frontiers | Research Topics

An update on neurological disorders post COVID-19 infection

Edited by
Beatrice Paradiso, Nuran Abdullayev, Clara Limback, Tao Su, Weiping Liao and Anastasios Mpotsaris

Published in
Frontiers in Neurology

ISSN 1664-8714
ISBN 978-2-8325-3131-0
DOI 10.3389/978-2-8325-3131-0

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

An update on neurological disorders post COVID-19 infection

Topic editors

Beatrice Paradiso — University of Milan, Italy

Nuran Abdullayev — Department of Radiology and Neuroradiology, GFO Clinics Troisdorf, Germany

Clara Limback — Oxford University Hospitals, United Kingdom

Tao Su — Guangzhou Medical University, China

Weiping Liao — Second Affiliated Hospital of Guangzhou Medical University, China

Anastasios Mpotsaris — München Hospital, Germany

Citation

Paradiso, B., Abdullayev, N., Limback, C., Su, T., Liao, W., Mpotsaris, A., eds. (2023). *An update on neurological disorders post COVID-19 infection*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3131-0

Table of contents

frontiers | Frontiers in Neurology

TYPE Editorial
PUBLISHED 13 July 2023
DOI 10.3389/fneur.2023.1229843

OPEN ACCESS

EDITED AND REVIEWED BY
Christina M. Marra,
University of Washington, United States

*CORRESPONDENCE
Beatrice Paradiso
✉ beatrice.paradiso@unife.it

RECEIVED 27 May 2023
ACCEPTED 03 July 2023
PUBLISHED 13 July 2023

CITATION
Paradiso B, Limback C, Su T, Liao W and Mpotsaris A (2023) Editorial: An update on neurological disorders post COVID-19 infection. *Front. Neurol.* 14:1229843. doi: 10.3389/fneur.2023.1229843

Editorial: An update on neurological disorders post COVID-19 infection

Beatrice Paradiso[1,2]*, Clara Limback[3], Tao Su[4], Weiping Liao[4] and Anastasios Mpotsaris[5,6]

[1]Department of Biomedical, Surgical and Dental Sciences, Faculty of Medicine and Surgery, Lino Rossi Research Center, University of Milan, Milan, Italy, [2]Anatomic Pathology Unit, Dolo Hospital Venice, Venice, Italy, [3]Department of Neuropathology and Ocular Pathology, Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, [4]Key Laboratory of Neurogenetics and Channelopathies of Guangdong Province and the Ministry of Education of China, The Second Affiliated Hospital, Institute of Neuroscience, Guangzhou Medical University, Guangzhou, China, [5]München Hospital, Munich, Germany, [6]Faculty of Medicine, University Hospital Magdeburg, Magdeburg, Germany

KEYWORDS

SARS-CoV-2, long-COVID syndrome, post-acute sequelae of COVID-19 (PASC), neuro-PASC, neuroinvasion, immunity, blood-brain barrier (BBB), brainstem nuclei

Editorial on the Research Topic
An update on neurological disorders post COVID-19 infection

A new zoonotic coronavirus epidemic began in December 2019 in the city of Wuhan, China, and has affected almost the entire world. The World Health Organization (WHO) named this coronavirus 2019-nCoV, and COVID-19 the disease caused by it. On 11 March 2020, WHO declared the COVID-19 outbreak a global pandemic (1).

Globally, as of 12 April 2023, WHO reported 762,791,152 confirmed cases of COVID-19, including 6,897,025 deaths. As of 11th April 2023, a total of 13,340,343,269 vaccine doses have been administered. Three million new cases and over 23,000 deaths were reported in the previous 28 days (13 March to 9 April 2023), a decrease of 28% and 30%, respectively, compared to the previous 28 days (13 February to 12 March 2023). However, in opposition to the overall trend, important increases in reported cases and deaths were observed in the South-East Asia and Eastern Mediterranean regions and in numerous other countries (2). The world is not yet at the end of the COVID-19 epidemic because new virus variants are expected. However, on 4th March 2023, the head of WHO declared "with great hope" an end to COVID-19 as a global public health emergency, stressing that this does not mean that the disease is no longer a worldwide threat (3).

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes COVID-19, a form of atypical pneumonia with multiple organ dysfunction but also simple respiratory flu-like symptoms; infection can be prevented or attenuated by vaccination. As in most respiratory infections, including influenza, SARS-CoV-2 infection viral shedding reaches the highest level in the nasopharynx and the nasal cavity mucosa is one of the most relevant sites of viral activity. Spinato et al. suggest, in a preliminary study, good compliance and subjective satisfaction for nasal lavages with saline solution in patients with newly diagnosed SARS-CoV-2 infection. The treatment showed effectiveness in reducing nasal symptoms of SARS-CoV-2 infection, compared to the control group. Hence, the nasopharyngeal route of viral dissemination and the easy administration of nasal sprays explains the rationale of the intranasal vaccine models that are under investigation.

According to the systematic review by Purja et al., the neurological complications of COVID-19 are diverse, and direct viral neuroinvasion is rare. The authors identify 2,387 studies and include 167 studies in which SARS-CoV-2 CSF PCR assay was performed in 101 patients. The SARS-CoV-2 PCR assay was positive in only four CSF samples out of the 101 cases. Olfactory dysfunction was present in only two of these four cases. The central and peripheral neurological manifestations observed were heterogeneous. The most common neurological diagnoses were Guillain-Barré syndrome (GBS) and its variants (24%), followed by encephalopathy (21%).

SARS-CoV-2 infection is a global health challenge producing significant post-acute sequelae and 30% of COVID-19 patients reported persistent symptoms for up to 9 months after illness. Patients who recover from COVID-19 and experience symptoms that persist for a protracted period after the primary infection are defined as having long-COVID (4 weeks after the primary infection) or post-COVID syndrome (12 weeks after the primary infection) (Schulze et al.). These patients are given the diagnosis of long COVID, post-acute COVID-19 syndrome (PACS), or post-acute sequelae of COVID-19 (PASC). It remains unclear whether long-COVID is a different disease entity than COVID-19 with unclear pathophysiology or a spectrum of prolonged viral infection (4).

Long COVID is frequently accompanied by new-onset conditions, mainly cardiovascular, thrombotic, or cerebrovascular disease; type 2 diabetes; myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS); postural orthostatic tachycardia syndrome; and other dysautonomic events (Carmona-Torre et al.). There are no validated effective treatments yet and these disabling symptoms can last for years. In particular, ME/CFS and dysautonomia may be lifelong conditions.

There are several possible causes of long COVID. Several hypotheses regarding its pathogenesis have been considered, including the persistent reservoir of SARS-CoV-2 in tissues; immune dysregulation with or without reactivation of the primary pathogen; and herpesviruses such as Epstein–Barr virus (EBV) and human herpesvirus 6 (HHV-6). Furthermore, the impact of SARS-CoV-2 on the microbiota, and the virome in particular; autoimmunity and other dysregulation of the immune system; microclotting with endothelial dysfunction; alterations in brainstem signaling and/or vagus nerve; and genetic causes have been considered (5).

Neuro-PASC involves direct or indirect brain invasion by the virus. The virus can then cause brain dysfunction and neuronal damage through direct cytolysis or secondary inflammatory and immune responses (indirect effects).

Direct invasion is uncommon (6). The virus can infect the Peripheral Nervous System (PNS) or CNS by direct infection of nerve endings (including olfactory, trigeminal, optic, and vagus nerves) gaining access to the CNS via the transport machinery of nerves and ganglions (7). The indirect mechanism is more frequent and involves the infection of cells of the circulatory system which carry the infection through the blood-brain barrier (BBB) into the CNS.

There are three main mechanisms by which a virus may cross the BBB: transcellular migration, paracellular migration, and the "Trojan horse" strategy. During transcellular migration, viruses enter the host endothelial cells to cross the BBB. In paracellular migration, viruses invade tight junctions formed by the endothelial cells of the BBB. With the Trojan horse strategy, viral particles are phagocytized by neutrophils and macrophages (8). Recently, some viral specialized molecules, called fusogens, have been recognized that fuse the viral envelope with neuronal or glioneuronal cell membranes and enter cells producing syncytial units among neurons and glia. This still poorly characterized, difficult-to-detect event could explain some of the neurological consequences of viral infections of the nervous system (9).

Viral BBB crossing determines three principal pathogenetic events: endotheliopathy, inflammatory response, and immune activation. These trigger astrocyte and microglia activation, proinflammatory cytokine release (Mehboob et al.), and CNS-specific immune activation which may be responsible for neural tissue injury and neurological symptoms of neuro-Covid (10). The neurological sequelae of Covid infection are frequently immune-mediated (Vavougios et al.).

Although COVID-19 may affect the incidence of specific neurological diseases, it is still to be determined whether this differs from the risk following other respiratory viral and bacterial infections. Zarifkar et al., study the frequency of neurodegenerative, cerebrovascular, and immune-mediated neurological diseases in outpatients post-COVID-19 compared to healthy control individuals and those with other respiratory tract infections. The risk of specific neurodegenerative and cerebrovascular, but not neuroimmune, disorders was increased in individuals with previous COVID-19 compared to healthy controls. However, with the exception of ischemic stroke, most neurological disorders were not more frequent after COVID-19 than after Influenza A/B and bacterial pneumonia.

Respiratory distress in patients with acute Covid-19 or in those with post-COVID syndrome is not exclusively due to atypical pneumonia. Both Vecchio et al. and Jareonsettasin et al. in 2022 demonstrated that inappropriate ventilatory homeostatic responses in individuals with acute COVID-19 may be related to direct brainstem involvement with overlapping indirect inflammatory mechanisms (8, 10–12) acting on the peripheral nervous system (Figure 1 by Jareonsettasin et al.). Weich et al. analyze the symptom of motor fatigue in post-COVID syndrome. All the patients included in Weich's clinical trial were not initially hospitalized and at the beginning displayed mild symptoms. Although they presented absolute values of oxygen uptake and ventilation within the normal range, they manifested mild anomalies in ventilation and chronic fatigue. These symptoms were not caused by organic lesions of the central motor system. In this study, Weich et al. do not exclude potential organic causes for chronic fatigue in long Covid disease such as mitochondrial

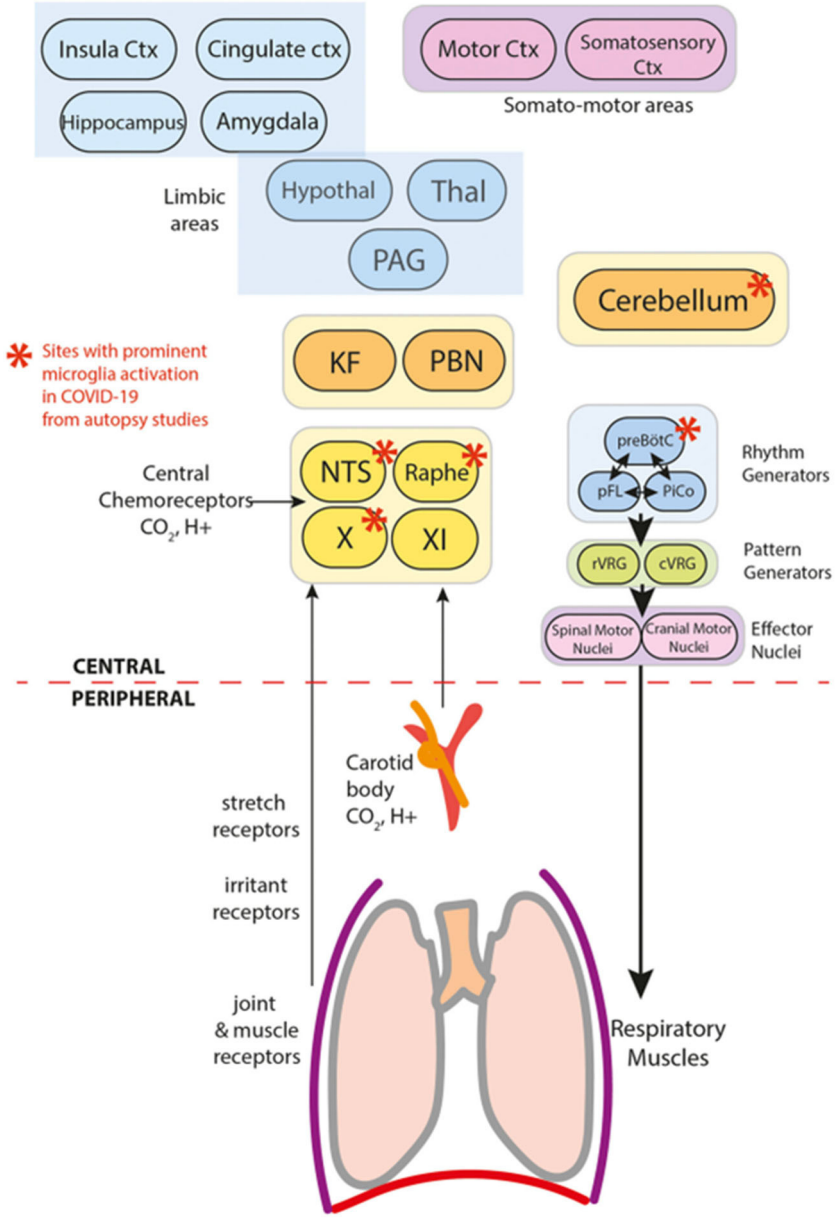


FIGURE 1
Components of breathing control in the context of COVID-19 (Jareonsettasin et al.).

dysfunction, endothelial dysfunction, chronic inflammation, autoimmunity, dysregulation of specific cytokines, or psychiatric and psychosomatic comorbidities, but rule out involvement of the central motor nuclei and cardiac and pulmonary deficit. Furthermore, it is not clear whether the mild anomalies in ventilation were caused by metabolic or psychogenic alterations too. Additional investigations of the psychiatric disorders in post-COVID-19 syndrome are necessary to understand the frequent association observed by Lier et al. between long COVID disease and a particular subset of patients with predominant fatigue, somatization, and depression. These patients present minor or no post-COVID cardiopulmonary distress and prominent psychiatric manifestations.

COVID-19 is a complex syndrome and a complex of syndromes with early complications and late sequelae involving the brain, the brainstem, and the autonomic peripheral system, all of which are still poorly characterized.

For this reason, the Neuroinfectious Diseases section in Frontiers in Neurology "opens the door" to new "Frontiers" in scientific adventures (An Update on Neurological Disorders Post COVID-19 Infection Vol 2: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions).

Author contributions

BP has made a substantial contribution to the concept of the article. CL revised it critically for important intellectual content. TS, WL, and AM approved the version to be published. All authors contributed to the article and approved the submitted version.

Acknowledgments

We want to thank Giulia Ottaviani, of the Lino Rossi Research Center, Faculty of Medicine and Surgery, University of Milan, Milan, Italy, for helping us draft the Editorial and for contributing to the preparation of the new Research Topic: An Update on Neurological Disorders Post COVID-19 Infection Vol 2: cardiovascular effects, neuro-cardiac and neuro-respiratory autonomic dysfunctions.

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. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus disease 2019 (COVID-19): a review. *JAMA*. (2020) 324:782–93. doi: 10.1001/jama.2020.12839

2. WHO Coronavirus (COVID-19) Dashboard. Available online at: https://covid19.who.int (accessed July 5, 2023).

3. COVID-19 Emergency Committee Statement. Available online at: https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic (accessed May 5, 2023).

4. Yachou Y, El Idrissi A, Belapasov V, Ait Benali S. Neuroinvasion, neurotropic, and neuroinflammatory events of SARS-CoV-2: understanding the neurological manifestations in COVID-19 patients. *Neurol Sci*. (2020) 41:2657–69. doi: 10.1007/s10072-020-04575-3

5. Martínez-Mármol R, Giordano-Santini R, Kaulich E, Cho AN, Przybyla M, Riyadh MA, et al. SARS-CoV-2 infection and viral fusogens cause neuronal and glial fusion that compromises neuronal activity. *Sci Adv*. (2023) 9:eadg2248. doi: 10.1126/sciadv.adg2248

6. Priyal, Sehgal V, Kapila S, Taneja R, Mehmi P, Gulati N. Review of neurological manifestations of SARS-CoV-2. *Cureus*. (2023) 15:e38194. doi: 10.7759/cureus.38194

7. Afshar H, Yassin Z, Kalantari S, Aloosh O, Lotfi T, Moghaddasi M, et al. Evolution and resolution of brain involvement associated with SARS- CoV2 infection: a close Clinical—Paraclinical follow up study of a case. *Mult Scler Relat Disord*. (2020) 43:102216. doi: 10.1016/j.msard.2020.102216

8. Pattanaik A, Bhandarkar BS, Lodha L, Marate S. SARS-CoV-2 and the nervous system: current perspectives. *Arch Virol*. (2023) 168:171. doi: 10.1007/s00705-023-05801-x

9. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. (2023) 21:133–46. doi: 10.1038/s41579-022-00846-2

10. Thaweethai T, Jolley SE, Karlson EW, Levitan EB, Levy B, McComsey GA, et al. Development of a definition of post-acute sequelae of SARS-CoV-2 infection. *JAMA*. (2023) 329:1934–46. doi: 10.1001/jama.2023.8823

11. Lewis A, Frontera J, Placantonakis DG, Lighter J, Galetta S, Balcer L, et al. Cerebrospinal fluid in COVID-19: a systematic review of the literature. *J Neurol Sci*. (2021) 421:117316. doi: 10.1016/j.jns.2021.117316

12. Achar A, Ghosh C. COVID-19-associated neurological disorders: the potential route of CNS invasion and blood-brain relevance. *Cells*. (2020) 9:2360. doi: 10.3390/cells9112360

frontiers in Neurology

ORIGINAL RESEARCH
published: 06 December 2021
doi: 10.3389/fneur.2021.794471

The Effect of Isotonic Saline Nasal Lavages in Improving Symptoms in SARS-CoV-2 Infection: A Case-Control Study

Giacomo Spinato[1], Cristoforo Fabbris[2], Giulio Costantini[3], Federica Conte[3], Pier Giorgio Scotton[2], Francesco Cinetto[1], Rosalba De Siati[1], Alessandro Matarazzo[1], Marco Citterio[1], Giacomo Contro[1], Cosimo De Filippis[1], Carlo Agostini[1], Enzo Emanuelli[2], Paolo Boscolo-Rizzo[4] and Daniele Frezza[2]*

[1] Department of Neurosciences, University of Padua, Padua, Italy, [2] Department of Otolaryngology, Ospedale di Treviso, Treviso, Italy, [3] Department of Psychology, University of Milano-Bicocca, Milan, Italy, [4] Department of Otolaryngology, University of Trieste, Trieste, Italy

OPEN ACCESS

Edited by:
Beatrice Paradiso, Dolo Hospital, Italy

Reviewed by:
Doriano Politi, Azienda ULSS 3 Serenissima, Italy
Alessandra Fioretti, European Hospital, Italy
Riffat Mehboob, King Edward Medical University, Pakistan

***Correspondence:**
Giacomo Spinato
giacomo.spinato@unipd.it

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: *13 October 2021*
Accepted: *17 November 2021*
Published: *06 December 2021*

Citation:
Spinato G, Fabbris C, Costantini G, Conte F, Scotton PG, Cinetto F, De Siati R, Matarazzo A, Citterio M, Contro G, De Filippis C, Agostini C, Emanuelli E, Boscolo-Rizzo P and Frezza D (2021) The Effect of Isotonic Saline Nasal Lavages in Improving Symptoms in SARS-CoV-2 Infection: A Case-Control Study. Front. Neurol. 12:794471. doi: 10.3389/fneur.2021.794471

Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) mainly colonizes nasopharynx. In upper airways acute infections, e.g., the common cold, saline nasal irrigations have a significant efficacy in reducing symptoms. The present study aimed to test the efficacy of nasal lavages in upper airways symptoms of Coronavirus Disease 2019 (COVID-19).

Methods: A series of consecutive adult subjects who tested positive for SARS-CoV-2 from December 2020 to February 2021 performed daily nasal lavages with saline solution (Lavonase®—Purling, Lugo di Romagna, Italy) for 12 days, starting on the day after the SARS-CoV-2 positive swab. A control group included a historical series of patients who were infected in February-March 2020 and who did not perform lavages. An *ad hoc* questionnaire regarding symptoms was administered to each subjects at base-line and 10 days after diagnosis (i.e., on the same day of the control swab) in both cases and controls.

Results: A total of 140 subjects were enrolled. 68 participants in the treatment group and 72 in the control group were included. 90% of respondents declared the lavages were simple to use and 70% declared they were satisfied. Symptoms of blocked nose, runny nose, or sneezing decreased by an average of 24.7% after the treatment. Blocked nose and sneezing increased in the same period of time in the control group. Ears and eyes symptoms, anosmia/ageusia symptoms, and infection duration (10.53 days in the treatment group and 10.48 days in the control group) didn't vary significantly among the two groups.

Conclusion: Nasal lavages resulted to significantly decrease nasal symptoms in newly diagnosed SARS-CoV-2 patients. These devices proved to be well-tolerated and easy to be used. Further studies on a larger number of subjects are needed in order to possibly confirm these preliminary results.

Keywords: SARS-CoV-2 infection, COVID-19, nasal lavage, upper airways infection, nasal swab

INTRODUCTION

During the last year, the current pandemic situation has brought clinicians to an ongoing quest toward the identification of novel tools to manage Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. In particular, the management of asymptomatic or oligosymptomatic patients represents a challenge, also in terms of development of prophylactic strategies to prevent the manifestation or worsening of clinically relevant symptoms, as well as to reduce the viral transmission (1, 2).

As in most of the respiratory infections, including influenza, also in SARS-CoV-2 viral shedding reaches the highest level in the nasopharynx, being also nasal cavity mucosa as one of the most relevant sites of viral activity (3–5). In previous studies on other respiratory infections, including common cold, saline nasal irrigations have been applied as a topical treatment approach, showing a significant efficacy in reducing symptom burden and decreasing viral shedding (6). This observation has led clinicians to focus their interest on the feasibility of a topical management of SARS-CoV-2 infection, based on the reduction of viral load in the nasal cavities and into the upper airways. However, although some trials are currently ongoing (7, 8), to date few and sparse evidence supporting topical preventive or therapeutic strategies in managing SARS-CoV-2 infection are available in the literature (9, 10).

Recently, three Cochrane reviews explored the evidence supporting the use of antimicrobial mouthwashes and nasal spray as a preventive tool to protect healthcare workers when performing aerosol-generating procedures (11) and when assisting suspected or confirmed Coronavirus Disease 2019 (COVID-19) cases (12), or as a therapeutic strategy to improve the outcome of patients with SARS-CoV-2 infection (13). However, none of these meta-analyses found in the literature provided sufficient evidence to support such strategies. Moreover, the large majority of the available reports on topical treatment of SARS-CoV-2 infection regards the local administration of antimicrobial solutions. On the other hand, the preventive and therapeutic role of isotonic saline solution nasal lavages has yet to be extensively explored.

The rationale of proposing isotonic saline solution lavages in SARS-CoV-2 infection resides not only in the mechanical action of the injected fluid which clears the viral particles out of the nasal fossae (14), but also, as recently reported (15), in a direct anti-microbial effect of saline solution, which may allow the epithelial cells to produce hypochlorous acid (HOCl).

Based on this rationale, and given the potential therapeutic relevance of this practice the principle aim of the present investigation was to evaluate the effectiveness of isotonic saline nasal lavages in improving symptoms of COVID-19. Secondary aims were to verify whether nasal lavages may reduce the incidence of symptoms in patients with asymptomatic SARS-CoV-2 infection and to evaluate the compliance to the use of a nasal-lavage device.

MATERIALS AND METHODS

Study Design

The present study was approved by the ethics committee of Treviso and Belluno provinces (ethic vote: 871/CESC). All patients included in this study received specific information material and signed a detailed informed consent form.

This study was a non-randomized controlled trial. The treatment group included a series of consecutive patients which underwent nasal lavages (see also paragraph "***Treatment***"), while the control group included an historical cohort, matched for age, sex, and base-line symptoms.

The date of the first negative test was also collected for each patient.

Treatment Group

A series of consecutive subjects who received diagnosis of SARS-CoV-2 infection in a period from December 9th 2020 to February 25th 2021 were included in the treatment group.

Inclusion criteria were:

1. positive molecular test for SARS-CoV-2 infection,
2. age $\geq$18 years,
3. capability of self-performing nasal lavages.

Exclusion criteria were:

1. clinical conditions preventing self-administration of nasal lavages,
2. clinical conditions preventing administration of the symptom questionnaire,
3. refusal to take part in the study.

Treatment

Nasal lavages were self-performed by each patient in the treatment group by the mean of a device, Lavonase® (Purling, Lugo di Romagna, Italy), which injected the saline solution into a nasal fossa, allowing it to enter the nasopharynx and to be evacuated from the other nasal fossa. Each nasal lavage administrated 250 ml of saline isotonic solution (NaCl 0.9%). The treatment schedule included one daily nasal lavage for 12 days, starting on the day after the molecular diagnosis of SARS-CoV-2 infection.

Symptom Questionnaire

The COVID-Q questionnaire on SARS-CoV-2 infection symptoms (16) was administered to each patient, at base-line and 10 days after diagnosis. The questionnaire included questions on the main clinical presentation patterns of SARS-CoV-2 infection: asthenia, influenza-like symptoms, ear and nose symptoms, breathing issues, throat symptoms, and altered sense of smell or taste (16). From those data, symptoms regarding the otolaryngologic field were considered and analyzed. Among questions about patients' history, one regarding "other not previously specified" was clearly asked, including sinonasal diseases.

When repeated 10 days after diagnosis, two further questions were added, regarding the ease of use of the device and the subjective satisfaction after treatment.

Control Group

The control study included a historical series of patients who tested positive for SARS-CoV-2 in a period from February 19th to March 23rd 2020 and who answered the COVID-Q questionnaire on the following day and on the 10th day since diagnosis.

This series was statistically comparable with the treatment group according to age, sex, and base-line symptoms.

Patients in both treatment and control group underwent a control molecular test for SARS-CoV-2 10 days after diagnosis. If they still tested positive at day 10, they would receive another test 7 days later.

In line with other studies in the field, the sample size was estimated according to a sensitivity analysis, which showed that 70 subjects provided 80% power to detect an effect size as low as dz = 0.307 in a one-tailed Wilcoxon signed-rank test, at the conventional alpha level of 0.05. One-hundred-forty subjects provided 80% power to detect an effect size as small as $d = 0.42$ in a one-tailed *t*-test, at the conventional alpha level of 0.05.

Statistical Analysis

The aim of the study was to examine the impact of nasal lavages on COVID-19, with regards to symptom frequency. First, participants' experiences with the intervention were assessed, testing for age and sex effects. Given the ordinal scale of the compliance variables, sex differences were investigated through the non-parametric Mann-Whitney U test, and age effects through Spearman's rank-order correlations.

COVID-19 symptoms have been shown to follow different trajectories during the infection. Therefore, each symptom was analyzed individually. Baseline symptoms were compared between the treatment and the control group. Next, change in symptom frequency across occasions was analyzed for each group separately using Wilcoxon's signed-rank test. The results also report the proportion of participants experiencing symptoms in the two groups.

Finally, an independent sample *t*-test was used compare the duration of the infection in the case and in the control group.

RESULTS

140 Subjects Were Enrolled and Divided Into two Groups. The Treatment Group Included 68 Participants (35 Males and 33 Females; Mean age 49.2 Years, Range 18–75 Years). The Control Group Comprehended 72 Subjects (29 Males and 43 Females; Mean age 49.2 Years, Range 21–75 Years). As Intended, There were no Significant Differences in the Mean age or sex Composition of the two Groups. In the Overall Sample, Women Were on Average 4.6 Years Younger than men: $t\ (209) = -2.21$, $p = 0.028$. The Mean age was 46.7 Years in Women and 51.3 in men.

Participants in the treatment group were asked to report on ease of use and satisfaction with the treatment. Sixty out of 68 participants answered the questions. The lavages appeared simple to use, with 90% ($N = 54$) of respondents marking them as "easy" or "extremely easy". Furthermore, the answers indicated a good satisfaction with the treatment, with 70% ($N = 42$) of participants declaring themselves "satisfied", "very satisfied" or saying they "would suggest [the lavages] to others". Mann-Whitney U test showed that the experience did not vary significantly according to sex ($W = 471.5$, $p = 0.709$ for ease of use, $W = 553.5$, $p = 0.113$ for satisfaction), nor did it correlate significantly with age ($r = 0.11$, $p = 0.376$ for ease of use, $r = 0.10$, $p = 0.437$ for satisfaction).

TABLE 1 | Reported symptom frequency.

	First assessment							Second assessment						
	0	**1**	**2**	**3**	**4**	**5**	**prop symptom**	**0**	**1**	**2**	**3**	**4**	**5**	**prop symptom**
Intervention														
Painful pressure in ears[1]	51	16	1	–	–	–	0.25	57	11	0	–	–	–	0.16
Blocked nose[1]	31	32	5	–	–	–	0.54	52	15	1	–	–	–	0.24
Runny nose[1]	40	26	2	–	–	–	0.41	58	9	1	–	–	–	0.15
Sneezing[1]	49	18	1	–	–	–	0.28	61	7	0	–	–	–	0.10
Watery eyes[1]	61	7	0	–	–	–	0.10	65	3	0	–	–	–	0.04
Altered sense of smell or taste[2]	33	8	7	5	6	9	0.73	41	6	7	4	3	7	0.50
Control														
Painful pressure in ears[1]	66	6	0	–	–	–	0.08	61	8	3	–	–	–	0.15
Blocked nose[1]	63	7	2	–	–	–	0.13	38	28	6	–	–	–	0.47
Runny nose[1]	56	16	0	–	–	–	0.22	52	16	4	–	–	–	0.28
Sneezing[1]	58	14	0	–	–	–	0.19	46	22	4	–	–	–	0.36
Watery eyes[1]	66	6	0	–	–	–	0.08	60	11	1	–	–	–	0.17
Altered sense of smell or taste[2]	56	0	1	1	2	12	0.28	61	0	0	3	2	6	0.18

Note. Prop symptom, proportion of patients scoring 1 or higher on the symptom frequency over total number of patients in the group.

[1]*Symptom Frequency Assessed on a 0–2 Scale, 0 = not Experienced, 1 = Experienced a Little, 2 = Experienced a lot.*

[2]*Symptom Frequency Assessed on a 0–5 Scale, 0 = not Experienced, 1 = Experienced Barely, 2 = Experienced a Little, 3 = Experienced Moderately, 4 = Experiences a lot, 5 = Complete Loss of Smell or Taste.*

TABLE 2 | Test of between-group differences in symptom frequency at first assessment.

	U	p-value
Painful pressure in ear	2,859	0.008
Blocked nose	3,459.5	0.000
Runny nose	2,928	0.013
Sneezing	2,663	0.224
Watery eyes	2,496	0.694
Altered sense of smell or taste	3,003	0.007

Note. U, U statistic from Mann-Whitney U test.

TABLE 3 | Test of within-group differences in symptom frequency between assessments.

	Intervention		Control	
	V	*p*-value	V	*p*-value
Painful pressure in ear	60	0.078	32.5	0.103
Blocked nose	510	0.000	39	0.000
Runny nose	264	0.000	203	0.217
Sneezing	198	0.010	170.5	0.018
Watery eyes	27	0.182	40	0.115
Altered sense of smell or taste	442.5	0.084	175.5	0.247

Note. V, V statistic from Wilcoxon's signed-rank test.

None of the patients reported sinonasal diseases or others possibly having an influence on nasal function, previous to infection. **Table 1** reports symptom frequency at the first and second assessment for the treatment and control group. Group differences in baseline symptoms were analyzed using Mann-Whitney U test (**Table 2**).

The change in symptoms across time was investigated within each group separately. **Table 3** reports statistics and significance levels from Wilcoxon's signed-rank test and **Figure 1** illustrates the proportion of patients experiencing symptoms. The frequency of the blocked nose and sneezing symptoms varied significantly in both the treatment and the control group. In the treatment group, the proportion of participants experiencing a blocked nose, either occasionally or frequently, decreased by 30.9% (i.e., from 54.4 to 23.5%), and patients experiencing sneezing decreased by 17.6% (i.e., from 27.9 to 10.3%). The control group showed the opposite trend, as the number of people reporting symptoms increased significantly across occasion: 24.2% more patients reported a blocked nose and 16.7% more patients reported sneezing (i.e., increasing from 13.2 to 37.4% and from 19.4 to 36.1%, respectively) (**Table 1**).

The runny nose symptom showed a significant decline (i.e., from 41.2 to 15.1% of participants) with the treatment, and no significant change in the control group. The painful pressure in ears, watery eyes, and anosmia/ageusia symptoms did not vary significantly across occasions in either the case or the control group (**Table 1**, **Figure 1**).

The infection lasted on average 10.53 days (range = 7–26, sd = 3.5) in the treatment group and 10.48 days (range = 6–31, sd = 3.95) in the control group. The *t*-test for independent samples confirmed that there was no significant difference between the mean infection duration in the two groups ($t(137) = 0.08$, C.I. $= -1.202; 1.304$, $p = 0.936$).

Follow-up molecular test at 10 days resulted negative among 62 cases (91.1 %) and in 2 controls (2.8%), with a statistically significant difference ($p < 0.00001$).

DISCUSSION

The present work was a pilot study investigating the effect of nasal lavages on COVID-19 symptoms. Our analysis showed that nasal lavages can significantly reduce the frequency of nose-related symptoms. Specifically, the proportion of patients experiencing a blocked nose, runny nose, or sneezing decreased by an average of 24.7% after the treatment. Conversely, over the same period of time, blocked nose and sneezing became more frequent in patients who did not perform the lavages. Thus, our results suggest that the treatment can offer a substantial relief from COVID-19-symptoms affecting the nose.

On the other hand, our study did not identify a significant difference in the evolution of non-nasal symptoms over time between patients who performed nasal lavages and those who did not. This seems to be in line with available evidence on other upper respiratory tract infections, not related with SARS-CoV-2, stating that nasal saline irrigation may be beneficial for nasal symptoms but not respiratory symptoms (17).

It is worth noting that, in our study population, although nasal symptoms seemed to worsen over time in the absence of treatment, they significantly improved in patients who performed nasal irrigation. This can be interpreted in view of both the well-known efficacy of saline nasal irrigations on symptoms of chronic sino-nasal inflammatory conditions and a possible direct effect in reducing the local viral load into the upper airways.

Literature reports that saline irrigation may improve the patient-reported severity of allergic rhinitis symptoms compared with no saline treatment in children and adults, both on the short-term (up to 4 weeks) and on the medium-term (4 weeks to 6 months) (18). Similar data emerged also from reports on non-allergic chronic sino-nasal inflammatory conditions (19). The effectiveness of nasal irrigation on chronic inflammatory sino-nasal symptoms has been described for isotonic (20), hypertonic (21), and mineral-enriched saline (22) solutions.

Regarding the effect of nasal irrigations on controlling the pathogen load in sino-nasal cavities, evidences seem to support the idea that saline solution alone may be as beneficial as direct antimicrobial agents (23), probably due to a possible direct antimicrobial effect of the hypochlorous acid, produced by the epithelial cells based on sodium chloride (15).

Other previously published papers studied the effectiveness of antimicrobial solutions (e.g., Amphotericin B) on sinonasal diseases (24, 25). Accordingly, no relevant reduction of chronic rhinosinusitis symptoms were obtained. Moreover, by comparing antimicrobial and saline solution, effects were not statistically

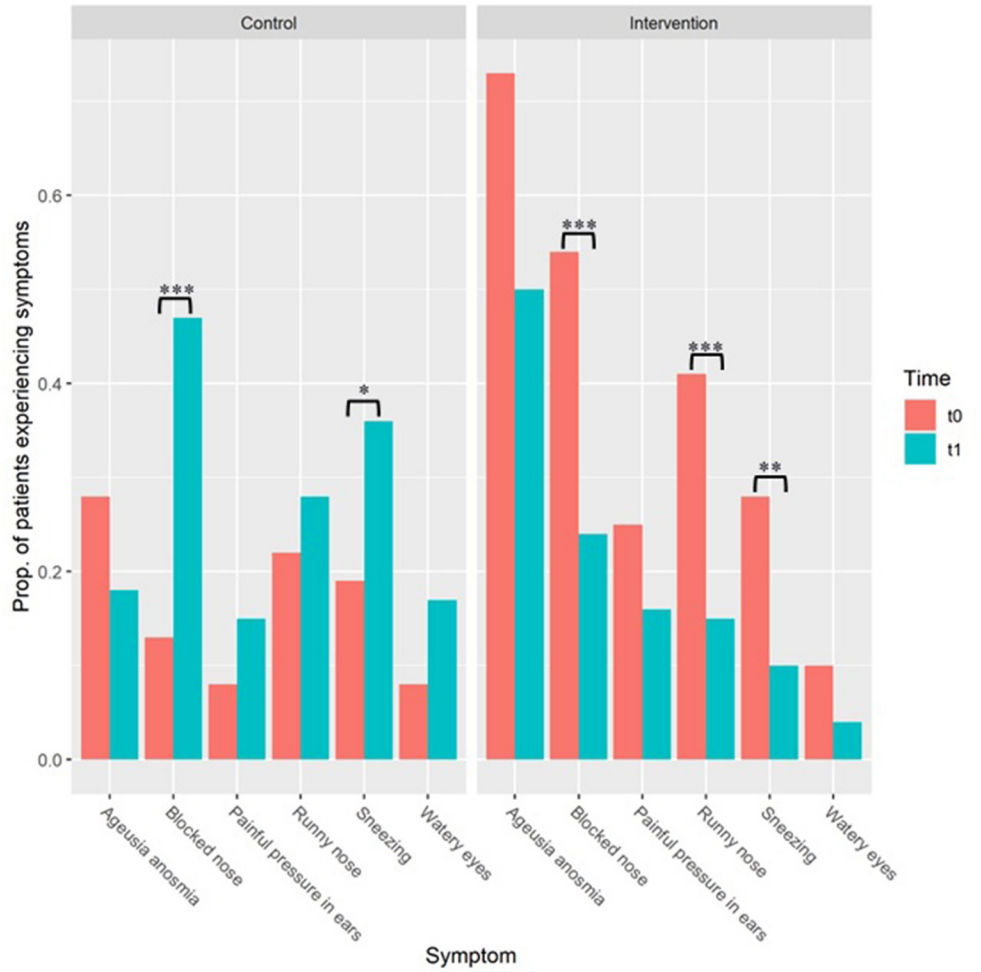


FIGURE 1 | Proportion ("Prop." within the figure) of patients reporting otolaryngologic COVID-19 symptoms. "t0" refers to baseline. "t1" refers to follow-up period after 10 days. Results of the control group are reported on the left ("Control") and those of the treatment group are reported on the right ("Treatment"). Note. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

different. Based on these results, antimicrobial properties of nasal irrigation seems not to be essential, thus confirming suitability of the saline solution we used in this study.

Our findings confirm previous literature with regards to the evolution of individual symptoms (26–28). Indeed, we observed that the frequency of anosmia and ageusia (i.e., loss of smell and taste), painful pressure in the ears, and watery eyes did not change significantly across measurement occasions, neither in the treatment nor in the control group. On the other hand, blocked nose and sneezing symptoms showed a greater, significant change in the observed time-span.

The nasal lavage treatment did not appear to affect the duration of the infection, as the range and mean infection duration did not differ significantly between the treatment and the control group. A statistically significant difference was obtained by comparing the rates of negative swabs among cases and controls at 10-day follow-up, showing a clearly higher rate among subjects who performed nasal lavages. However, such a comparison may be weakened by the fact that microbiological data were available only at fixed times, whereas a daily test might have detected subtler differences between the two groups in time to negativization.

Another weakness of this study concerns the relatively limited number of cases considered. However, based on the preliminary sample size analysis, it was deemed suitable to address this study's primary endpoint. Also, the modalities of treatment administration prevented the possibility of blinding, which might potentially reduce biases in patient's reports on symptoms.

On the other hand, the main strengths of this investigation lie in its controlled design and in the homogeneity of the series of patients considered because: only new diagnoses of SARS-CoV-2 infection were considered; all treated patients received the material for nasal lavage within 24 h from the diagnosis; the control group was comparable regarding age, sex and symptoms at the baseline.

