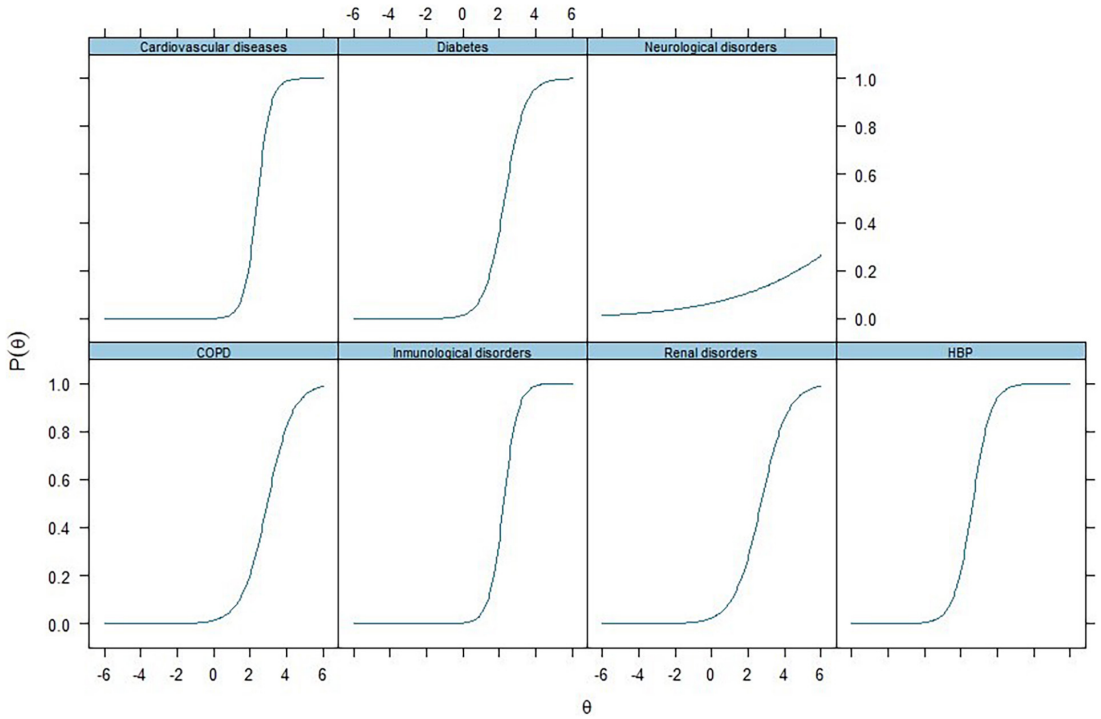


FIGURE 6
Item characteristic curves for pre-health conditions (PHC) via item response theory analysis (IRT). Each item's response indicates a certain degree of loadings on the latent traits. Simply put, the latent variable (θ) influences and distinguishes the likelihood of reporting positive on the items in pre-health conditions.

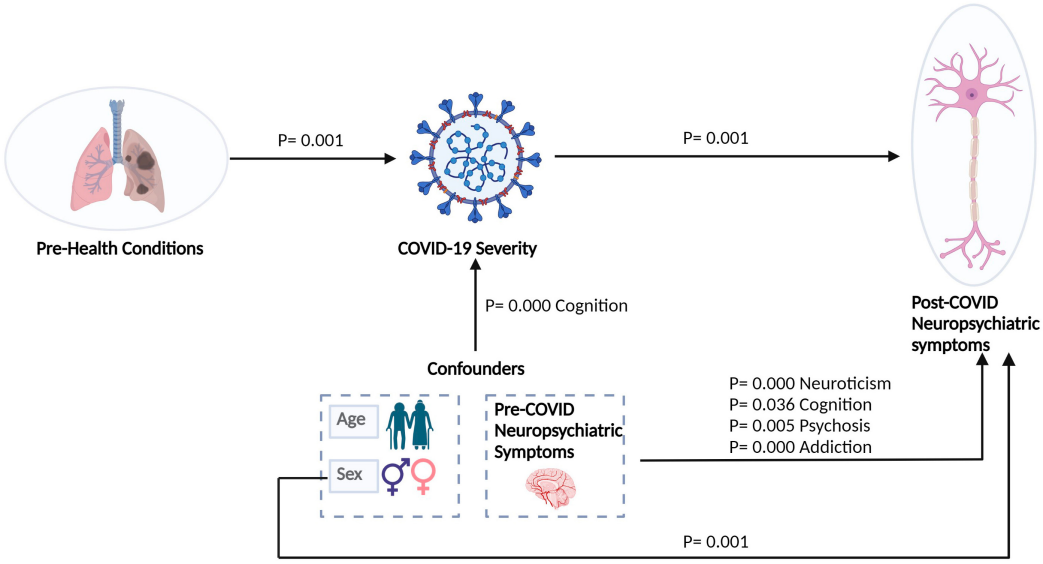


FIGURE 7
Relationship of COVID-19 with post-COVID-NPS of COVID-induced brain dysfunctions (CIBD) shown by structural equation modeling (SEM). A circle denotes a latent variable. A square denotes a measured variable. Directed arrows denote putative causal relations.

impairments significantly affect post-COVID-NPS in our study sample. Few neuropsychiatric manifestations of COVID-19 may cause severity in post-COVID neuropsychiatric symptoms in patients still recovering from SARS-CoV-2 infection and even those who recovered once and yet have some late health consequences (Charlton et al., 2021; Sampogna et al., 2022). COVID-19 increases the risk for neuropsychiatric sequelae, including psychosis (Chacko et al., 2020). An altered mental state is one of the most common neuropsychiatric conditions directly impacting effect, behavior (e.g., agitation), and cognition (Helms and Meziani, 2020). A study by Taquet et al. showed that there are many psychiatric conditions, such as bipolar disorder

TABLE 3 Pre-health conditions (PHC) Scores from item response theory (IRT).

Pre-health conditions	Loadings
Cardiovascular diseases	0.852
Immunological disorders	0.833
High blood pressure (HBP)	0.759
Diabetes	0.731
Chronic obstructive pulmonary disease (COPD)	0.645
Renal disorders	0.626
Neurological diseases	0.157

TABLE 4 Post-COVID-NPS scores from factor analysis.