In conclusion, data from this preliminary study showed a good compliance and subjective satisfaction for nasal lavages in patients with newly diagnosed SARS-CoV-2 infection. The treatment showed effectiveness in reducing nasal symptoms of SARS-CoV-2 infection, compared to the control group. However, further studies on larger scale are advocated to better characterize the effectiveness of this treatment on non-nasal symptoms and on the time to microbiological remission.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Ospedaliero di Treviso e Belluno. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GS and CF: conceptualization, manuscript drafting, and manuscript supervision. GCos and FCo: data analysis and manuscript drafting. PS, FCi, RD, AM, MC, GCon, CD, and CA: data collection and manuscript drafting. EE, PB-R, and DF: data collection, manuscript drafting, and manuscript supervision. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Spinato G, Gaudioso P, Boscolo Rizzo P, Fabbris C, Menegaldo A, Mularoni F, et al. Risk management during COVID-19: safety procedures for otolaryngologists. *Acta Biomed.* (2021) 92:e2021105. doi: 10.23750/abm.v92i1.11281
2. Volo T, Stritoni P, Battel I, Zennaro B, Lazzari F, Bellin M, et al. Elective tracheostomy during COVID-19 outbreak: to whom, when, how? Early experience from Venice, Italy. *Eur Arch Otorhinolaryngol.* (2021) 278:781–9. doi: 10.1007/s00405-020-06190-6
3. Spinato G, Fabbris C, Menegaldo A, Marciani S, Gaudioso P, Da Mosto MC, et al. Correct execution of the nasopharyngeal swab: a fundamental method to improve diagnosis of SARS-CoV-2 infection. *J Dr Nurs Pract.* (2021) 9:JDNP-D-20-00040. doi: 10.1891/JDNP-D-20-00040
4. Fabbris C, Cestaro W, Menegaldo A, Spinato G, Frezza D, Vijendren A, et al. Is oro/nasopharyngeal swab for SARS-CoV-2 detection a safe procedure? Complications observed among a case series of 4876 consecutive swabs. *Am J Otolaryngol.* (2021) 42:102758. doi: 10.1016/j.amjoto.2020.102758
5. Capriotti V, Mattioli F, Guida F, Marcuzzo AV, Lo Manto A, Martone A, et al. COVID-19 in the tonsillectomised population. *Acta Otorhinolaryngol Ital.* (2021) 41:197–205. doi: 10.14639/0392-100X-N1436
6. Ramalingam S, Graham C, Dove J, Morrice L, Sheikh A. A pilot, open labelled, randomised controlled trial of hypertonic saline nasal irrigation and gargling for the common cold. *Sci Rep.* (2019) 9:1015. doi: 10.1038/s41598-018-37703-3
7. Khan FR, Kazmi SMR, Iqbal NT, Iqbal J, Ali ST, Abbas SA, et al. Quadruple blind, randomised controlled trial of gargling agents in reducing intraoral viral load among hospitalised COVID-19 patients: a structured summary of a study protocol for a randomised controlled trial. *Trials.* (2020) 21:785. doi: 10.1186/s13063-020-04634-2
8. Kimura KS, Freeman MH, Wessinger BC, et al. Interim analysis of an open-label randomized controlled trial evaluating nasal irrigations in non-hospitalized patients with coronavirus disease 2019. *Int Forum Allergy Rhinol.* (2020) 10:1325–8. doi: 10.1002/alr.22703
9. Baruah B. Could simultaneous nasal and oral irrigation be a nontherapeutic tool against SARS-CoV-2? *ACS Chem Neurosci.* (2021) 12:2–4. doi: 10.1021/acschemneuro.0c00740
10. Farrell NF, Klatt-Cromwell C, Schneider JS. Benefits and safety of nasal saline irrigations in a pandemic-washing COVID-19 away. *JAMA Otolaryngol Head Neck Surg.* (2020) 46:787–8. doi: 10.1001/jamaoto.2020.1622
11. Burton MJ, Clarkson JE, Goulao B, Glenny AM, McBain AJ, Schilder AG, et al. Antimicrobial mouthwashes (gargling) and nasal sprays to protect healthcare workers when undertaking aerosol-generating procedures (AGPs) on patients without suspected or confirmed COVID-19 infection. *Cochrane Database Syst Rev.* (2020) 9:CD013628. doi: 10.1002/14651858.CD013628.pub2
12. Burton MJ, Clarkson JE, Goulao B, Glenny AM, McBain AJ, Schilder AG, et al. Use of antimicrobial mouthwashes (gargling) and nasal sprays by healthcare workers to protect them when treating patients with suspected or confirmed COVID-19 infection. *Cochrane Database Syst Rev.* (2020) 9:CD013626. doi: 10.1002/14651858.CD013626.pub2
13. Burton MJ, Clarkson JE, Goulao B, Glenny AM, McBain AJ, Schilder AG, et al. Antimicrobial mouthwashes (gargling) and nasal sprays administered to patients with suspected or confirmed COVID-19 infection to improve patient outcomes and to protect healthcare workers treating them. *Cochrane Database Syst Rev.* (2020) 9:CD013627. doi: 10.1002/14651858.CD013627.pub2
14. Frezza D, Fabbris C, Franz L, Vian E, Rigoli R, De Siati R, et al. A Severe Acute Respiratory Syndrome Coronavirus 2 detection method based on nasal and nasopharyngeal lavage fluid: a pilot feasibility study. *Laryngoscope Investig Otolaryngol.* (2021) 6:646–9. doi: 10.1002/lio2.625
15. Ramalingam S, Cai B, Wong J, Twomey M, Chen R, Fu RM, et al. Antiviral innate immune response in non-myeloid cells is augmented by chloride ions *via* an increase in intracellular hypochlorous acid levels. *Sci Rep.* (2018) 8:13630. doi: 10.1038/s41598-018-31936-y
16. Spinato G, Fabbris C, Conte F, Menegaldo A, Franz L, Gaudioso P, et al. COVID-Q: validation of the first COVID-19 questionnaire based on patient-rated symptom gravity. *medRxiv.* (2021) 12:e14829. doi: 10.22541/au.162144233.34223358/v1
17. Cabaillot A, Vorilhon P, Roca M, Boussageon R, Eschalier B, Pereirad B. Saline nasal irrigation for acute upper respiratory tract infections in infants and children: A systematic review and meta-analysis. *Paediatr Respir Rev.* (2020) 36:151–8. doi: 10.1016/j.prrv.2019.11.003
18. Head K, Snidvongs K, Glew S, Scadding G, Schilder AG, Philpott C, et al. Saline irrigation for allergic rhinitis. *Cochrane Database Syst Rev.* (2018) 6:CD012597. doi: 10.1002/14651858.CD012597.pub2
19. Giotakis AI, Karow EM, Scheithauer MO, Weber R, Riechelmann H. Saline irrigations following sinus surgery—a controlled, single blinded, randomized trial. *Rhinology.* (2016) 54:302–10. doi: 10.4193/Rhin16.026
20. Barberi S, D Auria E, Bernardo L, Pinto F, Pietra B, Ciprandi G. Isotonic saline in children with perennial allergic rhinitis. *J Biol Regul Homeost Agents.* (2016) 30:605–8.
21. Liu L, Pan M, Li Y, Tan G, Yang Y. Efficacy of nasal irrigation with hypertonic saline on chronic rhinosinusitis: systematic review and meta-analysis. *Braz J Otorhinolaryngol.* (2020) 86:639–46. doi: 10.1016/j.bjorl.2020.03.008
22. Franz L, Manica P, Claudatus J, Frigo AC, Marioni G, Staffieri A. Sulfurous-arsenical-ferruginous thermal water nasal inhalation and irrigation in children with recurrent upper respiratory tract infections: Clinical outcomes and predictive factors. *Am J Otolaryngol.* (2021) 42:103083. doi: 10.1016/j.amjoto.2021.103083
23. Ragab A, Farahat T, Al-Hendawy G, Samaka R, Ragab S, El-Ghobashy A. Nasal saline irrigation with or without systemic antibiotics in treatment of children with acute rhinosinusitis. *Int J Pediatr Otorhinolaryngol.* (2015) 79:2178–86. doi: 10.1016/j.ijporl.2015.09.045

24. Ebbens FA, Scadding GK, Badia L, Hellings PW, Jorissen M, Mullol J, et al. Amphotericin B nasal lavages: not a solution for patients with chronic rhinosinusitis. *J Allergy Clin Immunol.* (2006) 118:1149–56. doi: 10.1016/j.jaci.2006.07.058
25. Jiang RS, Hsu SH, Liang KL. Amphotericin B nasal irrigation as an adjuvant therapy after functional endoscopic sinus surgery. *Am J Rhinol Allergy.* (2015) 29:435–40. doi: 10.2500/ajra.2015.29.4246
26. Boscolo-Rizzo P, Polesel J, Spinato G, Menegaldo A, Fabbris C, Calvanese L, et al. Predominance of an altered sense of smell or taste among long-lasting symptoms in patients with mildly symptomatic COVID-19. *Rhinology.* (2020) 58:524–5. doi: 10.4193/Rhin20.263
27. Boscolo-Rizzo P, Menegaldo A, Fabbris C, Spinato G, Borsetto D, Vaira LA, et al. Six-month psychophysical evaluation of olfactory dysfunction in patients with COVID-19. *Chem Senses.* (2021) 46:bjab006. doi: 10.1093/chemse/bjab006
28. Spinato G, Costantini G, Fabbris C, Menegaldo A, Mularoni F, Gaudioso P, et al. The importance of early detection of ENT symptoms in mild-to-moderate COVID- 19. *Acta Otorhinolaryngol Ital.* (2021) 41:101–7. doi: 10.14639/0392-100X-N1038

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.

frontiers | Frontiers in Neurology

BRIEF RESEARCH REPORT
published: 16 May 2022
doi: 10.3389/fneur.2022.868538

Neurophysiological Aspects in SARS-CoV-2–Induced Acute Respiratory Distress Syndrome

Eleonora Vecchio[1], Lara Gallicchio[1], Nicola Caporusso[2], Valentina Recchia[1], Luigi Didonna[1], Giancarlo Pezzuto[2], Luigi Pisani[2], Antonella Petruzzellis[1], Vito Delmonte[2] and Filippo Tamma[1]*

[1] Department of Neurology, General Regional Hospital "F. Miulli", Acquaviva delle Fonti, Bari, Italy, [2] Department of Intensive Care, General Regional Hospital "F. Miulli", Acquaviva delle Fonti, Bari, Italy

Patients with coronavirus disease 2019 (COVID-19) often develop acute respiratory failure and acute respiratory distress syndrome (ARDS) that requires intensive care unit (ICU) hospitalization and invasive mechanical ventilation, associated with a high mortality rate. In addition, many patients fail early weaning attempts, further increasing ICU length of stay and mortality. COVID-19 related ARDS can be complicated by neurological involvement with mechanisms of direct central nervous system (CNS) infection and with overlapping para-infective mechanisms of the peripheral nervous system (PNS). We aimed to evaluate the possible involvement of the brainstem and PNS in patients with COVID-19 related ARDS and difficulty in weaning from mechanical ventilation. We evaluated electroencephalogram (EEG), brainstem auditory evoked potentials (BAEPs), electroneurography of the four limbs and the phrenic nerve in 10 patients with respiratory insufficiency due to SARS-CoV-2. All were admitted to intensive care unit and were facing prolonged weaning from mechanical ventilation. All ten patients showed a mild diffuse non-specific slowing of brain electrical activity on the EEG. Four patients had an acute motor axonal neuropathy with absent or reduced amplitude phrenic nerve CMAP while four patients showed impairment of the BAEPs. A patient with peripheral nerve impairment suggestive of Guillain-Barré syndrome (GBS) underwent an intravenous immunoglobulin (IVIg) cycle that led to an improvement in the weaning process and progressive motor improvement. The inclusion of a comprehensive neurological evaluation in COVID-19 patients in ICU facilitated the early identification and effective management of Nervous System involvement.

Keywords: COVID-19, ARDS, brainstem, nervous system involvement, Guillain-Barré syndrome

OPEN ACCESS

Edited by:
Beatrice Paradiso, Dolo Hospital, Italy

Reviewed by:
Giuseppe Lanza, University of Catania, Italy
Samir Abu Rumeileh, Martin Luther University of Halle-Wittenberg, Germany
Arianna Sartori, Neurology Unit, ASUGI, Italy

****Correspondence:***
Eleonora Vecchio
e.vecchio@miulli.it

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: *02 February 2022*
Accepted: *11 April 2022*
Published: *16 May 2022*

Citation:
Vecchio E, Gallicchio L, Caporusso N, Recchia V, Didonna L, Pezzuto G, Pisani L, Petruzzellis A, Delmonte V and Tamma F (2022) Neurophysiological Aspects in SARS-CoV-2–Induced Acute Respiratory Distress Syndrome. Front. Neurol. 13:868538. doi: 10.3389/fneur.2022.868538

INTRODUCTION

Interstitial pneumonia due to SARS-CoV-2 can be complicated by possible neurological involvement with mechanisms of direct CNS infection and/or with para-infective mechanisms of the peripheral nervous system (PNS), shown by some neuropathological findings of COVID-19 patients, as recently reviewed (1).

Respiratory failure appears to be one of the most worrying complications due to SARS-CoV-2 infection. Patients can develop severe pneumonia that requires invasive mechanical ventilation that

leads to death in a significant percentage of them. Furthermore, many patients fail early weaning attempts, thus prolonging the length of stay in the intensive care unit (ICU) and increasing in that way complications, morbidity, and mortality. In some cases, there appears to be a discrepancy between the severity of lung involvement and respiratory function. Severe COVID-19 leads to death through multiple mechanisms, including myocardial damage, renal failure, shock, and disseminated intravascular coagulopathy (2, 3). It has also been suggested that the brain stem could play a role in the severe respiratory failure of COVID-19 patients (4). This hypothesis comes from animal models infected with other coronaviruses that have shown that the brainstem is severely affected and in particular the respiratory center (i.e., the nucleus of the solitary tract in the medulla oblongata) (5). In a small case series of an Italian group, this hypothesis is taken into consideration in patients with poor recovery of respiratory function when SARS-CoV-2 pneumonia improves (6). The EEG of these patients showed a diffuse slowing while the brain CT or MRI evaluation was substantially normal (6). In a neurophysiological evaluation using Blink-Reflex in 11 patients with typical interstitial pneumonia due to COVID-19 and severe respiratory failure, the authors highlighted the absence or alteration of the RII component, suggesting a possible involvement of the brainstem, especially at the level of the bulb (7).

Recent reports describe a Guillain-Barré syndrome related to SARS-CoV-2, characterized mainly by axonal impairment with early involvement of cranial nerves that could lead to severe respiratory failure (8).

Our aim was to consider the possible overlap of central and peripheral nervous system involvement in patients with respiratory insufficiency due to COVID-19 and difficulty in weaning from mechanical ventilation.

MATERIALS AND METHODS

We present data about patients with respiratory failure due to SARS-CoV-2 infection admitted to the intensive care unit of the *Miulli Hospital in Acquaviva delle Fonti* in the period from 1 March to 30 May 2021, evaluated because they were facing prolonged weaning from mechanical ventilation, despite the improvement in pulmonary conditions. All patients underwent electroencephalogram (EEG), brainstem auditory evoked potentials (BAEPs), electroneurography of the four limbs and the phrenic nerve. A standard 20 min EEG was recorded according to the 10–20 International system of electrode placement. BAEPs were recorded following auditory stimulation by a 100-μs 85-dB ± click applied to one ear, with a (−20 dB) contralateral masking using "white noise." The recurrence frequency was 11 Hz (bandpass, 150–1,500 Hz; sweep time, 10 ms). Two sets of 2,000 sweeps were averaged. BAEPs were picked up in Cz. The reference electrode was placed at the earlobe ipsilateral to the stimulated ear. Nerve conduction studies were performed according to standardized techniques. Distal motor latency, amplitude and duration of negative peak of compound muscle action potential (CMAP), motor conduction velocity and minimal F-wave latency were measured from different stimulation sites (median, ulnar, peroneal, tibial, and phrenic nerves). Sensory studies were performed anti-dramatically in median, ulnar and sural nerves and amplitude of sensory nerve action potential was measured baseline to negative peak. Patients with previous pathology of the central and peripheral nervous system were not considered. At the time of the evaluation, patients were subjected to only mild sedation with dexmedetomidine.

RESULTS

We evaluated 10 patients, 5 male, aged 53–75 years (mean 66.1); the duration of Covid, from the first detection of SARS-CoV-2 RNA in respiratory specimen (Swab Nasopharyngeal) until the neurophysiological evaluation, was 6–50 days (mean 25.5). In all subjects, the onset of symptoms was on the same day or the day before arrival in ED and the diagnosis of SARS-CoV-2 infection.The main comorbidities presented by the patients were hypertension (present in the 80% of subjects), obesity (60%), Chronic kidney Disease (20%), diabetes (20%). All ten patients showed a mild diffuse non-specific slowing of brain electrical activity on the EEG. Three patients showed normal BAEPs, while in two patients we found

TABLE 1 | Brainstem evoked potential results obtained in the 10 patients evaluated (normal values referred to normative values of our laboratory).

	Lat. I (R/L) (ms)	Lat. III (R/L) (ms)	Lat. V (R/L) (ms)	I-III (R/L) (ms)	III-V (R/L) (ms)	I-V (R/L) (ms)
Normal values	1.7 ± 0.15	4.5 ± 0.2	5.7 ± 0.25	2.1± 0.15	1.9 ± 0.18	4 ± 3SD
Pat. 1	1.61/1.81	3.85/3.89	5.92/6.23	2.24/2.08	2.07/2.34	4.31/4.42
Pat.2	1.88/1.86	4/3.95	6.14/6.12	2.12/2.09	2.14/2.17	4.26/4.26
Pat. 3	1.73/1.79	3.93/3.67	5.79/ab	2.2/1.88	1.86/ab	4.06/ab
Pat. 4	ab/1.79	ab/3.26	ab/5.08	ab/1.47	ab/1.82	ab/3.29
Pat. 5	1.94/1.73	4.0/4.0	6.85/6.05	2.27/2.06	2.85/2.05	4.91/4.32
Pat 6	2.05/1.83	3.29/3.99	6.42/5.94	1.24/2.16	3.13/1.95	4.37/4.11
Pat 7	1.98/ab	4.46/ab	6.9/ab	2.48/ab	2.44/ab	4.92/ab
Pat. 8	1.74/1.65	3.8/3.25	5.76/5.39	2.06/1.6	1.96/2.14	4.02/3.74
Pat. 9	1.75/1.6	4.34/4.3	6.3/6.1	2.59/2.7	1.96/1.8	4.55/4.5
Pat. 10	1.7/1.5	3.9/3.9	5.9/5.8	2.2/2.4	2/1.9	4.2/4.3

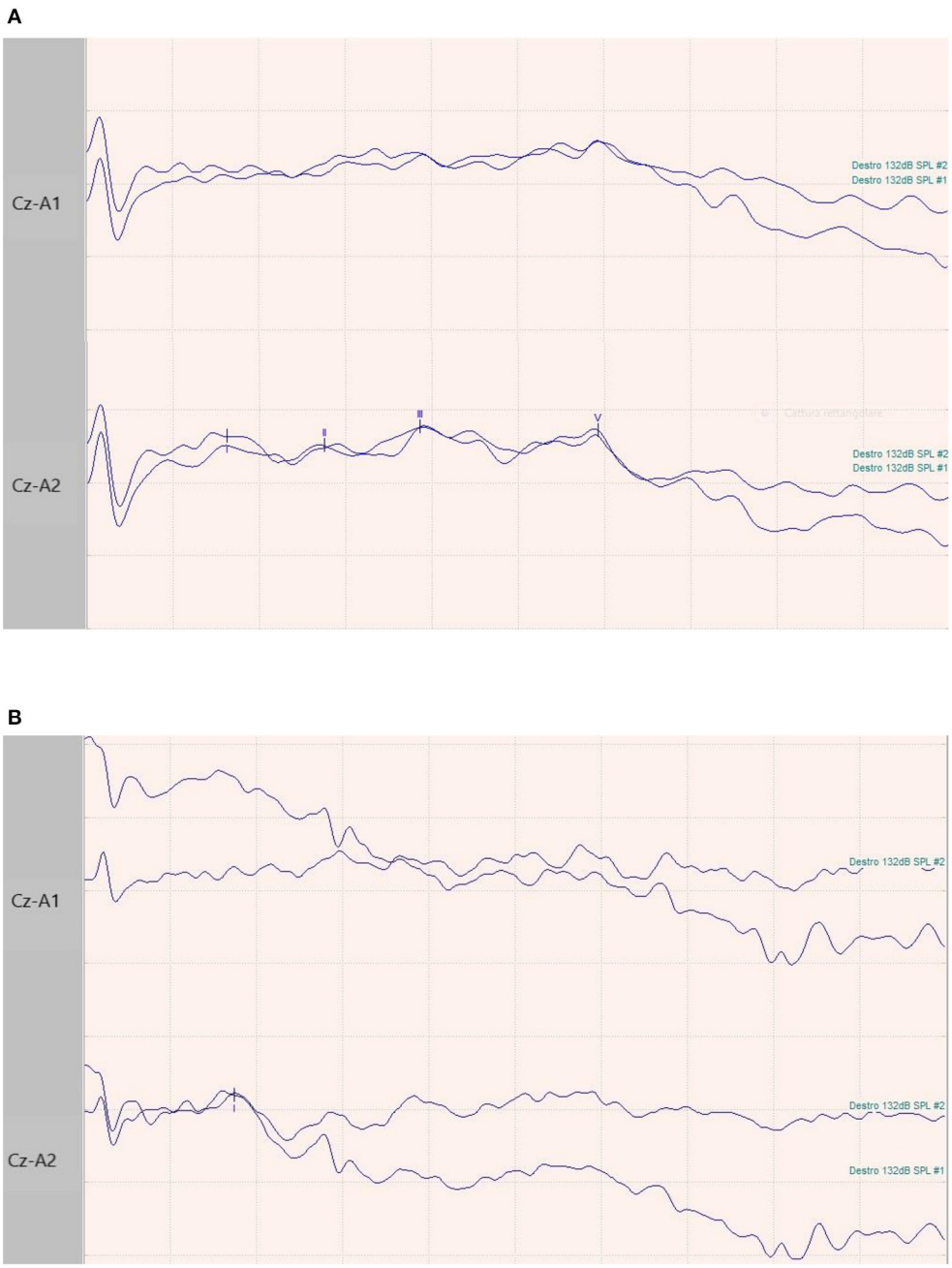


FIGURE 1 | BAEPs recordings in two patients. **(A)** 65-year-old male patient with normal I-III-V complex latency and amplitude and related inter-peak times. **(B)** 73-year-old female patient with normal latency and amplitude of I complex, and absence of III and V waves.

non-evocable responses and in 5 patients increased interpeak III-V wave latency monolaterally (**Table 1**, **Figure 1**). We performed extensive electrophysiological examination in all patients. Six of the patients examined show substantially normal or not significant findings.

In 6 patients the neurological evaluation showed no specific or significant findings. In these patients the electroneurographic study showed normal findings or abnormal studies that did not allow a specific electrodiagnostic classification (**Figure 2**). Four of the 10 subjects showed rapidly progressive tetraparesis with areflexia. In these, the nerve conduction studies showed low or absent motor responses with preserved sensory responses (**Supplementary Table 2**, **Figure 2**), whilst needle EMG findings were consistent with intense and diffuse denervation. In these patients also the CMAP of the phrenic nerve was bilaterally not evocable. These findings suggested a clinical

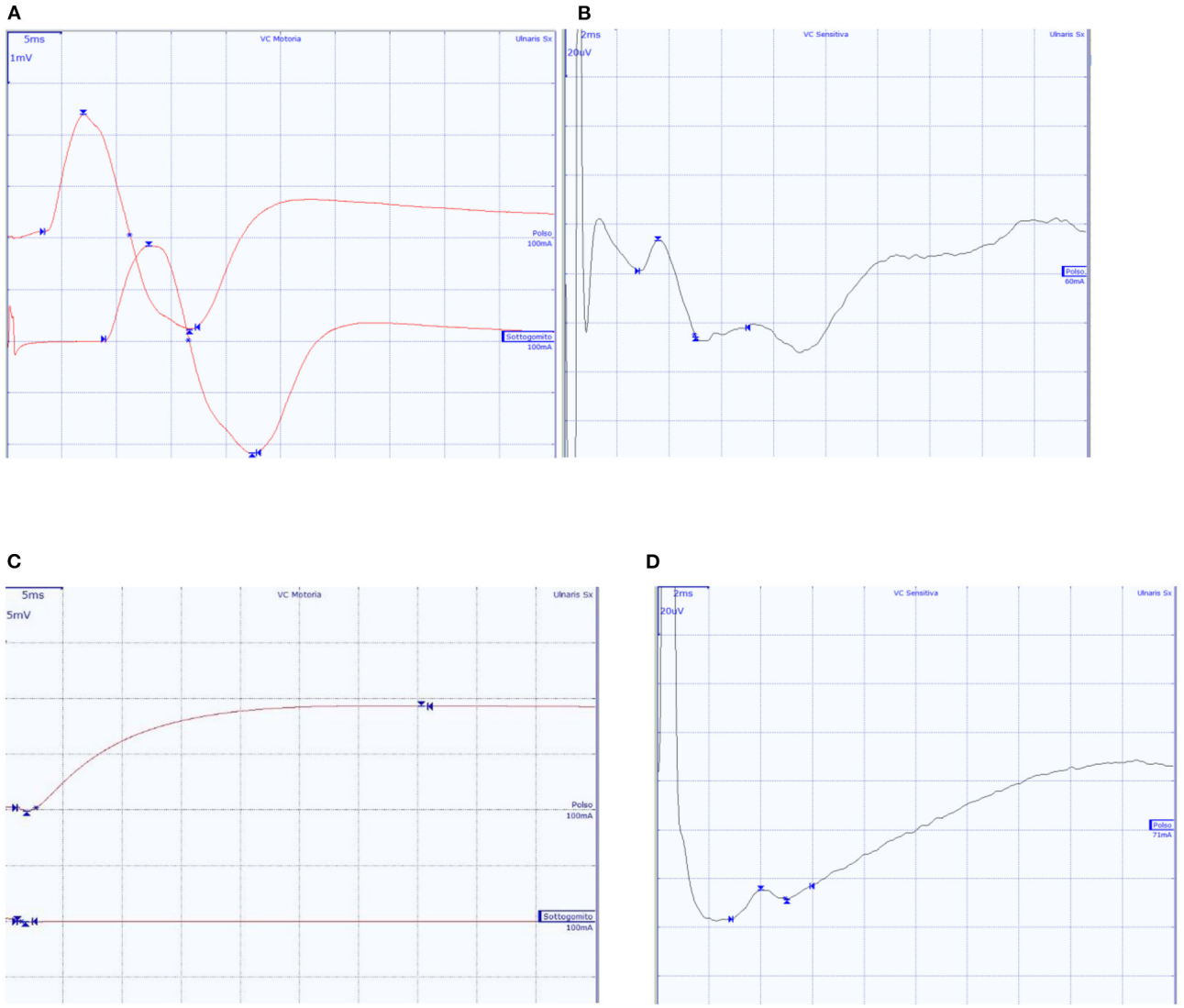


FIGURE 2 | Ulnar nerve CMAP and SAP of two subjects. **(A,B)** A 65-year-old male patient with normal findings; **(C,D)** A 57-year-old patient with diffuse axonal neuropathy, with absent ulnar nerve CMAP, and SAP within normal values.

and neurophysiological diagnosis of acute motor axonal neuropathy (AMAN) (**Supplementary Table 2**), according to the Rajabally criteria (9). We managed to treat one of these four patients with an IVIg cycle (2 g/kg) which led to an improvement in the weaning process and progressive motor improvement. This patient was then discharged at home after 105 days because of renal complications. In the remaining three patients with GBS profile, two of them died, respectively, after 29 and 75 days; the third patient was transferred to the COVID-19 Respiratory sub-intensive unit, and, after 38 days of hospitalization, was sent to a rehabilitation center. The patients with non-GBS profile were sent to rehabilitation centers after a mean of 46 days of hospitalization.

DISCUSSION

Whilst respiratory failure in COVID-19 arises from severe interstitial lung involvement (10), SARS-CoV-2 likely spreads also through the nervous system. It might spread cell-to-cell in a prion-like way (5, 11, 12) along the vagus nerve, reaching respiratory centers in the brainstem, possibly adding a neurogenic component to the respiratory failure (4, 5). Seven of our studied patients presented alterations of the BAEPs, but in 3 of them due to non-evocable responses, it was not possible to exclude a preexisting hearing loss, suggesting a previous peripheral acoustic nerve disorder or technical pitfalls. The remaining four subjects showed a prolonged III–V inter-peak latency, suggesting changes between caudal

pons and midbrain. These neurophysiological findings may be suggestive of SARS-CoV-2-related brainstem involvement in severe COVID-19 patients. EEG findings were non-specific and possibly related to the hypoxic and metabolic conditions of the patients, in addition to a possible pharmacological effect induced by dexmedetomidine.

Another important aspect that emerged from our evaluation in patients with COVID-19 is the need to consider a Guillain-Barré syndrome associated with severe respiratory impairment. Typically, GBS is a post-infectious condition with symptom onset for 76% of the patients occurring in about 4 weeks after the preceding respiratory or gastrointestinal infection (8). The para-infectious profile like the one described in our series, is an atypical feature that was only recently reported among patients infected with the Zika virus and SARS-CoV-2 (8). In other, larger series of patients with GBS associated with COVID-19, emerged a higher frequency of subjects in which GBS started while COVID-19 symptoms were still ongoing (13–15). Furthermore, COVID-GBS patients had respiratory symptoms at presentation to the ED, and the length of these symptoms was significantly longer than in COVID–non-GBS patient (14). The diagnosis of SARS-CoV-2 infection, the absence of any other immunological or microbiological explanation, and the epidemiological finding of increased relative frequency and standardized incidence of GBS in the COVID-19 patients in some studies, suggest that SARS-CoV-2 may have been responsible for the development of GBS in these patients (14, 15). The detection of a relatively high incidence of GBS cases, and in particular of AMAN subtype in our series, may therefore derive from the selection of patient only with a more severe COVID-19. This association, in subjects not specifically treated, revealed a worse outcome in our series. Accurate identification and categorization of GBS patients are very important, since the para-infectious profile is associated with a concurrent manifestation of COVID-19 and GBS symptoms, which can complicate the treatment and may be associated with a worse prognosis. Indeed, the patients with a para-infectious profile were more likely to have a poor prognosis (8, 13).

This study has several limitations. First, the number of cases was very small. Second, it is a retrospective study and some findings such as antiganglioside antibody titres and cerebrospinal fluid analysis were not available. However, it should be considered that these patients were studied in a pandemic context and under the pressure of and exceptional health emergency in a hospital of southern Italy. Therefore, the interpretation of our findings should be made with caution and should be interpreted as hypothesis-generating.

CONCLUSION

Overall, our results suggest that a central, mainly at brainstem level, and peripheral nervous system involvement likely contributes to respiratory failure in COVID-19 patients. The inclusion of a comprehensive neurological evaluation in SARS-CoV-2 patients with clinical and radiological lung amelioration, but difficulty in weaning from mechanical ventilation, facilitated the identification and effective treatment of neurological involvement.

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. Patient's next of kin provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EV, LG, and NC: conception, design, and drafting article. VR, LD, GP, and LP: acquisition of data. EV and AP: analysis and interpretation of data. FT and VD: study supervision. All authors critically revised the article and reviewed final version of the manuscript and approved it for submission.

ACKNOWLEDGMENTS

We would like to thank Dr. Grigorios Katsouras for his valuable comments that greatly improved the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.868538/full#supplementary-material

REFERENCES

1. Fisicaro F, Di Napoli M, Liberto A, Fanella M, Di Stasio F, Pennisi M, et al. Neurological sequelae in patients with COVID-19: a histopathological perspective. *Int J Environ Res Public Health.* (2021) 18:1415. doi: 10.3390/ijerph18041415
2. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* (2020) 323:1061–2. doi: 10.1001/jama.2020.1585
3. Guo T, Fan Y, Chen M, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* (2020) 5:811–8. doi: 10.1001/jamacardio.2020.1017
4. Tassorelli C, Mojoli F, Baldanti F, Bruno R, Benazzo M. COVID-19: what if the brain had a role in causing the deaths? *Eur J Neurol.* (2020) 27:e41–e42. doi: 10.1111/ene.14275
5. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol.* (2020) 92:552–5. doi: 10.1002/jmv.25728

6. Manganelli F, Vargas M, Iovino A, Iacovazzo C, Santoro L, Servillo G. Brainstem involvement and respiratory failure in COVID-19. *Neurol Sci.* (2020) 41:1663–5. doi: 10.1007/s10072-020-04487-2
7. Bocci T, Bulfamante G, Campiglio L, Coppola S, Falleni M, Chiumello D, et al. Brainstem clinical and neurophysiological involvement in COVID-19. *J Neurol*. (2021) 268:3598–600. doi: 10.1007/s00415-021-10474-0
8. Kajumba M, Kolls BJ, Koltai DC, Kaddumukasa M, Kaddumukasa M, Laskowitz DT. COVID-19-associated guillain-barre syndrome: atypical para-infectious profile, symptom overlap, and increased risk of severe neurological complications. *SN Compr Clin Med*. (2020) 2:2702–14. doi: 10.1007/s42399-020-00646-w
9. Rajabally YA, Durand MC, Mitchell J, Orlikowski D, Nicolas G. Electrophysiological diagnosis of Guillain–Barré syndrome subtype: could a single study suffice? *J Neurol Neurosurg Psychiatry*. (2015) 86:115–9. doi: 10.1136/jnnp-2014-307815
10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* (2020) 579:270–3. doi: 10.1038/s41586-020-2012-7
11. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 virus targeting the CNS: tissue distribution, host-virus interaction, and proposed neurotropic mechanisms. *ACS Chem Neurosci.* (2020) 11:995–8. doi: 10.1021/acschemneuro.0c00122
12. Dube M, Le Coupanec A, Wong AHM, Rini JM, Desforges M, Talbot PJ. Axonal transport enables neuron-to-neuron propagation of human coronavirus OC43. *J Virol.* (2018) 92:e00404–00418. doi: 10.1128/JVI.00404-18
13. Filosto M, Cotti Piccinelli S, Gazzina S, Foresti C, Frigeni B, Servalli MC, et al. Guillain-Barré syndrome and COVID-19: an observational multicentre study from two Italian hotspot regions. *J Neurol Neurosurg Psychiatry*. (2021) 92:751–6. doi: 10.1136/jnnp-2020-324837
14. Fragiel M, Miró O., Llorens P, Jiménez S, Piñera P, Burillo G, et al. Incidence, clinical, risk factors and outcomes of Guillain-Barré in Covid-19. *Ann Neurol.* (2021) 89:598–603. doi: 10.1002/ana.25987
15. Abu-Rumeileh S, Abdelhak A, Foschi M, Tumani H, Otto M. Guillain–Barré syndrome spectrum associated with COVID-19: an up-to-date systematic review of 73 cases. *J Neurol.* (2021) 268:1133–70. doi: 10.1007/s00415-020-10124-x

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.

frontiers | Frontiers in Neurology

REVIEW
published: 27 May 2022
doi: 10.3389/fneur.2022.886609

Dysautonomia in COVID-19 Patients: A Narrative Review on Clinical Course, Diagnostic and Therapeutic Strategies

Francisco Carmona-Torre[1,2,3], Ane Mínguez-Olaondo[4,5,6,7,8], Alba López-Bravo[9,10], Beatriz Tijero[7,11,12], Vesselina Grozeva[13], Michaela Walcker[5], Harkaitz Azkune-Galparsoro[6,14,15], Adolfo López de Munain[4,5,6,7,8,15], Ana Belen Alcaide[2,16], Jorge Quiroga[2,3,17,18], Jose Luis del Pozo[1,2,3†] and Juan Carlos Gómez-Esteban[5,7,8,11,12,15†]*

[1] Infectious Disease Service, University Clinic of Navarra, Pamplona, Spain, [2] COVID-19 Department, University Clinic of Navarra, Pamplona, Spain, [3] Immune and Infectious Inflammatory Diseases Research, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain, [4] Neurology Department, Donostia University Hospital-OSAKIDETZA, San Sebastián, Spain, [5] ATHENEA Neuroclinics, Policlínica Gipuzkoa Grupo Quironsalud, Donostia, Spain, [6] Neuroscience Area, Biodonostia Research Institute, San Sebastián, Spain, [7] Neurology Department, Faculty of Medicine, University of Deusto, Bilbao, Spain, [8] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Institute Carlos III, Madrid, Spain, [9] Neurology Department, Hospital Reina Sofía de Tudela-OSASUNBIDEA, Tudela, Spain, [10] Aragon Institute for Health Research (IIS-A), Zaragoza, Spain, [11] Neurodegenerative Diseases Group Biocruces Bizkaia Health Research Institute, Barakaldo, Spain, [12] Neurology Department, Cruces University Hospital-OSAKIDETZA, Barakaldo, Spain, [13] Neurology Practice, Polyclinic Mladost 1, Sofia, Bulgaria, [14] Infectious Disease Department, Donostia University Hospital-OSAKIDETZA, San Sebastián, Spain, [15] Department of Neurosciences, University of the Basque Country (UPV/EHU), Leioa, Spain, [16] Pulmonary Department, University Clinic of Navarra, Pamplona, Spain, [17] Internal Medicine Department, University Clinic of Navarra, Pamplona, Spain, [18] Centro de Investigación Biomédica en Red en Enfermedades Hepáticas y Digestivas (CIBEREHD), Institute Carlos III, Madrid, Spain

OPEN ACCESS

Edited by:
Beatrice Paradiso,
Dolo Hospital, Italy

Reviewed by:
Philip L. Mar,
Saint Louis University, United States
Anna Hohler,
St. Elizabeth's Medical Center,
United States

***Correspondence:**
Ane Mínguez-Olaondo
aminguezolaondo@gmail.com

[†]These authors share senior authorship

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: 28 February 2022
Accepted: 28 March 2022
Published: 27 May 2022

Citation:
Carmona-Torre F, Mínguez-Olaondo A, López-Bravo A, Tijero B, Grozeva V, Walcker M, Azkune-Galparsoro H, López de Munain A, Alcaide AB, Quiroga J, del Pozo JL and Gómez-Esteban JC (2022) Dysautonomia in COVID-19 Patients: A Narrative Review on Clinical Course, Diagnostic and Therapeutic Strategies. Front. Neurol. 13:886609. doi: 10.3389/fneur.2022.886609

Introduction: On March 11, 2020, the World Health Organization sounded the COVID-19 pandemic alarm. While efforts in the first few months focused on reducing the mortality of infected patients, there is increasing data on the effects of long-term infection (Post-COVID-19 condition). Among the different symptoms described after acute infection, those derived from autonomic dysfunction are especially frequent and limiting.

Objective: To conduct a narrative review synthesizing current evidence of the signs and symptoms of dysautonomia in patients diagnosed with COVID-19, together with a compilation of available treatment guidelines.

Results: Autonomic dysfunction associated with SARS-CoV-2 infection occurs at different temporal stages. Some of the proposed pathophysiological mechanisms include direct tissue damage, immune dysregulation, hormonal disturbances, elevated cytokine levels, and persistent low-grade infection. Acute autonomic dysfunction has a direct impact on the mortality risk, given its repercussions on the respiratory, cardiovascular, and neurological systems. Iatrogenic autonomic dysfunction is a side effect caused by the drugs used and/or admission to the intensive care unit. Finally, late dysautonomia occurs in 2.5% of patients with Post-COVID-19 condition. While orthostatic hypotension and neurally-mediated syncope should be considered, postural orthostatic tachycardia syndrome (POTS) appears to be the most

common autonomic phenotype among these patients. A review of diagnostic and treatment guidelines focused on each type of dysautonomic condition was done.

Conclusion: Symptoms deriving from autonomic dysfunction involvement are common in those affected by COVID-19. These symptoms have a great impact on the quality of life both in the short and medium to long term. A better understanding of the pathophysiological mechanisms of Post-COVID manifestations that affect the autonomic nervous system, and targeted therapeutic management could help reduce the sequelae of COVID-19, especially if we act in the earliest phases of the disease.

Keywords: dysautonomia, Post-COVID-19 condition, socioeconomic impact, orthostatic intolerance syndromes, POTS, diagnosis, management

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has created a pandemic, generally known as the coronavirus disease 2019 (COVID-19) pandemic, with devastating effect on the health and economy of the entire world population. The first cases of COVID-19 were reported in Wuhan, China, in November 2019, and the first cases in North America and Europe in January 2020 (1). By February 2022, more than 430 million confirmed cases of COVID-19 and more than 5.9 million deaths had been reported to the World Health Organization (WHO) (https://covid19.who.int/, as of February 27, 2022). About 80% of COVID-19 cases are paucisymptomatic and mild, and many patients recover within 2–4 weeks. However, severe pneumonia and critical multi-organ failure may occur in 15 and 5% of cases, respectively (2). Although there is a wealth of information on the clinical manifestations, therapeutic management and short-term consequences of the infection, there is less information on the residual symptoms that occur and persist in patients who have overcome acute infection but experience long-term multiorgan complications (3). These manifestations were detected from the very outset of the pandemic, and indeed, the existence of persistent symptoms (i.e., long COVID-19) after acute infection has been noted since April 2020 (4). Post-COVID symptoms are very heterogeneous and affect and involve multiple systems. Numerous pathophysiological mechanisms have been proposed that include, but are not limited to, direct or indirect invasion of the virus into the brain, immune dysregulation, hormonal disturbances, elevated cytokine levels due to immune reaction leading to chronic inflammation, direct tissue damage, and persistent low-grade infection.

The actual number of those affected who manifest symptoms after the acute episode of COVID-19 is unknown; however, in a survey carried out by the UK Government Office for National Statistics in November 2020, around 20% of patients diagnosed with COVID-19 reported symptoms that persisted 5 weeks or more after acute infection, and 10% reported symptoms lasting 12 weeks or more (3).

Frequently reported residual effects of the SARS-CoV-2 virus include a wide array of pulmonary and extrapulmonary clinical manifestations, including nervous system and neurocognitive disorders, mental health disorders, cardiovascular disorders, gastrointestinal disorders, skin disorders, and signs and symptoms associated with poor general wellbeing, including malaise, fatigue, musculoskeletal pain, and reduced quality of life. The most common neurocognitive symptoms reported are difficulties concentrating, memory deficits and cognitive impairment (5). Follow-ups conducted in Germany and the United Kingdom found post–COVID-19 neuropsychiatric symptoms in 20–70% of patients, including young adults (6). Systemic and neurocognitive deficits may last only weeks but can potentially lead to lifelong disability (2).

In a prospective study conducted in 3,762 participants from 56 countries with confirmed (diagnostic/antibody-positive; 1,020) or suspected (diagnostic/antibody-negative or untested; 2,742) COVID-19, it was found that more than 91% of participants continued to have symptoms at 7-month follow-up, mainly systemic and neurologic/cognitive symptoms. The most frequent symptoms after month 6 were fatigue, Post-exertional malaise, and cognitive dysfunction. Relapse or recurrence of symptoms, triggered primarily by exercise, physical or mental activity, and stress (7), were experienced by 85.9% of participants (95% CI, 84.8–87.0%).

In individuals at low risk of COVID-19 mortality with ongoing symptoms, 70% have impairment in one or more organs 4 months after the initial COVID-19 symptoms, including the

Abbreviations: ACE2, Angiotensin-converting enzyme 2; ADH, Vasopressin; AIDP, Acute inflammatory demyelinating neuropathy; AMAN, Acute motor axonal neuropathy; AMSAN, Acute motor and sensory axonal neuropathy; ANS, Autonomic nervous system; AT1-R, Angiotensin II receptor type 1; BBB, Blood-brain barrier; BP, Blood pressure (mmHg); CI, Confidence Interval; CNS, Central nervous system; COMPASS-31, Composite Autonomic Symptom Scale 31; COVID-19, Coronavirus disease 2019; CT, Computed tomography; EAS, Extended autonomic system; ECG, Electrocardiogram; ESC, Electrochemical skin conductance; g-AChR, Ganglionic neuronal nicotinic acetylcholine receptor; GBS, Guillain Barré Syndrome; GPCR, G-protein-coupled receptor; HR, Heart rate; HT, Hypertensive; hT, Hypotensive; hTO, Orthostatic hypotension; ICU, Intensive care unit; MasR, Mas receptor; MERS, Middle East respiratory syndrome; min, Minutes; MRI, Magnetic resonance imaging; MSA, Multiple system atrophy; NA, Noradrenaline; NICE, The United Kingdom National Institute for Health and Care Excellence; NOH, Neurogenic Orthostatic hypotension; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PASC, Post-acute sequelae of COVID; PLR, Pupillary light reflex; PNC, Polyneuritis cranialis; PNS, Peripheral nervous system; POTS, Postural tachycardia syndrome; QSART, Quantitative sudomotor axon reflex testing; RAAS, The renin-angiotensin-aldosterone system; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SARS, Severe acute respiratory syndrome; SNS, Sympathetic nervous system; WHO, World Health Organization.

heart (26%), lungs (11%), kidneys (4%), liver (28%), pancreas (40%) and spleen (4%) (8). Furthermore, persistent symptoms (>6 weeks) have been reported in 19% of 39 fully vaccinated healthcare workers after breakthrough infections (9).

Some studies indicate that disease severity correlates with worse and more prolonged neurologic symptoms (10, 11), while other studies have found no such correlation (5, 12).

Several symptoms are impacted by the autonomic nervous system, with fatigue described as one of the major clinical features of dysautonomia in patients with COVID-19 (13). Dysautonomia has been broadly defined as a condition where changes in the functioning of one or more components of the autonomic nervous system adversely affect health (14). Despite its prevalence, the relationship between Post-COVID symptoms and dysautonomic features has not been well-studied.

BOX 1 | Clinical case definition of Post-COVID-19 condition.

Post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of COVID-19 with symptoms that last for at least 2 months and cannot be explained by an alternative diagnosis. Common symptoms include, but are not limited to, fatigue, shortness of breath, and cognitive dysfunction, and generally have an impact on everyday functioning. Symptoms might be new onset following initial recovery from an acute COVID-19 episode or persist from the initial illness. Symptoms might also fluctuate or relapse over time. A separate definition might be applicable for children.

Notes:

There is no minimum number of symptoms required for diagnosis; symptoms involving different organs systems and clusters have been described.

Changes in the Criteria for the Classification of the Symptomatic Phases of COVID-19

The United Kingdom National Institute for Health and Care Excellence (NICE) (15) has defined several symptomatic phases of COVID-19 useful for the conduction and comparison of different studies, and established the following operational definitions based on the timing of signs and symptoms after illness onset. These are as follows:

- *Acute COVID-19:* signs and symptoms present up to 4 weeks after illness onset.
- *Ongoing symptomatic COVID-19*: signs and symptoms of COVID-19 persist from 4 to 12 weeks after illness onset.
- *Post-COVID-19 syndrome*: signs and symptoms that develop during or after an infection compatible with COVID-19, continue beyond 12 weeks and are not explained by an alternative diagnosis once active infection or reinfection has been ruled out.
- *Prolonged COVID/"long-COVID"/"Post-acute sequelae" of COVID (PASC)* includes both ongoing symptomatic COVID-19 (4–12 weeks) and Post-COVID-19 syndrome (12 weeks or more).

Diagnostic criteria for Post-acute phase sequelae (PASC) of SARS-CoV-2 infection, which may affect 20–60% of patients (16), were subsequently proposed (17). The term "neuro-PASC" refers to diagnostic criteria related to neurologic sequelae, including dysautonomia mentioned above. In this context, the neurologic symptoms or development of sequelae due to SARS-CoV-2 infection persist beyond 4 weeks after the onset of acute symptoms. Subacute neuro-PASC corresponds to neurologic symptoms and abnormalities present from 4 to 12 weeks after the acute phase of COVID-19, while chronic neuro-PACS refers to neurologic symptoms and abnormalities persisting or present beyond 12 weeks and not attributable to alternative diagnoses (2).

Finally, in the last consensus communication published on 6 October 2021, the WHO using a robust Delphi methodology, published a clinical case definition of the Post-COVID-19 condition reached by Delphi consensus (18) (**Box 1**).

Despite these efforts to define the picture, there is a clear need at the neurologic level to acquire a better understanding of the underlying pathophysiology of these symptoms in order to improve the therapeutic management of the different clinical pictures, in which autonomic dysfunction triggered in patients after COVID-19 (19) is of special interest.

Dysautonomia Definition

The autonomic nervous system, which innervates all organs of the body, maintains biological homeostasis at rest and in response to stress through an intricate network of central and peripheral neurons that work automatically. Autonomic disorders can manifest in a variety of ways: deafferentation of the central autonomic centers can alter the degree or timing of peripheral autonomic effectors; autonomic efferent neuron lesions can reduce or suppress autonomic responses; and drugs or antibodies acting on autonomic neuron receptors can produce a variety of physiological phenomena ranging from hyperfunction to hypofunction and loss of function (20).

Since the autonomic nervous system is an integrative system, the clinical approach to autonomic disorders should be holistic. Rarely does the patient present with a single, clearly explained and easily identifiable symptom (20).

The search for autonomic disorders requires a careful and thorough medical history. The goals of the assessment are to identify whether there is an autonomic disorder, to locate and define its distribution, and to measure its severity. It is especially important to detect severe and treatable disorders (20).

Socio-Economic Impact of Autonomic Dysfunction in COVID-19

The number of people with the Post-COVID-19 condition remains uncertain. Recent reports indicate that ∼20–60% of COVID-19 patients experience persistent symptoms, as stated above (16), which means that between 86 and 258 million of the more than 430 million confirmed cases of COVID-19 (https://covid19.who.int/, as of February 27, 2022) would have persistent symptoms. This gives us an idea of the enormous impact of the Post-COVID-19 condition. Assuming dysautonomic symptoms occur in 2.5% of Post-COVID-19 patients (21, 22), ∼2.15–6.45 million people experience Post-COVID-19 dysautonomia worldwide. This chilling statistic gives some idea of the

significance of this symptomatology and of the need to deepen our understanding of it.

The socio-economic impact of COVID-19 is largely related to the development of Post-COVID fatigue, autonomic and neurohemodynamic impairment (13). The potential scale of Post-COVID-19 syndrome in lower-risk individuals, who represent up to 80% of the population, calls for urgent policies in all countries to monitor and treat the long-term implications of COVID-19 and to mitigate its impact on healthcare utilization and the economy (8). According to data from an online survey of people with suspected and confirmed COVID-19, at 7 months after suspected or confirmed COVID-19 infection, 45.2% of patients required a reduction in working hours and 22.3% were not working due to illness (7).

There is a strong association between fatigue and Post-COVID anxiety, even in the absence of a preexisting diagnosis of depression or anxiety (23).

Little is known about the prognosis of postural orthostatic tachycardia syndrome (POTS). It is estimated that about 80% of all patients with POTS improved and 60% had minimal residual symptoms during ∼5 years (24). Addressing the needs of Post-COVID-19 patients will therefore require a significant investment in resources and funding both for clinical care and research. Action is needed during this window of opportunity in the interest of reducing or shortening the impact of symptoms in these patients (25) and thus promoting their earliest possible social and occupational reintegration.

This article reviews the available scientific evidence on dysautonomic symptoms during the disease, as well as the evidence on the management of the most prevalent syndromes.

METHODOLOGY

Methodological differences in the assessment of dysautonomia in the different published studies, as well as the variations in study design, prevent us from comparing them directly. We have performed a critical narrative review with a synthesis of the current publications on the subject.

We conducted a Non-systematic literature search of the PubMed database in January 2022 for published manuscripts on dysautonomia and COVID-19. The research strategy included the key terms "dysautonomia AND COVID-19" and "autonomic symptoms AND COVID-19". Six of the authors (F C-T, A M-O, A L-B, BT, VG, MW) independently reviewed the publications and selected those that met the inclusion criteria. Duplicate publications were removed by manual checking. Studies eligible for inclusion were all types of articles published in English or Spanish, human-centered, with well-defined COVID-19-related descriptions of dysautonomic signs and symptoms.

Studies lacking a clear description of the diagnostic criteria for dysautonomia or COVID-19 were excluded. Study protocols, publications that did not specifically mention dysautonomia or did not focus on dysautonomia in COVID-19, as well as those published in a language other than English or Spanish were excluded (**Figure 1**). The text has been completed with publications obtained from PubMed that were considered relevant. **Figures 2**, **3** were prepared using the BioRender.com tool.

POSSIBLE ROUTES OF ENTRY OF THE VIRUS

It is thought that part of the neurologic symptomatology may be due to invasion of the central nervous system (CNS) by SARS-CoV-2. The same hypothesis was considered in the 1918 influenza pandemic when an association between influenza, encephalitis lethargica, and postencephalitic parkinsonism was observed (28). It is known that SARS-CoV-2 penetrates the olfactory mucosa, causing loss of smell, and may invade the brain tissue by migrating from the cribriform plate along the olfactory tract, or by the vagal or trigeminal pathways (26, 29) (**Figure 2**).

Another hypothesis is that the virus could cross the blood-brain barrier (BBB) which is disrupted or becomes more permeable through the action of inflammatory cytokines and/or monocytes (30). The virus can reach the brain tissue through the circumventricular organs (midline structures around the third and fourth ventricles).

Once inside the CNS, SARS-CoV-2-related neuronal damage can be induced either by direct cell invasion, mediated by a virus protein binding to the endothelial acetylcholine receptor, or by a cytokine-mediated dysimmune mechanism (31). Low-grade inflammation in small vessels is also thought to play a role (32). This is most likely facilitated by the inflammatory reflex and the autonomic brainstem reflex (27, 33).

THE ROLE OF DYSAUTONOMIA IN THE CLINICAL COURSE OF COVID-19

COVID-19 is especially life-threatening in the elderly and in those with any of a variety of chronic medical conditions. Pneumonitis and pulmonary dysfunction usually dominate the clinical picture, but it is clear that COVID-19 significantly affects other body organ systems, including the heart, gut, kidneys, and brain (14, 34). One hypothesis contends that this heightened risk may be caused by the development of dysautonomia (14).

It is not clear whether infection-associated dysautonomia is the direct result of the action of the virus on autonomic nervous system (ANS) structures or a consequence of postinfectious immune-mediated processes (34, 35).

The ANS has traditionally been viewed as consisting of the sympathetic nervous system, the parasympathetic nervous system, and the enteric nervous system. Over the past century, however, the neuroendocrine and neuroimmune systems have come to the fore, prompting a change of nomenclature to "extended autonomic system (EAS)". Additional facets include the sympathetic adrenergic system, for which adrenaline is the key effector; the hypothalamic-pituitary-adrenocortical axis; arginine vasopressin; the renin-angiotensin-aldosterone system, with angiotensin II and aldosterone as the main effectors; and the cholinergic anti-inflammatory and sympathetic inflammasome pathways. A hierarchical brain network—the central autonomic network—regulates these systems; embedded within it are components of the Chrousos/Gold "stress system" (14).

Acute, coordinated alterations in homeostatic settings (allostasis) can be crucial for surviving stressors. Allostatic states however also increase wear and tear on both the effectors

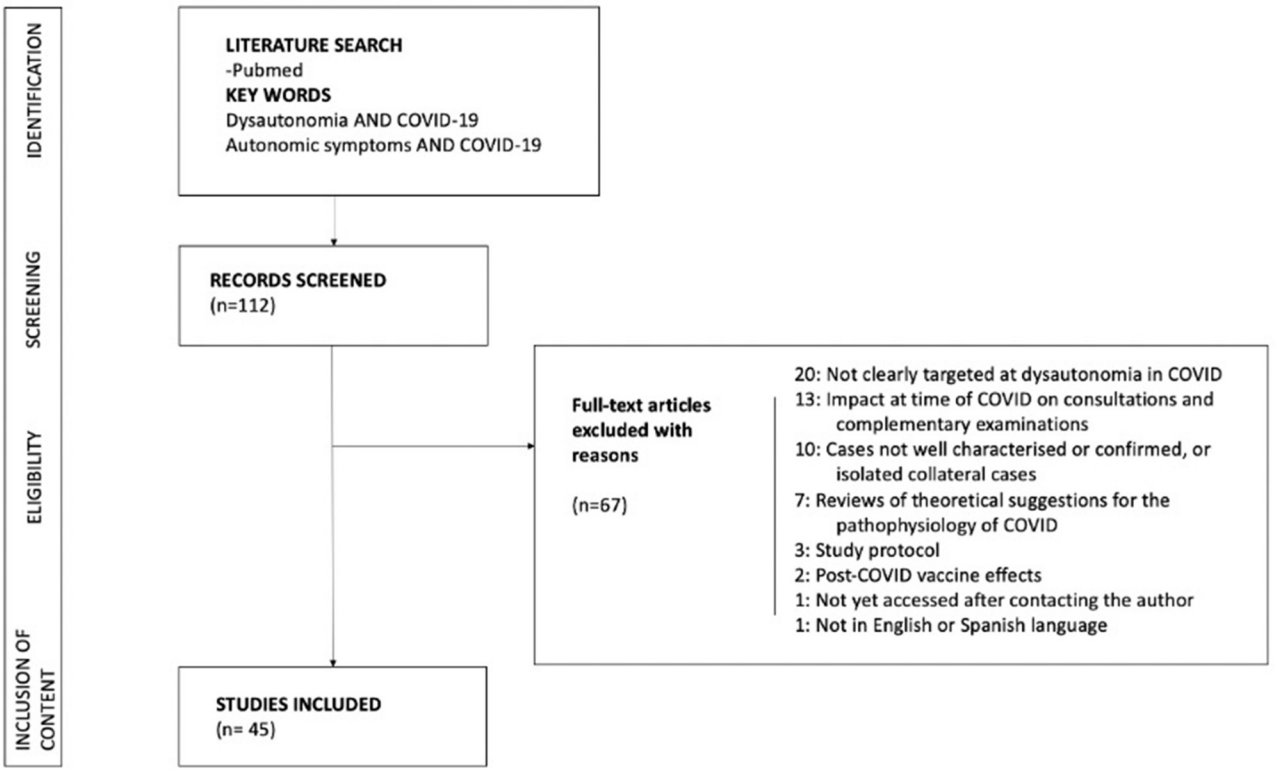


FIGURE 1 | Flow chart of the study.

and the target organs. Intense or long-term EAS activation in the setting of chronically decreased homeostatic efficiencies (dyshomeostasis), associated with aging and chronic disorders, can prolong or intensify allostatic load, and eventually lower the thresholds for a variety of vicious cycles (positive feedback loops) that can be lethal. This phenomenon could explain the close correlation of COVID-19 mortality with age and multiple organ involvement in the disease (14).

Orthostatic intolerance, sudomotor, gastrointestinal and pupillomotor disorders are described as common complications of COVID-19, together with low tolerance for environmental conditions, and sexual dysfunction (36). **Figure 3** shows the most prevalent symptoms of dysautonomia in severe COVID-19 (27).

Autonomic dysfunction associated with SARS-CoV-2 infection occurs at different temporal stages. Acute autonomic dysfunction is due to axonal damage or cardiopulmonary involvement. Iatrogenic autonomic dysfunction is a side effect caused by the drugs used and/or admission to the intensive care unit (37). Finally, late dysautonomia occurs in the *Post-COVID-19 condition*. Cardiovascular involvement, especially POTS, and to a lesser extent neurogenic orthostatic hypotension (NOH), has been more frequently observed in these latter patients. The aspects that we consider to be the most important in each phase are, in chronological order:

Acute-Subacute Autonomic Dysfunction

Among other things, activation of the EAS in the context of acute COVID-19 increases myocardial oxygen consumption and glucose levels, depletes energy, lowers thresholds for arrhythmias, induces hypokalemia and hyponatremia, may promote renal ischemic injury and intravascular thrombosis, and can induce a form of stress cardiomyopathy. Imbalances in the inflammasome system can contribute to cytokine storms (14, 34). All these changes, facilitated by dysautonomia, generate a series of manifestations at different levels. We summarize below the neurologic, cardiovascular and respiratory manifestations.

Neurologic Manifestations

Like Post-Chikungunya syndrome in 2006, as well as other viral infections and vaccines, SARS-CoV-2 could trigger an immune response leading to Guillain Barré Syndrome (GBS) or other neurologic manifestations of an autoimmune nature. By the end of 2020, at least 220 patients with GBS or its variants following COVID-19 infection have been reported. GBS subtype was specified in 152 as acute inflammatory demyelinating neuropathy (AIDP), 118 cases; acute motor axonal neuropathy (AMAN) in 13; acute motor and sensory axonal neuropathy (AMSAN) in 11; Miller-Fisher Syndrome in 7; polyneuritis cranialis (PNC) in 2; and the pharyngeal-cervical-brachial variant in 1. No cases of Bickerstaff encephalitis were found (38).

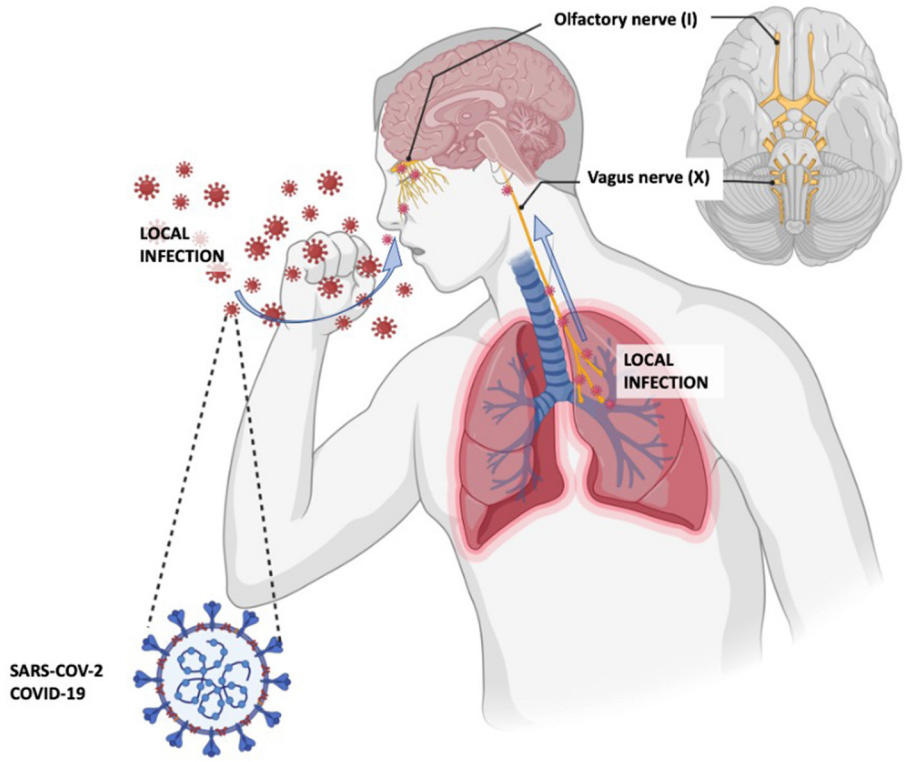


FIGURE 2 | Proposed COVID-19 pathways to the central nervous system. Adapted from the article by Yachou Y et al. (26).

It has been shown that antibodies to SARS-CoV-2 can cross-react with peripheral myelin causing GBS (39). GBS usually manifests with a florid picture of clinical dysautonomia that includes the presence of hemodynamic instability, urinary retention, gastroplegia, paralytic ileus or refractory hypertension (40).

In most patients who develop GBS, the time gap between COVID-19 infection and GBS is very short, which not only complicates treatment, but could also result in a poor prognostic clinical sign for development of severe autonomic dysfunction (41) without early detection and appropriate therapeutic management.

It is speculated that the pathogenesis of Miller Fisher syndrome following SARS-CoV-2 infection is mediated by neurotropism or aberrant activation of the immune system, with production of circulating antibodies similar to GDq1B in idiopathic Miller Fisher syndrome (42).

An increased incidence of acute motor and sensory and axonal neuropathy (AMSAN) and acute inflammatory demyelinating polyneuropathy (AIDP) is also associated with COVID-19 infection, which may present with autonomic dysfunction, especially in cases with greater axonal involvement (25, 43, 44).

Pupillary light reflex (PLR) is under the control of the autonomic nervous system. Pupil dilation is innervated by the sympathetic nervous system and pupil constriction by the parasympathetic nervous system (45). Using PRL to assess autonomic dysfunction in patients with acute COVID-19 infection varies according to the severity of clinical presentation. In an observational, cross-sectional study, higher values of pupillary dilation velocity and baseline pupil diameter were reported in 20 Non-critically-ill COVID-19 patients in the acute phase of the disease (31). Regarding critically-ill COVID-19 patients, a study in 18 patients with respiratory failure requiring mechanical ventilation for >48 h did not find, after statistical correction for possible confounders (i.e., sedation), significant differences in PLR dynamics between SARS-CoV-2-infected patients and those suffering from respiratory failure due to other causes (46).

Cardiovascular Manifestations

Characteristics of cardiovascular involvement in patients with COVID-19 may include myocardial lesions (myocarditis), vasculitis-like syndromes, atherothrombotic manifestations and autonomic dysfunction (47).

Maladaptive functions of the renin–angiotensin–aldosterone system (RAAS) constitute another plausible pathophysiological mechanism of SARS-CoV-2 infection–related tissue damage, probably related to central autonomic network dysfunction mentioned above. The RAAS is composed of a cascade of regulatory peptides that participate in key physiological processes in the body, including fluid and electrolyte balance, blood pressure regulation, vascular permeability, and tissue growth. Angiotensin-converting enzyme 2 (ACE2) has emerged as a potent counter-regulator of the RAAS pathway. ACE2

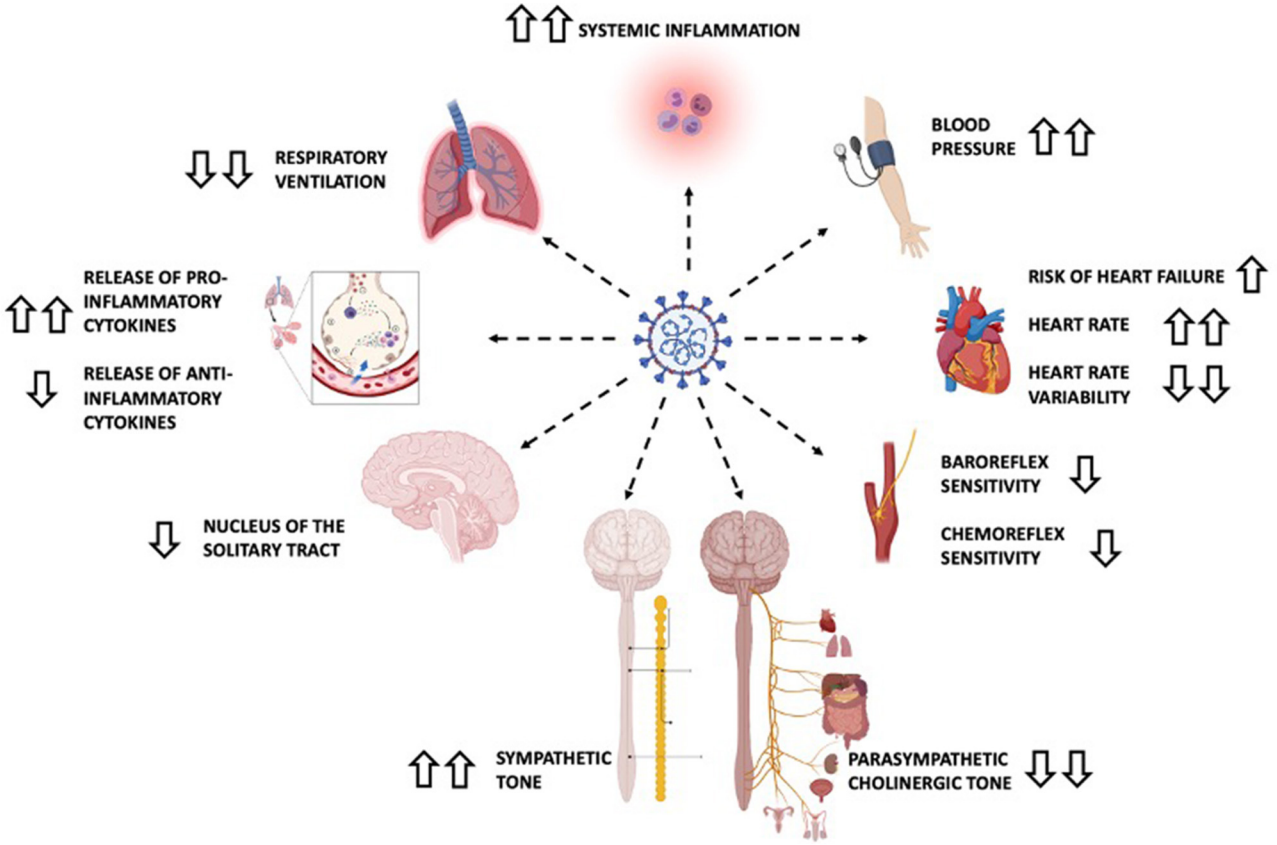


FIGURE 3 | Main dysautonomic changes in severe COVID-19 infection. Information extracted from the text and based on the article by Rangon et al. (27). Upward and downward pointing arrows indicate increase and decrease, respectively. Double arrows indicate important variations. Recording hemodynamic changes and detailed neurologic examinations are both standard clinical practice but are of the utmost importance in patients with COVID-19 and manifestations suggestive of autonomic dysfunction.

cleaves angiotensin I into inactive angiotensin 1–9 and degrades angiotensin II to angiotensin 1–7, which has vasodilatory, antiproliferative, antifibrotic, anti-inflammatory and sympathoinhibitory effects through binding to the Mas receptor (14, 48–50). Angiotensin II promotes vasoconstriction, fibrosis, hypertrophy and inflammation by binding to angiotensin II receptor type 1 (AT1-R) and mediating sympathoexcitation. Internalization of SARS-CoV-2 leads to inhibition of ACE2 activity and progressive depletion of membrane-bound ACE2, with ACE1/ACE2 imbalance and increased angiotensin II (50).

Fluctuating blood pressure could be explained by acute dysautonomia secondary to afferent baroreflex failure, a syndrome characterized by very labile blood pressures in which severe hypertensive crises alternate with hypotensive episodes. This phenomenon has previously been observed as a consequence of radiation therapy of the cervical region or more rarely after surgery for *Glomus caroticum* or brainstem tumors (51, 52). SARS-CoV-2 is known to have a tropism for the medullary structures of the CNS, including the ventrolateral part and the nucleus tractus solitarius, where the ACE2 receptor is highly expressed (53).

The pathophysiology of COVID-19–related myocarditis is thought to be a combination of direct viral injury and cardiac damage due to the host immune response (54). In addition, toxic effects of endogenous or exogenous catecholamines may show a pattern of Takotsubo cardiomyopathy (14).

Cardiac arrhythmias, including new-onset atrial fibrillation, heart block, and ventricular arrhythmias, are prevalent, occurring in 17% of hospitalized patients and 44% of patients in the ICU setting (34). Atrial arrhythmias are more common among patients who required mechanical ventilation than among those who did not (17.7 vs. 1.9%) (34).

Respiratory Manifestations

Impaired exercise tolerance is multifactorial and related to cardiac sympathetic predominance, decreased response to both sympathetic and parasympathetic stimuli that alter cardiovascular and pulmonary function, muscle tone, and impaired exercise tolerance (55). Airway sensory receptors channel information to the central nervous system, which regulates breathing and other parameters of lung function. This degree of crosstalk is achieved through three distinct airway

receptors: C-fiber receptors, rapidly adapting receptors, and slowly adapting receptors. There are also deflation-activated receptors (mechano-receptors) (55).

The parasympathetic nervous system regulates pulmonary mucous production, airway smooth muscle tone, ciliary motility and transport, mucous secretion, cough reflexes and also local pulmonary inflammation and immunity (55).

SARS-CoV-2 infection-induced stress can activate the sympathetic nervous system (SNS) leading to neurohormonal stimulation and activation of pro-inflammatory cytokines with further development of sympathetic storm. Sympathetic overactivation in COVID-19 is correlated with increase in capillary pulmonary leakage, alveolar damage, and development of acute respiratory distress syndrome (56). However, it is very likely that respiratory distress is not only the result of inflammation and structural lung damage, but also of damage caused by the virus to the respiratory centers of the brain, making the management of these patients difficult (26).

The exact pathophysiological mechanism behind the **"happy" hypoxemia** phenomenon is still unknown. Patients with severe glossopharyngeal or vagus nerve lesions due to neck tumors or congenital neuropathies have reported a disconnect between the perceived degree of hypoxia and dyspnea after the development of pneumonia. Possible damage to hypoxia-sensitive afferent neurons in persons with COVID-19 could be due to cytokine storm or the direct effect of SARS-CoV-2 on mitochondria or nerve fibers. The brain magnetic resonance imaging (MRI) findings and pathoanatomic studies in fatal cases of COVID-19 are so far inconsistent in this regard and do not provide a pathophysiological correlate to satisfactorily explain the absence of dyspnea in these patients (57).

Other Manifestations

The incidence of gastrointestinal manifestations has ranged from 12 to 61% in patients with COVID-19 (34). In a recent meta-analysis of 29 studies, the pooled prevalence of individual symptoms was reported to include that of anorexia (21%), nausea and/or vomiting (7%), diarrhea (9%), and abdominal pain (3%) (58).

In hospitalized COVID-19 patients, hypokalemia is frequent and is also associated with increased mortality. Low serum potassium may reflect increased aldosterone-mediated sodium/potassium exchange in the kidneys, as well as endogenous and exogenously administered epinephrine (14). ACE2 expression has been reported in the endocrine pancreas, albeit inconsistently. Direct binding of SARS-CoV-2 to ACE2 on β-cells could contribute to insulin deficiency and hyperglycemia. The increase in counterregulatory hormones that contributes to hepatic glucose production, decreased insulin secretion, ketogenesis and insulin resistance (34) promotes hyperglycemia in patients with COVID-19 at the time of hospitalization and has been related to adverse prognosis (14).

Drug-Induced Autonomic Impairment and/or ICU Admission

Cardiac arrhythmias, including atrial fibrillation and life-threatening atrioventricular block, can be induced by drugs used in the treatment of COVID-19. Among the drugs used, especially at the beginning of the pandemic, are chloroquine/hydroxychloroquine, macrolides (azithromycin) and quinolones that can cause *Torsades de Pointes*-type arrhythmias or other lethal arrhythmias as a potential consequence of QT prolongation. Other drugs with arrhythmogenic potential include other antiviral agents such as lopinavir/ritonavir, favipiravir, immunomodulatory treatments as tocilizumab, fingolimod, the anesthetic propofol, the antiemetic domperidone, class IA and III antiarrhythmics and the antipsychotic haloperidol, used in the initial phases of the pandemic mainly to combat the so-called cytokine storm. Drug combinations, especially QT-prolonging agents, used in the early stages may have induced increased arrhythmogenicity and secondary lethality (59). We suspect that all these events are facilitated by disorders in the autonomic system.

Many critically ill COVID-19 patients often have previous comorbidities, which together with acute comorbidities such as electrolyte disturbances (hypokalemia, hypomagnesemia), fever, systemic inflammation and excess autonomic lability contributes to increased cardiovascular morbidity and mortality (47, 59).

Dysautonomia in Post-COVID-19 Condition

Dysautonomic symptoms observed after SARS-CoV-2 infection are similar to those described after other viral infections such as mumps, human immunodeficiency virus, hepatitis C, Epstein-Barr, or Coxsackie type B virus (60, 61). In the severe acute respiratory syndrome (SARS) epidemic of 2002–2004 [8,422 cases and 916 deaths (11% mortality)], one study reported that 40% of patients (67% female) still had chronic fatigue nearly 2 years after infection (62). Studies utilizing autonomic reflex testing in post-SARS syndrome are scarce. One study of 14 patients (85% female) demonstrated an abnormal 30:15 ratio on active stand testing in 4/14 (29%) patients at 6 months Post-infection, with three reporting orthostatic intolerance (63). The 2012 coronavirus epidemic caused by the Middle East respiratory syndrome virus (MERS) was more limited and resulted in 2,468 cases and 851 deaths (34% mortality), but to our knowledge there are no reports of autonomic impairment following MERS (1).

Approximately 2.5% of patients with infection suffered Post-COVID-19 autonomic dysfunction (21, 22). In an observational cohort study involving 205 patients with confirmed or probable COVID-19 infection who met specific eligibility criteria (hospitalization, life-limiting symptoms beyond 12 weeks, desaturation <=95% on a Harvard step test, or chest pain with electrocardiographic changes during acute illness) a high prevalence (25%) of Post-COVID dysautonomia (64) was shown. Dysautonomia was defined as a resting heart rate (HR) >75 bpm, HR increase with exercise <89 bpm, and HR recovery <25 bpm 1 min after exercise (64) and was associated with objective functional limitations (reduced work rate and peak oxygen consumption and a steeper V_E/V_{CO2} slope), but was not associated with subjective symptoms or limitations (64).

Orthostatic Intolerance Syndromes

It has been proposed that some symptoms of the *post COVID-19 condition* may be related to a virus- or immune-mediated disruption to the autonomic nervous system, resulting in transient or long-term orthostatic intolerance syndromes. It is

well established that some cases of autonomic disorders such as NOH and POTS are associated with autoantibodies against α-/β-adrenoceptors and muscarinic receptors (65–70).

When a healthy person stands upright, blood pools in the pelvis and legs, reducing venous return to the heart. This is detected by cardiac and aortic baroreceptors, which respond by increasing sympathetic and adrenergic tone (mediated by noradrenaline and epinephrine/adrenaline, respectively). This results in tachycardia to compensate for the reduction in stroke volume and is followed by vasoconstriction in the splanchnic vascular bed, which increases the venous return to the heart (69).

Orthostatic intolerance is the inability to tolerate the upright posture because of symptoms of cerebral hypoperfusion or sympathetic activation, or both, which are relieved by recumbency (71). In orthostatic intolerance, the release of epinephrine and norepinephrine causes pronounced tachycardia, which is experienced as palpitations, breathlessness, and chest pain. Very high catecholamine levels can lead to paradoxical vasodilatation, sympathetic activity withdrawal and activation of the vagus nerve, resulting in hypotension, dizziness and ultimately, syncope (69).

Orthostatic intolerance syndromes include neurogenic orthostatic hypotension, neuromediated syncope and postural orthostatic tachycardia syndrome, and even orthostatic hypotension and neurally-mediated syncope should be considered. Since POTS appears to be the most common autonomic phenotype among PACS patients (1), the explanation of this feature has been expanded.

Neurogenic Orthostatic Hypotension (NOH)

Defined as a reduction of systolic blood pressure of at least 20 mmHg or a reduction in diastolic blood pressure of at least 10 mmHg within 3 min of active standing or head-up tilt on a tilt table (71–73). Approximately one third of persistent orthostatic hypotension is neurogenic (20). It is due to reduced norepinephrine release from postganglionic sympathetic nerves, resulting in defective vasoconstriction when assuming the upright position. It is most frequent in patients with diabetes mellitus, neurodegenerative disorders and small fiber neuropathies (73).

Neuromediated Syncope (Particularly Vasovagal Syncope)

This is the most common cause of syncope. The median number of episodes over a lifetime is 3, with a recurrence rate of 30% at 30 months (74).