Post-COVID-NPS symptoms	Loadings
Pain	0.82
Autonomic symptoms	0.78
Cardiovascular symptoms	0.77
Fatigue syndrome	0.74
Neurological symptoms	0.74
Sleep problems	0.73
Anxiety generalized symptoms	0.73
Depressed mood and ideation	0.72
Gastrointestinal symptoms	0.68
Trauma-related symptoms	0.66
Panic symptoms	0.57
Urogenital symptoms	0.55
Adjustment disorder	0.53
Sexual dysfunction	0.38

(BPD), major depressive disorder (MDD), panic disorder (PD), generalized anxiety disorder (GAD), or post-traumatic stress disorder (PTSD), and cognition disorders such as Alzheimer's disease which could be worsened by the COVID-19 infection or fear of getting an infection (Taquet et al., 2021).

We treated age, sex, and pre-COVID-NPS as confounders to check the effect of COVID-19 severity on post-COVID-NPS. Even after adjusting for confounders, we still got a significant $p < 0.001$ value between COVID-19 severity and post-COVID-NPS. See preliminary results at Yasir et al. (2023). Another study also provides evidence that COVID-19 infection is associated with a significant risk of various autoimmune diseases, such as rheumatoid arthritis, sclerosis, etc., in COVID-19 patients. They have analyzed the study with demographic covariates, age, sex, race, socioeconomic determinants of health, lifestyle-related problems, and health comorbidities (Type 2 diabetes mellitus, depression, chronic kidney disease, sleep disorder, and psychoactive substance use) in different ethnicities (Chang et al., 2023).

Poor general health, pre-pandemic mental health, and sociodemographic characteristics have emerged as significant determinants in various COVID-19 patient studies (Stefano et al., 2022). Symptoms related to fatigue, dyspnea, headache, and anosmia were characterized as long-COVID conditions in patients and were more likely severe with increasing age, BMI, and female sex (Sudre et al., 2021). Different factors affect COVID-19 severity and post-recovery symptoms in patients. Female sex, increasing age, obesity, smoking, vaping, hospitalization with COVID-19, and being a healthcare worker were directly associated with a higher probability of persistent symptoms (Whitaker et al., 2022). However, in our case, age is not directly relevant to the severity of COVID-19 infection or post-COVID consequences because we have treated it as a confounder. A previous study reported that people who had recovered from COVID-19 with no longer reporting symptoms still experienced significant cognitive deficits while controlling for age, sex, education, pre-existing medical disorders, tiredness, depression, and anxiety (Hampshire et al., 2020).

There is a large body of evidence that COVID-19 affects the brain; it may infect and damage specific brain cells directly, resulting in memory loss, strokes, and other brain-related disorders (Edison, 2021b; Marshall, 2021; Nalbandian et al., 2021). Past studies have shown that many COVID-19 patients have experienced COVID-Induced Brain Dysfunction (CIBD) 6 months to 1 year after recovery (De Berardis et al., 2022). Apart from direct CIBD resulting from SARS-CoV-2 infection, prolonged pre-health conditions, disease-associated factors, work-related stress, social distancing, and quarantine (isolation) have affected the mental health of many infected and recovered people. Therefore, complete follow-up on COVID-19 patients (critically ill or recovered) is mandatory to lessen the effects of long-CIBD (Valdes-Sosa et al., 2021). Post-COVID-NPS analysis shows that critically ill or severe patients, after recovery, may still have few complications related to neuropsychiatric symptoms. Our study's healthy controls experienced some post-COVID neuropsychiatric symptoms like anxiety and depression due to isolation and quarantine. In our sample size, the highest loadings obtained from factor analysis for post-COVID-NPS are for pain, autonomic dysfunctionalities, cardiovascular disorders, fatigue syndrome, and neurological symptoms, with a threshold ranging from 0.74 to 0.82. However, in this study, we have not classified or differentiated the origin of the post-COVID-symptoms, i.e., fatigue syndrome (organic or immunological) and restricted our analysis to behavioral measures. We are working on more biological variables in a further publication by studying the quantitative electroencephalogram (qEEG), which is based on a causal analysis to check qEEG as a proxy for brain functions that mediate the effect of Post-COVID-NPS in COVID-19 patients. Preliminary results showed that qEEG is a mediator and can be a biomarker to understand the effects of COVID-19 severity on post-COVID neuropsychiatric symptoms (Jin et al., 2023). On the other hand, a recent paper by Tauqet et al. studied blood biomarkers in 1837 COVID-19 patients to analyze the cognitive brain deficits where fatigue is the mediator for the biomarker profile, which clearly states that it is linked with post-COVID neuropsychiatric (cognitive) consequences in COVID-19 patients (Taquet et al., 2023).

COVID-19 infection, especially long-COVID, is associated with increased short and long-term risks of cardiovascular diseases in recovered patients (Chilazi et al., 2021). A follow-up on signs and symptoms of developing these cardiovascular complications post-COVID diagnosis or recovery up to a year or two may benefit infected patients with severe illness (Inciardi et al., 2020; Ramadan et al., 2021; Wan et al., 2023). Douaud et al. (2022) carried out one of the largest studies on COVID-19 and compared two

TABLE 5 Effect of COVID-19 severity and post-COVID-NPS on age, sex, and pre-COVID-NPS via structural equation modeling (SEM).