Postural Orthostatic Tachycardia Syndrome (POTS)

POTS is a disorder in which patients frequently experience symptoms of orthostatic intolerance in response to postural stressors, despite autonomic reflexes that are generally preserved (71). The main POTS mechanisms are impaired sympathetically- mediated vasoconstriction in the lower limbs (neuropathic POTS), excessive cardiac sympathoexcitation response (hyperadrenergic POTS), volume dysregulation, joint hypermobility, and physical deconditioning. POTS is characterized by an increase of 30 bpm or more over baseline or a sustained heart rate of more than 120 bpm, according to current standing criteria, and symptoms associated with orthostatic intolerance without a drop in blood pressure (**Appendix 1**) (71). There may be an overlap between POTS and other disorders, in particular, orthostatic hypotension, vasovagal syncope, panic disorders, psychogenic pseudosyncope, chronic fatigue syndrome, Ehlers–Danlos syndrome, mast cell activation disorder and cardiac arrhythmias and should be considered in complex cases (71, 75).

Symptoms of cerebral hypoperfusion that may occur with any of the disorders of orthostatic intolerance include lightheadedness, dizziness, presyncope, vision and hearing changes, lower limb or generalized weakness, and cognitive difficulties (often vaguely termed brain fog). Symptoms of sympathoexcitation that distinguish POTS from orthostatic hypotension include palpitations, chest pain, dyspnea, tremulousness, sweating, pallor, nausea, diarrhea, and coldness of the extremities (**Appendix 2**) (76).

The affected population is usually young and predominantly female (77). Prevalence estimates are imprecise and there are no European data available to our knowledge. In the USA, estimates range from 0.2 to 1.0% in the general population. The onset of POTS may be precipitated by typical immunological stressors such as viral infection (20–50% of patients), frequently of the upper respiratory or gastrointestinal tract, vaccination (70), trauma, pregnancy, surgery, cardiovascular deconditioning (78) or even after a period of intense psychosocial stress. However, in a considerable number of patients with POTS, there is no clear identifiable trigger (79). Cardiac symptoms include chest pain, palpitations, exercise intolerance and orthostatic intolerance. More than 90% of patients with POTS have at least one gastrointestinal (GI) symptom, with nausea, abdominal pain, and bloating being the most common (80). Other symptoms that frequently accompany POTS include fatigue, "mental confusion", headache, temporomandibular joint disorder, fibromyalgia and sleep disturbances (81) and others listed by organ system in **Appendix 3**. It is recommended that all patients presenting with signs or symptoms of POTS should be evaluated to rule out the diagnosis of POTS (7, 82).

These syndromes may be exacerbated by hypovolemia resulting either from the initial infection or physical deconditioning following prolonged bed rest in the intensive care unit; prolonged bed rest leads to reduced cardiac output and stroke volume, hypovolemia, baroreflex dysfunction and decreased sympathetic responsiveness (69).

Other Symptoms

Other more Non-specific symptoms such as palpitations, tachycardia during mild exertion, "resting heart rate increase" (11%), chest pains/discomfort (16%), labile blood pressure, new-onset hypertension (1%), gastrointestinal symptoms (e.g., abdominal pain, bloating, nausea/vomiting (16%), gastroparesis, constipation or loose stools), sleep disorders (11%), flushing (5%), peripheral vasoconstriction, sweating abnormalities (17%), temperature intolerance and even unexplained low-grade fever are also thought to be due to autonomic dysfunction (1, 83) and their presence in any person after SARS CoV2 infection should prompt a thorough examination for possible autonomic dysfunction (69).

Sinus tachycardia, episodic sinus bradycardia and sinus pauses have been described as manifestations of autonomic dysfunction in patients with COVID-19 infection (84). Pupillary responses were impaired in patients recovering from COVID-19 vs. healthy controls, showing a larger resting-state pupil diameter and higher pupil contraction velocity, and lower values of dilatation latency and duration of pupil constriction (45).

DETECTION AND DIAGNOSIS OF DYSAUTONOMIA IN COVID-19

A very detailed anamnesis with a thorough medical history is essential to obtain all the necessary information from the patient. Therefore, questions should be asked about different aspects. Frequent comorbidities include migraine and other headaches, inappropriate sinus tachycardia, visceral hypersensitivity, gastrointestinal dysmotility, chronic fatigue, insomnia, fibromyalgia, and often autoimmune diseases (71, 85). The histories of SARS-CoV-2 survivors with persistent autonomic dysfunction may reveal frequent episodes of fainting, dizziness, lightheadedness, and/or palpitations, revealing underlying hypotensive susceptibility or prior orthostatic intolerance syndrome (85, 86). Unexplained dyspnea, fatigue, chest pain, persistent dizziness, diarrhea, recurrent presyncope episodes, anxiety, panic attacks with low-threshold emotional triggers or symptoms of irritable bowel syndrome, among others, have been observed (69, 71). These patterns may be explained by autonomic instability and may be a consequence of deconditioning, hypovolemia and immune-mediated or viral neuropathy (69). Although the exact etiology is unknown, it is thought that patients with dysautonomia have a less favorable body composition compared to those without dysautonomia (higher body mass index and waist circumference) (64).

The quantity and diversity of the symptoms mentioned are the reason why, among the proposed Neuro-PASC diagnostic criteria, orthostatic intolerance and cardiovascular, respiratory, gastrointestinal, and genitourinary manifestations are regarded as significant and related to autonomic dysfunction (2).

In Post-COVID patients with suspected dysautonomia, the Composite Autonomic Symptom Scale 31 (COMPASS-31) questionnaire is a sensitive tool to test the likelihood of autonomic dysfunction. This questionnaire has been previously applied to COVID-19 survivors, who showed significantly higher scores than controls, with an optimal cut-point for ruling out cardiovascular autonomic dysfunction of 13.25 (85).

Parameters related to **heart rate variability and blood pressure** in sitting and standing positions seem to be another key element in the detection of dysautonomia in patients with COVID-19 (20). Sinus tachycardia, episodic sinus bradycardia, and sinus pauses have been described as autonomic dysfunction manifestations in patients with COVID-19 infection (84). **Table 1** offers a simple guideline for monitoring blood pressure and heart rate in this context.

Blood tests (including complete blood count, renal function, B-type natriuretic peptide, electrolytes, thyroid stimulating hormone, and morning cortisol), resting 12-lead ECG, and the 6-min walking test should be routinely evaluated (85).

Holter ECG monitoring, 24 h ambulatory blood pressure monitoring, cardiothoracic imaging (chest X-ray, chest Computed Tomography, echocardiography, and cardiac magnetic resonance) and exercise testing are also invaluable diagnostic tools for the study of Post-acute sequelae of SARS-CoV-2 and COVID-19 complications (61, 85). It has been suggested that remote electrophysiological monitoring or long-term telemonitoring could be a very useful tracking tool after hospital discharge, especially in patients who have been critically ill (47, 59).

Active standing and/or head-up tilt tests are very useful for evaluating PASC patients, especially in individuals with inappropriate/orthostatic tachycardia, unexplained syncope, or syndromes of orthostatic intolerance. Other autonomic function tests include the Valsalva maneuver, deep breathing, and sweat function testing (85). Sudomotor function is an indirect index of sympathetic cholinergic Non-myelinated C-fiber activity, since sweat glands lack parasympathetic innervation (31). It can be assessed using *Sudoscan*, which allows estimation of electrochemical skin conductance (ESC) (31, 87). Abnormal ESC results suggest autonomic small fiber neuropathy and require confirmation with other validated techniques of sudomotor function, such as QSART testing and skin biopsy (1).

TABLE 1 | Interpretation of blood pressure and heart rate measurements in the event of clinical suspicion of orthostatic hypotension (20) and after differential diagnosis with vertigo, postural instability, ataxia, weakness of leg muscles, and osteoarthritis with weight-bearing musculoskeletal pain.

After 5′ supine position	*1st measurement*	If BP: >140/90 mmHg → **Probable NOH**
1′ standing	*2nd measurement*	If BP: ↓*c*+20/10 mmHg → **hTO** [If BP: ↓ +30/15 mmHg → assess whether MSA phenotype exists]
2′-5′ standing	*3rd–4th measurements*	In case of high clinical suspicion without objective proof of hTO in the measurements, carry out several repetitions in this range until: - BP: ↓ +20/10 mmHg → **hTO** [if also not so pronounced HR ↑ → **NOH**]
10′ standing (or head up tilt)	*5th measurement*	Sustained HR ↑+30 lpm* without hTO → **POTS**

hTO, Orthostatic hypotension; NOH, neurogenic orthostatic hypotension; BP, blood pressure (mmHg); MSA, multiple system atrophy; HR, heart rate; POTS, Postural orthostatic tachycardia syndrome; ↓, decrease; ↑, increase.

**For individuals between 12 and 19 years old, ↑+40 lpm is required.*

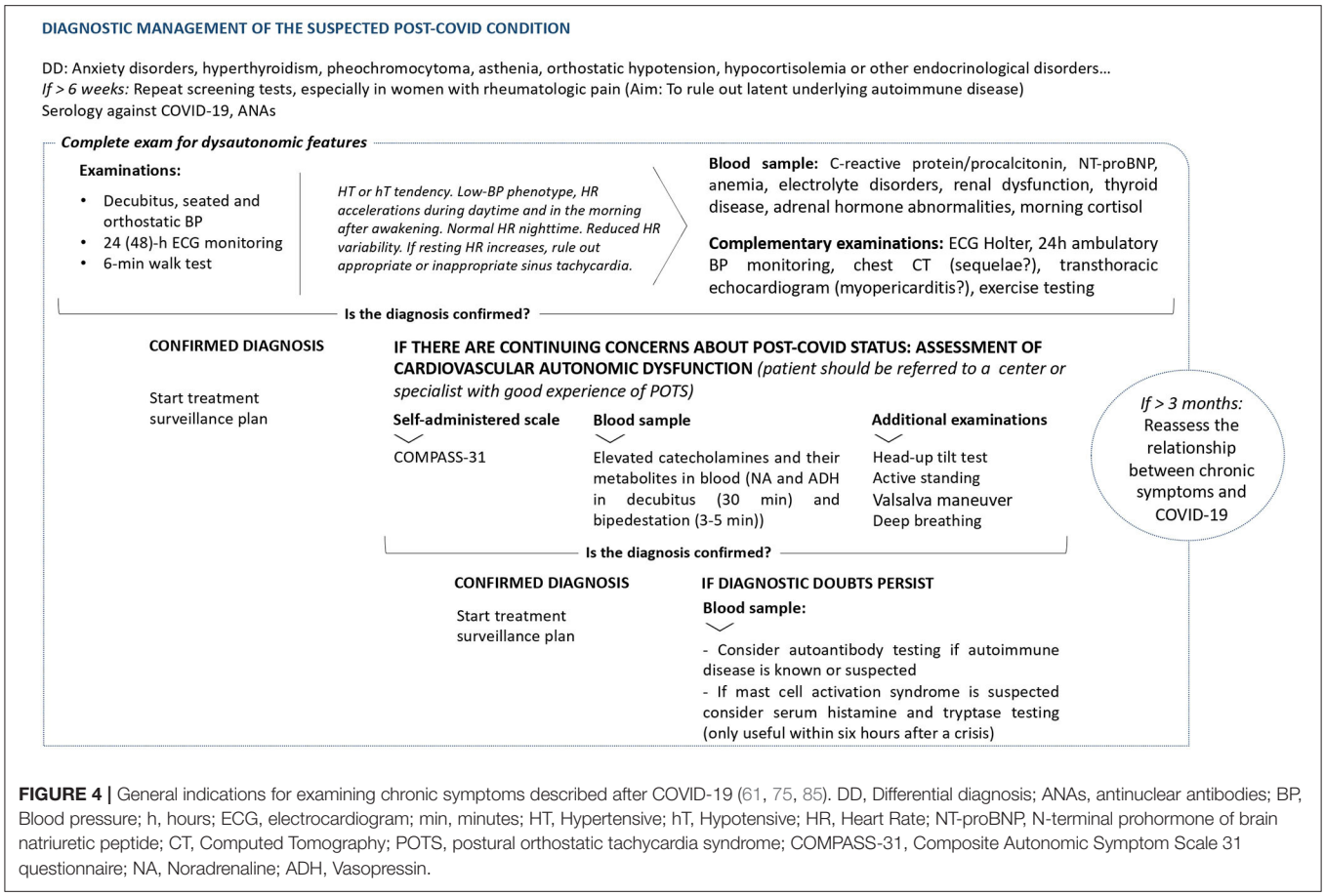


FIGURE 4 | General indications for examining chronic symptoms described after COVID-19 (61, 75, 85). DD, Differential diagnosis; ANAs, antinuclear antibodies; BP, Blood pressure; h, hours; ECG, electrocardiogram; min, minutes; HT, Hypertensive; hT, Hypotensive; HR, Heart Rate; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; CT, Computed Tomography; POTS, postural orthostatic tachycardia syndrome; COMPASS-31, Composite Autonomic Symptom Scale 31 questionnaire; NA, Noradrenaline; ADH, Vasopressin.

A feature of PASC patients with POTS-like symptoms is a high prevalence of specific circulating autoantibodies, including G-protein-coupled receptor (GPCR) antibodies (such as adrenergic, muscarinic and angiotensin II type-1 receptors) and the ganglionic neuronal nicotinic acetylcholine receptor (g-AChR) (75). Other recognized autoantibodies in POTS include circulating anti-nuclear, anti-thyroid, anti-NMDA-type glutamate receptor, anti-cardiac protein, anti-phospholipid, and Sjögren's antibodies (61, 71, 75, 85). Although neither sensitive nor specific, autoantibody testing can be helpful in selected cases. Specific tests for mast cell activation syndrome may also be considered in PASC patients with flushing episodes, frequent headaches, and persistent gastrointestinal symptoms (85).

Schematic summary of the diagnostic management of a suspected Post-COVID condition is shown in **Figure 4**.

POSSIBLE TREATMENTS FOR DYSAUTONOMIA IN COVID-19

Cardiovascular Dysautonomic Involvement

When there is cardiovascular dysautonomia, the following order is recommended (55, 85, 88).

1/Physical reconditioning:

- Progressive aerobic exercise training programs.
- Use of compression accessories.
- Water and salt intake.
- Drinking water before getting up in the morning.
- Sleeping with the head raised in bed.
- Avoid situations that may exacerbate symptoms (sleep deprivation, exposure to heat, alcohol intake or large meals).

RECOMMENDATION: At the onset of prodromal symptoms and to delay/prevent vasovagal syncope, perform physical maneuvers such as crossing legs, tensing muscles and squatting.

2/ In case of insufficient or complementary Non-pharmacological measures in patients with severe refractory symptoms (**Table 2**).

Because of the tendency for blood pressure to fluctuate, as explained above, it is important to maintain a euvolemic state and avoid excessive fluid administration during episodes of hypotension, with gradual titration of vasopressors to avoid excessive blood pressure, and to use short-acting antihypertensive drugs in hypertensive crises (89).

Postural Orthostatic Tachycardia Syndrome

There are some more specific management protocols available to help treat POTS. The aspects mentioned below represent a summary of the nonpharmacologic and pharmacologic therapeutic options available for the management of POTS. For

TABLE 2 | Therapeutic options in case of insufficient non-pharmacological measures or as a supplement in patients with serious refractory symptoms.

Volume expanders	Fludrocortisone, desmopressin, and intravenous saline
Heart rate inhibitors	Propranolol, ivabradine, and pyridostigmine
Vasoconstrictors	Midodrine, octreotide, methylphenidate, and droxidopa
Sympatholytic drugs	Clonidine and methyldopa

more detail, it is advisable to consult documents that address these issues in greater depth (90, 91).

- *Non-pharmacologic measures*: There is no Class I recommendation. The Class IIA recommendation is based on physical training to avoid chronicity of symptoms. Indications based on the management of orthostatic intolerance syndromes, such as educating the patient about the pathology, simple isometric, aerobic and resistance exercises, ensuring fluid replacement (2–3 liters of water per day, avoiding caffeine and alcohol) and one or two additional teaspoons of salt per day are maintained (Class IIB), as well as moving carefully from a lying or sitting to a standing position and avoiding exacerbating factors such as prolonged standing, hot environments and dehydration, using waist-high compression garments and assessing the need for fluid expanders if hypovolemia is considered to be a dominant symptom, among others (69, 90). Long-term repeated saline infusions are not recommended (78).
- *Pharmacologic measures:* Midodrine, beta-blockers, fludrocortisone, pyridostigmine, clonidine and alpha-methyldopa (Class IIB) (69). The only drugs that have demonstrated benefits in randomized trials are propranolol and pyridostigmine (71). Since there is no good evidence in this regard, polypharmacy is frequent; the overall effects of drug therapy however are modest. Likewise, it is recommended to discontinue the intake of noradrenaline reuptake inhibitors such as duloxetine, nortriptyline, and tapentadol. It should also be considered whether the indication of fludrocortisone, midodrine, clonidine methyldopa or propranolol is necessary, bearing in mind that these drugs are not usually very well tolerated (69, 91).

A schematic proposal for the management of orthostatic intolerance is set out in **Table 3**.

TABLE 3 | Therapeutic proposal for orthostatic intolerance and intended effects (71, 73).

Treatment	Mechanism
Non-pharmacologic	
Increase water and sodium intake	Avoids hypovolemia
Compression and physical countermaneuvers	Reduces venous pooling
Physical exercise training, including gradual resistance and lower extremity resistance training	Improves physical deconditioning and reduces venous pooling
Pharmacologic	
Propranolol: 10 mg 1–3 times/day	Reduces standing heart rate and improves orthostatic symptoms, especially in hyperadrenergic patients with POTS
Midodrine: 2.5–15 mg 2–3 times/day (3–4 h before going to bed)	Reduces venous pooling and orthostatic hypotension, especially in neuropathic patients with POTS. Patients should be advised not to lie flat for at least 4 h after any dose of midodrine to avoid supine hypertension
Pyridostigmine: 30–60 mg 2–3 times/day	Reduces orthostatic tachycardia and improves chronic symptoms without worsening supine hypertension. Use should be limited in case of diarrhea, abdominal cramps, pain, nausea, urinary frequency and urgency
Fludrocortisone: 0.05–0.2 mg once/day	The effect only lasts 1–2 days, avoid prolonged use due to renal and cardiac involvement
Ivabradine: 5–10 mg	Reduces heart rate without affecting blood pressure
IV fluid therapy (saline)	Improves symptoms quickly although the effect lasts a short time. It is considered a bridging therapy
Others: - Droxidopa 100–600 mg 3 times/day (3–4 h before bedtime) - Atomoxetine 10–18 mg 2 times/day	

Other Therapeutic Options

Other therapeutic options include Non-invasive neuromodulation (especially transcranial direct current stimulation, repetitive transcranial magnetic stimulation and vagus nerve stimulation) which could be used in patients with COVID-19 and autonomic dysfunction (92). It seems that it may on the one hand reduce the impact of the infection by stimulating regions involved in the regulation of systemic anti-inflammatory and/or autonomic responses, prevent neuroinflammation and aid recovery of breathing, and, on the other, improve the symptoms of musculoskeletal pain, systemic fatigue, physical and cognitive rehabilitation after the disease, even if it has been critical, as well as treat the distress generated by the disease (92).

KEY POINTS

- Dysautonomia, present in at least 2.5% of COVID-19 patients, is clinically similar to dysautonomia secondary to other viral infections. The prevalence of Post-COVID dysautonomia could rise to 25% in those patients who met specific eligibility criteria (hospitalization, life-limiting symptoms beyond 12 weeks and so on).
- Potentials mechanism of autonomic impairment caused by SARS CoV2 are based on direct tissue damage, immune dysregulation, hormonal disturbances, elevated cytokine levels due to immune reaction leading to chronic inflammation, and persistent low-grade infection. The EAS with allostasis

and dyshomeostasis may partially explain the mortality and multi-organ involvement in COVID-19 patients.
- The major socioeconomic impact of symptom persistence after COVID-19 infection stems from fatigue, autonomic and neurohemodynamic involvement, hence the need for early intervention in these areas.
- Dysautonomic involvement secondary to COVID-19 may be acute-subacute, caused by drugs and/or ICU admission, and chronic, as in the Post-COVID-19 condition, due to orthostatic intolerance syndromes of autoimmune origin.
- A careful neurologic assessment is necessary in any patient with findings compatible with autonomic dysfunction following SARS CoV-2 infection.
- Protocols for the diagnostic and therapeutic management of autonomic dysfunction are mainly aimed at avoiding triggers of orthostatic intolerance by means of pharmacologic and Non-pharmacologic measures.

LIMITATIONS

Some limitations need to be acknowledged in the interpretation of our results. First, this is not a systematic review. We limited our scope to selected articles which we believed could be the most representative ones. Secondly, we relied on PubMed only for our search strategy. Among the strengths of our study, we analyzed an important quantity of articles highlighting the most relevant research on COVID-19 and dysautonomia.

CONCLUSION

Two years after the declaration of the COVID-19 pandemic, patients affected by this disease continue to manifest patterns of neurological involvement attributable to autonomic dysfunction. This could be the result of a multifactorial etiology deriving from physical deconditioning after time spent isolated in home, hospital wards or intensive care units, hypovolemia, virus-mediated neuropathy, or an immune response secondary to infection. One of the consequences is that a high percentage of patients with COVID-19 do not make a full return to work due to residual symptoms. The socioeconomic impact is considerable and could be significantly reduced with an appropriate diagnostic and therapeutic protocol for the underlying autonomic dysfunction. Considering the wide dissemination of COVID-19 worldwide and the extraordinary dissemination of the SARS-CoV2 omicron variant and its emerging subvariants, it would appear to be imperative to adopt measures that, in addition to containing the spread of the virus, also help improve the acute management of infected patients and prevent and/or reduce the long-term sequelae of the infection.

Among these measures, one would be improving access to autonomic testing for early diagnosis of autonomic dysfunction. This would allow early treatment, reducing the associated morbidity and mortality and thus containing its personal and socioeconomic impact.

At the same time, the sheer scale of the infection and of the Post-COVID-19 syndrome presents a unique opportunity to add to our knowledge and understanding of the specific mechanisms responsible for orthostatic intolerance, POTS-like symptoms, and their duration. It is particularly noteworthy that the comprehension of the mechanisms of self-immunity could generate new pathophysiological hypotheses applicable to other disorders with which could share clinical similarities such as chronic fatigue syndrome or fibromyalgia. This improvement in awareness may turn out to help ameliorate diagnostic accuracy in these entities, currently very diffuse and poorly managed. Hence the value of studying in depth the clinical pictures found in patients with the Post-COVID-19 syndrome, especially Post-COVID-19-POTS condition, for which a special effort is required in terms of clinical care and resources devoted to their research.

A better recognition of dysautonomia will help to improve the management of COVID-19 in all its phases, providing information for possible diagnostic and therapeutic tools applicable not only to these patients, but also to those affected by other pathologies with physiopathological similarities (other viral conditions, Alzheimer's disease, Parkinson's disease, and so on). We therefore recommend further studies to explore the prevalence, pathophysiology, clinical features, and treatment approach in patients with COVID-19-related dysautonomia.

AUTHOR CONTRIBUTIONS

FC-T and AM-O: literature review, data processing and analyses, interpretation of results, language editing, review and drafting of the first manuscript, and interpretation of results. AL-B, BT, VG, and MW: literature review, data processing, and interpretation of results. HA-G, AL, AA, JQ, and JP: critical revision of the manuscript. JG-E: conception and design of the article, literature review, interpretation of results, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to dedicate this article to all our healthcare colleagues, with whom we continue to work tirelessly during this COVID-19 pandemic. We hope that this review will facilitate understanding and the management of patients with dysautonomia in the context of COVID-19. Also, we would like to express our gratitude to our family members for their support and understanding of our dedication to our daily work and patients.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.886609/full#supplementary-material

REFERENCES

1. Larsen NW, Stiles LE, Miglis MG. Preparing for the long-haul: autonomic complications of COVID-19. *Auton Neurosci.* (2021) 235:102841. doi: 10.1016/j.autneu.2021.102841
2. Moghimi N, di Napoli M, Biller J, Siegler JE, Shekhar R, McCullough LD, et al. The neurological manifestations of post-acute sequelae of SARS-CoV-2 infection. *Curr Neurol Neurosci Rep.* (2021) 21:44. doi: 10.1007/s11910-021-01130-1
3. Venkatesan P. NICE guideline on long COVID. *Lancet Respir Med.* (2021) 9:129–38. doi: 10.1016/S2213-2600(21)00031-X
4. Carfì A, Bernabei R, Landi F, Gemelli Against C-P-ACSG. Persistent symptoms in patients after acute COVID-19. *JAMA.* (2020) 324:603–5. doi: 10.1001/jama.2020.12603
5. Groff D, Sun A, Ssentongo AE, Ba DM, Parsons N, Poudel GR, et al. Short-term and long-term rates of postacute sequelae of SARS-CoV-2 infection: a systematic review. *JAMA Netw Open.* (2021) 4:e2128568. doi: 10.1001/jamanetworkopen.2021.28568
6. Boldrini M, Canoll PD, Klein RS. How COVID-19 affects the brain. *JAMA Psychiatry.* (2021) 78:682–3. doi: 10.1001/jamapsychiatry.2021.0500
7. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ, Re'em Y, et al.. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EClinicalMedicine.* (2021) 38:101019. doi: 10.1016/j.eclinm.2021.101019
8. Dennis A, Wamil M, Alberts J, Oben J, Cuthbertson DJ, Wootton D, et al. Multiorgan impairment in low-risk individuals with Post-COVID-19 syndrome: a prospective, community-based study. *BMJ Open.* (2021) 11:e048391. doi: 10.1136/bmjopen-2020-048391
9. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in vaccinated health care workers. *N Engl J Med.* (2021) 385:1474–84. doi: 10.1056/NEJMoa2109072
10. Halpin SJ, McIvor C, Whyatt G, Adams A, Harvey O, McLean L, et al. Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: a cross-sectional evaluation. *J Med Virol.* (2021) 93:1013–22. doi: 10.1002/jmv.26368
11. LaVergne SM, Stromberg S, Baxter BA, Webb TL, Dutt TS, Berry K, et al. A longitudinal SARS-CoV-2 biorepository for COVID-19 survivors with and without post-acute sequelae. *BMC Infect Dis.* (2021) 21:677. doi: 10.1186/s12879-021-06359-2
12. Moreno-Perez O, Merino E, Leon-Ramirez JM, Andres M, Ramos JM, Arenas-Jimenez J, et al. Post-acute COVID-19 syndrome. Incidence and risk factors: a mediterranean cohort study. *J Infect.* (2021) 82:378–83. doi: 10.1016/j.jinf.2021.01.004
13. Lo YL. COVID-19, fatigue, and dysautonomia. *J Med Virol.* (2021) 93:1213. doi: 10.1002/jmv.26552
14. Goldstein DS. The extended autonomic system, dyshomeostasis, and COVID-19. *Clin Auton Res.* (2020) 30:299–315. doi: 10.1007/s10286-020-00714-0
15. (NICE) NIfHaCE. *COVID-19 Rapid Guideline: Managing The Long-Term Effects of COVID-19.* (2021). Available online at: https://www.nice.org.uk/guidance/ng188 (accessed June, 2021).
16. Dixit NM, Churchill A, Nsair A, Hsu JJ. Post-acute COVID-19 syndrome and the cardiovascular system: what is known? *Am Heart J Plus.* (2021) 5:100025. doi: 10.1016/j.ahjo.2021.100025
17. Kalter L. Fauci introduces new acronym for long COVID at white house briefing. *Medscape.* (2021, February 24). Available online at: https://www.medscape.com/viewarticle/946419 (accessed April 8, 2022).
18. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of Post-COVID-19 condition by a Delphi consensus. *Lancet Infect Dis.* (2021). doi: 10.1016/S1473-3099(21)00703-9
19. Miglis MG, Goodman BP, Chemali KR, Stiles L. Re: 'Post-COVID-19 chronic symptoms' by Davido et al. *Clin Microbiol Infect.* (2021) 27:494. doi: 10.1016/j.cmi.2020.08.028
20. Cheshire WP, Jr. Autonomic history, examination, and laboratory evaluation. *Continuum.* (2020) 26:25–43. doi: 10.1212/CON.0000000000000815
21. Romero-Sanchez CM, Diaz-Maroto I, Fernandez-Diaz E, Sanchez-Larsen A, Layos-Romero A, Garcia-Garcia J, et al. Neurologic manifestations in hospitalized patients with COVID-19: the ALBACOVID registry. *Neurology.* (2020) 95:e1060–e70. doi: 10.1212/WNL.0000000000009937
22. Misra S, Kolappa K, Prasad M, Radhakrishnan D, Thakur KT, Solomon T, et al. Frequency of neurologic manifestations in COVID-19: a systematic review and meta-analysis. *Neurology.* (2021) 97:e2269–e81. doi: 10.1212/WNL.0000000000012930
23. Townsend L, Moloney D, Finucane C, McCarthy K, Bergin C, Bannan C, et al. Fatigue following COVID-19 infection is not associated with autonomic dysfunction. *PLoS One.* (2021) 16:e0247280. doi: 10.1371/journal.pone.0247280
24. Sandroni P, Opfer-Gehrking TL, McPhee BR, Low PA. Postural tachycardia syndrome: clinical features and follow-up study. *Mayo Clin Proc.* (1999) 74:1106–10. doi: 10.4065/74.11.1106
25. Raj SR, Arnold AC, Barboi A, Claydon VE, Limberg JK, Lucci VM, et al. Long-COVID postural tachycardia syndrome: an American autonomic society statement. *Clin Auton Res.* (2021) 31:365–8. doi: 10.1007/s10286-021-00798-2
26. Yachou Y, El Idrissi A, Belapasov V, Ait Benali S. Neuroinvasion, neurotropic, and neuroinflammatory events of SARS-CoV-2: understanding the neurological manifestations in COVID-19 patients. *Neurol Sci.* (2020) 41:2657–69. doi: 10.1007/s10072-020-04575-3
27. Rangon CM, Krantic S, Moyse E, Fougere B. The vagal autonomic pathway of COVID-19 at the crossroad of Alzheimer's disease and aging: a review of knowledge. *J Alzheimers Dis Rep.* (2020) 4:537–51. doi: 10.3233/ADR-200273
28. Cocoros NM, Svensson E, Szpligeti SK, Vestergaard SV, Szentkuti P, Thomsen RW, et al. Long-term risk of parkinson disease following influenza and other infections. *JAMA Neurol.* (2021) 78:1461–70. doi: 10.1001/jamaneurol.2021.3895
29. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci.* (2021) 24:168–75. doi: 10.1038/s41593-020-00758-5
30. Synowiec A, Szczepa?ski A, Barreto-Duran E, Lie LK, Pyrc K. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): a Systemic Infection. *Clin Microbiol Rev.* (2021) 34:e00133–20. doi: 10.1128/CMR.00133-20
31. Bellavia S, Scala I, Luigetti M, Brunetti V, Gabrielli M, Verme LZD, et al. Instrumental evaluation of COVID-19 related dysautonomia in non-critically-ill patients: An observational, cross-sectional study. *J Clin Med.* (2021) 10:586. doi: 10.3390/jcm10245861
32. Novak P, Mukerji SS, Alabsi HS, Systrom D, Marciano SP, Felsenstein D, et al. Multisystem Involvement in Post-Acute Sequelae of Coronavirus Disease 19. *Ann Neurol.* (2022) 9:367–79. doi: 10.1002/ana.26286
33. Tracey KJ. The inflammatory reflex. *Nature.* (2002) 420:853–9. doi: 10.1038/nature01321
34. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al. Extrapulmonary manifestations of COVID-19. *Nat Med.* (2020) 26:1017–32. doi: 10.1038/s41591-020-0968-3
35. Vallée A. Dysautonomia and implications for anosmia in long COVID-19 disease. *J Clin Med.* (2021) 10:5514. doi: 10.3390/jcm10235514
36. Buoite Stella A, Furlanis G, Frezza NA, Valentinotti R, Ajcevic M, Manganotti P. Autonomic dysfunction in Post-COVID patients with and witfhout neurological symptoms: a prospective multidomain observational study. *J Neurol.* (2022) 269:587–96. doi: 10.1007/s00415-021-10735-y
37. Stanbro M, Gray BH, Kellicut DC. Carotidynia: revisiting an unfamiliar entity. *Ann Vasc Surg.* (2011) 25:1144–53. doi: 10.1016/j.avsg.2011.06.006
38. Finsterer J, Scorza FA. Guillain-Barre syndrome in 220 patients with COVID-19. *Egypt J Neurol Psychiatr Neurosurg.* (2021) 57:55. doi: 10.1186/s41983-021-00310-7
39. Seixas R, Campoamor D, Lopes J, Bernardo T, Nzwalo H, Pascoalinho D. Occurrence of guillain-barre syndrome during the initial symptomatic phase of COVID-19 disease: coincidence or consequence? *Cureus.* (2021) 13:e19655. doi: 10.7759/cureus.19655
40. Uncini A, Vallat JM, Jacobs BC. Guillain-barre syndrome in SARS-CoV-2 infection: an instant systematic review of the first six months of pandemic. *J Neurol Neurosurg Psychiatry.* (2020) 91:1105–10. doi: 10.1136/jnnp-2020-324491
41. Kajumba MM, Kolls BJ, Koltai DC, Kaddumukasa M, Kaddumukasa M, Laskowitz DT. COVID-19-associated guillain-barre syndrome: Atypical

para-infectious profile, symptom overlap, and increased risk of severe neurological complications. *SN Compr Clin Med*. (2020) 2:2702–14. doi: 10.1007/s42399-020-00646-w
42. Biswas S, Ghosh R, Mandal A, Pandit A, Roy D, Sengupta S, et al. COVID-19 Induced miller fisher syndrome presenting with autonomic dysfunction: a unique case report and review of literature. *Neurohospitalist.* (2022) 12:111–6. doi: 10.1177/19418744211016709
43. Filosto M, Cotti Piccinelli S, Gazzina S, Foresti C, Frigeni B, Servalli MC, et al. Guillain-Barre syndrome and COVID-19: an observational multicentre study from two Italian hotspot regions. *J Neurol Neurosurg Psychiatry.* (2021) 92:751–6. doi: 10.1136/jnnp-2020-324837
44. Arcila-Londono X, Lewis RA. Guillain-Barre syndrome. *Semin Neurol.* (2012) 32:179–86. doi: 10.1055/s-0032-1329196
45. Karahan M, Demirtas AA, Hazar L, Erdem S, Ava S, Dursun ME, et al. Autonomic dysfunction detection by an automatic pupillometer as a non-invasive test in patients recovered from COVID-19. *Graefes Arch Clin Exp Ophthalmol.* (2021) 259:2821–6. doi: 10.1007/s00417-021-05209-w
46. Vrettou CS, Korompoki E, Sarri K, Papachatzakis I, Theodorakopoulou M, Chrysanthopoulou E, et al. Pupillometry in critically ill patients with COVID-19: a prospective study. *Clin Auton Res.* (2020) 30:563–5. doi: 10.1007/s10286-020-00737-7
47. Briguglio M, Porta M, Zuffada F, Bona AR, Crespi T, Pino F, et al. SARS-CoV-2 Aiming for the heart: a multicenter italian perspective about cardiovascular issues in COVID-19. *Front Physiol.* (2020) 11:571367. doi: 10.3389/fphys.2020.571367
48. Kunutsor SK, Whitehouse MR, Blom AW, Board T, Kay P, Wroblewski BM, et al. One- and two-stage surgical revision of peri-prosthetic joint infection of the hip: a pooled individual participant data analysis of 44 cohort studies. *Eur J Epidemiol.* (2018) 33:933–46. doi: 10.1007/s10654-018-0377-9
49. Xie Y, Xu E, Bowe B, Al-Aly Z. Long-term cardiovascular outcomes of COVID-19. *Nat Med.* (2022) 28:583–90. doi: 10.1038/s41591-022-01689-3
50. Porzionato A, Emmi A, Barbon S, Boscolo-Berto R, Stecco C, Stocco E, et al. Sympathetic activation: a potential link between comorbidities and COVID-19. *FEBS J.* (2020) 287:3681–8. doi: 10.1111/febs.15481
51. Biaggioni I, Shibao CA, Diedrich A, Muldowney JAS, 3rd, Laffer CL, Jordan J. Blood pressure management in afferent baroreflex failure: JACC review topic of the week. *J Am Coll Cardiol.* (2019) 74:2939–47. doi: 10.1016/j.jacc.2019.10.027
52. Kaufmann H, Norcliffe-Kaufmann L, Palma JA. Baroreflex dysfunction. *N Engl J Med.* (2020) 382:163–78. doi: 10.1056/NEJMra1509723
53. Montalvan V, Lee J, Bueso T, de Toledo J, Rivas K. Neurological manifestations of COVID-19 and other coronavirus infections: a systematic review. *Clin Neurol Neurosurg.* (2020) 194:105921. doi: 10.1016/j.clineuro.2020.105921
54. Siripanthong B, Nazarian S, Muser D, Deo R, Santangeli P, Khanji MY, et al. Recognizing COVID-19-related myocarditis: The possible pathophysiology and proposed guideline for diagnosis and management. *Heart Rhythm.* (2020) 17:1463–71. doi: 10.1016/j.hrthm.2020.05.001
55. Becker RC. Autonomic dysfunction in SARS-COV-2 infection acute and long-term implications COVID-19 editor's page series. *J Thromb Thrombolysis.* (2021) 52:692–707. doi: 10.1007/s11239-021-02549-6
56. Al-Kuraishy HM, Al-Gareeb AI, Qusti S, Alshammari EM, Gyebi GA, Batiha GE. Covid-19-induced dysautonomia: a menace of sympathetic storm. *ASN Neuro.* (2021) 13:17590914211057635. doi: 10.1177/17590914211057635
57. Gonzalez-Duarte A, Norcliffe-Kaufmann L. Is 'happy hypoxia' in COVID-19 a disorder of autonomic interoception? A hypothesis. *Clin Auton Res.* (2020) 30:331–3. doi: 10.1007/s10286-020-00715-z
58. Mao R, Qiu Y, He JS, Tan JY, Li XH, Liang J, et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* (2020) 5:667–78. doi: 10.1016/S2468-1253(20)30126-6
59. Manolis AS, Manolis AA, Manolis TA, Apostolopoulos EJ, Papatheou D, Melita H. COVID-19 infection and cardiac arrhythmias. *Trends Cardiovasc Med.* (2020) 30:451–60. doi: 10.1016/j.tcm.2020.08.002
60. Billman GE. The LF/HF ratio does not accurately measure cardiac sympatho-vagal balance. *Front Physiol.* (2013) 4:26. doi: 10.3389/fphys.2013.00026
61. Davido B, Seang S, Tubiana R, de Truchis P. Post-COVID-19 chronic symptoms: a postinfectious entity? *Clin Microbiol Infect.* (2020) 26:1448–9. doi: 10.1016/j.cmi.2020.07.028
62. Lam MH, Wing YK, Yu MW, Leung CM, Ma RC, Kong AP, et al. Mental morbidities and chronic fatigue in severe acute respiratory syndrome survivors: long-term follow-up. *Arch Intern Med.* (2009) 169:2142–7. doi: 10.1001/archinternmed.2009.384
63. Lau ST, Yu WC, Mok NS, Tsui PT, Tong WL, Cheng SW. Tachycardia amongst subjects recovering from severe acute respiratory syndrome (SARS). *Int J Cardiol.* (2005) 100:167–9. doi: 10.1016/j.ijcard.2004.06.022
64. Ladlow P, O'Sullivan O, Houston A, Barker-Davies R, May S, Mills D, et al. Dysautonomia following COVID-19 is not associated with subjective limitations or symptoms but is associated with objective functional limitations. *Heart Rhythm.* (2021). doi: 10.1016/j.hrthm.2021.12.005
65. Li H, Kem DC, Reim S, Khan M, Vanderlinde-Wood M, Zillner C, et al. Agonistic autoantibodies as vasodilators in orthostatic hypotension: a new mechanism. *Hypertension.* (2012) 59:402–8. doi: 10.1161/HYPERTENSIONAHA.111.184937
66. Fedorowski A, Li H, Yu X, Koelsch KA, Harris VM, Liles C, et al. Antiadrenergic autoimmunity in postural tachycardia syndrome. *Europace.* (2017) 19:1211–9. doi: 10.1093/europace/euw154
67. Li H, Yu X, Liles C, Khan M, Vanderlinde-Wood M, Galloway A, et al. Autoimmune basis for postural tachycardia syndrome. *J Am Heart Assoc.* (2014) 3:e000755. doi: 10.1161/JAHA.113.000755
68. Yu X, Stavrakis S, Hill MA, Huang S, Reim S, Li H, et al. Autoantibody activation of beta-adrenergic and muscarinic receptors contributes to an "autoimmune" orthostatic hypotension. *J Am Soc Hypertens.* (2012) 6:40–7. doi: 10.1016/j.jash.2011.10.003
69. Dani M, Dirksen A, Taraborrelli P, Torocastro M, Panagopoulos D, Sutton R, et al. Autonomic dysfunction in 'long COVID': rationale, physiology and management strategies. *Clin Med.* (2021) 21:e63–e7. doi: 10.7861/clinmed.2020-0896
70. Ruzieh M, Batizy L, Dasa O, Oostra C, Grubb B. The role of autoantibodies in the syndromes of orthostatic intolerance: a systematic review. *Scand Cardiovasc J.* (2017) 51:243–7. doi: 10.1080/14017431.2017.1355068
71. Cutsforth-Gregory JK. Postural tachycardia syndrome and neurally mediated syncope. *Continuum.* (2020) 26:93–115. doi: 10.1212/CON.0000000000000818
72. Francois C, Shibao CA, Biaggioni I, Duhig AM, McLeod K, Ogbonnaya A, et al. Six-month use of droxidopa for neurogenic orthostatic hypotension. *Mov Disord Clin Pract.* (2019) 6:235–42. doi: 10.1002/mdc3.12726
73. Palma JA, Kaufmann H. Management of orthostatic hypotension. *Continuum.* (2020) 26:154–77. doi: 10.1212/CON.0000000000000816
74. Baron-Esquivias G, Morillo CA. Definitive pacing therapy in patients with neuromediated syncope. Lessons from the SPAIN study. *Rev Esp Cardiol.* (2018) 71:320–2. doi: 10.1016/j.rec.2017.10.037
75. Fedorowski A. Postural orthostatic tachycardia syndrome: clinical presentation, aetiology and management. *J Intern Med.* (2019) 285:352–66. doi: 10.1111/joim.12852
76. Johansson M, Stahlberg M, Runold M, Nygren-Bonnier M, Nilsson J, Olshansky B, et al. Long-Haul Post-COVID-19 symptoms presenting as a variant of postural orthostatic tachycardia syndrome: the Swedish experience. *JACC Case Rep.* (2021) 3:573–80. doi: 10.1016/j.jaccas.2021.01.009
77. Thieben MJ, Sandroni P, Sletten DM, Benrud-Larson LM, Fealey RD, Vernino S, et al. Postural orthostatic tachycardia syndrome: the Mayo clinic experience. *Mayo Clin Proc.* (2007) 82:308–13. doi: 10.1016/S0025-6196(11)61027-6
78. Bryarly M, Phillips LT, Fu Q, Vernino S, Levine BD. Postural orthostatic tachycardia syndrome: JACC focus seminar. *J Am Coll Cardiol.* (2019) 73:1207–28. doi: 10.1016/j.jacc.2018.11.059
79. Olshansky B, Cannom D, Fedorowski A, Stewart J, Gibbons C, Sutton R, et al. Postural Orthostatic Tachycardia Syndrome (POTS): a critical assessment. *Prog Cardiovasc Dis.* (2020) 63:263–70. doi: 10.1016/j.pcad.2020.03.010
80. Tu Y, Abell TL, Raj SR, Mar PL. Mechanisms and management of gastrointestinal symptoms in postural orthostatic tachycardia syndrome. *Neurogastroenterol Motil.* (2020) 32:e14031. doi: 10.1111/nmo.14031
81. Goldstein DS. The possible association between COVID-19 and postural tachycardia syndrome. *Heart Rhythm.* (2021) 18:508–9. doi: 10.1016/j.hrthm.2020.12.007