CIBD determinants	Estimate	$P(>\|z\|)$
Severity~		
PHC	0.293	0.003**
Age	0.008	0.143
Sex	0.008	0.955
Neuroticism	0.006	0.534
Cognitive	2.051	0.000***
Psychosis	0.684	0.266
Addiction	0.821	0.187
NPS~		
Severity	0.236	0.000***
Age	−0.001	0.775
Sex	0.413	0.001**
Neuroticism	0.667	0.000***
Cognitive	1.071	0.036*
Psychosis	1.637	0.005**
Addiction	2.285	0.000***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

groups (COVID and non-COVID patients). They found several significant longitudinal effects, including a severe reduction in the texture of gray matter. Moreover, SARS-CoV-2-infected individuals displayed a larger average cognitive impairment between the two-time points (Douaud et al., 2022). A recent study of one of the largest cohorts (n = 1733) on COVID-19 patients reported that at 6 months after symptom onset, patients discharged from the hospital or recovering from COVID-19 still have symptoms of fatigue, including muscle weakness, sleep difficulties, and anxiety or depression (Bourmistrova, 2022). Around 68% of patients reported at least one symptom, and such patients can be a primary target population for a follow-up and long-term recovery protocol (Huang et al., 2023). Two studies showed that the most frequent neuropsychiatric symptoms were sleep disturbance/disruption, followed by fatigue, objective cognitive impairment, anxiety, and post-traumatic stress (Badenoch et al., 2022; Sampogna et al., 2022), as analyzed in our results.

SARS-CoV-2 infection is commonly associated with a wide range of persistent, long-lasting symptoms, now known as post-COVID or long-COVID (Choutka et al., 2022). Many studies (Davis et al., 2021, 2023; Al-Aly et al., 2022) from the past determine which symptoms are associated with confirmed SARS-CoV-2 infection (post-recovery) in non-hospitalized individuals, as well as the risk factors for developing long-lasting symptoms in COVID-19 patients. Most frequent long-COVID symptoms include anosmia, shortness of breath at rest, chest pain, obesity, and other comorbidities such as COPD (Subramanian et al., 2022). Neuropsychiatric symptoms were common up to 5–6 months after initial hospitalization in hospitalized patients and recovered patients with no symptoms (Castro et al., 2021). A systematic review of psychiatric and neuropsychiatric sequelae of COVID-19 patients states that post-COVID symptoms such as post-traumatic stress disorder (PTSD), cognitive deficits, and sleep disturbances are persistent after hospital discharge till 7–9 months (Renaud-Charest et al., 2021). Risk factors associated with COVID-19 were disease severity and duration of symptoms (Schou et al., 2021). Kyzar et al. (2021) reported findings from a study of 52 patients recruited from New York City following acute COVID-19 infection and found a high level of correlation between psychiatric symptoms in participants. Many participants had clinically significant insomnia, depression, and post-traumatic stress at follow-up compared to baseline, indicating that post-COVID neuropsychiatric symptoms may grow over time (Kyzar et al., 2021).

As indicated earlier, fatigue, muscle weakness, sleep difficulties, and anxiety or depression were common, even 6 months after symptom onset (Badenoch et al., 2022). This result is consistent with studies from previous SARS long-term follow-ups in patients. Canadian researchers found that most SARS survivors had good physical recovery after illness, but 1 year later, 33% reported a significant decline in mental health. A follow-up study of SARS survivors showed that 40% of patients still had a chronic fatigue problem for a mean period of 41·3 months after SARS infection (Tansey, 2007; Lam, 2009). Similarly, our study analyzes the post-COVID-NPS in COVID-19 patients, which can help us better understand the long COVID-19 symptoms in future Research. The causal association between COVID-19 severity and post-COVID-NPS was studied through SEM, and the hypothetical causal relations are based on anatomically plausible connections between them. The strength of each association is specified by a path coefficient, which, by analogy to a partial regression coefficient, indicates how the variance of one region depends on the variance of another region if all other influences are held constant.

Long-COVID or post-COVID syndrome is poorly understood because it affects COVID-19 survivors at all disease severity levels, including younger people, children, and those not hospitalized (Bourmistrova et al., 2022; Frontera and Simon, 2022). Notably, neuropsychiatric deficits in long-COVID have been mainly associated with ACE2-rich brain areas, affecting inflammatory, metabolic, and degenerative processes (Guedj, 2021). The most typical symptoms following acute COVID-19 (long-COVID) are cognitive and mental disabilities, heart palpitations, fatigue, cough, headache, gastric, and cardiovascular diseases, all possible long-term effects (Efstathiou et al., 2022). Female sex and prior psychiatric problems can be risk factors for getting long-term COVID-19, although additional study is needed to support such risk factors (Yong, 2021). However, there are a few factors that contribute to the heterogeneity in long-COVID prevalence estimates, such as disparities in vaccinations, SARS-CoV-2 variants, pre-existing health comorbidities, study sample size, and use of varying non-COVID-19 control groups bring bias and appear to drive heterogeneity in prevalence estimates of long-COVID (Raman et al., 2022). Nevertheless, these factors help analyze the risk factors associated with long-term post-COVID symptoms (long-COVID). Our study has a small data set, but patients with any pre-health or pre-neuropsychiatric conditions have more chances of severe COVID-19 with enhanced post-COVID neuropsychiatric disorders. It can help us better evaluate long-COVID symptoms in late COVID-19 stages of recovered patients, as indicated in **Figure 7**. The output of the Lavaan Model for our hypothesis is as follows:

- Lavaan Model found that PHC and Pre-COVID-NPS affect the severity of SARS-CoV-2 infection that eventually affects post-COVID-NPS in COVID-19 patients.
- Pre-COVID-NPS (neuroticism, cognitive impairments, psychosis, addiction, and sex directly affect post-COVID-NPS in COVID-19 patients). It is to be noted that in our sample, the female sex is more significantly related to post-COVID-NPS rather than the male. The reason could be that few post-COVID neuropsychiatric symptom categories/sub-items from SCAN 2.1 are more related to female problems/disorders. On the other hand, Pre-COVID-NPS (specifically cognitive impairments) has a significant *p*-value, directly affecting the severity of COVID-19. So, people with cognitive impairment issues may face more severe COVID-19 symptoms.
- Even after controlling for confounders such as age, sex, and pre-COVID-NPS, the severity of COVID-19 is still significantly associated with post-COVID-NPS.

5.1 Summary

Early in the pandemic, concerns about the neurological and neuropsychiatric outcomes in COVID-19 patients and survivors were raised. Understanding the effect of SARS-CoV-2 infection and the fear of getting an infection (subject to isolation) on the brain remains unclear despite all the clinical investigations available. The significant gap lies in the mid to long-term neuropathogenic effects of the SARS-COV-2 infection due to the wide variety of mechanisms of viral entry into the brain.