82. Agarwal AK, Garg R, Ritch A, Sarkar P. Postural orthostatic tachycardia syndrome. *Postgrad Med J.* (2007) 83:478–80. doi: 10.1136/pgmj.2006.055046
83. Nath A. Neurologic manifestations of severe acute respiratory syndrome coronavirus 2 infection. *Continuum.* (2021) 27:1051–65. doi: 10.1212/CON.0000000000000992
84. Kanjwal K, Jamal S, Kichloo A, Grubb BP. New-onset postural orthostatic tachycardia syndrome following coronavirus disease 2019 infection. *J Innov Card Rhythm Manag.* (2020) 11:4302–4. doi: 10.19102/icrm.2020.111102
85. Bisaccia G, Ricci F, Recce V, Serio A, Iannetti G, Chahal AA, et al. Post-acute sequelae of COVID-19 and cardiovascular autonomic dysfunction: what do we know? *J Cardiovasc Dev Dis.* (2021) 8:156. doi: 10.3390/jcdd8110156
86. Shouman K, Vanichkachorn G, Cheshire WP, Suarez MD, Shelly S, Lamotte GJ, et al. Autonomic dysfunction following COVID-19 infection: an early experience. *Clin Auton Res.* (2021) 31:385–94. doi: 10.1007/s10286-021-00803-8
87. Hinduja A, Moutairou A, Calvet JH. Sudomotor dysfunction in patients recovered from COVID-19. *Neurophysiol Clin.* (2021) 51:193–6. doi: 10.1016/j.neucli.2021.01.003
88. Chilazi M, Duffy EY, Thakkar A, Michos ED. COVID and Cardiovascular disease: what we know in 2021. *Curr Atheroscler Rep.* (2021) 23:37. doi: 10.1007/s11883-021-00935-2
89. Eshak N, Abdelnabi M, Ball S, Elgwairi E, Creed K, Test V, et al. Dysautonomia: an overlooked neurological manifestation in a critically ill COVID-19 patient. *Am J Med Sci.* (2020) 360:427–9. doi: 10.1016/j.amjms.2020.07.022
90. Fu Q, Levine BD. Exercise and non-pharmacological treatment of POTS. *Auton Neurosci.* (2018) 215:20–7. doi: 10.1016/j.autneu.2018.07.001
91. Miller AJ, Raj SR. Pharmacotherapy for postural tachycardia syndrome. *Auton Neurosci.* (2018) 215:28–36. doi: 10.1016/j.autneu.2018.04.008
92. Baptista AF, Baltar A, Okano AH, Moreira A, Campos ACP, Fernandes AM, et al. Applications of non-invasive neuromodulation for the management of disorders related to COVID-19. *Front Neurol.* (2020) 11:573718. doi: 10.3389/fneur.2020.573718

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.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 15 June 2022
doi: 10.3389/fneur.2022.909915

Inappropriate Ventilatory Homeostatic Responses in Hospitalized COVID-19 Patients

Prem Jareonsettasin[1,2†], Claudia Zeicu[1,2†], Beate Diehl[1,3], Ronald M. Harper[4‡] and Rónan Astin[2,5‡]*

[1] Department of Clinical and Experimental Epilepsy, Queen Square Institute of Neurology, University College London, London, United Kingdom, [2] Division of Medical Specialties, University College London Hospitals NHS Foundation Trust, London, United Kingdom, [3] Department of Clinical Neurophysiology, University College London Hospitals NHS Foundation Trust National Hospital for Neurology and Neurosurgery, London, United Kingdom, [4] Department of Neurobiology and the Brain Research Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, United States, [5] NIHR University College London Hospitals Biomedical Research Centre, London, United Kingdom

OPEN ACCESS

Edited by:
Beatrice Paradiso, Dolo Hospital, Venice, Italy

Reviewed by:
Miguel García-Grimshaw, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico
Francesco Turco, University of Pisa, Italy

***Correspondence:**
Prem Jareonsettasin
p.jareonsettasin@ucl.ac.uk

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

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: *31 March 2022*
Accepted: *19 May 2022*
Published: *15 June 2022*

Citation:
Jareonsettasin P, Zeicu C, Diehl B, Harper RM and Astin R (2022) Inappropriate Ventilatory Homeostatic Responses in Hospitalized COVID-19 Patients. Front. Neurol. 13:909915. doi: 10.3389/fneur.2022.909915

Background: The clinical presentation of COVID-19 suggests altered breathing control - tachypnoea, relative lack of dyspnoea, and often a discrepancy between severity of clinical and radiological findings. Few studies characterize and analyse the contribution of breathing drivers and their ventilatory and perceptual responses.

Aim: To establish the prevalence of inappropriate ventilatory and perceptual response in COVID-19, by characterizing the relationships between respiratory rate (RR), dyspnoea and arterial blood gas (ABG) in a cohort of COVID-19 patients at presentation to hospital, and their post-Covid respiratory sequelae at follow-up.

Methods: We conducted a retrospective cohort study including consecutive adult patients admitted to hospital with confirmed COVID-19 between 1st March 2020 and 30th April 2020. In those with concurrent ABG, RR and documented dyspnoea status on presentation, we documented patient characteristics, disease severity, and outcomes at hospital and 6-week post-discharge.

Results: Of 492 admissions, 194 patients met the inclusion criteria. Tachypnoea was present in 75% pronounced (RR>30) in 36%, and persisted during sleep. RR correlated with heart rate (HR) ($r = 0.2674$), temperature ($r = 0.2824$), CRP ($r = 0.2561$), Alveolar-arterial (A-a) gradient ($r = 0.4189$), and lower PaO_2/FiO_2 (PF) ratio ($r = -0.3636$). RR was not correlated with any neurological symptoms. Dyspnoea was correlated with RR ($r = 0.2932$), A-a gradient ($r = 0.1723$), and lower PF ratio ($r = -0.1914$), but not correlated with PaO_2 ($r = -0.1095$), $PaCO_2$ ($r = -0.0598$) or any recorded neurological symptom except for altered consciousness. Impaired ventilatory homeostatic control of $pH/PaCO_2$ [tachypnoea (RR>20), hypocapnia ($PaCO_2$ <4.6 kPa), and alkalosis (pH>7.45)] was observed in 29%. This group, of which 37% reported no dyspnoea, had more severe respiratory disease (A-a gradient 38.9 vs. 12.4 mmHg; PF ratio 120 vs. 238), and higher prevalence of anosmia (21 vs. 15%), dysgeusia (25 vs. 12%), headache (33 vs. 23%) and nausea (33 vs. 14%) with similar rates of new anxiety/depression (26 vs. 23%), but lower incidence of past neurological or psychiatric diagnoses (5 vs. 21%)

compared to appropriate responders. Only 5% had hypoxia sufficiently severe to drive breathing (i.e. PaO_2 <6.6 kPa). At 6 weeks post-discharge, 24% (8/34) showed a new breathing pattern disorder with no other neurological findings, nor previous respiratory, neurological, or psychiatric disorder diagnoses.

Conclusions: Impaired homeostatic control of ventilation i.e., tachypnoea, despite hypocapnia to the point of alkalosis appears prevalent in patients admitted to hospital with COVID-19, a finding typically accompanying more severe disease. Tachypnoea prevalence was between 12 and 29%. Data suggest that excessive tachypnoea is driven by both peripheral and central mechanisms, but not hypoxia. Over a third of patients with impaired homeostatic ventilatory control did not experience dyspnoea despite tachypnoea. A subset of followed-up patients developed post-covid breathing pattern disorder.

Keywords: ventilation, impaired homeostasis, COVID-19, breathing pattern disorder, dyspnea, post-covid breathing pattern dysfunction

INTRODUCTION

Early descriptive studies of COVID-19 clinical presentations found tachypnoea, a relative lack of dyspnoea, and often a discrepancy between severity of respiratory clinical signs and radiological findings. Some have described the combination of phenomena as "silent" or "happy" hypoxemia (1–4), inferring dysfunctional regulatory breathing mechanisms.

However, data on the nature of breathing control in COVID-19 are lacking. A few previous studies investigated blood gas analysis in hospitalized COVID-19 patients (5–8), but none concurrently assessed arterial blood gases (ABG), respiratory rate and perception of dyspnoea, and therefore could not directly comment on appropriateness of physiological and breathing perception responses, which we attempt to do here.

Various physiological mechanisms control breathing. Hypercapnia/acidosis drives automatic breathing in a negative feedback loop, while hypoxia only drives breathing when severe (i.e., PaO_2 < 6.6 kPa) (9–11). Additional drives include thermal, emotional, somatosensory, pulmonary afferents, wakefulness-related signals, and conscious volition. Their neural substrates span all levels of the neuraxis, from the periphery to rostral brain areas, overlapping many areas that process smell, taste, emotion, and arousal.

Neurological symptoms, predominantly anosmia, dysgeusia and altered mental status, occur in many COVID-19 patients (12). Accumulating evidence points toward infection of vascular and immune cells, but not CNS neurons, particularly not those of the brainstem and cerebellum (13) – areas where major breathing control sites lie, raising the possibility of altered breathing control during COVID-19 infection due to direct injury to ancillary support areas by the virus.

In this study, our aim was to establish the prevalence of inappropriate ventilatory and perceptual responses in COVID-19, by characterizing breathing responses during acute infection through investigating the relationship between respiratory rate (RR), dyspnoea and ABG in COVID-19 patients at presentation to hospital, their relationship to neurological symptoms and autonomic control dysfunction, and their post-Covid respiratory sequelae at follow-up.

METHODS

We retrospectively collected data from electronic medical records (EPIC, Milky Way, Verona, WI, USA) of consecutive patients with a nasopharyngeal PCR-positive COVID-19 diagnosis who presented to the Emergency Department (ED) of University College Hospital, London between 1st March 2020 and 30th April 2020. Excluded patients were those transferred from another hospital, those without ABG results within 4 h of presentation, undocumented dyspnoea status, and patients who were immediately intubated on arrival. All patients had a respiratory presentation of COVID-19 as their main reason for admission. There were no secondary diagnoses, but co-morbidities are listed in **Table 2**. Clinical data, including RR, HR and ABGs were part of the ED initial evaluation and used for analysis. Dyspnoea status was assigned as positive if any of the following were documented by the clerking clinician: dyspnoea, breathlessness, shortness of breath, air hunger, respiratory discomfort or respiratory distress. Arterial blood samples were processed on ABL90 FLEX gas analysers (Radiometer, Crawley, UK).

Physiological breathing response was considered inappropriate ("Inappropriate Responders") in those who were simultaneously tachypnoeic (RR>20), hypocapnic ($PaCO2$<4.6 kPa) and alkalotic (pH>7.45) (15). Otherwise, the response was considered appropriate ("Appropriate Responders"). No patients had RR<12, which would suggest a deficient respiratory response. Hypoxia was defined as PaO_2 <10 kPa. Hypoxia sufficient to stimulate respiratory drive was defined as PaO_2 <6.6 kPa. The breathing pattern assessment tool (BPAT) provides a validated score used to grade the severity and make the diagnosis of breathing pattern disorder (BPD), with a BPAT score of 4

or more corresponding to a diagnosis of BPD (16). BPD was assessed using the BPAT for patients who attended face-to-face follow-up appointments.

Correlations between continuous variables were evaluated using Pearson's correlation and described using median and inter-quartile range (IQR); mortality rates between groups were compared using the $\chi 2$ test. Data were analyzed using Prism 8 (GraphPad, San Diego, CA, USA). The study was approved by the Westminster Research Ethics Committee (NHS Health Research Authority, IRAS no: 284088).

RESULTS

Of 492 patients admitted with COVID-19 during the study period, 194 had concurrent ABG, RR and documented dyspnoea status, and were therefore included in the study.

Tachypnoea was common and pronounced: 75% (146/194) exhibited RR>20 breaths per minute (bpm), RR>25 bpm in 57% (69/194), RR>30 bpm in 36% (42/194), RR>35 bpm in 22% (42/194), and RR>40 bpm in 8%(16/194) (**Figure 1A**). RR at 1 and 2 h following arterial blood sampling varied little (average standard deviation: 2.8 bpm). Notably, tachypnoea was maintained with little variation both day and night over the 96 h following admission, which included sleep periods (**Figure 1B**). No patients in our cohort had RR<12, which would indicate depressed respiratory drive.

RR correlated with HR ($r = 0.267$, $p < 0.001$), temperature ($r = 0.282$, $p < 0.001$), CRP ($r = 0.256$, $p < 0.001$), A-a gradient ($r = 0.419$, $p < 0.001$), and inversely correlated with PF ratio ($r = -0.363$, $p < 0.001$). RR did not correlate with mortality ($r = 0.0319$, $p = 0.692$), mean arterial pressure (MAP) (r-0.113, $p = 0.115$), anosmia, dysgeusia, headache, dizziness, altered consciousness, nausea, seizure, new anxiety or depression (**Table 1**). There was no shift in the oxygen dissociation curve in our cohort (**Figure 1E**).

Dyspnoea was present in 44% (86/194) of all patients, 48% (48/101) of those with hypoxia ($PaO_2 < 10$ kPa), but only 33% (4/12) of those with hypoxia sufficient to stimulate respiratory drive ($PaO_2 < 6.7$kPa) (**Figures 1C,D**). Dyspnoea was weakly correlated with RR ($r = 0.293$, $p < 0.001$), temperature ($r =$

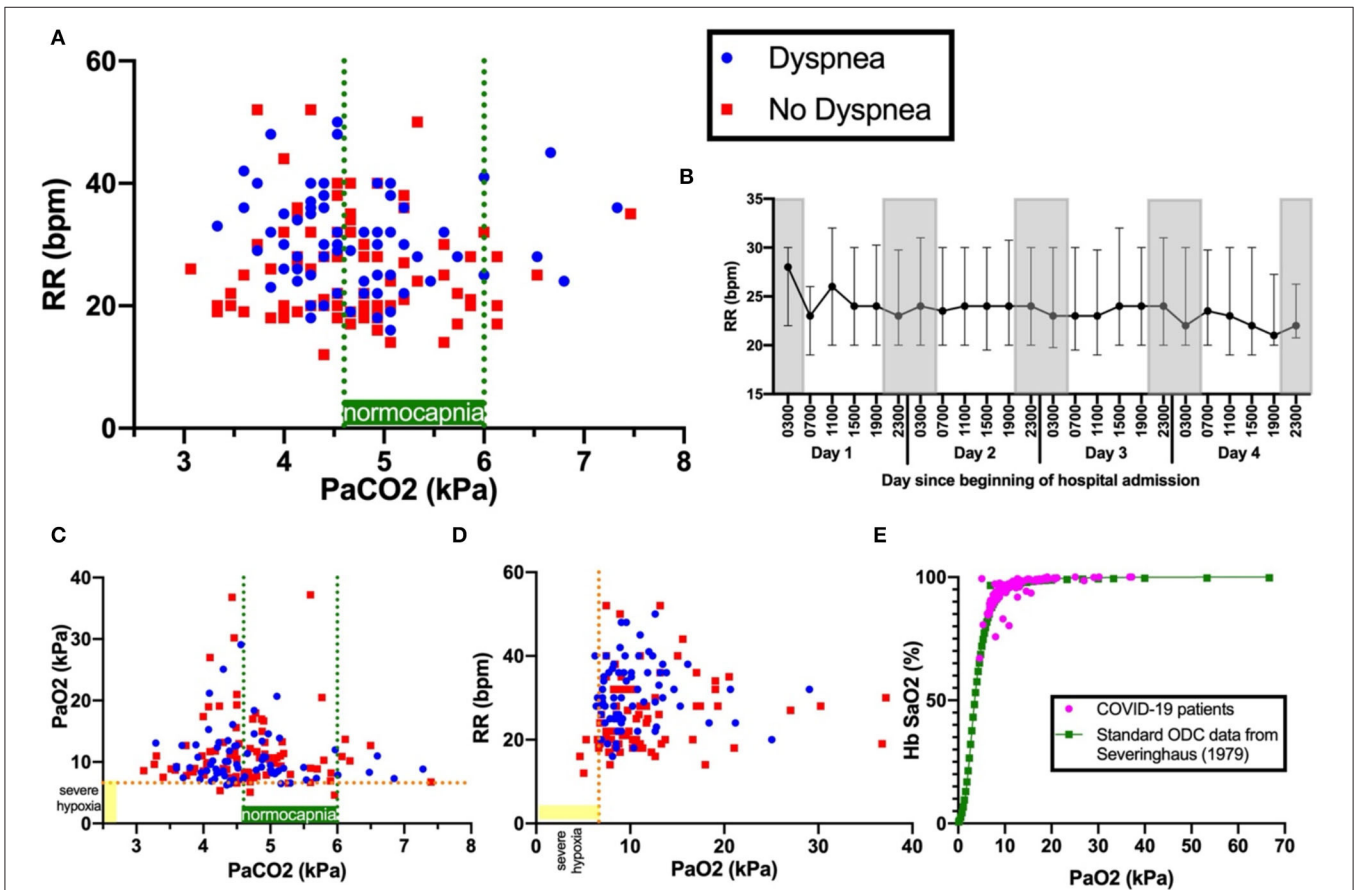


FIGURE 1 | Panel **(A)** shows PaCO2 versus RR. Blue dots indicate patients with dyspnoea; red squares, patients with no dyspnoea. Dashed green lines indicate boundaries of normocapnia (4.6kPa and 6.0kPa). Dashed orange line indicates severe hypoxia at 6.6kPa, a level sufficient to drive ventilation. Panel **(B)** shows the 4-hourly RR (median and interquartile range) over the first 96 hours since admission. Gray-shaded boxes indicate night. Panels **(C)** show $PaCO_2$ versus PaO_2 and **(D)**$PaCO_2$ versus RR. Panel **(E)** shows the oxygen dissociation curve (ODC) of Covid-19 patients in this cohort plotted against standard human ODC data from (14), showing no shift.

TABLE 1 | Relationship between neurological, autonomic, biochemical variables and RR and dyspnea.

	RR (Pearson's correlation)		Dyspnea status		
	r-value	*p*-value	Dyspnea (*n* = 86) (%)	No Dyspnea (*n* = 108) (%)	*p*-value (Chi-square)
Neurological symptoms					
Anosmia	0.055	0.4446	15 (17)	12 (11)	0.2057
Dysgeusia	0.041	0.5735	18 (21)	13 (12)	0.0878
Headache	−0.009	0.9025	24 (28)	27 (25)	0.6477
Dizziness	−0.058	0.4238	3 (3)	7 (6)	0.3489
Nausea	0.025	0.7270	20 (23)	20 (19)	0.4178
Altered consciousness	−0.114	0.1128	3 (3)	25 (23)	**0.0001**
Seizure	0.109	0.1309	1 (1)	2 (2)	0.6992
Psychiatric (New anxiety or new depression)	−0.043	0.5510	21 (24)	26 (24)	0.9556
	RR (Pearson's correlation)		**Dysnea (Pearson's correlation)**		
	r-value	***p*-value**	**r-value**		***p*-value**
Autonomic variables					
HR	0.2674	**0.0002**	0.1300		0.0708
MAP	0.1134	0.1153	0.1319		0.0668
Temperature	0.2824	**<0.0001**	0.1673		**0.0198**
RR	n/a	n/a	0.2932		**<0.0001**
Respiratory variables					
pH	−0.0568	0.4312	0.0988		0.1703
PaCO2	−0.1242	0.0844	−0.0598		0.4078
PaO2	−0.1142	0.1128	−0.1095		0.1284
PF	−0.3636	**<0.0001**	−0.1914		**0.0075**
A-a gradient	0.4189	**<0.0001**	0.1723		**0.0163**
Laboratory variables					
Hb	0.0838	0.2489	0.1492		**0.0395**
Lym	0.1316	0.088	−0.0918		0.2353
CRP	0.2561	**0.0008**	0.1232		0.1106
D-dimer	0.1556	0.1111	0.1979		**0.042**
LDH	0.1688	0.0666	0.1668		0.0698
Ferritin	−0.0172	0.8471	0.0208		0.8154
Mortality	0.0319	0.692	−0.0412		0.5686

Bold values meant statistically significant.

0.167, $p = 0.020$), A-a gradient (r = 0.172, $p = 0.016$), Hb (r = 0.149, $p = 0.040$) and D-dimer (r = 0.198, $p = 0.042$). Dyspnoea was not correlated with pH (r = 0.099, *p*-0.170), $PaCO_2$ ($r = -0.060$, $p = 0.408$) or PaO_2 (r = −0.110, $p = 0.128$), nor mortality (r = −0.041, $p = 0.569$). Dyspnoea was weakly inversely correlated to PF ratio (r = −0.191, $p = 0.008$). Except for altered consciousness (p=0.002), dyspnoea was not correlated with other neurological symptoms.

Inappropriate responders accounted for 29% (57/194). Respiratory alkalosis was associated with more severe respiratory disease; higher FiO_2 (0.60 vs. 0.32, $p < 0.001$), greater A-a gradient (38.9 vs. 12.4 mmHg, $p = 0.002$), and lower PF ratio (120 vs. 238, $p = 0.002$).Markers of inflammation were also higher in this group [LDH (498 vs. 386 IU/L, $p < 0.001$), Ferritin (1,430 vs. 948 ug/L, $p = 0.018$)], but no significant difference in mortality was observed (30 vs. 40%, $p = 0.195$). The prevalence of severe hypoxia was low and similar in both groups (5 vs. 4%). However, inappropriate responders had higher rates of supplemental oxygen use (84 vs. 66%) indicating a higher level of underlying pre-hospital hypoxia that was immediately corrected on admission. Anosmia (21 vs. 11%), dysgeusia (25 vs. 12%), headache (33 vs. 23%), nausea (33 vs. 14%) were more prevalent in inappropriate responders. There were no differences in new anxiety or depression (26 vs. 23%), and past neurological or psychiatric diagnoses were less prevalent (5 vs. 21%) in inappropriate responders. The two groups did not differ significantly in age, sex, BMI, ethnicity, cardiovascular and respiratory co-morbidities. Though inappropriate responders had higher rates of dyspnoea (63 vs. 36%), 37% did not report dyspnoea (**Table 2**).

Of the total cohort, 38% (74/194), had a 6-week follow-up after hospital discharge, of which 34 (17.5%) were face-to-face consultations which allowed full clinical assessment. A new diagnosis of breathing pattern disorder (BPD) was made in 23.5%

TABLE 2 | Characteristics of appropriate vs inappropriate responders.

Characteristics	Appropriate Responders [$n = 137$]	Inappropriate Responders (Tacyhpnoeic RR>20, hypocapnic PaCO2<4.6kPa alkalotic pH>7.45) [$n = 57$]	*p*-value
Age (median + IQR)	68 (51–80)	63 (50–74)	0.0708
Female (%)	47 (34)	17 (30)	0.6166
BMI (median + IQR)	26.7 (22.8–30.6)	28.0 (25.4–31.6)	0.0682
Ethnicity (%)			
White	73 (53)	33 (58)	0.6355
Black	19 (14)	10 (18)	0.5137
Asian	26 (19)	6 (11)	0.2025
Other Ethnic Background	7 (4)	6 (3)	0.2082
Unknown	12 (9)	2 (4)	0.2401
Co-morbidities (%)			
Cardiovascular Disorders	85 (62)	41 (72)	0.2475
Respiratory Disorders	43 (31)	12 (21)	0.1648
Asthma	22 (16)	5 (9)	
COPD	14 (10)	2 (4)	
ILD	2 (1)	2 (4)	
OSA	2 (2)	4 (1)	
Other respiratory disorders	2 (1)	1 (2)	
Neurological/Psychiatric Disorders	29 (21)	3 (5)	**0.0055**
Other co-morbidities	59 (43)	14 (25)	**0.0222**
Neurological/Psychiatric Symptoms (%)			
Anosmia	15 (11)	12 (21)	0.0719
Dysgeusia	17 (12)	14 (25)	0.0514
Headache	32 (23)	19 (33)	0.1567
Dizziness	6 (4)	4 (7)	0.4835
Nausea	21 (14)	19 (33)	**0.0065**
Altered consciousness	24 (18)	4 (7)	0.0726
Seizure	3 (2)	0 (0)	0.5568
New Anxiety or Depression	32 (23)	15 (26)	0.7140
Any neurological or psychiatric symptom	92 (67)	41 (72)	0.6113
Respiratory Characteristics (median + IQR)			
Respiratory rate (bpm)	25 (20–32)	30 (26–36)	**<0.0001**
pH	7.44 (7.40–7.62)	7.49 (7.48–7.41)	**<0.0001**
PaCO2 (kPa)	4.87 (4.40–5.29)	4.10 (3.78–4.36)	**<0.0001**
PaO2 (kPa)	10.10 (8.18–12.85)	9.02 (7.76–12.20)	0.2711
BE (mEq/L)	1.10 (−2.85–4.55)	0.70 (−1.00–2.25)	0.3848
FiO2	0.32 (0.21–0.60)	0.60 (0.32–0.90)	**0.0011**
Supplemental Oxygen (%)	90 (66)	48 (84)	**0.0094**
A-a gradient (mmHg)	12.4 (5.3–44.5)	38.9 (12.3–73.0)	**0.0001**
PF ratio	238 (134–328)	120 (74–276)	**0.0019**
Dyspnoea (%)	50 (36%)	36 (63)	**0.0008**
Severely hypoxemic (PaO2<6.6kPa) (%)	6 (4)	3 (5)	0.7237
CXR severity			
Mild (%)	34 (25)	14 (25)	1.0000
Moderate (%)	29 (21)	22 (39)	**0.0192**
Severe (%)	31 (23)	16 (28)	0.4634
Unknown (%)	43 (31)	5 (9)	**0.0008**
Other Clinical Observations (median + IQR)			
Heart Rate (bpm)	93 (78–105)	102 (86–115)	**0.0048**
Mean Arterial Pressure (mmHg)	90 (78–104)	94 (86–102)	0.1547
Temperature (°C)	37.2 (36.6–38.0)	37.8 (37.2–38.7)	**0.0006**

(Continued)

TABLE 2 | Continued

Characteristics	Appropriate Responders [$n = 137$]	Inappropriate Responders (Tacyhpnoeic RR>20, hypocapnic PaCO2<4.6kPa alkalotic pH>7.45) [$n = 57$]	*p*-value
Admission Bloods (median + IQR)			
Hb (g/L)	129 (110–140)	134 (123–144)	0.0733
Lym (×10∧9/L)	1.03 (0.64–1.46)	0.96 (0.73–1.39)	0.9053
CRP (mg/L)	98 (42–192)	102 (66–238)	0.1299
D-dimer (mg/L)	1.68 (0.69–2.94)	1.28 (0.69–4.0)	0.721
Troponin T (ng/L)	22 (10–45)	16 (10–23)	0.596
LDH (IU/L)	386 (295–510)	498 (391–600)	**0.0007**
Ferritin (ug/L)	948 (406–1,814)	1,430 (793–2,491)	**0.018**
Mortality (%)	55 (40)	17 (30)	0.1948

Bold values meant statistically significant.

(8/34), with a BPAT score of 6 (5–7). None had a past medical history of respiratory, neurological or psychiatric disorder. None had other focal neurological findings on examination. 62.5% (5/8) were in the inappropriate responders group. All eight patients with BPD had 6-month follow-up, of which three still had BPD, with a BPAT score of 2.5 (3–5). Five of the eight patients attended 12-month follow-up, of which one had BPD, with a BPAT score of 2 (2, 3) (**Figure 2**).

DISCUSSION

Nearly a third of the patients in this study demonstrated impaired homeostatic control of ventilation i.e., tachypnoea, despite hypocapnia to the point of alkalosis, a finding accompanying more severe disease (as evidenced by worse physiological (higher FiO_2, greater A-a gradient, and lower PF ratio) and inflammatory markers). Over a third showed a reduced dyspnoeic response to tachypnoea.

The prevalence of inappropriate responders is consistent with estimates of respiratory alkalosis in 28.7–55.4% from other studies that investigated blood gas analysis in hospitalized COVID-19 patients [(5) (55.4%); (6) (28.7%); (7) (30.3%); (8) (40.4%)]. Wu et al. (6) found higher inflammatory markers in respiratory alkalosis group, and other studies corroborate admission hypocapnia as a marker of severe disease (17, 18).

No previous study concurrently assessed ABG, respiratory rate and perception of dyspnoea, and therefore could not directly comment on appropriateness of physiological and perception response, which is a unique aspect here.

Outside the setting of COVID-19, few studies characterize and analyse the ventilatory response in the context of respiratory infection. Although a few studies report prevalence of hypocapnia in community-acquired pneumonia (19–21), none, to our knowledge, report the prevalence of hypocapnia to the extent of alkalosis (PaCO2<4.6kPa and pH>7.45); therefore, those findings cannot be directly compared.

Profound hypocapnia is found in the context of critical illness, and when prolonged, may adversely influence outcome (15). However, most critically ill patients with abnormal ventilatory responses present with insufficiency (i.e. with acidemia) rather than excessiveness (22). Inappropriate perception of dyspnea is even less well studied. Impaired perception of dypsnoea is more often found in patients with a history of near fatal asthma (23–25), and is associated with impaired chemosensitivity (23, 26) and downregulation of insular activity (27).

We also explored the potential processes that drive excessive tachypnoea in the inappropriate responders group, as well as the impaired perception of dyspnoea to these breathing patterns, and how these acute findings relate to post-covid syndrome.

What Drives Excessive Tachypnoea?

Breathing is controlled by various physiological mechanisms. At a systems level, hypercapnia (increase in $PaCO_2$) and acidosis are the principal chemical drivers of spontaneous automatic breathing, while hypoxia drives breathing only at severe hypoxemia (i.e. $PaO_2 < 6.6$ kPa). In addition, thermal, peripheral pulmonary afferents, sympathetic, emotional and somatosensory drives provide adaptive value for particular situations. When awake, further signals provide wakefulness-related drive to breath which underlies why hypocapnia during wakefulness, but not sleep or anesthesia, does not cause apnoea (28). At the highest level, we can voluntarily control breathing through top-down influence of lower breathing centers.

At a biological level, these physiological mechanisms span all levels of the neuraxis from the periphery to central areas illustrated in **Figure 3**. These drivers interact in a complex non-linear fashion across large-scale neural networks that control breathing. The final common downstream pathway includes central rhythm generators (pre-Botzinger, parafacial respiratory group, and post-inspiratory complex) that output to the central pattern generators (rostral and caudal ventral respiratory group) and then onwards to spinal and cranial motor nuclei and their neuromuscular efferent arm (29).

The effects of COVID-19 on breathing control can occur at multiple levels of regulatory control. Here, we explore evidence of COVID-19 effects at various planes, and document disruption inferred from these data.

Hypoxic Drive

Severe hypoxemia (i.e. $PaO_2 < 6.6$kPa) drives breathing through the hypoxic ventilatory reflex (HVR) (9–11). In the periphery,

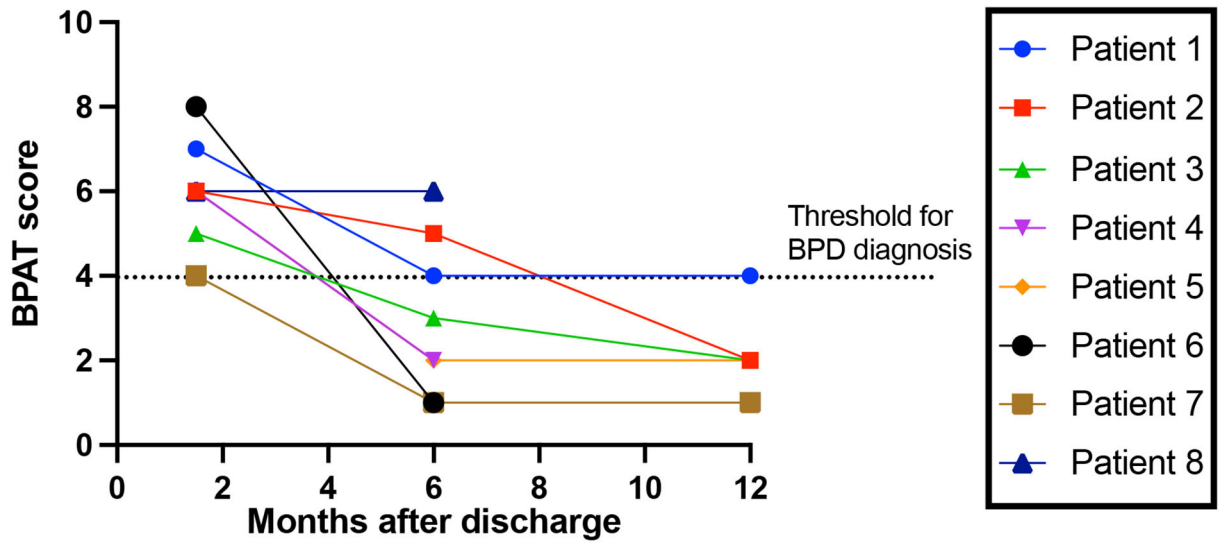


FIGURE 2 | Breathing Pattern Disorder severity over time. Breathing Pattern Assessment Tool score to rate breathing pattern disorder (BPD) severity, over time since discharge from hospital. Threshold for BPD diagnosis is a score of 4 or more (16).

arterial chemoreceptors located in the carotid bodies (CB) (**Figure 3**) sense PaO_2. Aortic bodies play a minimal role, except when carotid bodies are impaired (ref). Oxygen-regulating cells are also present centrally in the caudal hypothalamus, posterior thalamus, periaqueductal gray, nucleus tactus solitarii (NTS) and the rostral ventrolateral medulla (RVLM)(ref). The CB provide the dominant drive for the HVR, since CB denervation significantly attenuates the response (ref). Central oxygen sensors play a role in severe hypoxia-induced tachypnoea in animal models of carotid-deafferented animals (30).

Given that O_2-sensing glomus cells express ACE2 (a SARS-CoV-2 receptor), direct infection and impairment of the carotid body could either abolish the HVR or cause abnormal excitability resulting in excessive CB-driven HVR.

However, our results do not support that scenario, since very few patients (5%) had sufficient hypoxemia to drive ventilation. Additionally, hypocapnia observed in our patients would further blunt the HVR.

We cannot exclude pre-hospital periods of sustained severe hypoxia, which could sensitize the CB. Such sensitization results in hyperventilation and increased sympathetic activity that is sustained even after reversal of sustained hypoxic insult, and slowly declines over a few days (31). That more patients in the inappropriate responder group required supplemental oxygen on admission, suggests that this group had higher rates of pre-hospital hypoxia which could sensitize the CB.

CO_2 and pH Homeostasis

Central chemoreceptors that sense $PaCO_2$ and pH, are found in the brainstem, cerebellum, hypothalamus and midbrain (32). Those sensors monitor brain interstitial pH, which reflects the integration of $PaCO_2$, cerebral blood flow (CBF), and cerebral metabolic rate. CBF itself responds to changes in $PaCO_2$ (cerebral autoregulation). A set-point exists which keeps $PaCO_2$ and pH in a relatively narrow range. Hypercapnia ($PaCO_2$ >4.6kPa) or acidosis (pH <7.35) drives hyperventilation. Conversely, hypocapnia ($PaCO_2$ <4.6kPa) or alkalosis (pH >7.45) drives hypoventilation. The inappropriate responders group showed hypocapnia *and* alkalosis, which should cause hypoventilation (RR<12), yet they paradoxically are hyperventilating (RR>20). This disturbance in normal homeostasis requires explanation.

PCR-positive SARS-CoV-2 is present at autopsy in the brainstem and cerebellum, specifically in vascular and glial cells, but not neurons, along with activated microglia and evidence of secondary neuronal damage in chemosensitive areas. Specific affected areas include CN X, NTS, dorsal raphe nuclei and cerebellum (13, 33–36) (**Figure 3**). As such, dysfunction in this redundant network of chemoreceptors appears plausible. Serotonergic neurons of the dorsal raphe, with their extensive projections to motor and respiratory regulatory areas, especially to the cerebellum, are of particular concern.

Two possible mechanisms are conceivable:

First is rheostasis – i.e., shifting the setpoint lower such that hyperventilation is driven by lower $PaCO_2$ than the normal 4.6 kPa. **Figure 4** shows that lowering the threshold at which $PaCO_2$ drives breathing (i.e., lower than the normal set-point/threshold of 4.6 kPa), lowers the proportion of inappropriate responders i.e., those who still have simultaneous tachypnoea (RR>20) and alkalosis (pH>7.45) at the new $PaCO_2$ threshold. Rheostasis is normally an adaptive process in homeostatic systems to a sustained change in the environment, such as increased core temperature setpoint during infections, or vestibular-ocular reflex set-points after prolonged stimulation or imbalance in vestibular input (37). Supportive evidence for this hypothesis predicts a rebound hypoventilation when the setpoint returns to normal after the acute insult is

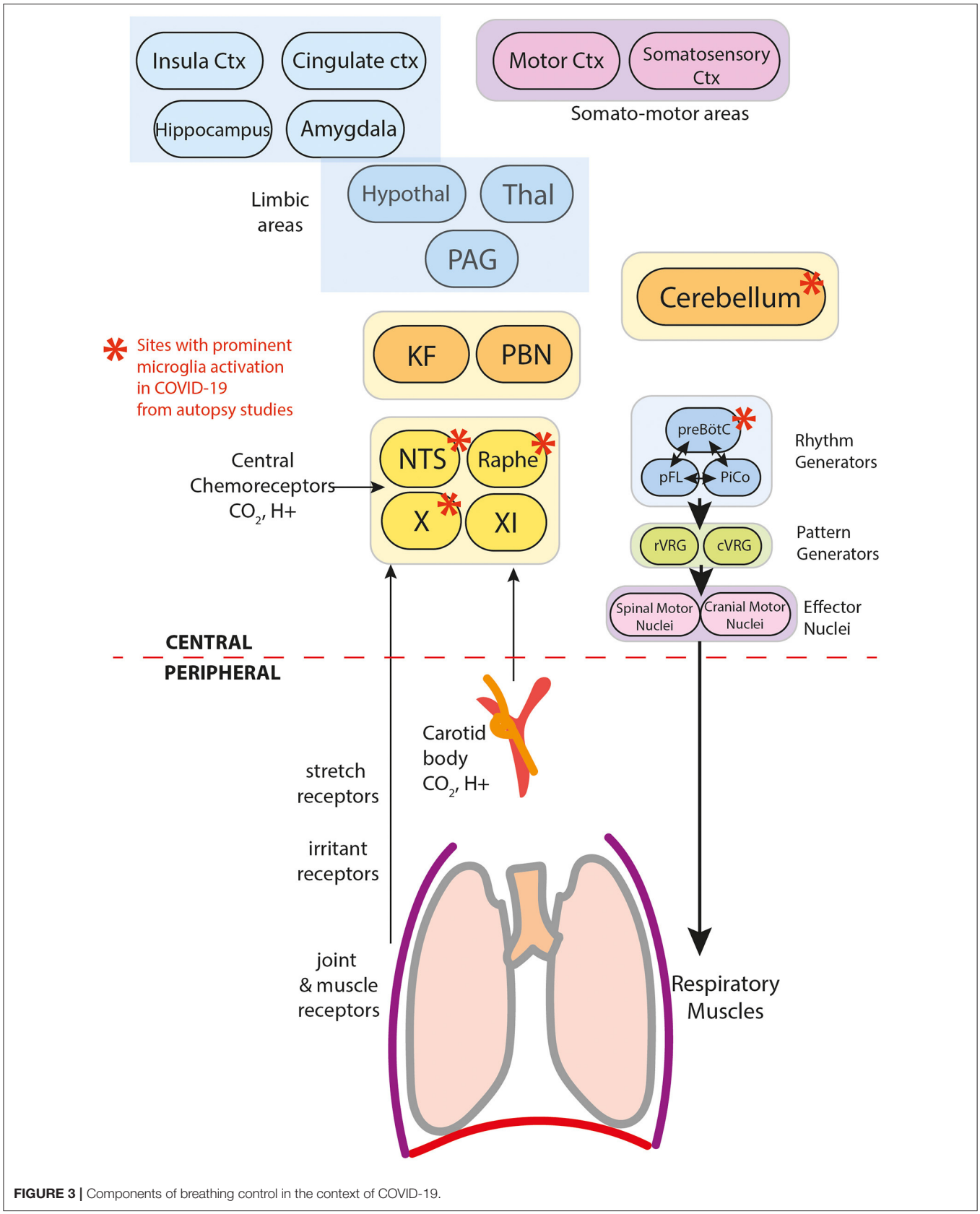


FIGURE 3 | Components of breathing control in the context of COVID-19.

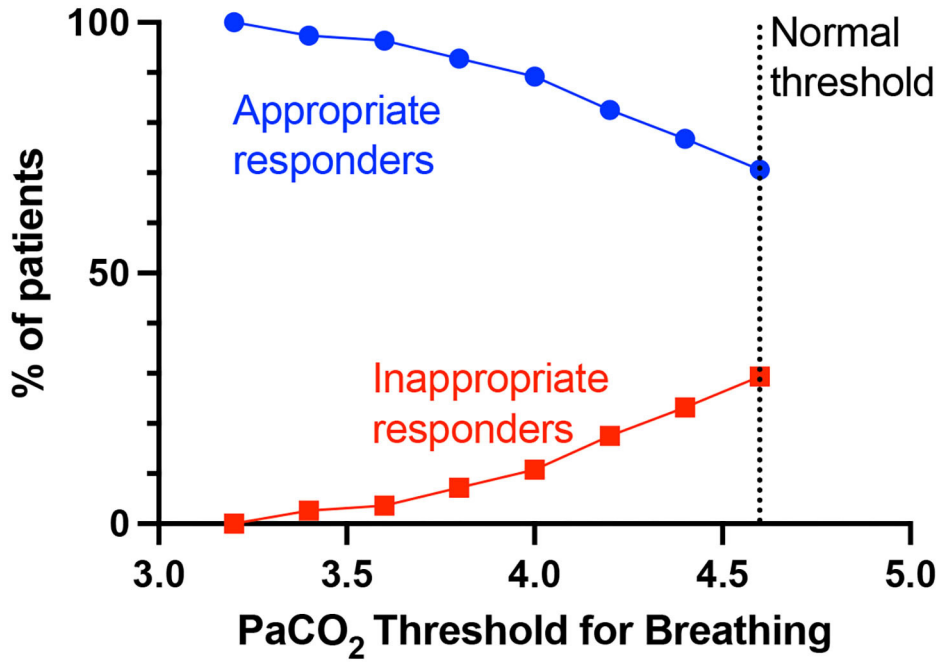


FIGURE 4 | $PaCO_2$ thresholds for breathing and % appropriate and inappropriate responders. The normal $PaCO_2$ threshold for breathing is >4.6 kPa, below which $PaCO_2$ as a breathing drive would be suppressed. Lowering the $PaCO_2$ threshold (set-point) for breathing, will decrease the proportion of patients who are considered inappropriate responders for that particular $PaCO_2$ threshold i.e., still simultaneously have tachypnoea (RR>20) and alkalosis (pH>7.45).

removed; that possibility has not been tested in COVID-19 patients.

A second potential mechanism is added bias/additional ventilatory drives.

Aberrant Peripheral Sensing

Peripheral drivers via pulmonary vagal C-fibers and slow-adapting mechanoreceptors (SARS) provide sensory feedback to central respiratory centers on local chemical and mechanical conditions. Pulmonary vagal C-fibers fibers are sensitive to inflammatory mediators (including histamine, bradykinin, and prostaglandins), and are consistently activated in lung oedema (38) and experimental acute lung injury (39). These C-fibers can modulate ventilation (increase RR and decrease tidal volume) (40), possibly through vagally-mediated cytokine release in the brainstem (41).

Slow-adapting mechanoreceptors are normally activated by lung inflation, and inhibit central chemoreception (42). Peripheral drives from these sensors may explain hyperventilation in pulmonary oedema, pulmonary fibrosis and pulmonary embolism which persists in the absence of hypercapnia or severe hypoxemia (43).

Our data support a role for these peripheral receptors. This inference is based on the observation of a higher prevalence of more-severe lung disease in the hypocapnic group. Acute respiratory distress syndrome increases RR before impairing gas exchange in rodent models, suggesting an initial role for peripheral afferent stimulation. The acute lung inflammation found in COVID-19 would be expected to stimulate SARs in a similar manner.

Other Breathing Drives

Thermal Drive

Animals can regulate their body temperature with an increase of core temperature by 1°C, triggering hyperventilation to induce heat loss (44). Our data, showing a correlation between temperature and RR, support this relationship. The significantly higher temperature in the inappropriate responder group suggests a contribution of thermal drive to tachypnoea.

Diminished "Higher" Drivers of Breathing

Normally, higher brain centers influence breathing to allow flexible control of breathing with emotion, experience and context, and provide signals involved in the "wakefulness drive to breathe" (28). Our study assessed measures of breathing throughout the day and night. A remarkable finding was that little change in tachypnoea was found in sleeping periods. Although classification of sleep states was unavailable, the data indicate that the normal slowing of respiratory rates with quiet sleep did not occur. That finding is significant, since it points to an abolition of the descending brain influences that mediate control of breathing during sleep states. Although descending limbic and thalamic drives, such as airflow, olfactory or temperature influences may be exerting timing effects, the timing influences that normally slow breathing during sleep appear to be ineffective.

Systemic Inflammation

Systemic inflammation itself increases respiratory drive. Our data support greater hyperinflammation in the inappropriate responder group. Hypocapnia is seen in critically ill patients, systemic inflammatory response syndrome, and liver failure (45). In COVID-19, hyperinflammatory responses contribute to disease severity and mortality (46).

Central Neurogenic Hyperventilation

In the inappropriate responders group, we found a higher prevalence of anosmia (21 vs. 15%), dysgeusia (25 vs. 12%), headache (33 vs. 23%) and nausea (33 vs. 14%) with similar rates of new anxiety/depression (26 vs. 23%), but a lower incidence of past neurological or psychiatric diagnoses (5 vs. 21%) compared to appropriate responders. These findings warrant exploration of possible central neurogenic contributions to hyperventilation.

The demonstration of COVID-19 influences on the olfactory apparatus (36) and the role of those structures on sensing CO_2 and other aspects of air passage, as well as the known injury to the amygdala and other limbic structures mediating taste and drive to respiratory phase switching areas of the parabrachial pons (47) and thus, respiratory rate, provide a number of potential central mechanisms to mediate the findings here. Central neurogenic hyperventilation has been reported in other conditions involving immune dysfunction, including multiple sclerosis (48), anti-NMDA receptor encephalitis (49), neuro-Behcet's (50) and Bickerstaff encephalitis (51). Interestingly, marked tachypnoea was the predominant respiratory phenotype of the 1918–1925 epidemic of encephalitis lethargica (52), a disease with recent evidence suggestive of an immune-mediated pathogenesis (53).

Anosmia and dysgeusia are the most prevalent neurological symptoms in COVID-19, suggesting key roles for forebrain limbic structures (**Figure 3**), particularly olfactory and amygdala structures. However, the weight of evidence supports an interpretation that anosmia results predominantly from SARS-CoV-2 infection of non-neuronal cells in the olfactory epithelium and olfactory bulb (54), and dysgeusia more likely results from peripheral damage to ACE-2-expressing cells of taste buds and peripheral chemoreceptors, or cranial nerves responsible for gustation (CN VII, IX, or X) (55).

Centrally, olfaction is processed by multiple cortical and subcortical regions (56), in particular temporal lobe areas, including the piriform and entorhinal cortex, hippocampus, parahippocampus, amygdala and extra-temporal areas such as the orbitofrontal cortex. Amongst these structures, the hippocampus and amygdala are critical subcortical structures controlling breathing (57, 58).

Central gustatory areas include the NTS, parabrachial nucleus, gustatory thalamus (ventropostero-medial nucleus), amygdala basolateral nucleus and central nucleus, insula cortex, orbitofrontal cortex and anterior cingulate cortex. Among these structures, the NTS and parabrachial nuclei are chemosensitive brainstem structures or receive afferent signals mediating control of breathing. The higher prevalence of nausea in the inappropriate responder group supports involvement of the NTS and parabrachial nuclei. Of interest, the nausea finding is corroborated by the significantly higher prevalence of vomiting in the respiratory alkalosis group (21.2%) compared to the non-respiratory alkalosis group (7.3%) in a previous study (6).

Headache as a symptom has no specific localization, but hints at involvement of CN V (trigeminal nuclei). Another study of hospitalized COVID-19 patients identified the presence of new-onset headache in those presenting without dyspnoea, who also presented earlier (4). This finding raises the possibility of early activation of the trigeminal-vascular system, a concept supported by neuropathological studies showing neuroinvasive potential of SARS-CoV-2 to the brainstem (36). CN V plays a major role in respiratory timing through airflow receptors in the nasal and oral cavities and motor activation of the upper airway musculature (59). These timing roles are especially important for preventing obstructive apnea and maintaining appropriate coordination of cerebellar and pontine respiratory timing circuitry through airflow and thermal afferent activity to the parabrachial pons, a major site of respiratory phase switching (and thus, respiratory timing). The thermal role can be readily demonstrated through cold water facial immersion, which results in immediate apnea, while warming results in tachypnoea and panting.

The amygdala, insula and anterior cingulate cortex, all injured in COVID-19, also serve critical respiratory roles, integrating afferent input from a wide range of receptors and sending projections to other amygdala structures and the hypothalamus; the central nucleus of the amygdala has prominent projections to the parabrachial pons and can influence respiratory rate, even to the point of pacing inspiratory efforts (60). The hypothalamus provides substantial thermal drive to breathing, perhaps influencing the significant role we found for breathing rate and temperature.

Autopsy reports in COVID-19 indicate local immune-mediated activity in the brainstem and cerebellum (13). The cerebellum plays a critical role in respiratory timing, coordinating afferent stimuli from multiple somatic and vascular sites and essential timing circuitry with the parabrachial pons. The cerebellar fastigial nuclei are particularly important in these ventilatory roles, specifically during chemical stress and not during eupnoea. Injury to the fastigial nuclei, such as in Central Congenital Hypoventilation Syndrome or heart failure patients, distorts both amplitude and timing to ventilatory and blood pressure challenges (61, 62).

Sensitization of the Efferent Arm

A possible source of hyperventilation lies in the efferent arm. There is no evidence to suggest dysfunction in the motor nuclei, motor neurons or muscles. Nevertheless, pre-admission sustained hypoxia could centrally sensitize motor neurons driving the phrenic nerves, enhancing phrenic output. However, the principal findings suggest a timing dysfunction, i.e., a rate, not motor effort, issue.

Overall, the data presented here suggest that tachypnoea was driven by both peripheral and central mechanisms, but not hypoxia.

What Drives Impaired Perception of Dyspnoea to Tachypnoea?

Increased afferent feedback from chest wall mechanoreceptors and muscle stretch receptors with increased RR is usually perceived as breathlessness (63). It is abnormal that over a third of patients in the inappropriate responder group had reduced dyspnoeic response to tachypnoea.

First, neuromechanical coupling may be maintained in this COVID-19 cohort due to relatively preserved lung compliance (64). This coupling is unusual for most disorders that lead to acute lung injury. To a large degree, this interpretation explains the lack of dyspnoea, because the mechanoreceptor activity should continue to be proportional to the predicted activity from a given motor signal that drives ventilation. Therefore, there should not be an error signal, which should indicate no dyspnoea. This possibility has been supported by others (64, 65).

Secondly, interruptions in central processes that compare expected consequences of (breathing) motor commands and the actual consequences (feedback from periphery) may occur. Normally the "error" signal generated from a mismatch between these expected and actual consequences would generate the dyspnoeic perception of "increased work of breathing" (66). Both the cerebellum and insula play major roles in the perception of dyspnoea (67), as well as their aforementioned roles in control of ventilation. Damage to the cerebellum could impair gain and timing of these signals. Alternatively, a shift in setpoint to a higher threshold for dyspnoea perception would require a higher RR to perceive dyspnoea. Here, one possibility is a downregulation of insula activity. In patients with asthma, downregulation of affect-related insula cortex activity correlates with blunted perception of dyspnoea (68). Lesions in the right insular cortex are associated with blunted dyspnoea (69).

How Do These Acute Findings Relate to Post-COVID Syndrome in a Subset of Patients?

The prevalence of breathing pattern disorder (BPD) at 6 weeks post-discharge was (24%) and in the absence of other neurological findings, or previous respiratory, neurological, or psychiatric disorder diagnoses. Notably, most patients recovered over time. The pathophysiology of breathing pattern disorder is poorly understood, but involves abnormal breathing rate, pattern and inappropriate dyspnoea. The neural mechanisms underlying the recovery are not understood.

Whether mechanisms of post-Covid breathing pattern disorder can be inferred from our data is unclear. Only 62.5% of patients who had BPD were inappropriate responders in the acute phase – for this group, rheostasis may be explanatory– a shift in set-point during the acute phase to a higher state. Such a shift is likely followed by a resetting after the acute illness that disturbs breathing perception and results in the high prevalence of breathing disorder found in our cohort. Our data suggest that by 1 year after the acute insult, the set-point has reset to its pre-Covid state.

Future work should focus on prospective cohort studies of hospitalized COVID-19 patients, with an emphasis on gathering more objective respiratory rate using wearable devices, more quantitative measures of perception of dyspnea over multiple intervals from admission to discharge, and prolonged follow-up. Correlation of respiratory patterning with cardiovascular changes would also be useful. Determination of respiratory patterning during the normally short-lasting periods of rapid eye movement sleep would help differentiate whether COVID-19 impacts breathing differently during that state, thus helping to determine abberant influences. Additional functional neuroimaging of subsets of patients with impaired ventilatory and perceptual response would further mechanistic understanding. More broadly, it remains unanswered whether the phenomena we observe here are unique to COVID-19 or are found in other respiratory conditions – further studies are needed.

Clinical Implications

We show (1) that dyspnoea alone poorly correlates with disease severity or degree of hypoxia, despite its inclusion in many severity triage scoring systems; (2) tachypnoea appears to be a more useful clinical marker, as it is common, and correlated with more severe pulmonary disease; (3) our study supports the use of early blood gas analysis - with hypocapnia and respiratory alkalosis being of particular concern, because this group has more severe disease; (4) we suggest that acute impairment in breathing control may lead to dysfunctional breathing that is prolonged, but will likely resolve by 1 year. The unresponsiveness of control mechanisms to extreme values in pH and oxygenation mandate further studies into processes mediating disruption of sensory, integrative central processing, and motor output on respiration, and the activities underlying recovery of longer-term effects of COVID-19 on breathing control.

Limitations of the Study

The limitations include a relatively small number of subjects, and that the data are derived from a single center. However, the study is from a geographical location with a highly heterogenous population, providing a wide representation of physiological presentation. The study is also a retrospective design, and therefore, no formal protocolised assessment of dyspnoea was available, nor were comments on hyperpnoea. Not unique to the study is the difficulty of counting RR in clinical situations. However, the persistence of tachypnoea over multiple recordings argues for the validity of the data. Inherently, the perception of dyspnoea is subjective and multi-dimensional – but our inclusion criteria for recording dyspnoea covers these multi-dimensional descriptors. We also had limited detailed data on other autonomic aspects including cardiac patterning. We had no neuropsychometric assessment, for practical reasons during that phase of the COVID-19 pandemic which may have revealed subtler psychological localisable deficits. Although we note that nearly a third of patients in our study had impaired homeostatic control of ventilation, the study only included 194 (who met the inclusion criteria)

out of the 492 patients admitted. Therefore, the lowest bound of the prevalence estimate would be 11.6% (57/492). Finally, the number of patients who attended follow-up appointment was low, which limited inferences of post-acute Covid effects.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and the study was approved by the Westminster Research Ethics Committee (NHS Health Research Authority, IRAS no: 284088). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

PJ, CZ, RH, and RA: acquisition and analysis or interpretation of data. PJ, RH, and RA: drafting of the manuscript, administrative, and technical or material support. PJ: statistical analysis. RH and RA: obtained funding and supervision. All authors: concept and design and critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported in part by the Fidelity Charitable Nancy Adams and Scott Schoen Fund and the Kraig and Linda Kupiec Family Trust.

REFERENCES

1. Tobin MJ, Laghi F, and Jubran A. Why COVID-19 silent hypoxemia is baffling to physicians. *Am J Respir Crit Care Med*. (2020) 202:356–60. doi: 10.1164/rccm.202006-2157CP
2. Brouqui P, Amrane S, Million M, Cortaredona S, Parola P, Lagier JC, et al. Asymptomatic hypoxia in COVID-19 is associated with poor outcome. *Int J Infect Dis.* (2021) 102:233–8. doi: 10.1016/j.ijid.2020.10.067
3. Busana M, Gasperetti A, Giosa L, Forleo GB, Schiavone M, Mitacchione G, et al. Prevalence and outcome of silent hypoxemia in COVID-19. *Minerva Anestesiol.* (2021) 87:325–33. doi: 10.23736/S0375-9393.21.15245-9
4. García-Grimshaw M, Flores-Silva FD, Chiquete E, Cantú-Brito C, Michel-Chávez A, Vigueras-Hernández AP, et al. Characteristics and predictors for silent hypoxemia in a cohort of hospitalized COVID-19 patients. *Auton Neurosci.* (2021) 235:102855. doi: 10.1016/j.autneu.2021.102855
5. Mondal S, Das TK, Bhattacharya S, Banerjee S, Hazra D. Blood gas analysis among COVID-19 patients: a single centre retrospective observational study. *J Clin Diagn Res.* (2021) 3:1–4. doi: 10.7860/JCDR/2021/49835.15185
6. Wu C, Wang G, Zhang Q, Yu B, Lv J, Zhang S, et al. Association Between Respiratory Alkalosis and the Prognosis of COVID-19 Patients. *Front Med.* (2021) 8:6–11. doi: 10.3389/fmed.2021.564635
7. Alfano G, Fontana F, Mori G, Giaroni F, Ferrari A, Giovanella S, et al. Acid base disorders in patients with COVID-19. *Int Urol Nephrol.* (2022) 54:405–10. doi: 10.1007/s11255-021-02855-1
8. Chiumello D, Pozzi T, Fratti I, Modafferi L, Montante M, Papa GF, et al. Acid-base disorders in COVID-19 patients with acute respiratory distress syndrome. *J Clin Med*. (2022) 11:2093. doi: 10.3390/jcm11082093
9. Weil JV, Byrne-Quinn E, Sodal IE, Friesen WO, Underhill B, Filley GF. et al. Hypoxic ventilatory drive in normal man. *J Clin Investig.* (1970) 49:1061–72. doi: 10.1172/JCI106322
10. Mohan R, Duffin J. The effect of hypoxia on the ventilatory response to carbon dioxide in man. *Respir Physiol.* (1997) 108:101–15. doi: 10.1016/S0034-5687(97)00024-8
11. Moosavi SH, Golestanian E, Binks AP, Lansing RW, Brown R, Banzett RB. Hypoxic and hypercapnic drives to breathe generate equivalent levels of air hunger in humans. *J Appl Physiol.* (2003) 94:141–54. doi: 10.1152/japplphysiol.00594.2002
12. Ellul MA, Benjamin L, Singh B, Lant S, Michael BD, Easton A, et al. Neurological associations of COVID-19. *Lancet Neurol.* (2020) 19:767–83. doi: 10.1016/S1474-4422(20)30221-0
13. Solomon T. Neurological infection with SARS-CoV-2 - the story so far. *Nat Rev Neurol.* (2021) 17:65–6. doi: 10.1038/s41582-020-00453-w
14. Severinghaus JW. Simple, accurate equations for human blood O2 dissociation computations. *J Appl Physiol.* (1979) 46:599–602.
15. Laffey JG, Kavanagh BP. Hypocapnia. *N Engl J Med.* (2002) 347:43–53. doi: 10.1056/NEJMra012457
16. Todd S, Walsted ES, Grillo L, Livingston R, Menzies-Gow A, Hull JH. Novel assessment tool to detect breathing pattern disorder in patients with refractory asthma. *Respirology.* (2018) 23:284–90. doi: 10.1111/resp.13173
17. Turcato G, Panebianco L, Zaboli A, Scheurer C, Ausserhofer D, Wieser A, et al. Correlation between arterial blood gas and CT volumetry in patients with SARS-CoV-2 in the emergency department. *Int J Infect Dis.* (2020) 97:233–5. doi: 10.1016/j.ijid.2020.06.033
18. Jain P, Sinha N, Prasad M, Padole V. Clinical and laboratory profile of COVID-19 patients admitted at a tertiary care center in New Delhi and assessment of factors predicting disease severity. *Indian J Med Spec.* (2021) 12:59–63. doi: 10.4103/injms.injms_158_20
19. Sin DD, Man SFP, Marrie TJ. Arterial carbon dioxide tension on admission as a marker of in-hospital mortality in community-acquired pneumonia. *Am J Med.* (2005) 118:145–50. doi: 10.1016/j.amjmed.2004.10.014
20. Laserna E, Sibila O, Aguilar PR, Mortensen EM, Anzueto A, Blanquer JM, et al. Hypocapnia and Hypercapnia Are Predictors for ICU Admission and Mortality in Hospitalized Patients With Community-Acquired Pneumonia. *Chest.* (2012) 142:1193–9. doi: 10.1378/chest.12-0576
21. Yassin Z, Saadat M, Abtahi H, Rahimi Foroushani A, Peiman S. Prognostic value of on admission arterial PCO(2) in hospitalized patients with community-acquired pneumonia. *J Thorac Dis.* (2016) 8:2765–71. doi: 10.21037/jtd.2016.10.21
22. Jung B, Rimmele T, Le Goff C, Chanques G, Corne P, Jonquet O, et al. Severe metabolic or mixed acidemia on intensive care unit admission: incidence, prognosis and administration of buffer therapy. a prospective, multiple-center study. *Crit Care.* (2011) 15:R238. doi: 10.1186/cc10487
23. Kikuchi Y, Okabe S, Tamura G, Hida W, Homma M, Shirato K, et al. Chemosensitivity and Perception of Dyspnea in Patients with a History of Near-Fatal Asthma. *N Engl J Med.* (1994) 330:1329–34. doi: 10.1056/NEJM199405123301901
24. Martínez-Moragón E, Perpi,ñá M, Fullana J, Macián V, Lloris A, and Belloch A.. [Perception of dyspnea and treatment adherence in asthmatic patients]. *Arch Bronconeumol.* (2008) 44:459–463. doi: 10.1016/S1579-2129(08)60083-X
25. Barnes PJ, Szefler SJ, Reddel HK, Chipps BE. Symptoms and perception of airway obstruction in asthmatic patients: clinical implications for use of reliever medications. *J Allergy Clin Immunol.* (2019) 144:1180–6. doi: 10.1016/j.jaci.2019.06.040

26. Chang KC, Morrill CG, Chai H. Impaired response to hypoxia after bilateral carotid body resection for treatment of bronchial asthma. *Chest.* (1978) 73:667–9. doi: 10.1378/chest.73.5.667
27. von Leupoldt A, Sommer T, Kegat S, Eippert F, Baumann H, Klose H, et al. Down-regulation of insular cortex responses to dyspnea and pain in asthma. *Am J Respir Crit Care Med.* (2009) 180:232–8. doi: 10.1164/rccm.200902-0300OC
28. Dubois M, Chenivesse C, Raux M, Morales-Robles A, Nierat M-C, Garcia G, et al. Neurophysiological evidence for a cortical contribution to the wakefulness-related drive to breathe explaining hypocapnia-resistant ventilation in humans. *J Neurosci.* (2016) 36:10673–82. doi: 10.1523/JNEUROSCI.2376-16.2016
29. Del Negro CA, Funk GD, Feldman JL. Breathing matters. *Nat Rev Neurosci.* (2018) 19:351–67. doi: 10.1038/s41583-018-0003-6
30. Teppema LJ, Dahan A. The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiol Rev.* (2010) 90:675–754. doi: 10.1152/physrev.00012.2009
31. Hansen J, Sander M. Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol.* (2003) 546(Pt 3):921–929. doi: 10.1113/jphysiol.2002.031765
32. Nattie E, Li A. Central chemoreceptors: locations and functions. *Compr Physiol.* (2012) 2:221–54. doi: 10.1002/cphy.c100083
33. Matschke J, Lütgehetmann M, Hagel C, Sperhake JP, Schröder AS, Edler C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* (2020) 19:919–29. doi: 10.1016/S1474-4422(20)30308-2
34. von Weyhern C.H., Kaufmann I, Neff F, and Kremer M. Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *The Lancet.* (2020) 395. doi: 10.1016/S0140-6736(20)31282-4
35. Lee JC, Nallani R, Cass L, Bhalla V, Chiu AG, Villwock JA, et al. Systematic review of the neuropathologic findings of post-viral olfactory dysfunction: implications and novel insight for the COVID-19 pandemic. *Am J Rhinol Allergy.* (2021) 35:323–33. doi: 10.1177/1945892420957853
36. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci.* (2021) 24:168–75. doi: 10.1038/s41593-020-00758-5
37. Jareonsettasin P, Otero-Millan J, Ward BKBK, Roberts DCDC, Schubert MC, Zee DSDS. Multiple time courses of vestibular set-point adaptation revealed by sustained magnetic field stimulation of the labyrinth. *Curr Biol.* (2016) 26:1359–66. doi: 10.1016/j.cub.2016.03.066
38. Paintal AS. Vagal sensory receptors and their reflex effects. *Physiol Rev.* (1973) 53:159–227. doi: 10.1152/physrev.1973.53.1.159
39. Lin RL, Gu Q, Lee LY. Hypersensitivity of vagal pulmonary afferents induced by tumor necrosis factor alpha in mice. *Front Physiol.* (2017) 8:411. doi: 10.3389/fphys.2017.00411
40. Lee LY, Pisarri TE. Afferent properties and reflex functions of bronchopulmonary C-fibers. *Respir Physiol.* (2001) 125:47–65. doi: 10.1016/S0034-5687(00)00204-8
41. Jacono FJ, Mayer CA, Hsieh YH, Wilson CG, Dick TE. Lung and brainstem cytokine levels are associated with breathing pattern changes in a rodent model of acute lung injury. *Respir Physiol Neurobiol.* (2011) 178:429–38. doi: 10.1016/j.resp.2011.04.022
42. Kubin L, Alheid GF, Zuperku EJ, and McCrimmon DR. Central pathways of pulmonary and lower airway vagal afferents. *J Appl Physiol.* (2006) 101:618–627. doi: 10.1152/japplphysiol.00252.2006
43. Jonkman AH, de Vries HJ, Heunks LMA. Physiology of the respiratory drive in ICU patients: implications for diagnosis and treatment. *Crit Care.* (2020) 24:104. doi: 10.1186/s13054-020-2776-z
44. White MD. Components and mechanisms of thermal hyperpnea. *J Appl Physiol.* (2006) 101:655–63. doi: 10.1152/japplphysiol.00210.2006
45. Vaporidi K, Akoumianaki E, Telias I, Goligher EC, Brochard L, Georgopoulos D. Respiratory drive in critically Ill patients. Pathophysiology and clinical implications. *Am J Respir Crit Care Med.* (2019) 201:20–32. doi: 10.1164/rccm.201903-0596SO
46. 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
47. Hopkins DA, Holstege G. Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. *Exp Brain Res.* (1978) 32:529–47. doi: 10.1007/BF00239551
48. Takahashi M, Tsunemi T, Miyayosi T, Mizusawa H. Reversible central neurogenic hyperventilation in an awake patient with multiple sclerosis. *J Neurol.* (2007) 254:1763–4. doi: 10.1007/s00415-007-0662-0
49. Vural A, Arsava EM, Dericioglu N, Topcuoglu MA. Central neurogenic hyperventilation in anti-NMDA receptor encephalitis. *Intern Med.* (2012) 51:2789–92. doi: 10.2169/internalmedicine.51.8215
50. Alkhachroum AM, Saeed S, Kaur J, Shams T, DeGeorgia MA. A case of neuro-behcet's disease presenting with central neurogenic hyperventilation. *Am J Case Rep.* (2016) 17:154–9. doi: 10.12659/AJCR.895382
51. Nystad D, Salvesen R, Nielsen EW. Brain stem encephalitis with central neurogenic hyperventilation. *J Neurol Neurosurg Psychiatry.* (2007) 78:107–8. doi: 10.1136/jnnp.2006.094375
52. Turner WA, Critchley M. Respiratory disorders in epidemic encephalitis. *Brain.* (1925) 48:72–104. doi: 10.1093/brain/48.1.72
53. Dale RC, Church AJ, Surtees RAH, Lees AJ, Adcock JE, Harding B, et al. Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity. *Brain.* (2004) 127(Pt 1):21–33. doi: 10.1093/brain/awh008
54. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, Van den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Sci Adv.* (2020) 6:eabc5801. doi: 10.1126/sciadv.abc5801
55. Lozada-Nur F, Chainani-Wu N, Fortuna G, Sroussi H. Dysgeusia in COVID-19: Possible Mechanisms and Implications. *Oral Surg Oral Med Oral Pathol Oral Radiol.* (2020) 130:344–6. doi: 10.1016/j.oooo.2020.06.016
56. Fjaeldstad AW, Stiller-Stut F, Gleesborg C, Kringelbach ML, Hummel T, Fernandes HM. Validation of olfactory network based on brain structural connectivity and its association with olfactory test scores. *Front Syst Neurosci.* (2021) 15:638053. doi: 10.3389/fnsys.2021.638053
57. Harper RM, Poe GR, Rector DM, Kristensen MP. Relationships between hippocampal activity and breathing patterns. *Neurosci Biobehav Rev.* (1998) 22:233–6. doi: 10.1016/S0149-7634(97)00010-9
58. Nobis WP, González Otárula KA, Templer JW, Gerard EE, VanHaerents S, Lane G, et al. The effect of seizure spread to the amygdala on respiration and onset of ictal central apnea. *J Neurosurg.* (2019) 132:1313–23. doi: 10.3171/2019.1.JNS183157
59. Koizumi H, Nomura K, Yokota Y, Enomoto A, Yamanishi T, Iida S, et al. Regulation of trigeminal respiratory motor activity in the brainstem. *J Dent Res.* (2009) 88:1048–53. doi: 10.1177/0022034509345998
60. Harper RM, Frysinger RC, Trelease RB, Marks JD. State-dependent alteration of respiratory cycle timing by stimulation of the central nucleus of the amygdala. *Brain Res.* (1984) 306:1–8. doi: 10.1016/0006-8993(84)90350-0
61. Ogren JA, Macey PM, Kumar R, Woo MA, Harper RM. Central autonomic regulation in congenital central hypoventilation syndrome. *Neuroscience.* (2010) 167:1249–56. doi: 10.1016/j.neuroscience.2010.02.078
62. Ogren JA, Macey PM, Kumar R, Fonarow GC, Hamilton MA, Harper RM. et al. Impaired cerebellar and limbic responses to the valsalva maneuver in heart failure. *Cerebellum.* (2012) 11:931–8. doi: 10.1007/s12311-012-0361-y
63. Burki NK, Lee LY. Mechanisms of dyspnea. *Chest.* (2010) 138:1196–201. doi: 10.1378/chest.10-0534
64. Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, and Chiumello D. Covid-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome'. *Am J Respir Crit Care Med.* (2020) 201:1299–300. doi: 10.1164/rccm.202003-0817LE
65. Marini JJ, Gattinoni L. Management of COVID-19 respiratory distress. *JAMA.* (2020) 323:2329–30. doi: 10.1001/jama.2020.6825
66. Laviolette L, Laveneziana P. Dyspnoea: a multidimensional and multidisciplinary approach. *Eur Respir J.* (2014) 43:1750–62. doi: 10.1183/09031936.00092613
67. Banzett RB, O'Donnell CR, Guilfoyle TE, Parshall MB, Schwartzstein RM, Meek PM, et al. Multidimensional Dyspnea Profile: an instrument

for clinical and laboratory research. *Eur Respir J.* (2015) 45:1681–91. doi: 10.1183/09031936.00038914
68. von Leupoldt A, Sommer T, Kegat S. Baumann HJ, Klose H, Dahme B, et al. The Unpleasantness of perceived dyspnea is processed in the anterior insula and amygdala. *Am J Respir Crit Care Med.* (2008) 177:1026–32. doi: 10.1164/rccm.200712-1821OC
69. Schön D, Rosenkranz M, Regelsberger J, Dahme B, Büchel C, von Leupoldt A. Reduced perception of dyspnea and pain after right insular cortex lesions. *Am J Respir Crit Care Med.* (2008) 178:1173–9. doi: 10.1164/rccm.200805-731OC

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.

frontiers | Frontiers in Neurology

BRIEF RESEARCH REPORT
published: 23 June 2022
doi: 10.3389/fneur.2022.904796

Frequency of Neurological Diseases After COVID-19, Influenza A/B and Bacterial Pneumonia

Pardis Zarifkar[1], *Costanza Peinkhofer*[1], *Michael E. Benros*[2,3]* *and Daniel Kondziella*[1,4]*

[1] Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, [2] Copenhagen Research Center for Mental Health–CORE, Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark, [3] Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark, [4] Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

Introduction: COVID-19 might affect the incidence of specific neurological diseases, but it is unknown if this differs from the risk following other infections. Here, we characterized the frequency of neurodegenerative, cerebrovascular, and immune-mediated neurological diseases after COVID-19 compared to individuals without COVID-19 and those with other respiratory tract infections.

Methods: This population-based cohort study utilized electronic health records covering ∼50% of Denmark's population (n = 2,972,192). Between 02/2020 and 11/2021, we included individuals tested for COVID-19 or diagnosed with community-acquired bacterial pneumonia in hospital-based facilities. Additionally, we included individuals tested for influenza in the corresponding pre-pandemic period between 02/ 2018 and 11/2019. We stratified cohorts for in- and outpatient status, age, sex, and comorbidities.

Results: In total, 919,731 individuals were tested for COVID-19, of whom 43,375 tested positive (35,362 outpatients, 8,013 inpatients). Compared to COVID-negative outpatients, COVID-19 positive outpatients had an increased RR of Alzheimer's disease (RR = 3.5; 95%CI: 2.2–5.5) and Parkinson's disease (RR = 2.6; 95%CI: 1.7–4.0), ischemic stroke (RR = 2.7; 95%CI: 2.3–3.2) and intracerebral hemorrhage (RR = 4.8; 95%CI: 1.8–12.9). However, when comparing to other respiratory tract infections, only the RR for ischemic stroke was increased among inpatients with COVID-19 when comparing to inpatients with influenza (RR = 1.7; 95%CI: 1.2–2.4) and only for those >80 years of age when comparing to inpatients with bacterial pneumonia (RR = 2.7; 95%CI: 1.2–6.2). Frequencies of multiple sclerosis, myasthenia gravis, Guillain-Barré syndrome and narcolepsy did not differ after COVID-19, influenza and bacterial pneumonia.

Conclusion: The risk of neurodegenerative and cerebrovascular, but not neuroimmune, disorders was increased among COVID-19 positive outpatients compared to COVID-negative outpatients. However, except for ischemic stroke, most neurological disorders were not more frequent after COVID-19 than after other respiratory infections.

Keywords: COVID-19, SARS-CoV-2, bacterial pneumonia, Alzheimer's disease (AD), Parkinson's disease (PD), ischemic stroke (IS), auto-immune

OPEN ACCESS

Edited by:
Erwin Chiquete, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico

Reviewed by:
Martin Rakuša, Maribor University Medical Centre, Slovenia
Marialuisa Zedde, IRCCS Local Health Authority of Reggio Emilia, Italy

***Correspondence:**
Michael E. Benros
Michael.eriksen.benros@regionh.dk
Daniel Kondziella
daniel.kondziella@regionh.dk

Specialty section:
This article was submitted to Neuroepidemiology, a section of the journal Frontiers in Neurology

Received: *25 March 2022*
Accepted: *26 May 2022*
Published: *23 June 2022*

Citation:
Zarifkar P, Peinkhofer C, Benros ME and Kondziella D (2022) Frequency of Neurological Diseases After COVID-19, Influenza A/B and Bacterial Pneumonia. Front. Neurol. 13:904796. doi: 10.3389/fneur.2022.904796

INTRODUCTION

Neurological symptoms, including headache and anosmia, are present in more than 80% of hospitalized COVID-19 patients (1, 2). There is also evidence of an inflammatory hypercoagulable state with subsequent cerebrovascular incidents, (3–8) and case descriptions exist of Guillain-Barré syndrome (GBS) and Parkinson's disease following COVID-19 (9, 10). To our knowledge, however, epidemiologic studies investigating the incidence of specific neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease or auto-immune disorders (e.g., multiple sclerosis, narcolepsy, and myasthenia gravis) after COVID-19 are still missing.