The present study protocol is a longitudinal study of brain disorders in Cuban patients and survivors of COVID-19. It aims to characterize the post-COVID brain dysfunctions in all hospitalized subjects (patients and controls). The healthy subjects (controls) were initially hospitalized for being in close contact with the COVID-19 positive patients and later discharged upon having negative PCR reports. The general result of our analysis depicts that according to psychiatric assessments in SCAN 2.1 (Schützwohl et al., 2007), COVID-19 illness, along with a level of severity, leads to psychiatric manifestations. The hospitalized COVID-19 convalescents/patients showed higher somatomorphic symptomatology and autonomic dysfunctionalities than the healthy controls/non-COVID-19 patients. The convalescence phase of COVID-19 is characterized by a varied spectrum of specific neuropsychiatric manifestations that are intensified in subjects with psychiatric histories or pre-health comorbidities. It will eventually increase the incidence of post-COVID-related neuropsychiatric symptoms.

We created a latent variable combining all the prior-health symptoms using Item Response Theory (IRT), a statistical approach suitable for scaling numerous health outcomes along a single severity continuum (latent trait modeling). This latent variable was used further to investigate the relationship with post-COVID-neuropsychiatric symptomatology in COVID-19 patients. Finally, we studied the association of the latent variables with COVID-19 severity and post-COVID-NPS through Structural equation modeling. Compared with other studies on brain disorders in long-COVID, our study has some strengths that make it unique. The first advantage is the homogeneity in the clinical management of the cases because Cuba uses a single medical action protocol for the whole country so that the variability in the treatment and other medical procedures will be reduced. In addition, we studied a control group of people with negative PCR who were isolated in hospital institutions and were contacts of confirmed cases. It will minimize the intergroup differences explained by the negative psychological effect associated with the receipt or suspicion of a positive PCR. Another advantage is that our study involves a face-to-face evaluation of the subjects, promoting safer and more objective data collection compared to other studies conducted online or through telephone questionnaires. We have been powered to evaluate whether factors such as sex, pre-existing health comorbidities, admission to hospital because of COVID-19, and pre-neuropsychiatric conditions modify the risk of long-term neuropsychiatric sequelae after the acute infection. Even after adjusting for confounding factors like sex, age, pre-health conditions and pre-neuropsychiatric conditions, the most common post-COVID-neuropsychiatric symptoms in our study include pain, fatigue syndrome, autonomic dysfunctionalities, cardiovascular disorders, and neurological symptoms. The recurrence of these post-COVID symptoms is supported by many other studies stated in the discussion session of this article. These findings support the idea that there is a significant degree of co-occurrence of multiple post-recovery symptoms associated with pre-health and pre-neuropsychiatric conditions in COVID-19 patients assessed several months after hospitalization, implying that common underlying pathological mechanisms may influence the persistence of long-term post-COVID-neuropsychiatric consequences.

5.2 Limitations

The current sample size shows strong and reliable results for COVID-19 patients to have more post-COVID consequences if they have prior health comorbidities. However, the sample size is small and biased as few patients have severe symptomatic consequences after COVID-19. It is a cross-sectional rather than a longitudinal study, so the results need further validation. The statistical power of our sample is 0.984 for an effect size of 0.7 and 0.999 for an effect size of 0.5, respectively.

On the other hand, SARS-CoV-2 is a virus affecting the immune system. However, we didn't use any immunological quantitative measures or other biomarkers to distinguish between the origin of organic and psychological symptoms. We only focused on the subjective reports by COVID-19 patients and neuropsychiatric assessments by the psychiatrists.

5.3 Possible future direction

The severity of COVID-19 is affected by many factors that may lead to long-term post-COVID-NPS. Long-COVID is a multisystemic illness that impacts multiple organ systems in the human body. There are many potential risk factors associated with long-term neuropsychiatric sequelae of COVID-19. Older age, pre-health status, and psychological factors significantly

enhance the severity of COVID-19 and its consequences. They might trigger either the acute onset of neuropsychological manifestations or the worsening of the existing neuropsychiatric conditions. So, future research is needed to lessen the chances of getting severe COVID-19 or post-COVID-NPS in critically ill or recovered patients.

6 Conclusion

Patients with pre-health comorbidities and pre-neuropsychiatric conditions have a high risk of getting more severe COVID-19. It demonstrates how the severity of COVID-19 had a causal relationship with post-COVID neuropsychiatric symptomatology, even after adjusting for confounders, i.e., age, sex, and pre-COVID-19 neuropsychiatric symptoms. So, our results present evidence from a small but controlled cohort explaining that neuropsychiatric symptoms may worsen over time, particularly in COVID-19 patients and survivors with prior health comorbidities and neuropsychiatric disorders.

On the other hand, our study has a valuable sample of those COVID-19 subjects who were hospitalized at the same time as PCR-positive patients but later got negative PCR tests and were discharged. However, the sample suffered from the same emotional and psychological impact as the COVID-19 patients due to hospitalization, isolation and other environmental factors, thereby exhibiting a unique sample to eliminate the confounding variables from the analysis and isolate the COVID-19 effects. Future studies should continue to investigate and follow up in broader populations while exploring the potential mechanisms that may help to understand the neuropsychiatric pathology after SARS-CoV-2 infection.

Data availability statement

The dataset supporting the conclusions of this article will be made available by the co-authors affiliated with Servicio de Psiquiatria and The Cuban Neuroscience Center upon reasonable request.

Ethics statement

The studies involving humans were approved by the Local Research Ethics Committee of the Cuban Centre for Neurosciences and the National Hospital "Enrique Cabrera." approved this study. All eligible participants were informed of the research purpose and confidential data processing, and they signed the consent form before the beginning of the experiments. All procedures were according to the Helsinki Declaration. The patients/participants provided their written informed consent to participate in this study. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SY and YJ curated the CUBAN COVID-19 data and carried out statistical analyses. SY worked on the clinical data/analysis and YJ is working on the EEG analysis in another manuscript. PV-S directed the study with FR and MB-V including formulating the hypotheses, selecting the research approach, supervising results, and revising the manuscript. FR guided statistical analysis. RR-L, AC-M, PV-S, and MV-S designed the Cuban cohort study, with RR-L and AC-M directing the data collection. LG-G contributed to the organization and curation of the databases. PR gave valuable suggestions for data analysis and manuscript revision. SY wrote the original draft of the manuscript and worked on reviews and revisions. All authors contributed and approved the submitted final version of the article.

Funding

This study was funded by the MOST Chengdu city grant 2022-GH02-00042-HZ 5 and the National Program of Neuroscience of Neurotechnology in Cuba (project grant: PN305LH013-016).

Acknowledgments

We thank all COVID-19 patients and controls for their willingness to participate in the study. We also thank the group of psychiatrists who participated in the data collection and the Ministry of Public Health of Cuba for their contribution to the study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1249282/full#supplementary-material

References

Adab, P., Haroon, S., O'Hara, M. E., and Jordan, R. E. (2022). Comorbidities and covid-19. *BMJ* 377:o1431. doi: 10.1136/bmj.o1431

Al-Aly, Z., Bowe, B., and Xie, Y. (2022). Long COVID after breakthrough SARS-CoV-2 infection. *Nat. Med.* 28, 1461–1467. doi: 10.1038/s41591-022-01840-0

Augustin, M., Schommers, P., Stecher, M., Dewald, F., Gieselmann, L., Gruell, H., et al. (2021). Post-COVID syndrome in non-hospitalized patients with COVID-19: a longitudinal prospective cohort study. *Lancet Reg. Health Europe* 6:100122. doi: 10.1016/j.lanepe.2021.100122

Badenoch, J. B., Rengasamy, E. R., Watson, C., Jansen, K., Chakraborty, S., Sundaram, R. D., et al. (2022). Persistent neuropsychiatric symptoms after COVID-19: a systematic review and meta-analysis. *Brain Commun.* 4:fcab297. doi: 10.1093/braincomms/fcab297

Beran, T. N., and Violato, C. (2010). Structural equation modeling in medical research: a primer. *BMC Res. Notes* 3:267. doi: 10.1186/1756-0500-3-267

Bigdelou, B., Sepand, M. R., Najafikhoshnoo, S., Negrete, J. A. T., Sharaf, M., Ho, J. Q., et al. (2022). COVID-19 and Preexisting Comorbidities: risks, synergies, and clinical outcomes. *Front. Immunol.* 13:890517. doi: 10.3389/fimmu.2022.890517

Bourmistrova, N. W. (2022). Long-term effects of COVID-19 on mental health: a systematic review. *J. Affect. Disord.* 299, 118–125. doi: 10.1016/j.jad.2021.11.031

Bourmistrova, N. W., Solomon, T., Braude, P., Strawbridge, R., and Carter, B. (2022). Long-term effects of COVID-19 on mental health: a systematic review. *J. Affect. Disord.* 299, 118–125.

Busatto, G. F., De Araujo, A. L., Castaldelli-Maia, J. M., Damiano, R. F., Imamura, M., Guedes, B. F., et al. (2022). Post-acute sequelae of SARS-CoV-2 infection: relationship of central nervous system manifestations with physical disability and systemic inflammation. *Psychol. Med.* 52, 2387–2398. doi: 10.1017/S0033291722001374

Calixto-Calderón, B., Vázquez-González, M. F., Martínez Peláez, R., Bermeo-Escalona, J. R., García, V., Mena, L. J., et al. (2021). Pre-existing comorbidity, the highest risk factor for poor prognosis of COVID-19 among the Mexican population. *Nova Sci.* 13.

Castro, V. M., Rosand, J., Giacino, J. T., McCoy, T. H., & Perlis, R. H. (2021). *Case-control study of neuropsychiatric symptoms following COVID-19 hospitalization in 2 academic health systems* [Preprint]. *Psychiatry Clin. Psychol.* doi: 10.1101/2021.07.09.2125235

Chacko, M., Job, A., Caston, F., George, P., Yacoub, A., and Cáceda, R. (2020). COVID-19-induced psychosis and suicidal behavior: case report. *SN Compr. Clin. Med.* 2, 2391–2395. doi: 10.1007/s42399-020-00530-7

Chalmers, R. P. (2012). mirt: A multidimensional item response theory package for the R environment. *J. Stat. Softw.* 48, 1-29. doi: 10.18637/jss.v048.i06

Chang, R., Yen-Ting Chen, T., Wang, S.-I., Hung, Y.-M., Chen, H.-Y., and Wei, C.-C. J. (2023). Risk of autoimmune diseases in patients with COVID-19: a retrospective cohort study. *EClinicalMedicine* 56:101783. doi: 10.1016/j.eclinm.2022.101783

Charlton, E., Nainar, F., and Reyes-Beaman, S. (2021). Neuropsychiatric disorders and COVID-19. *Lancet Psychiatry* 8:563. doi: 10.1016/S2215-0366(21)00177-2

Chilazi, M., Duffy, E. Y., Thakkar, A., and Michos, E. D. (2021). COVID and cardiovascular disease: what we know in 2021. *Curr. Atheroscler. Rep.* 23:37. doi: 10.1007/s11883-021-00935-2

Choutka, J., Jansari, V., Hornig, M., and Iwasaki, A. (2022). Unexplained post-acute infection syndromes. *Nat. Med.* 28, 911–923. doi: 10.1038/s41591-022-01810-6

Cunningham, J. W., Vaduganathan, M., Claggett, B. L., Jering, K. S., Bhatt, A. S., Rosenthal, N., et al. (2021). Clinical outcomes in young us adults hospitalized with COVID-19. *JAMA Internal Med.* 181:379. doi: 10.1001/jamainternmed.2020.5313

Daugherty, S. E., Guo, Y., Heath, K., Dasmariñas, M. C., Jubilo, K. G., Samranvedhya, J., et al. (2021). Risk of clinical sequelae after the acute phase of SARS-CoV-2 infection: retrospective cohort study. *BMJ* 373:n1098. doi: 10.1136/bmj.n1098

Davis, H. E., Assaf, G. S., McCorkell, L., Wei, H., Low, R. J., Re'em, Y., et al. (2021). Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EClinicalMedicine* 38:101019. doi: 10.1016/j.eclinm.2021.101019