The aim of this study was to provide the first broad investigation into the influence of COVID-19 on neurological diseases, providing a rapid glimpse based on the electronic health record data currently available while awaiting more detailed longitudinal nationwide registry studies. Specifically, we aimed to (1) characterize the frequency and relative risk (RR) of neurodegenerative, cerebrovascular, and immune-mediated diseases in patients with COVID-19, and (2) to compare the risk of being diagnosed with a neurological disease after COVID-19 to the risk after influenza A/B and community-acquired bacterial pneumonia.

METHODS

Study Population

Using previously published methods, (5) we extracted patient data from electronic health records covering 2,972,192 individuals, equating to ∼50% of the Danish population from two (of five in total) well-defined administrative regions in Denmark, i.e., the Capital Region (Greater Copenhagen and Bornholm) and Region Zealand. The electronic health records (EPIC, version 2021, Wisconsin, USA) Slicer-Dicer function, were searched from implementation in 2016 to November 27, 2021. All individuals ≥ 18 years who were tested in a hospital setting for COVID-19, influenza A/B (referred to as influenza) or diagnosed with community-acquired bacterial pneumonia (referred to as bacterial pneumonia) were followed for new-onset neurological diseases up to 12 months later. Included individuals were (1) hospitalized patients tested for COVID-19, influenza, or diagnosed with bacterial pneumonia during admission (referred to as "inpatients"), and (2) non-hospitalized patients tested during ambulatory visits, or healthy individuals tested in hospital-based facilities that serve the general population (referred to as "outpatients"). Individuals tested for COVID-19 in the community setting (e.g., over-the-counter antigen tests or PCR tests from private providers and primary care settings) were not captured. We also collected anonymized aggregated data on age, sex, smoking, pre-existing comorbidities, laboratory data, medical prescriptions, and history of neurological disorders. Data extraction and analysis were conducted in consultation with EPIC data experts from our institution (Rigshospitalet, Copenhagen University Hospital) according to previous publications by our group (5).

Slicer Dicer search strategies are detailed in **Supplementary Table 1**.

Study Period

The study period spanned from February 27, 2018 to November 27, 2021. COVID-19 and bacterial pneumonia patients were included from February 27, 2020 (the first reported case of COVID-19 in Denmark) (11) to November 27, 2021 (the day before the first reported case of the omicron variant in Denmark) (12), and influenza patients from February 27, 2018, to November 27, 2019 (the corresponding 2-year pre-pandemic period).

Assessment of Infection Exposure

COVID-19 or influenza positive cases were determined by positive reverse-transcriptase polymerase chain reaction assays of nasal, pharyngeal, or tracheal samples. We defined COVID-19 or influenza negative cases as having negative laboratory test results and (for those tested more than once) no previous history of positive laboratory tests.

Assessment of Neurological Outcomes

Using ICD-10 diagnoses, we identified individuals with neurodegenerative (Alzheimer's disease, Parkinson's disease), cerebrovascular (ischemic stroke, intracerebral hemorrhage, subarachnoid hemorrhage), and immune-mediated (multiple sclerosis, GBS, myasthenia gravis, and narcolepsy) disorders. ICD-10 diagnosis codes are detailed in **Supplementary Table 1**.

Statistical Analyses

We calculated the risk of new-onset neurological diagnoses in the acute (1 month), subacute (3 and 6 months), and chronic (12 months) phases after a diagnosis of COVID-19, influenza, or bacterial pneumonia. Specifically, we calculated the relative risk (RR) of diagnosis rates with 95% confidence intervals (CI) and stratified the study population across admittance status (inpatients and outpatients), age (18–39, 40–59, 60–79, and ≥80 years), and sex (male and female), using R studio (2021 Vienna, Austria). To reduce the risk of type II errors, statistical analyses were only conducted for diseases with ≥4 cases in each group. Hospitalization and delirium [which occurs at higher rates in COVID-19 patients (**Table 1**)] can lead to cognitive decline and aggravate neurodegenerative diseases (13–16). Thus, to best balance recovery from hospitalization and allow for reliable diagnoses, Alzheimer's disease and Parkinson's disease patients diagnosed within the first 3 months after admission were excluded from 6 and 12-month assessments (14–16).

Sensitivity Analyses

To search for possible bias related to restricted access to diagnostic work-up during the pandemic, the prevalence of disease-specific diagnostic procedures (including cerebral fluorodeoxyglucose (FDG)- positron emission tomography (PET)-18 for Alzheimer's disease and single-photon emission computerized tomography (SPECT) for Parkinson's disease), medical prescriptions and common risk factors, including smoking status, and pre-existing comorbidities were compared across groups using chi-squared statistics with a Yates correction. Where there was a significant difference in risk factors

TABLE 1 | Clinical characteristics and demographics at baseline.

	Inpatient status at baseline				Outpatient status at baseline		
	COVID-19 positive (*n* = 8,013)	**COVID-19 negative (*n* = 230,686)**	**Influenza positive (*n* = 4,142)**	**Pneumonia (*n* = 1,474)**	**COVID-19 positive (*n* = 35,362)**	**COVID-19 negative (*n* = 645,670)**	**Influenza positive (*n* = 3,960)**
Age, *n* (%)							
Mean, years	66y	58y	68y	75y	48y	47y	52y
18–39	1,023 (12.8%)	65,333 (28%)	508 (12.3%)	44 (3%)	14,309 (40.1%)	258,412 (40%)	1,352 (34.1%)
40–59	1,841 (23%)	38,108 (20.9%)	854 (20.6%)	140 (9.5%)	12,526 (35.4%)	234,480 (36.3%)	1,482 (37.4%)
60–79	3,128 (39%)	76,865 (33.3%)	1,743 (42.08%)	671 (45.5%)	5,731 (16.2%)	128,382 (19.9%)	929 (23.4%)
≥80	2,021 (25.2%)	40,380 (17.5%)	1,037 (25%)	619 (42%)	2,796 (7.9%)	24,396 (3.8%)	197 (5%)
Sex, *n* (%)							
Females	3,567 (44.5%)	131,399 (57%)	2,257 (54%)	625 (42.4%)	20,913 (59.1%)	368,142 (57%)	2,374 (59.9%)
Smoking status, *n* (%)							
Current or history of smoking (%)	3,141 (39.2%)	93,283 (40.4%)	2,053 (49.6%)	829 (56.2%)	6,180 (17.5%)	117,505 (18.2%)	931 (23.5%)
Pre-existing comorbidities, *n* (%)							
Celiac disease	11 (0.1%)	370 (0.2%)	1 (0.02%)	1 (0.07%)	51 (0.1%)	1,071 (0.2%)	3 (0.08%)
Delirium	149 (1.9%)	1,335 (1%)	19 (0.5%)	33 (2.2%)	127 (0.4%)	429 (0.1%)	1 (0.03%)
Diabetes mellitus, type 1	30 (0.4%)	719 (0.3%)	16 (0.4%)	7 (0.5%)	66 (0.2%)	974 (0.2%)	9 (0.2%)
Diabetes mellitus, type 2	501 (6.2%)	7,880 (3.4%)	142 (3.4%)	102 (6.9%)	4,397 (1.4%)	5,335 (0.8%)	30 (0.8%)
Hashimoto's auto-immune thyroiditis	11 (0.14%)	417 (0.2%)	5 (0.1%)	0 (0%)	67 (0.2%)	1,103 (0.2%)	3 (0.08%)
Hypercholesterolemia	431 (5.4%)	9,571 (4.2%)	118 (2.9%)	83 (5.6 %)	560 (1.6%)	8,303 (1.3%)	39 (1%)
Hypertension	1,681 (21%)	36,754 (15.9%)	519 (12.5%)	411 (27.9%)	2,155 (6.1%)	29,935 (4.6%)	155 (4%)
Ischemic stroke	340 (4.2%)	10,030 (4.4%)	53 (1.3%)	96 (6.5%)	442 (1.3%)	3,829 (0.6%)	26 (0.7%)
Obesity	356 (4.4%)	10,962 (4.8%)	60 (1.5%)	36 (2.4%)	959 (2.7%)	15,465 (2.4%)	56 (1.4%)
Rheumatoid arthritis	46 (0.6%)	982 (0.4%)	22 (0.5%)	13 (0.9%)	66 (0.2%)	995 (0.2%)	10 (0.2%)
Transitory cerebral ischemia	130 (1.6%)	4,197 (1.8%)	24 (0.6%)	37 (2.5%)	220 (0.6%)	2,505 (0.4%)	7 (0.2%)

between groups, the populations at risk were excluded from comparative analyses.

Ethics and Data Availability Statement

The Scientific Ethics Committee of the Capital Region of Denmark waives approval for register-based studies on aggregated anonymized data (Section 14.2, Committee Act 2). The datasets included in this study are freely available to medical and administrative staff in Denmark with access to electronic health records in EPIC.

RESULTS

Between February 27, 2020 and November 27, 2021, a total of 919,731 individuals were tested for COVID-19 in a hospital-based facility. Of these, 43,375 individuals had a positive COVID-19 test (equating to 20% of the COVID-19 positive population in the surveyed areas) (17) and 876,356 had a negative COVID-19 test (40% of the COVID-negative population in these areas) (18). A total of 1,474 individuals were diagnosed with bacterial pneumonia in a hospital-based facility during the same period. Between February 27, 2018 and November 27, 2019, a total of

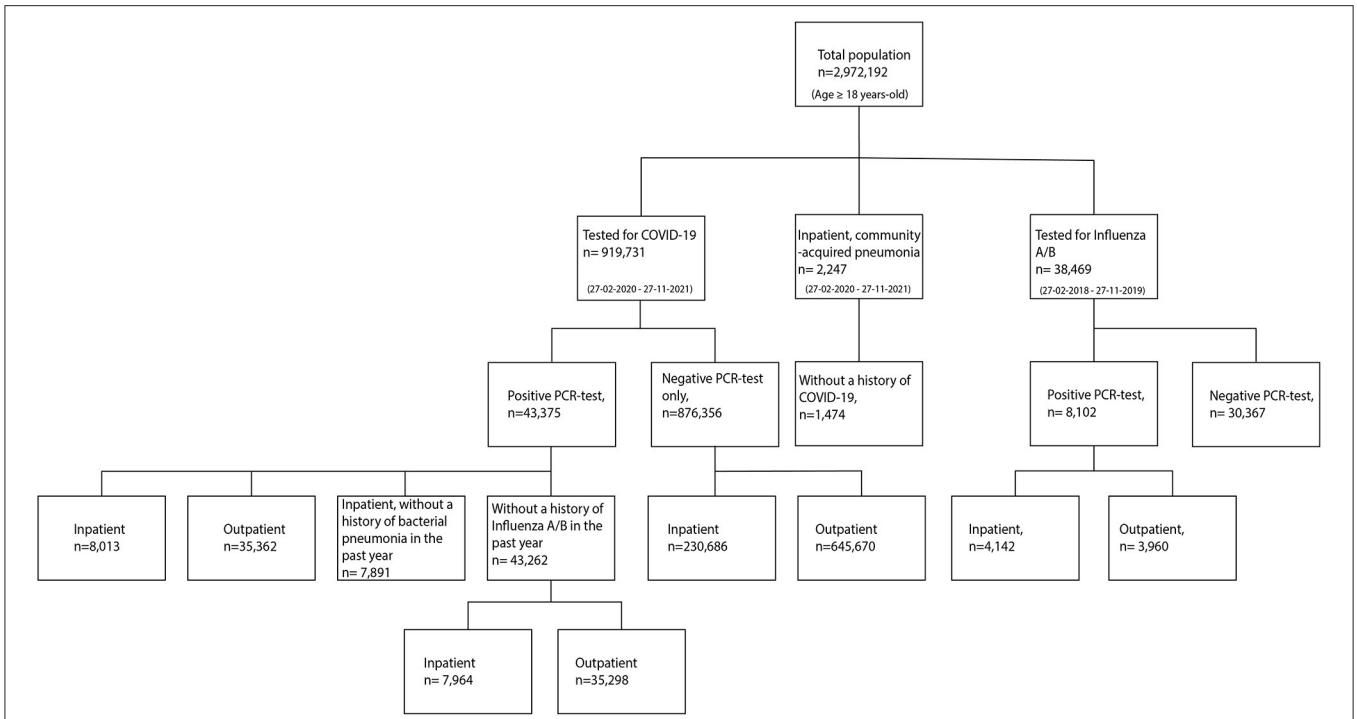


FIGURE 1 | Flowchart of individuals tested for COVID-19 or Influenza A/B, and diagnosed with community-acquired bacterial pneumonia.

8,102 individuals were tested positive for influenza. A flowchart of the study population is depicted in **Figure 1**, and demographic and clinical characteristics are detailed in **Table 1**.

Risk Factors at Baseline

The prevalence and comparative analyses of clinical baseline characteristics are detailed in **Table 1** and **Supplementary Table 2**. Compared to COVID-negative individuals (in- and outpatients separately and combined) and influenza inpatients, COVID-19 positive individuals carried higher rates of some pre-existing cerebrovascular risk factors, (19) including hypercholesterolemia, diabetes mellitus type 2 and hypertension. Compared to COVID-negative outpatients and influenza inpatients, COVID-19 positive individuals also had higher rates of obesity, and a history of transitory ischemic attack. By contrast, smoking rates were higher among COVID-negative individuals, and influenza and pneumonia inpatients. Pneumonia inpatients also had higher rates of past transitory ischemic attacks. There were no other differences in cerebrovascular risk factors, nor in the rates of pre-existing auto-immune disorders.

The Incidence of New-Onset Neurodegenerative, Cerebrovascular and Auto-Immune Disorders

The incidence, absolute risk, and RR of all neurological diseases in COVID-19 positive and COVID-negative individuals are depicted in **Figure 2** and **Table 2**. Stratifications by age and sex are detailed in **Supplementary Table 3**, and stratifications by in- and outpatient status are detailed in **Supplementary Table 4**.

The incidences, absolute risks, and RR's of all neurological diseases in COVID-19 positive, influenza positive, and bacterial pneumonia patients are depicted in **Table 3** and **Supplementary Table 5**.

Alzheimer's Disease and Parkinson's Disease

The RR of Alzheimer's disease was increased 6 and 12 months after a positive test in COVID-19 positive compared to COVID-negative individuals (in- and outpatients combined), and separately among in- and outpatients (in- and outpatients: RR = 3.5; 95%CI: 2.5–5.6 at 6 months and RR = 3.4; 95%CI: 2.3–5.1 at 12 months; inpatients: six (RR = 3.3; 95%CI: 1.7–9.3 at 6 months and RR = 3.7; 95%CI: 1.7–8.0 at 12 months; outpatients; RR = 3.6; 95%CI: 2.1–6.1 at 6 months and RR = 3.5, 95%CI: 2.2–5.5 at 12 months).

Notably, COVID-19 positive individuals had a higher frequency of delirium, an independent risk factor for dementia (20) (0.6 vs. 0.3%, $\chi^2 = 128.2$, $p < 0.00001$), compared to COVID-negative individuals. After exclusion of those with a history of delirium, the RR for Alzheimer's disease remained elevated in COVID-19 individuals (in- and outpatients combined) (**Supplementary Table 6**), and separately in both in- and outpatients. COVID-19 positive individuals also had a higher frequency of cerebrovascular

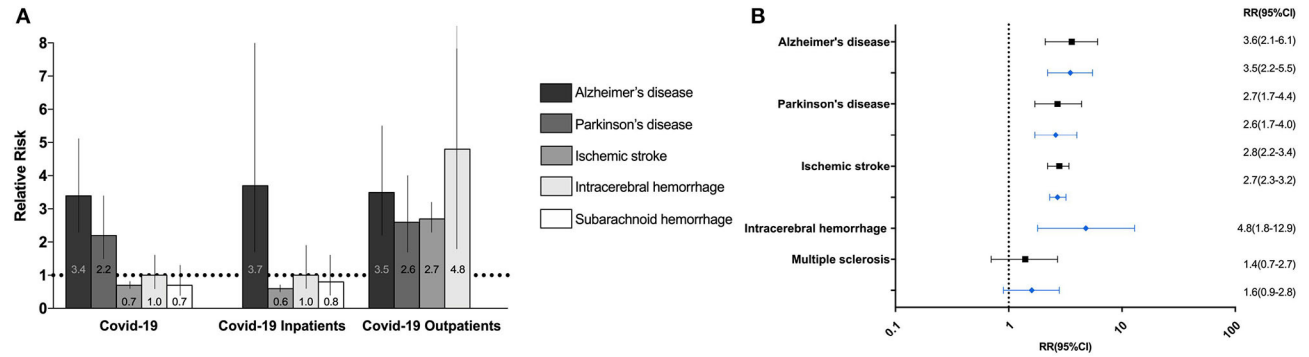


FIGURE 2 | Relative risk of neurodegenerative, cerebrovascular and neuroimmune neurological disorders after COVID-19 **(A)**. Bar chart of the relative risks (RR) of new-onset neurodegenerative disorders and cerebrovascular events after 12 months in COVID-19 positive versus COVID-negative individuals, inpatients, and outpatients. Barcharts depict RR with 95% confidence intervals. **(B)** Forest plot of the RR of new-onset neurodegenerative, cerebrovascular and neuroimmune disorders six (black) and twelve (blue) after COVID-19 in COVID-19 positive outpatients compared to negative outpatients.

risk factors (**Table 1** and **Supplementary Table 2**). After exclusion of those with cerebrovascular risk factors, the RR for Alzheimer's disease remained elevated in COVID-19 individuals (**Supplementary Table 6**). In the inpatient group, there were too few cases for statistical analyses.

The RR of Parkinson's disease was increased 6 and 12 months after a positive test in COVID-19 positive compared to COVID-negative individuals (in- and outpatients combined) and specifically in COVID-19 outpatients (in- and outpatients combined: RR = 2.4; 95%CI: 1.5–3.8 at 6 months and RR = 2.2; 95%CI: 1.5–3.4 at 12 months; outpatients: RR = 2.7; 95%CI: 1.7–4.4 at 6 months and RR = 2.6; 95% CI: 1.7–4.0. Among inpatients, there were not enough Parkinson's disease cases to conduct meaningful statistics. Finally, there was no excess risk of Alzheimer's disease or Parkinson's disease compared to influenza or bacterial pneumonia inpatients (**Table 3**).

From February 27, 2020 to November 27, 2021, 1,137 cerebral PET-FDG-18 scans were conducted in COVID-19 positive individuals and 23,889 in COVID-negative individuals, corresponding to a 3% scanning rate in each group ($\chi^2 = 1.7$, $p = 0.19$). Similarly, there was no difference in the number of SPECT scans among COVID-19 positive and negative individuals (0.04 vs. 0.03%, $\chi^2 = 2.1$, $p = 0.14$), indicating equal access to these diagnostic tools.

Ischemic Stroke

The frequency of new-onset ischemic stroke did not differ significantly between COVID-19 positive and COVID-negative individuals (in- and outpatients combined), nor between COVID-19 positive and COVID-negative inpatients (**Table 2** and **Supplementary Table 4**). Compared to COVID-negative outpatients, the RR of ischemic stroke was increased three, six, and 12 months after a positive test in COVID-19 positive outpatients but was insignificant within the first month (RR = 1.4, 95%CI: 1.0–2.0, $p = 0.08$ after 1 month, RR = 2.3; 95% CI: 1.8–3.0 after 3 months, RR = 2.8; 95%CI: 2.2–3.4 after 6 months and RR = 2.7; 95%CI: 2.3–3.2 after 12 months). Notably, age-specific stratifications showed that the relative risk was highest among younger patients between 40 and 59 years (**Supplementary Table 3**). After exclusion of cerebrovascular risk factors, the RR for ischemic stroke remained elevated in COVID-19 positive outpatients (RR = 1.8; 95%CI: 1.5–2.8 after 3 months, RR = 2.2; 95% CI:1.5–3.1 after 6 months, and RR = 2.1; 95% CI:1.5–2.8 after 12 months).

Compared to influenza positive inpatients, COVID-19 inpatients had an increased RR of ischemic stroke one, three, and 6 months after a positive test (RR = 1.7; 95%CI: 1.1–2.6 after 1 month; RR = 1.7; 95%CI: 1.2–2.5 after 3 months; RR = 1.7; 95%CI: 1.2–2.4 after 6 months). After 12 months, the RR between the two groups was decreased (RR = 1.3; 95%CI: 1.0–1.8, $p = 0.09$). After removal of cerebrovascular risk factors, the RR of ischemic stroke remained increased in COVID-19 inpatients (RR = 3.4; 95%CI: 1.4–8.2 after 1 month; RR = 3.0; 95%CI: 1.5–6.3 after 3 months; RR = 3.5; 95%CI: 1.7–7.2 after 6 months; RR = 2.8; 95%CI: 1.5–5.0 after 12 months; **Supplementary Table 6**).

The frequency of ischemic stroke did not differ significantly between COVID-19 positive and bacterial pneumonia inpatients (**Table 3**). After removal of individuals with significant cerebrovascular risk factors, there remained no significant difference between groups (**Supplementary Table 6**). After stratification for age, the incidence of ischemic stroke was increased in COVID-19 positive inpatients aged ≥ 80 (RR = 2.7; 95%CI: =1.2–6.2), but not in other age groups.

Intracerebral and Subarachnoid Hemorrhage

The RR of intracerebral hemorrhage was increased 12 months after a positive test in COVID-19 positive compared to COVID-negative outpatients (RR = 4.8; 95%CI: 1.8–12.9). There were no other differences in the rates of intracerebral and subarachnoid hemorrhage between groups (**Tables 2**, **3** and **Supplementary Tables 4, 5**). Notably, COVID-19 outpatients received higher rates of intravenous thrombolysis, a risk factor for medically induced intracerebral hemorrhage (21) (0.14% in COVID-19 positive vs. 0.02% in COVID-negative outpatients,

TABLE 2 | Relative risk of neurodegenerative, cerebrovascular and neuroimmune disorders in COVID-19 positive compared to COVID-negative individuals.

	COVID-19 positive (*n* = 43,375)	**COVID-19 negative (*n* = 876,356)**	**RR (95%CI)**	**COVID-19 positive (*n* = 43,375)**	**COVID-19 negative (*n* = 876,356)**	**RR (95%CI)**	**COVID-19 positive (*n* = 43,375)**	**COVID-19 negative (*n* = 876,356)**	**RR (95%CI)**	**COVID-19 positive (*n* = 43,375)**	**COVID-19 negative (*n* = 876,356)**	**RR (95%CI)**
	1 month, *n* (%)			**3 months, *n* (%)**			**6 months, *n* (%)**			**12 months, *n* (%)**		
Alzheimer's disease♦	-	-	-	-	-	-	21 (0.05%)	121 (0.01%)	**3.5 (2.2–5.6)***	29 (0.07%)	171 (0.02%)	**3.4 (2.3–5.1)***
Parkinson's disease♦	-	-	-	-	-	-	20 (0.05%)	169 (0.02%)	**2.4 (1.5–3.8)***	26 (0.06%)	234 (0.03%)	**2.2 (1.5–3.4)***
Ischemic stroke	117 (0.3%)	6,251 (0.7%)	**0.4 (0.3–0.5)***	180 (0.4%)	6,908 (0.8%)	**0.6 (0.5–0.7)***	227 (0.5%)	7,365 (0.8%)	**0.6 (0.5–0.7)***	281 (0.6%)	7,910 (0.9%)	0.7 (0.6–0.8)
Intracerebral hemorrhage	7 (0.02%)	250 (0.03%)	0.6 (0.3–1.2)	10 (0.02%)	280 (0.03%)	0.7 (0.4–1.4)	13 (0.03%)	306 (0.03%)	0.9 (0.5–1.5)	16 (0.04%)	330 (0.04%)	1.0 (0.6–1.6)
Subarachnoid hemorrhage	4 (0.01%)	201 (0.02%)	0.4 (0.1–1.1)	5 (0.01%)	233 (0.03%)	0.4 (0.2–1.1)	6 (0.01%)	254 (0.03%)	0.5 (0.2–1.1)	ll10 (0.02%)	289 (0.03%)	0.7 (0.4–1.3)
Guillain-Barré syndrome	1 (0.002%)	52 (0.006%)	N/A	2 (0.005%)	58 (0.007)	N/A	2 (0.005)	61 (0.007%)	N/A	2 (0.005)	64 (0.007%)	N/A
Multiple sclerosis	4 (0.01%)	185 (0.02%)	0.4 (0.2–1.2)	6 (0.01%)	246 (0.03%)	0.5 (0.2–1.1)	11 (0.03%)	293 (0.03%)	0.8 (0.4–1.4)	14 (0.03%)	332 (0.04%)	0.9 (0.5–1.5)
Myasthenia gravis	1 (0.002%)	44 (0.005%)	N/A	1 (0.002%)	59 (0.007%)	N/A	1 (0.002%)	61 (0.007%)	N/A	1 (0,002%)	71 (0.008%)	N/A
Narcolepsy	0 (0.0%)	19 (0.002%)	N/A	0 (0.0%)	30 (0.003%)	N/A	0 (0.0%)	37 (0.004%)	N/A	0 (0.0%)	41 (0.005%)	N/A

Statistical analyses were only conducted for diseases with ≥ 4 cases in each group.

**Statistically significant RR ($p < 0.05$) are highlighted in bold.*

♦*Excluding inpatient cases of Alzheimer's disease and Parkinson's disease the first three months after hospitalization with COVID-19.*

TABLE 3 | Relative risk of neurodegenerative, cerebrovascular and neuroimmune disorders in inpatients with COVID-19 compared to influenza inpatients and community-acquired bacterial pneumonia inpatients.

	COVID- 19 positive (*n* = 7,964)	**Influenza positive (*n* = 4,142)**	**RR (95%CI)**	**COVID-19 positive (*n* = 7,964)**	**Influenza positive (*n* = 4,142)**	**RR (95%CI)**	**COVID-19 positive (*n* = 7,891)**	**Pneumonia (*n* = 1,474)**	**RR (95%CI)**	**COVID-19 positive (*n* = 7,891)**	**Pneumonia (*n* = 1,474)**	**RR (95%CI)**
	1 month, *n* (%)			**3 months, *n* (%)**			**1 month, *n* (%)**			**3 months, *n* (%)**		
Ischemic stroke	85 (1.07%)	26 (0.63%)	**1.7 (1.1–2.6)***	113 (1.4%)	34 (0.8%)	**1.7 (1.2–2.5)***	79 (1.0%)	14 (0.9%)	1.1 (0.6–1.9)	107 (1.4%)	19 (1.3%)	1.18 (0.6–1.7)
Intracerebral hemorrhage	6 (0.08%)	0 (0.0%)	N/A	8 (0.1%)	0 (0.0%)	N/A	9 (0.1%)	0 (0.0%)	N/A	8 (0.1%)	0 (0.0%)	N/A
Subarachnoid hemorrhage	4 (0.05%)	0 (0.0%)	N/A	5 (0.06%)	0 (0.0%)	N/A	6 (0.08%)	0 (0.0%)	N/A	5 (0.06%)	0 (0.0%)	N/A
Guillain-Barré syndrome	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	1 (0.07%)	N/A	1 (0.01%)	1 (0.07%)	N/A
Multiple sclerosis	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	1 (0.02%)	N/A	1 (0.01%)	2 (0.1%)	N/A	1 (0.01%)	2 (0.1%)	N/A
Myasthenia gravis	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A
Narcolepsy	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A
	6 months, *n* (%)			**12 months, *n* (%)**			**6 months, *n* (%)**			**12 months, *n* (%)**		
Alzheimer's disease	4 (0.05%)	1 (0.02%)	N/A	7 (0.09%)	3 (0.07%)	N/A	4 (0.05%)	0	N/A	7 (0.09%)	0 (0.0%)	N/A
Parkinson's disease	0 (0.0%)	0 (0.0%)	N/A	2 (0.03%)	4 (0.1%)	N/A	1 (0.01%)	0	N/A	3 (0.04%)	3 (0.2%)	N/A
Ischemic stroke	128 (1.6%)	39 (0.9%)	**1.7 (1.2–2.4)***	145 (1.8%)	58 (1.4%)	1.3 (1.0–1.8)	121 (1.5%)	23 (1.6%)	1.0 (0.6–1.5)	139 (1.8%)	28 (1.9%)	0.9 (0.6–1.4)
Intracerebral hemorrhage	10 (0.1%)	0 (0.0%)	N/A	11 (0.14%)	1 (0.02%)	N/A	10 (0.1%)	0 (0.0%)	N/A	11 (0.1%)	0 (0.0%)	N/A
Subarachnoid hemorrhage	5 (0.06%)	0 (0.0%)	N/A	7 (0.09%)	0	N/A	5 (0.1%)	0 (0.0%)	N/A	7 (0.1%)	0 (0.0%)	N/A
Guillain-Barré syndrome	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	1 (0.07%)	N/A	1 (0.01%)	1 (0.07%)	N/A
Multiple sclerosis	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	2 (0.05%)	N/A	1 (0.01%)	2 (0.1%)	N/A	1 (0.01%)	2 (0.1%)	N/A
Myasthenia gravis	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A	1 (0.01%)	0 (0.0%)	N/A
Narcolepsy	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A	0 (0.0%)	0 (0.0%)	N/A

Statistical analyses were only conducted for diseases with ≥ 4 cases in each group.

**Statistically significant RR ($p < 0.05$) are highlighted in bold.*

$\chi^2 = 177.6$, $p < 0.0001$). After exclusion of those treated with intravenous thrombolysis, the RR of intracerebral hemorrhage remained elevated after 12 months (RR = 4.4, 95%CI 1.6–11.5). There were too few cases to carry out meaningful statistics after 1–6 months.

Multiple Sclerosis and Other Auto-Immune Disorders

The frequency of new-onset multiple sclerosis did not differ significantly between COVID-19 positive and COVID-negative individuals (in- and outpatients combined), nor separately across in- and outpatients (**Table 2** and **Supplementary Table 3**). There was also no significant difference in multiple sclerosis rates between COVID-19 positive inpatients and influenza inpatients (**Table 3**), and there were not enough cases to conduct meaningful statistics in pneumonia inpatients.

Among 43,375 COVID-19 individuals, one developed Guillain-Barré syndrome within 1 month (0.002%) and two (0.005%) within 3 months. One individual (0.002%) developed myasthenia gravis one through 12 months, and none (0.0%) developed narcolepsy (**Tables 2**, **3**). There were not enough cases to conduct meaningful comparisons between groups.

DISCUSSION

Key findings from this population-based cohort study covering roughly half of Denmark's population include an increased frequency of new-onset neurodegenerative and cerebrovascular (but not neuroimmune) disorders in COVID-19 positive compared to COVID-negative individuals. However, when comparing the frequencies of these disorders after COVID-19 with those after influenza and community-acquired pneumonia, we found no significant differences, except for ischemic stroke.

Neurodegenerative Diseases

Alzheimer's disease was 3.4 times more frequent and Parkinson's disease was 2.2 times more frequent in COVID-19 positive than COVID-negative individuals, 12 months after a COVID-19 test. These findings should be considered in light of the prolonged temporal course and the complex pathophysiology of these disorders, including a possible role for neuroinflammation: it is hypothesized that the body's innate response and subsequent inflammatory processes can induce a toxic cycle of accumulating β-amyloid and alpha-synuclein peptides (the pathologic hallmarks of Alzheimer's and Parkinson's diseases) (22–26). In support of this, unexpectedly high amounts of β-amyloid peptides have been discovered in brain autopsies of young deceased patients with COVID-19 (27). Other factors such as fatigue, depression, and anxiety after COVID-19 may also contribute to the development of neurodegenerative disorders (20, 28–34). Moreover, it is uncertain if the risk of Alzheimer's disease and Parkinson's disease differs after COVID-19 compared to after influenza and bacterial pneumonia. Finally, the scientific focus on long-term sequelae after COVID-19 may have led to increased recognition by clinicians and hence earlier diagnosis, perhaps explaining some of the observed increase in neurodegenerative diagnoses.

Cerebrovascular Disorders

Ischemic Stroke

New-onset ischemic stroke was 2.3 times more frequent in COVID-19 positive than COVID-negative outpatients after 3 months. Ischemic stroke was also 1.7 times more frequent in COVID-19 inpatients compared to influenza inpatients in the early and subacute phases after a positive test, as supported by previous retrospective studies (albeit with shorter observation periods) (5, 35). Ischemic stroke was also 2.7 times more frequent in COVID-19 inpatients compared to bacterial pneumonia among the elderly. In our study, the overall incidence of ischemic stroke in COVID-19 positive inpatients (1.8%) is well in line with previously reported data (0.4-2.7%) (36–39). Of note, age-specific stratifications showed that the relative risk for ischemic stroke was highest amongst patients between 40 and 59 years. A recent study of 37,379 Medicare fee-for-service beneficiaries aged ≥65 years diagnosed with COVID-19 (36) and a multi-center study involving a further 423 patients (40) similarly found an increase in ischemic stroke among younger patients when compared to population studies before the pandemic.

Increased rates of ischemic stroke in COVID-19 patients may occur for several reasons. In line with an inflammatory etiology, there were minimal differences in cerebrovascular events between COVID-19 positive and community-acquired pneumonia inpatients in our study, except for elderly patients, who generally have a weaker inflammatory response (41). It is unknown if the increased risk of thromboembolic events in COVID-19 patients can be directly attributed to unique properties of the virus, or if it is a consequence of a more pronounced inflammatory state (41). Moreover, given the association of COVID-19 with cardiac disorders, including myocarditis, arrhythmias, heart failure, and myocardial infarction, cardiac embolism is also a potential mechanism (42–44). It should be noted that COVID-19 patients had a slightly higher rate of certain pre-existing risk factors for ischemic stroke, including hypercholesterolemia, diabetes mellitus, and hypertension, as have previously been reported (3, 45). However, even when these cerebrovascular risk factors were excluded from analysis, the COVID-19 population maintained a higher risk of ischemic stroke. Finally, factors such as immobilization during hospital admission may increase stroke risk as well (44).

Intracerebral and Subarachnoid Hemorrhage

The 1-month incidence of intracerebral hemorrhage among COVID-19 inpatients was 0.1%, similar to previously published studies (46). The frequency was 4.8 times higher in COVID-19 positive compared to negative outpatients. There was, however, no excess risk compared to patients with influenza or community-acquired bacterial pneumonia. Some authors have argued that a subset of intracerebral hemorrhages may be due to hemorrhagic conversion of ischemic events, particularly after anticoagulation therapy (47–49). In two recent studies, 76% (25 of 33) and 60% (6 out 10) of patients developed intracerebral hemorrhage after low- or high-dose anticoagulation therapy (47, 48). Besides anticoagulation, a systematic review of 94 studies found that older age, mechanical ventilation and extracorporeal membrane oxygenation also increased the risk of intracranial

hemorrhage in COVID-19 patients (46). In our study, the risk of intracerebral hemorrhage remained elevated after removal of patients who received intravenous thrombolysis, indicating an independent COVID-19 related risk.

In our study of over 43,000 COVID-19 patients, only four individuals developed subarachnoid hemorrhage within the first month, and 10 within 12 months. This does not represent an excess risk compared to COVID-negative individuals and patients with influenza or bacterial pneumonia. Our results confirm findings from another large study with 85,645 COVID-19 patients, in which 86 developed SAH, without an excess risk compared to COVID-negative patients (50).

Auto-Immune Neurological Diseases

Guillain-Barré Syndrome

In our study, only two patients developed GBS. In a study of 1,200 COVID-19 patients from Italy (51) and a study of 3,927 COVID-19 patients from India, there were five cases of GBS each, (52) which appears to be an order of magnitude higher than our data. Another epidemiologic study showed that the incidence of GBS was lower during the pandemic than the corresponding months in the four preceding pre-pandemic years (53). However, precautionary measures intended to reduce the risk of COVID-19 transmission might also have reduced the rate of other infectious diseases associated with GBS (54).

Multiple Sclerosis

In the COVID-19 positive population, 14 of 43,375 individuals developed MS 12 months after a positive test, which did not represent an excess risk. Cases of multiple sclerosis after COVID-19 infection or vaccination have been reported, (55–59) but to our knowledge, no study has yet investigated the incidence of multiple sclerosis after COVID-19.

Myasthenia Gravis and Narcolepsy

In the COVID-19 cohort, only one individual developed myasthenia gravis, and none were diagnosed with narcolepsy, 12 months after a positive test. Only a few cases of new-onset myasthenia gravis following COVID-19 have been reported, (60, 61) and to our knowledge, none of narcolepsy. Based on our findings, it appears that COVID-19 does not increase the 1-year risk of myasthenia gravis or narcolepsy. It must, however, be kept in mind that the median age of new-diagnosed narcolepsy patients is 12 years (62). Given the inclusion criteria of adults $\geq$18 years, we may have missed a possible association between COVID-19 and narcolepsy. Longer follow-up studies in larger and younger COVID-19 populations are needed to exclude subsequent risks of myasthenia gravis and narcolepsy.

Strengths and Limitations

The strengths of this study include the large population and wide catchment area, constituting half of the Danish population. We were able to include all individuals irrespective of age, sex, ethnicity, lifestyle, and socioeconomic background without loss-to-follow-up. Sensitivity analyses showed no differences in rates of clinical work-ups utilizing cerebral PET-FDG-18 and SPECT for diagnoses of neurodegenerative disorders, nor in the rates of risk factors for auto-immune disorders.

Given the nature of aggregated data, several caveats need to be considered. First, we could not adjust for potential confounders such as socioeconomic, lifestyle, pre-existing comorbidities, and length of hospitalization. Instead, we stratified analyses by age, sex, smoking status and pre-existing comorbidities.

Second, we only captured a subset of the Danish population's absolute number of tested individuals, because only COVID-19 tests performed in hospital facilities are registered in the Danish electronic health record system, and not those performed in the community setting (including over-the-counter antigen tests or PCR tests from private providers and the primary care sector). Altogether, we captured ∼20% of the COVID-19 positive (17) and 40% of the COVID-negative (18) population in the Capital Region and Region Zealand (which together correspond to roughly half the population in Denmark).

To assess the representativeness of our study population, we compared the frequencies of neurological diseases in our COVID-negative population with those of the general Danish population. We found that the prevalence or incidences of Alzheimer's disease, Parkinson's disease, narcolepsy, and intracerebral hemorrhage were representative of the Danish and other Western populations (**Supplementary Table 7**) (63). However, the prevalence of ischemic stroke, subarachnoid hemorrhage, and GBS were higher than previous reports from Denmark (64–66). While these results may be surprising, they are in line with a recent Danish study of 23,688 individuals that showed an increase in ischemic stroke in the pandemic period from March 13, 2020 – February 28, 2021, (67) and another showing increasing rates of GBS from 2019 to 2020 (68). The incidence of multiple sclerosis was also higher than the reported yearly incidence in the Danish population, (69) and may be accounted for by the younger population in the Greater Copenhagen area (18) and, possibly, by greater air pollution in urban areas (70–72). Altogether, however, these figures suggest that our study cohorts are representative of the general Danish population.

Given the attention on COVID-19 in the medical community, the frequency of neurological diagnoses may have been increased during the pandemic, thereby artificially increasing the numbers in our study. Conversely, we may have missed the diagnosis of some neurologic cases given the nature of aggregated data from electronic health records and the one-year follow-up duration which arguably is too short to detect longer-term changes, as might be the case for multiple sclerosis after Ebstein-Barr virus infection (73).

CONCLUSION

In this population-based study covering ∼50% of the Danish population, we found support for an increased risk of neurodegenerative disorders (i.e., Alzheimer's disease and Parkinson's disease) and cerebrovascular disorders (i.e., ischemic stroke and intracerebral hemorrhage), in COVID-19 patients compared to individuals tested negative for COVID-19. While the risk of ischemic stroke was increased with COVID-19 compared to influenza, reassuringly, most neurological disorders do not appear to be more frequent after COVID-19 than after

influenza or community-acquired bacterial pneumonia. Future nationwide registry-based studies of pre-and post-pandemic disease rates with full nationwide follow-up are required to confirm these observations.