Davis, H. E., McCorkell, L., Vogel, J. M., and Topol, E. J. (2023). Long COVID: major findings, mechanisms and recommendations. *Nat. Rev. Microbiol.* 21, 133–146. doi: 10.1038/s41579-022-00846-2

De Berardis, D., Di Carlo, F., Di Giannantonio, M., and Pettorruso, M. (2022). Legacy of neuropsychiatric symptoms associated with past COVID-19 infection: a cause of concern. *World J. Psychiatry* 12, 773–778. doi: 10.5498/wjp.v12.i6.773

De Erausquin, G. A., Snyder, H., Brugha, T. S., Seshadri, S., Carrillo, M., Sagar, R., et al. (2022). Chronic neuropsychiatric sequelae of SARS-CoV-2: protocol and methods from the Alzheimer's Association Global Consortium. *Alzheimers Dement. Transl. Res. Clin. Interv.* 8:e12348. doi: 10.1002/trc2.12348

Douaud, G., Lee, S., Alfaro-Almagro, F., Arthofer, C., Wang, C., McCarthy, P., et al. (2022). SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature* 604, 697–707. doi: 10.1038/s41586-022-04569-5

Edison, P. (2021a). Brain connectivity: evaluating neurological complications in COVID-19. *Brain Connect.* 11, 692–694. doi: 10.1089/brain.2021.29028.ped

Edison, P. (2021b). COVID-19, network dysfunction and neurodegeneration. *Brain Connect.* 11, 785–787. doi: 10.1089/brain.2021.29030.ped

Efstathiou, V., Stefanou, M. I., Demetriou, M., Siafakas, N., Makris, M., Tsivgoulis, G., et al. (2022). Long COVID and neuropsychiatric manifestations (Review). *Exp. Ther. Med.* 23:363.

Ernst, A. F., and Albers, C. J. (2017). Regression assumptions in clinical psychology research practice—A systematic review of common misconceptions. *PeerJ* 5:e3323. doi: 10.7717/peerj.3323

Fahriani, M., Ilmawan, M., Fajar, J. K., Maliga, H. A., Frediansyah, A., Masyeni, S., et al. (2021). Persistence of long COVID symptoms in COVID-19 survivors worldwide and its potential pathogenesis—A systematic review and meta-analysis. *Narra J.* 1:e36. doi: 10.52225/narraj.v1i2.36

Fernández-de-Las-Peñas, C., Palacios-Ceña, D., Gómez-Mayordomo, V., Cuadrado, M. L., and Florencio, L. L. (2021). Defining post-COVID symptoms (post-acute COVID, long COVID, persistent post-COVID): an integrative classification. *Int. J. Environ. Res. Public Health* 18:2621. doi: 10.3390/ijerph18052621

Frontera, J. A., and Simon, N. M. (2022). Bridging knowledge gaps in the diagnosis and management of neuropsychiatric sequelae of COVID-19. *JAMA Psychiatry* 79:811. doi: 10.1001/jamapsychiatry.2022.1616

Guedj, E. (2021). 18F-FDG brain PET hypometabolism in patients with long COVID. *Eur. J. Nucl. Med. Mol. Imaging* 48, 2823–2833. doi: 10.1007/s00259-021-05215-4

Guzmán, M. G., Pérez, L., Tejero, Y., Mederos, D., Aguado, M. E., Pintos, Y., et al. (2022). Emergence and evolution of SARS-CoV-2 genetic variants during the Cuban epidemic. *J. Clin. Virol. Plus* 2:100104. doi: 10.1016/j.jcvp.2022.100104

Hampshire, A., Trender, W., Chamberlain, S. R., Jolly, A., Grant, J. E., Patrick, F., et al. (2020). Cognitive deficits in people who have recovered from COVID-19 relative to controls: an N=84,285 online study. *Psychiatry Clin. Psychol.* [preprint]. doi: 10.1101/2020.10.20.20215863

Helms, J., and Meziani, F. (2020). Neurologic features in severe SARS-CoV-2 infection. *N. Engl. J. Med.* 382, 2268–2270. doi: 10.1056/NEJMc2008597

Huang, C., Huang, L., Wang, Y., Li, X., Ren, L., Gu, X., et al. (2023). 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet* 401, e21–e33. doi: 10.1016/S0140-6736(23)00810-3

Huang, L., Li, X., Gu, X., Zhang, H., Ren, L., Guo, L., et al. (2022). Health outcomes in people 2 years after surviving hospitalization with COVID-19: a longitudinal cohort study. *Lancet Respir. Med.* 10, 863–876. doi: 10.1016/S2213-2600(22)00126-6

Inciardi, R. M., Lupi, L., Zaccone, G., Italia, L., Raffo, M., Tomasoni, D., et al. (2020). Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 5, 819–824. doi: 10.1001/jamacardio.2020.1096

Jin, Y., Yasir, S., Razzaq, F. A., Calzada, A., Virues, T., Caballero, A., et al. (2023). qEEG mediates the effect of infection severity on COVID-induced brain dysfunction. *Int. J. Psychophysiol.* 188, 46–47. doi: 10.1016/j.ijpsycho.2023.05.118

Jorgensen, T. D., Pornprasertmanit, S., Schoemann, A. M., and Rosseel, Y. (2022). *\texttt{semTools: useful tools for structural equation modeling}*. Available online at: https://CRAN.R-project.org/package=semTools (accessed April 28, 2023)

Kompaniyets, L., Pennington, A. F., Goodman, A. B., Rosenblum, H. G., Belay, B., Ko, J. Y., et al. (2021). Underlying medical conditions and severe illness among 540,667 adults hospitalized with COVID-19, March 2020–March 2021. *Prev. Chronic Dis.* 18:210123. doi: 10.5888/pcd18.210123

Komsta, L., and Novomestky, F. (2022). *moments: moments, cumulants, skewness, kurtosis and related tests*. Available online at: https://CRAN.R-project.org/package=moments (accessed April 25, 2023)

Kumar, S., Veldhuis, A., and Malhotra, T. (2021). Neuropsychiatric and cognitive sequelae of COVID-19. *Front. Psychol.* 12:577529. doi: 10.3389/fpsyg.2021.577529

Kyzar, E. J., Purpura, L. J., Shah, J., Cantos, A., Nordvig, A. S., and Yin, M. T. (2021). Anxiety, depression, insomnia, and trauma-related symptoms following COVID-19 infection at long-term follow-up. *Brain Behav. Immun. Health* 16:100315. doi: 10.1016/j.bbih.2021.100315

Lam, M. H.-B. (2009). Mental morbidities and chronic fatigue in severe acute respiratory syndrome survivors: long-term follow-up. *Arch. Internal Med.* 169:2142. doi: 10.1001/archinternmed.2009.384

Magnusson, K., Kristoffersen, D. T., Dell'Isola, A., Kiadaliri, A., Turkiewicz, A., Runhaar, J., et al. (2022). Post-covid medical complaints following infection with

SARS-CoV-2 Omicron vs Delta variants. *Nat. Commun.* 13:7363. doi: 10.1038/s41467-022-35240-2

Marshall, M. (2021). Covid and the brain: researchers zero in on how damage occurs. *Nature* 595, 484–485. doi: 10.1038/d41586-021-01693-6

Moshagen, M., and Bader, M. (2023). *semPower: power analyses for SEM*. Available online at: https://CRAN.R-project.org/package=semPower (accessed April 25, 2023)

Nalbandian, A., Desai, A. D., and Wan, E. Y. (2023). Post-COVID-19 condition. *Annu. Rev. Med.* 74, 55–64. doi: 10.1146/annurev-med-043021-030635

Nalbandian, A., Sehgal, K., Gupta, A., Madhavan, M. V., McGroder, C., Stevens, J. S., et al. (2021). Post-acute COVID-19 syndrome. *Nat. Med.* 27, 601–615. doi: 10.1038/s41591-021-01283-z

Ramadan, M. S., Bertolino, L., Zampino, R., Durante-Mangoni, E., and Monaldi Hospital Cardiovascular Infection Study Group (2021). Cardiac sequelae after coronavirus disease 2019 recovery: a systematic review. *Clin. Microbiol. Infect.* 27, 1250–1261. doi: 10.1016/j.cmi.2021.06.015

Raman, B., Bluemke, D. A., Lüscher, T. F., and Neubauer, S. (2022). Long COVID: post-acute sequelae of COVID-19 with a cardiovascular focus. *Eur. Heart J.* 43, 1157–1172. doi: 10.1093/eurheartj/ehac031

Renaud-Charest, O., Lui, L. M. W., Eskander, S., Ceban, F., Ho, R., Di Vincenzo, J. D., et al. (2021). Onset and frequency of depression in post-COVID-19 syndrome: a systematic review. *J. Psychiatr. Res.* 144, 129–137. doi: 10.1016/j.jpsychires.2021.09.054

Richardson, T. G., Fang, S., Mitchell, R. E., Holmes, M. V., and Davey Smith, G. (2021). Evaluating the effects of cardiometabolic exposures on circulating proteins which may contribute to severe SARS-CoV-2. *EBioMedicine* 64:103228. doi: 10.1016/j.ebiom.2021.103228

Rogers, J. P., Chesney, E., Oliver, D., Pollak, T. A., McGuire, P., Fusar-Poli, P., et al. (2020). Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. *Lancet Psychiatry* 7, 611–627. doi: 10.1016/S2215-0366(20)30203-0

Russell, C. D., Lone, N. I., and Baillie, J. K. (2023). Comorbidities, multimorbidity and COVID-19. *Nat. Med.* 29, 334–343. doi: 10.1038/s41591-022-02156-9

Sampogna, G., Di Vincenzo, M., Giallonardo, V., Perris, F., Volpicelli, A., Del Vecchio, V., et al. (2022). The psychiatric consequences of long-COVID: a scoping review. *J. Pers. Med.* 12:1767.

Sanyaolu, A., Okorie, C., Marinkovic, A., Patidar, R., Younis, K., Desai, P., et al. (2020). Comorbidity and its Impact on Patients with COVID-19. *SN Compr. Clin. Med.* 2, 1069–1076. doi: 10.1007/s42399-020-00363-4

Schou, T. M., Joca, S., Wegener, G., and Bay-Richter, C. (2021). Psychiatric and neuropsychiatric sequelae of COVID-19 – A systematic review. *Brain Behav. Immun.* 97, 328–348. doi: 10.1016/j.bbi.2021.07.018

Schützwohl, M., Kallert, T., and Jurjanz, L. (2007). Using the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) as a diagnostic interview providing dimensional measures: cross-national findings on the psychometric properties of psychopathology scales. *Eur. Psychiatry* 22, 229–238. doi: 10.1016/j.eurpsy.2006.10.005

Sharun, K., Tiwari, R., Yatoo, M. I., Natesan, S., Megawati, D., Singh, K. P., et al. (2022). A comprehensive review on pharmacologic agents, immunotherapies and supportive therapeutics for COVID-19. *Narra J.* 2:e92. doi: 10.52225/narra.v2i3.92

Stefano, G. B., Büttiker, P., Weissenberger, S., Ptacek, R., Wang, F., Esch, T., et al. (2022). Biomedical perspectives of acute and chronic neurological and neuropsychiatric sequelae of COVID-19. *Curr. Neuropharmacol.* 20, 1229–1240. doi: 10.2174/1570159X20666211223130228

Subramanian, A., Nirantharakumar, K., Hughes, S., Myles, P., Williams, T., Gokhale, K., et al. (2022). Assessment of 115 symptoms for Long COVID (post-COVID-19 condition) and their risk factors in non-hospitalized individuals: a retrospective matched cohort study in UK primary care. *Res. Square* [preprint]. doi: 10.21203/rs.3.rs-1343889/v1

Sudre, C. H., Murray, B., Varsavsky, T., Graham, M. S., Penfold, R. S., Bowyer, R. C., et al. (2021). Attributes and predictors of long COVID. *Nat. Med.* 27, 626–631. doi: 10.1038/s41591-021-01292-y

Tabachnick, B. G., Linda, S. F., and Ullman, J. B. (2007). *Using multivariate statistics*, Vol. 5. Boston, MA: Pearson. doi: 10.1515/snde-2014-0102