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 authors.

AUTHOR CONTRIBUTIONS

PZ, CP, MB, and DK contributed to the conception and design of the study. PZ and CP extracted data, performed statistical analyses, and drafted the first version of the manuscript. All authors contributed to manuscript revision and approved the submitted version.

FUNDING

This research was supported by grants from Novo Nordisk (Grant Number NNF21OC0067769) and the Lundbeck Foundation (Grant Number R349-2020-658).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.904796/full#supplementary-material

REFERENCES

1. Liotta EM, Batra A, Clark JR, Shlobin NA, Hoffman SC, Orban ZS, et al. Frequent neurologic manifestations and encephalopathy-associated morbidity in Covid-19 patients. *Ann Clin Transl Neurol.* (2020) 7:2221–30. doi: 10.1002/acn3.51210
2. Chou SH, Beghi E, Helbok R, Moro E, Sampson J, Altamirano V, et al. Global incidence of neurological manifestations among patients hospitalized with COVID-19-a report for the GCS-NeuroCOVID consortium and the ENERGY consortium. *JAMA Netw Open.* (2021) 4:e2112131. doi: 10.1001/jamanetworkopen.2021.12131
3. Qureshi AI, Baskett WI, Huang W, Shyu D, Myers D, Raju M, et al. Acute ischemic stroke and COVID-19: an analysis of 27 676 patients. *Stroke.* (2021) 52:905–12. doi: 10.1161/STROKEAHA.120.031786
4. Perry RJ, Smith CJ, Roffe C, Simister R, Narayanamoorthi S, Marigold R, et al. Characteristics and outcomes of COVID-19 associated stroke: a UK multicentre case-control study. *J Neurol Neurosurg Psychiatry.* (2021) 92:242–8. doi: 10.1136/jnnp-2020-324927
5. Nersesjan V, Amiri M, Christensen HK, Benros ME, Kondziella D. Thirty-day mortality and morbidity in COVID-19 positive vs. COVID-19 negative individuals and vs individuals tested for influenza A/B: a population-based study. *Front Med.* (2020) 7:598272. doi: 10.3389/fmed.2020.598272
6. Cui Y, Zhao B, Li T, Yang Z, Li S, Le W. Risk of ischemic stroke in patients with COVID-19 infection: a systematic review and meta-analysis. *Brain Res Bull.* (2022) 180:31–7. doi: 10.1016/j.brainresbull.2021.12.011
7. Connors JM, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19. *J Thromb Haemost.* (2020) 18:1559–61. doi: 10.1111/jth.14849
8. Bhattacharyya R, Iyer P, Phua GC, Lee JH. The interplay between coagulation and inflammation pathways in COVID-19-associated respiratory failure: a narrative review. *Pulm Ther.* (2020) 6:215–31. doi: 10.1007/s41030-020-00126-5
9. Zuberbuhler P, Conti ME, Leon-Cejas L, Maximiliano-Gonzalez F, Bonardo P, Miquelini A, et al. Guillain-barre syndrome associated to COVID-19 infection: a review of published case reports. *Rev Neurol.* (2021) 72:203–12. doi: 10.33588/rn.7206.2020487
10. Li WS, Chan LL, Chao YX, Tan EK. Parkinson's disease following COVID-19: causal link or chance occurrence? *J Transl Med.* (2020) 18:493. doi: 10.1186/s12967-020-02670-9
11. *Første dansker er bekræftet smittet med COVID-19 (ny coronavirus)*. Available online at: https://stps.dk/da/nyheder/2020/foerste-dansker-er-bekraeftet-smittet-med-covid-19-ny-coronavirus/ (accessed February 28, 2022).
12. *To mistænkte tilfælde af Omicron (B.1.1.529) er påvist i Danmark hos rejsende fra Sydafrika*. Available online at: https://sum.dk/nyheder/2021/november/to-mistaenkte-tilfaelde-af-omicron-b11529-er-paavist-i-danmark-hos-rejsende-fra-sydafrika (accessed February 28, 2022).
13. Kennedy M, Helfand BKI, Gou RY, Gartaganis SL, Webb M, Moccia JM, et al. Delirium in older patients with COVID-19 presenting to the emergency department. *JAMA Netw Open.* (2020) 3:e2029540. doi: 10.1001/jamanetworkopen.2020.29540
14. Ehlenbach WJ, Hough CL, Crane PK, Haneuse SJ, Carson SS, Curtis JR, et al. Association between acute care and critical illness hospitalization and cognitive function in older adults. *JAMA.* (2010) 303:763–70. doi: 10.1001/jama.2010.167
15. Phelan EA, Borson S, Grothaus L, Balch S, Larson EB. Association of incident dementia with hospitalizations. *JAMA.* (2012) 307:165–72. doi: 10.1001/jama.2011.1964
16. Davis DH, Muniz Terrera G, Keage H, Rahkonen T, Oinas M, Matthews FE, et al. Delirium is a strong risk factor for dementia in the oldest-old: a population-based cohort study. *Brain.* (2012) 135:2809–16. doi: 10.1093/brain/aws190
17. *Dagens Covid-19-Opgørelser*. Available online at: https://covid19.ssi.dk/overvagningsdata/download-fil-med-overvaagningdata (accessed February 28, 2022).
18. *FOLK1A: Folketal den 1. i kvartalet efter område, køn, alder og civilstand*. Available online at: https://www.statistikbanken.dk/statbank5a/selectvarval/define.asp?PLanguage=0&subword=tabsel&MainTable=FOLK1A&PXSId=199114&tablestyle=&ST=SD&buttons=0 (accessed February 28, 2022).
19. Alawneh KZ, Al Qawasmeh M, Raffee LA, Abuzayed B, Bani Hani DA, Abdalla KM, et al. A snapshot of ischemic stroke risk factors, subtypes, and its epidemiology: cohort study. *Ann Med Surg.* (2020) 59:101–5. doi: 10.1016/j.amsu.2020.09.016
20. Lotz SK, Blackhurst BM, Reagin KL, Funk KE. Microbial infections are a risk factor for neurodegenerative diseases. *Front Cell Neurosci.* (2021) 15:691136. doi: 10.3389/fncel.2021.691136
21. Whiteley WN, Emberson J, Lees KR, Blackwell L, Albers G, Bluhmki E, et al. Risk of intracerebral haemorrhage with alteplase after acute ischaemic stroke: a secondary analysis of an individual patient data meta-analysis. *Lancet Neurol.* (2016) 15:925–33. doi: 10.1016/S1474-4422(16)30076-X
22. Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, Rodriguez AS, Mitchell T, Washicosky KJ, et al. Alzheimer's disease-associated beta-amyloid is rapidly seeded by herpesviridae to protect against brain infection. *Neuron.* (2018) 100:1527–32. doi: 10.1016/j.neuron.2018.11.043
23. Kumar DK, Choi SH, Washicosky KJ, Eimer WA, Tucker S, Ghofrani J, et al. Amyloid-beta peptide protects against microbial infection in mouse and worm models of Alzheimer's disease. *Sci Transl Med.* (2016) 8:340ra372. doi: 10.1126/scitranslmed.aaf1059
24. Harris SA, Harris EA. Molecular mechanisms for herpes simplex virus type 1 pathogenesis in Alzheimer's disease. *Front Aging Neurosci.* (2018) 10:48. doi: 10.3389/fnagi.2018.00048
25. Caggiu E, Arru G, Hosseini S, Niegowska M, Sechi G, Zarbo IR, et al. Inflammation, infectious triggers, and Parkinson's disease. *Front Neurol.* (2019) 10:122. doi: 10.3389/fneur.2019.00122
26. Jang H, Boltz D, McClaren J, Pani AK, Smeyne M, Korff A, et al. Inflammatory effects of highly pathogenic H5N1 influenza virus infection in the CNS

of mice. *J Neurosci.* (2012) 32:1545–59. doi: 10.1523/JNEUROSCI.5123-11.2012
27. Harker RC and Priemer DS. *?-Amyloid Deposits in Young COVID Patients*. Available online at: https://ssrn.com/abstract=4003213 (accessed April 25 2022).
28. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* (2015) 14:388–405. doi: 10.1016/S1474-4422(15)70016-5
29. Henry J, Smeyne RJ, Jang H, Miller B, Okun MS. Parkinsonism and neurological manifestations of influenza throughout the 20th and 21st centuries. *Parkinsonism Relat Disord.* (2010) 16:566–71. doi: 10.1016/j.parkreldis.2010.06.012
30. Marreiros R, Muller-Schiffmann A, Trossbach SV, Prikulis I, Hansch S, Weidtkamp-Peters S, et al. Disruption of cellular proteostasis by H1N1 influenza A virus causes alpha-synuclein aggregation. *Proc Natl Acad Sci U S A.* (2020) 117:6741–51. doi: 10.1073/pnas.1906466117
31. Barnes LL, Capuano AW, Aiello AE, Turner AD, Yolken RH, Torrey EF, et al. Cytomegalovirus infection and risk of Alzheimer disease in older black and white individuals. *J Infect Dis.* (2015) 211:230–7. doi: 10.1093/infdis/jiu437
32. Becker JH, Lin JJ, Doernberg M, Stone K, Navis A, Festa JR, et al. Assessment of cognitive function in patients after COVID-19 infection. *JAMA Netw Open.* (2021) 4:e2130645. doi: 10.1001/jamanetworkopen.2021.30645
33. Hampshire A, Trender W, Chamberlain SR, Jolly AE, Grant JE, Patrick F, et al. Cognitive deficits in people who have recovered from COVID-19. *EClinicalMedicine.* (2021) 39:101044. doi: 10.1016/j.eclinm.2021.101044
34. Hurley LL, Tizabi Y. Neuroinflammation, neurodegeneration, and depression. *Neurotox Res.* (2013) 23:131–44. doi: 10.1007/s12640-012-9348-1
35. Yaghi S, Ishida K, Torres J, Mac Grory B, Raz E, Humbert K, et al. SARS-CoV-2 and stroke in a new york healthcare system. *Stroke.* (2020) 51:2002–11. doi: 10.1161/STROKEAHA.120.030335
36. Sluis WM, Linschoten M, Buijs JE, Biesbroek JM, den Hertog HM, Ribbers T, et al. Risk, clinical course, and outcome of ischemic stroke in patients hospitalized with COVID-19: a multicenter cohort study. *Stroke.* (2021) 52:3978–86. doi: 10.1161/STROKEAHA.121.034787
37. Luo W, Liu X, Bao K, Huang C. Ischemic stroke associated with COVID-19: a systematic review and meta-analysis. *J Neurol.* (2022) 269:1731–40. doi: 10.1007/s00415-021-10837-7
38. Misra S, Kolappa K, Prasad M, Radhakrishnan D, Thakur KT, Solomon T, et al. Frequency of neurologic manifestations in COVID-19: a systematic review and meta-analysis. *Neurology.* (2021) 97:e2269–81. doi: 10.1212/WNL.0000000000012930
39. Ramos-Araque ME, Siegler JE, Ribo M, Requena M, Lopez C, de Lera M, et al. Stroke etiologies in patients with COVID-19: the SVIN COVID-19 multinational registry. *BMC Neurol.* (2021) 21:43. doi: 10.1186/s12883-021-02075-1
40. Shahjouei S, Tsivgoulis G, Farahmand G, Koza E, Mowla A, Vafaei Sadr A, et al. SARS-CoV-2 and stroke characteristics: a report from the multinational COVID-19 stroke study group. *Stroke.* (2021) 52:e117–30. doi: 10.1161/str.52.suppl_1.P81
41. Tang X, Zheng F. A review of ischemic stroke in COVID-19: currently known pathophysiological mechanisms. *Neurol Sci.* (2022) 43:67–79. doi: 10.1007/s10072-021-05679-0
42. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med.* (2004) 351:2611–8. doi: 10.1056/NEJMoa041747
43. Patone M, Mei XW, Handunnetthi L, Dixon S, Zaccardi F, Shankar-Hari M, et al. Risks of myocarditis, pericarditis, and cardiac arrhythmias associated with COVID-19 vaccination or SARS-CoV-2 infection. *Nat Med.* (2022) 28:410–22. doi: 10.1038/s41591-021-01630-0
44. Aghayari Sheikh Neshin S, Shahjouei S, Koza E, Friedenberg I, Khodadadi F, Sabra M, et al. Stroke in SARS-CoV-2 infection: a pictorial overview of the pathoetiology. *Front Cardiovasc Med.* (2021) 8:649922. doi: 10.3389/fcvm.2021.649922
45. Finsterer J, Scorza FA, Scorza CA, Fiorini AC. Ischemic stroke in 455 COVID-19 patients. *Clinics.* (2022) 77:100012. doi: 10.1016/j.clinsp.2022.100012
46. Daly SR, Nguyen AV, Zhang Y, Feng D, Huang JH. The relationship between COVID-19 infection and intracranial hemorrhage: a systematic review. *Brain Hemorrhages.* (2021) 2:141–50. doi: 10.1016/j.hest.2021.11.003
47. Dogra S, Jain R, Cao M, Bilaloglu S, Zagzag D, Hochman S, et al. Hemorrhagic stroke and anticoagulation in COVID-19. *J Stroke Cerebrovasc Dis.* (2020) 29:104984. doi: 10.1016/j.jstrokecerebrovasdis.2020.104984
48. Lin E, Lantos JE, Strauss SB, Phillips CD, Campion TR Jr, Navi BB, et al. Brain Imaging of patients with COVID-19: findings at an academic institution during the height of the outbreak in New York City. *AJNR Am J Neuroradiol.* (2020) 41:2001–8. doi: 10.3174/ajnr.A6793
49. Ravindra VM, Grandhi R, Delic A, Hohmann S, Shippey E, Tirschwell D, et al. Impact of COVID-19 on the hospitalization, treatment, and outcomes of intracerebral and subarachnoid hemorrhage in the United States. *PLoS ONE.* (2021) 16:e0248728. doi: 10.1371/journal.pone.0248728
50. Qureshi AI, Baskett WI, Huang W, Shyu D, Myers D, Lobanova I, et al. Subarachnoid hemorrhage and COVID-19: an analysis of 282,718 patients. *World Neurosurg.* (2021) 151:e615–20. doi: 10.1016/j.wneu.2021.04.089
51. Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, et al. Guillain-barre syndrome associated with SARS-CoV-2. *N Engl J Med.* (2020) 382:2574–6. doi: 10.1056/NEJMc2009191
52. Khan F, Sharma P, Pandey S, Sharma D. V V, Kumar N, et al. COVID-19-associated guillain-barre syndrome: postinfectious alone or neuroinvasive too? *J Med Virol.* (2021) 93:6045–9. doi: 10.1002/jmv.27159
53. Keddie S, Pakpoor J, Mousele C, Pipis M, Machado PM, Foster M, et al. Epidemiological and cohort study finds no association between COVID-19 and Guillain-Barre syndrome. *Brain.* (2021) 144:682–93. doi: 10.1093/brain/awaa433
54. Dimachkie MM, Barohn RJ. Guillain-barre syndrome and variants. *Neurol Clin.* (2013) 31:491–510. doi: 10.1016/j.ncl.2013.01.005
55. Schirinzi T, Landi D, Liguori C. COVID-19: dealing with a potential risk factor for chronic neurological disorders. *J Neurol.* (2021) 268:1171–8. doi: 10.1007/s00415-020-10131-y
56. Palao M, Fernandez-Diaz E, Gracia-Gil J, Romero-Sanchez CM, Diaz-Maroto I, Segura T. Multiple sclerosis following SARS-CoV-2 infection. *Mult Scler Relat Disord.* (2020) 45:102377. doi: 10.1016/j.msard.2020.102377
57. Pignolo A, Aprile M, Gagliardo C, Giammanco GM, D'Amelio M, Aridon P, et al. Clinical onset and multiple sclerosis relapse after SARS-CoV-2 infection. *Neurol Int.* (2021) 13:695–700. doi: 10.3390/neurolint13040066
58. Havla J, Schultz Y, Zimmermann H, Hohlfeld R, Danek A, Kumpfel T. First manifestation of multiple sclerosis after immunization with the Pfizer-BioNTech COVID-19 vaccine. *J Neurol.* (2022) 269:55–8. doi: 10.1007/s00415-021-10648-w
59. Satheesh NJ, Salloum-Asfar S, Abdulla SA. The potential role of COVID-19 in the pathogenesis of multiple sclerosis-a preliminary report. *Viruses.* (2021) 13:2091. doi: 10.3390/v13102091
60. Restivo DA, Centonze D, Alesina A, Marchese-Ragona R. Myasthenia gravis associated with SARS-CoV-2 infection. *Ann Intern Med.* (2020) 173:1027–8. doi: 10.7326/L20-0845
61. Rodrigues CL, de Freitas HC, Lima PRO, de Oliveira Junior PH, Fernandes JMA, D'Almeida JAC, et al. Myasthenia gravis exacerbation and myasthenic crisis associated with COVID-19: case series and literature review. *Neurol Sci.* (2022) 43:2271–6. doi: 10.21203/rs.3.rs-790941/v1
62. Zhang M, Inocente CO, Villanueva C, Lecendreux M, Dauvilliers Y, Lin JS, et al. Narcolepsy with cataplexy: does age at diagnosis change the clinical picture? *CNS Neurosci Ther.* (2020) 26:1092–102. doi: 10.1111/cns.13438
63. Lioutas VA, Beiser AS, Aparicio HJ, Himali JJ, Selim MH, Romero JR, et al. Assessment of incidence and risk factors of intracerebral hemorrhage among participants in the framingham heart study between 1948 and 2016. *JAMA Neurol.* (2020) 77:1252–60. doi: 10.1001/jamaneurol.2020.1512
64. Sonne A, Andersen JB, Rasmussen LS. The positive predictive value of spontaneous subarachnoid hemorrhage diagnoses in the Danish national patient register. *Clin Epidemiol.* (2019) 11:323–31. doi: 10.2147/CLEP.S197251
65. Demant MN, Andersson C, Ahlehoff O, Charlot M, Olesen JB, Gjesing A, et al. Temporal trends in stroke admissions in Denmark 1997-2009. *BMC Neurol.* (2013) 13:156. doi: 10.1186/1471-2377-13-156
66. *Dansk Apopleksiregister*. Available online at: https://www.sundhed.dk/content/cms/69/4669_dap_aarsrapport-2020_240621.pdf (accessed February 28, 2022).

67. Drenck N, Grundtvig J, Christensen T, Iversen HK, Kruuse C, Truelsen T, et al. Stroke admissions and revascularization treatments in Denmark during COVID-19. *Acta Neurol Scand.* (2022) 145:160–70. doi: 10.1111/ane.13535
68. Filosto M, Cotti Piccinelli S, Gazzina S, Foresti C, Frigeni B, Servalli MC, et al. Guillain-Barre syndrome and COVID-19: an observational multicentre study from two Italian hotspot regions. *J Neurol Neurosurg Psychiatry.* (2021) 92:751–6. doi: 10.1136/jnnp-2020-324837
69. *Multipel Sklerose*. Available online at: https://sundhedsdatastyrelsen.dk/da/tal-og-analyser/analyser-og-rapporter/sygdomme-og-behandlinger/multipel_sklerose (accessed February 28, 2022).
70. Scartezzini A, Tateo F, Perini P, Benacchio L, Ermani M, Ferro A, et al. Association of multiple sclerosis with PM 2. 5 levels further evidence from the highly polluted area of Padua Province, Italy. *Mult Scler Relat Disord.* (2021) 48:102677. doi: 10.1016/j.msard.2020.102677
71. Turk Boru U, Boluk C, Tasdemir M, Gezer T, Serim VA. Air pollution, a possible risk factor for multiple sclerosis. *Acta Neurol Scand.* (2020) 141:431–7. doi: 10.1111/ane.13223
72. Bergamaschi R, Monti MC, Trivelli L, Mallucci G, Gerosa L, Pisoni E, et al. PM2. 5 exposure as a risk factor for multiple sclerosis an ecological study with a bayesian mapping approach. *Environ Sci Pollut Res Int*. (2021) 28:2804–9. doi: 10.1007/s11356-020-10595-5
73. Bjornevik K, Riise T, Casetta I, Drulovic J, Granieri E, Holmoy T, et al. Sun exposure and multiple sclerosis risk in Norway and Italy: the EnvIMS study. *Mult Scler.* (2014) 20:1042–9. doi: 10.1177/1352458513513968

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.

frontiers | Frontiers in Neurology

ORIGINAL RESEARCH
published: 01 July 2022
doi: 10.3389/fneur.2022.902502

Prominent Fatigue but No Motor Fatigability in Non-Hospitalized Patients With Post-COVID-Syndrome

Christian Weich[1,2], *Christian Dettmers*[2]*, *Romina Saile*[1,2], *Luise Schleicher*[1], *Manfred Vieten*[1] and *Michael Joebges*[1,2]

[1] Department of Sports Science, University of Konstanz, Konstanz, Germany, [2] Kliniken Schmieder, Konstanz, Germany

Objectives: Fatigue is a frequent and often disabling symptom in patients with post-COVID syndrome. To better understand and evaluate the symptom of motor fatigue in the context of the post-COVID syndrome, we conducted treadmill walking tests to detect the phenomenon of motor fatigability or to evaluate whether evidence of organic lesions of the motor system could be found, similar to patients with multiple sclerosis.

Method: Twenty-nine non-hospitalized patients with post-COVID syndrome completed the Fatigue Scale for Motor and Cognitive Function (FSMC) questionnaire to determine the trait component of subjective fatigue before they were tested on a treadmill walking at a moderate speed for up to 60 min or until exhaustion. During the walking test oxygen uptake, ventilation and acceleration data of both feet were collected. To determine motor performance fatigability, the Fatigue Index Kliniken Schmieder (FKS) was calculated using the attractor method.

Results: The average walking duration was 42.7 ± 18.6 min with 15 subjects stopping the walking test prematurely. The FSMC score revealed a severe cognitive (37.6 ± 8.2) and motor (37.1 ± 7.8) fatigue averaged over all subjects but only two subjects showed an FKS above the normal range (>4), representing performance fatigability. There was no significant correlation between subjective fatigue (FSMC) and FKS as well as walking time. Absolute values of oxygen uptake and ventilation were in the normal range reported in literature ($r = 0.9$, $p < 0.05$), although eight subjects did not produce a steady-state behavior.

Conclusion: Almost all patients with post-COVID syndrome and subjectively severe motor fatigue, did not show motor fatigability nor severe metabolic anomalies. This is argued against organic, permanent damage to the motor system, as is often seen in MS. Many of the patients were - to our and their own surprise - motorically more exertable than expected.

Keywords: post-COVID-syndrome, gait analysis, attractor method, motor fatigability, motor fatigue

OPEN ACCESS

Edited by:
Anastasios Mpotsaris, München Hospital, Germany

Reviewed by:
Jorge Tolivia, University of Oviedo, Spain
Kristian Borg, Karolinska Institutet Danderyds Sjukhus, Sweden

***Correspondence:**
Christian Dettmers
c.dettmers@kliniken-schmieder.de

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: *23 March 2022*
Accepted: *09 June 2022*
Published: *01 July 2022*

Citation:
Weich C, Dettmers C, Saile R, Schleicher L, Vieten M and Joebges M (2022) Prominent Fatigue but No Motor Fatigability in Non-Hospitalized Patients With Post-COVID-Syndrome. Front. Neurol. 13:902502. doi: 10.3389/fneur.2022.902502

INTRODUCTION

While in the beginning, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was thought to be a viral airway infection, lasting rarely longer than 14 days in mild cases, it is now realized that a considerable number of patients have long-lasting symptoms (1, 2). Post-COVID syndrome has arrived in the mainstream of medicine and is challenging the health system (3). Many patients were advised during the acute phase to stay at home in quarantine and not to visit their general practitioner. Many patients felt left alone with their disease and in the first few months of 2020, symptoms of post-COVID have been primarily described, exchanged, and advocated in patients' forums and social media. This encouraged publications stating that post-COVID is the first patient-made disease (4). Symptoms have been described and empathically shared on social media. This might have contributed to the fact that post-COVID syndrome has been taken up by scientists and later by health professionals and politicians for debate (5).

The first publications focused on the symptomatology, with most often cited symptoms of fatigue, myalgia, dyspnea, headache, sleep disturbances, cognitive disturbances ("brain fog"), and post-exertional fatigue (6). Symptomatology seems to be independent of the seriousness of the primary infection (1, 2). Patients are often on sick leave for months, and some of them have difficulties returning to work (3). Six months after the primary infection, an online survey showed that 22% were still not working and 45% required a reduced working schedule (7). Prevalence rates of symptoms and time course during the first 7 months have been elaborately described (7). The precise pathophysiology remains poorly understood (8). There seems to be no correlation between the degree of symptoms and biomarkers [like CRP, interleukin, etc. (6)], albeit there is evidence that they are linked to chronic (possibly autoimmune) inflammations (9). While publications on symptoms and prevalence rates grow rapidly, the precise etiology in individual patients and in single case studies and the contributing psychosocial risk factors remain obscure.

A different disease, in which fatigue is very prominent and often the most devastating symptom, is multiple sclerosis (MS). In the field of MS, discrimination between fatigue and fatigability has been introduced (10) and has been shown to be extremely helpful (11). Fatigue represents the subjective sensation of the patient, while fatigability represents the change in performance, which can be measured (10). In addition, state fatigue represents the short-lasting, momentary condition often depicted by a visual analog scale. Trait fatigue reflects a long-lasting condition, often regarding the last 4 weeks. It is most often captured in one of the many fatigue scales (12). Besides motor and cognitive fatigue, there is a third category termed emotional (or psychosocial) fatigue (13).

The advantage of the new terminology is that fatigability can be measured and observed. Many patients with MS, who suffer from motor fatigability, show increasing weakness during exhaustion, for instance, increasing foot drop or proximal weakness, which might also cause increasing spasticity or ataxia. If it is very prominent, the neurologist can observe motor fatigability by comparing the gait of the exhausted patient with his normal gait. More sophisticated are measurements using motion-sensitive (IMU) sensors fixed to the ankle in combination with an attractor-based evaluation (14–16). This change in gait performance is not found in, e.g., depressive disorders (17) and is interpreted as a demonstration of an organic lesion of the central nervous system, possibly comparable to a use-or activity-dependent conduction block (18). The correct discrimination between organic and psychological causes of fatigue and fatigability is helpful to define the best therapy in individual cases of MS.

Besides a sophisticated gait analysis, we also investigated oxygen uptake and ventilatory data to document an adequate load for the treadmill test for each individual patient. At the same time, these parameters allow the identification of a potential insufficient ventilatory capacity as a potential consequence of SARS-CoV-2 infection. Oxygen uptake during submaximal continuous exercise will initially increase monoexponentially from a resting state (∼3.5 ml/min/kg) until it finally settles in a steady state (after ∼3 min) when exercising at a constant, moderate load (walking speed) (19). The resulting intensity was proven to be suitable for gait exercises in a rehabilitative context (20). Normal values for oxygen consumption for a constant walking load on a treadmill for a common range of 3–6 km/h correspond to about 8–18 ml/min/kg when at a steady-state load (21). This corresponds comparably with minute ventilation (L/min), which facilitates an increased oxygen exchange. At light intensities, as defined above, a turnover of 25–40 L/min can be expected as the increase is predominantly due to adjustments to the tidal volume (22).

Motor fatigue is a very prominent finding in patients with post-COVID syndrome (7). The aim of our study was a first attempt to disentangle organic components of motor fatigue. We did not imply that we can rule out other potential phenomena like endothelial, mitochondrial, or other dysfunctions. In early 2021, our first approach was to exclude one potential mechanism of organic dysfunction knowing that this might not be the only or last option. To be more specific, the intention was to investigate whether we can identify changes in gait pattern, oxygen consumption, and ventilation during physical exertion as indications of an organic failure. We evaluated rather mildly affected patients – none of them had been hospitalized during the acute infection – suffering from motor fatigue after being infected with SARS-CoV-2 in an exertional test on a treadmill.

MATERIALS AND METHODS

Patient Demographics and Medical History

The inclusion criteria of our study were the diagnosis of SARS-CoV-2 (proven or suspected), initially non-hospitalized post-COVID syndrome, subjective fatigue, being able to walk on a treadmill without holding the side rails, and sick leave before admission for several months. A total of 29 patients with post-COVID syndrome, 24 women and five men, were included in the study between May 2021 and February 2022 during their rehabilitation at the Kliniken Schmieder Konstanz (Germany).

Patients were aged 47.6 (± 10.02) years and weighed 80 (± 18.92) kg. Most of our patients had a thorough cardiac and pulmonary investigation without any pathological finding, which might have explained their symptoms or sick leave before they were referred to our rehabilitation clinic. The majority of our patients were able to dress and bath themselves. They could follow their training schedule and attend the sessions on their own and did not rely on support from nurses (Barthel Index >70). Most of them had been referred to a rehabilitation setting by the Berufsgenossenschaft (the organization responsible for diseases and accidents caused by or during work), by their pension fund, by their health insurance company, or by their neurologist or general practitioner due to protracted sick leave. All patients suffered from a SARS-CoV-2 infection during the first month of 2020 (first wave), during the end of 2020/beginning of 2021 (second wave), or during spring 2021 (third wave). Twenty-five patients had been tested with a polymerase chain reaction (PCR) test during the course of the disease. None had been hospitalized during the acute phase. All of them were on sick leave before being admitted to our clinic. The average duration of sick leave was 8.75 (± 6.2) months. Twelve patients had returned to work after the period of acute illness, and nine had deteriorated at some stage and were unable to continue their work. Six had been continuously on sick leave since their acute phase. The study was approved by the local ethical committee of the University of Konstanz (Germany) under the RefNo: 44/2021. All of the participants filled out and signed informed consent.

Equipment

To acquire the raw data for the gait analyses, an inertial sensor (IMU) from RehaWatch (Magdeburg, Germany) was attached to each ankle of the patient with adhesive tape. The sensor was located directly above the lateral malleoli. Technically, the sensor works as a triaxial accelerometer with up to 16 G (1 G = 9.81 m/s^2), a triaxial gyroscope with up to 2,000°/s, and a magnetometer. The raw data were collected with a sampling rate of 500 Hz with the corresponding RehaGait app (version 1.3.14; Hasomed, Magdeburg, Germany) to be saved internally for later use.

To conduct the ventilatory measurements, a mobile spirometry device (VO_2Master, Vernon, Canada) with its associated app (version 0.22.10) was used. With this instrument (weight: 320 g; 200 g unit & 120 g mask), it was possible to determine the oxygen uptake as well as the ventilation breath-by-breath using a mask while walking. For all test sessions, the mouthpiece M, with a ventilation range of 15–180 L/min, was chosen. The walking tests were conducted on an HP COSMOS treadmill (model locomotion 150/50 DE med) equipped with a security harness.

Study Design and Clinical Setting

The study was undertaken as a prospective study in a neurological inpatient rehabilitation clinic. The clinic has the capacity for about 240 patients. The largest patient group encompasses patients with MS. The first patients with post-COVID-syndrome were admitted at the beginning of 2021. The therapeutic team consists of physiotherapists, occupational therapists, psychologists, speech pathologists, sports scientists, and vocational trainers. Patients stay for 4–5 weeks in the clinic. There is no acute neurological department, no intensive care, or early rehabilitation phase in our hospital. The test protocol was structured in three stages: First, all patients underwent a preliminary medical interview, followed by a ramp test, to determine the load level for the subsequent walking test, which had to be performed a few days later (see **Figure 1**).

Fatigue Scale (FSMC)

The Fatigue Scale for Motor and Cognition (FSMC) has been developed and introduced by Penner et al. (12). It is a questionnaire containing 20 items, which are answered on a Likert scale from one to five. The questionnaire is well evaluated compared to healthy people and is commonly used in patients with MS. Motor and cognitive fatigue can be separately evaluated; ranges are given for normal values (no fatigue), light motor and cognitive fatigue (>22 and >22, respectively), moderate motor and cognitive fatigue (>27 and >28), and severe motor and

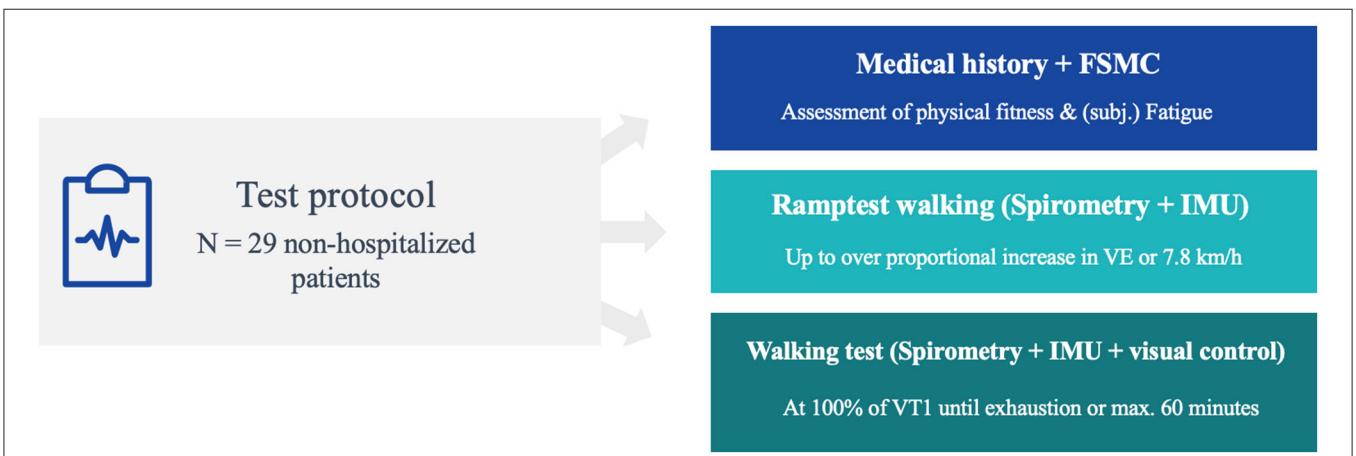


FIGURE 1 | Overview of test protocol consisting of three parts: clinical interview, ramp test and walking test.

cognitive fatigue (>32 and >34). Values are also given for the overall fatigue (>43; >53; >63).

Ramptest

To determine an adequate exertion for the walking (performance fatigability) test, a ramp test on a treadmill was performed prior to a separate day. Once the participants were fixed with the security harness, they were allowed to familiarize themselves with the treadmill at a self-chosen speed for 5 min. Afterward, they started to walk at 1.0 km/h with 1% inclination for half a minute when the speed was increased by 0.3 km/h every further 30s. The walking test was either stopped as soon as the subjects claimed that they could not walk any further or when a maximum speed of 7.8 km/h was attained. Holding the side rails was not allowed to ensure free walking. The onset of physical activity from a resting condition is always linked to rapid and sensitive adaptations of the physiological systems. The increased energetic expenditure must be compensated accordingly. Sports science literature separates three intensity zones that can be determined spirometrically: regenerative, extensive, and intensive activity. The transitions from one intensity to another are defined by ventilatory threshold 1 (VT 1 or AerT) and ventilatory threshold 2 (VT2 or AnT) (20, 23). These thresholds can be detected by analyzing the respiratory gases and ventilation. VT1 correlates with the first lactate threshold, which is accompanied by the bicarbonate buffering of protons (H^+). The increased release of carbon dioxide, also known as *excess* CO_2, leads to an over-proportional increase in ventilation, which can be detected in the collected (breath-by-breath) spirometry data. Because this inflection point is not always easy to detect, a graphical determination is, even today, still preferred over full computational methods like the one provided by Orr et al. (24). The best known is certainly the V-Slope method (25), which draws regression lines, one in the lower and one in the steeper part of the ventilation-speed relationship. Once the best fit is determined relying on the R^2 of each regression line, VT 1, the representative speed at VT 1 can be set at the intersection of both lines. The current test was always performed under medical supervision. Only subjects with spirometric data showing a clear VT1 were invited to participate in the performance fatigability test.

Continuous Walking Test (Performance Fatigability Test)

In order to adequately expose the patients to physical activity in the present study, an extensive continuous walking exercise was chosen to assess whether any motor fatigability symptoms or metabolic abnormalities occur. The test was undertaken within 1 week after the ramp test, with a walking speed set one step above where VT1 was detected (= VT1 + 0.3 km/h) and a treadmill inclination of 1%. By determining this workload level, the energy is predominantly derived from aerobic metabolism. This is to ensure that the intensity is sufficiently low so that a large number of metabolites (e.g., lactate) do not accumulate in the working muscles. It has been reported that this can promote a negative affective valence by increasing the effort perception (load-induced soreness), which can lead to an early termination of the test session (26). Subjects were instructed to walk until they felt they could no longer withstand the effort or for a maximal time of 60 min. To collect all motoric and metabolic raw data, the participants were equipped with an inertial measurement unit (IMU)-sensor attached to each ankle and a spirometer, as described earlier. The walking behavior was always visually observed, supported by a video recording from the back, and eventually, potential abnormalities, as well as the cause of termination, were written down. Spirometry and IMU data were collected throughout the entire session. The test was also performed under medical supervision. Since patients had been already familiarized with the treadmill in the first session (ramp test), the treadmill accelerated to the predetermined speed shortly after the start.

Data Analysis

For the final assessment of fatigue, as well as performance fatigability and spirometric measures, the recorded raw data were further processed. FSMC scores were evaluated as described by Penner et al. (12). To interpret the behavior of respiratory variables, oxygen uptake (ml/min/kg) and minute ventilation (L/min) were evaluated. To provide an informative statement about motor patterns and gait behavior, algorithms of the attractor method (15) were applied.

Attractor Method (Kinematic Analysis)

The attractor method allows the analysis of human movements, especially cyclic motions like walking, running, cycling, or skiing. The approach was first described in 2013 (15) and is still being further developed to this day (16, 27–29). In this regard, it represents a feasible application in which handy IMU sensors can be used to capture motion data and evaluate it with respect to its development over time. In addition to applications in the context of sports (30–32), the attractor method has been established especially in rehabilitative diagnostics (14, 33–37). Attractors, a kind of average value of the covered gait cycles, are calculated minute by minute in order to subsequently rank them in relation to each other. In this way, modifications of two measured events can be evaluated not only with respect to the motion pattern itself (deltaM) but also concerning the motion accuracy (deltaD) (15, 38). In 2014, a specific application of these parameters, the so-called Fatigue Index Kliniken Schmieder (FKS or deltaF), was developed for the diagnosis of motor performance fatigability (14, 39). In the original methodology for determining the FKS (39), deltaM and deltaD were first calculated between the initial and final minute of a multi-minute walk test (for example the 6-min walking test) to finally multiply both values to deltaF. Recently, it was suggested to compare the last minute with the second minute, instead of the first, in order to obtain a more stable assessment (40). The so-called *transient effect* causes much larger oscillations at the beginning of a walking session, which eventually settle down after a short time (28). The established threshold for the occurrence of motor performance fatigability is deltaF $\geq$4 (39). In the present study, the FKS was determined as a parameter for motor performance fatigability for the continuous walking test, comparing the gait behavior of the last minute before termination

and the second minute after the start of the test. For all fatigue indexes $\leq$4, the gait pattern can be assumed to be within the normal range.

Metabolic Assessment

Throughout the entire measurement, oxygen and ventilation data were collected via a portable spirometer (see above) on a breath-by-breath basis. First, an Augmented Dickey-Fuller test (41, 42) was performed for each data set (walking session) to check if it showed a clear steady-state signature. For each session, oxygen uptake and minute ventilation from the third minute after the start [respecting the initial *fast component* phase, see (19)] were analyzed. Subsequently, mean oxygen uptake (ml/min/kg) and ventilation (L/min) of the steady state [3 min until termination of effort] were determined for each case and checked with a paired sample *t*-test and a correlation analysis (IBM SPSS Version 28) if they are in accordance with literature-based norm values (21). In the context of our gait exertions, oxygen uptakes between 8–18 ml/min/kg (21) and minute ventilation of 20–40 L/min (22) can be expected (speed-dependent).

RESULTS

Fatigue Scale for Motor and Cognition (FSMC)

The $FSMC_{Motor}$, $FSMC_{Cognitive}$, and $FSMC