Tansey, C. M. (2007). One-year outcomes and health care utilization in survivors of severe acute respiratory syndrome. *Arch. Internal Med.* 167:1312. doi: 10.1001/archinte.167.12.1312

Taquet, M., Geddes, J. R., Husain, M., Luciano, S., and Harrison, P. J. (2021). 6-month neurological and psychiatric outcomes in 236 379 survivors of COVID-19: a retrospective cohort study using electronic health records. *Lancet Psychiatry* 8, 416–427. doi: 10.1016/S2215-0366(21)00084-5

Taquet, M., Sillett, R., Zhu, L., Mendel, J., Camplisson, I., Dercon, Q., et al. (2022). Neurological and psychiatric risk trajectories after SARS-CoV-2 infection: an analysis of 2-year retrospective cohort studies including 1 284 437 patients. *Lancet Psychiatry* 9, 815–827. doi: 10.1016/S2215-0366(22)00260-7

Taquet, M., Skorniewska, Z., Hampshire, A., Chalmers, J. D., Ho, L.-P., Horsley, A., et al. (2023). Acute blood biomarker profiles predict cognitive deficits 6 and 12 months after COVID-19 hospitalization. *Nat. Med.* 29, 2498–2508. doi: 10.1038/s41591-023-02525-y

Treskova-Schwarzbach, M., Haas, L., Reda, S., Pilic, A., Borodova, A., Karimi, K., et al. (2021). Pre-existing health conditions and severe COVID-19 outcomes: an umbrella review approach and meta-analysis of global evidence. *BMC Med.* 19:212. doi: 10.1186/s12916-021-02058-6

Valdes-Sosa, P. A., Evans, A. C., Valdes-Sosa, M. J., and Poo, M. (2021). A call for international research on COVID-19-induced brain dysfunctions. *Natl. Sci. Rev.* 8:nwab190. doi: 10.1093/nsr/nwab190

Wan, E. Y. F., Mathur, S., Zhang, R., Yan, V. K. C., Lai, F. T. T., Chui, C. S. L., et al. (2023). Association of COVID-19 with short- and long-term risk of cardiovascular disease and mortality: a prospective cohort in UK Biobank. *Cardiovasc. Res.* 119, 1718–1727. doi: 10.1093/cvr/cvac195

Wei, T., and Simko, V. (2021). *R package "corrplot" visualization of a correlation matrix*. Available online at: https://github.com/taiyun/corrplot (accessed May 05, 2023)

Whitaker, M., Elliott, J., Chadeau-Hyam, M., Riley, S., Darzi, A., Cooke, G., et al. (2022). Persistent COVID-19 symptoms in a community study of 606,434 people in England. *Nat. Commun.* 13:1957. doi: 10.1038/s41467-022-29521-z

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019). Welcome to the tidyverse. *J. Open Source Softw.* 4:1686. doi: 10.21105/joss.01686

Wilkinson, G. N., and Rogers, C. E. (1973). Symbolic description of factorial models for analysis of variance. *Appl. Stat.* 22:392. doi: 10.2307/2346786

Williams, M. N., Grajales, C. A. G., and Kurkiewicz, D. (2013). Assumptions of multiple regression: correcting two misconceptions. *Pract. Assess. Res. Eval.* 18, 1–14. doi: 10.7275/55HN-WK47

Yasir, S., Jin, Y., Razzaq, F. A., Rodriguez-Labrada, R., Valdes-Sosa, P. A., Bringas-Vega, M. L., et al. (2023). The determinants of covid-induced brain disorders after covid-19 infection. *Int. J. Psychophysiol.* 188, 134–135. doi: 10.1016/j.ijpsycho.2023.05.342

Yong, S. J. (2021). Long COVID or post-COVID-19 syndrome: putative pathophysiology, risk factors, and treatments. *Infect. Dis.* 53, 737–754. doi: 10.1080/23744235.2021.1924397

Yoshida, K., and Bartel, A. (2022). *tableone: create "Table 1" to describe baseline characteristics with or without propensity score weights*. Available online at: https://CRAN.R-project.org/package=tableone (accessed April 10, 2023)

Zanon, C., Hutz, C. S., Yoo, H., and Hambleton, R. K. (2016). An application of item response theory to psychological test development. *Psicol. Reflex. Crít.* 29:18. doi: 10.1186/s41155-016-0040-x

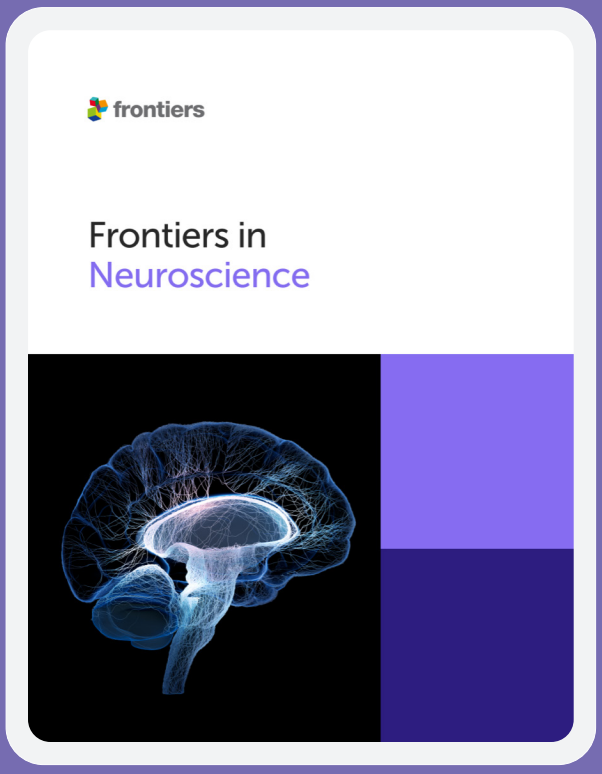
Frontiers in Neuroscience

Provides a holistic understanding of brain function from genes to behavior

Part of the most cited neuroscience journal series which explores the brain - from the new eras of causation and anatomical neurosciences to neuroeconomics and neuroenergetics.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact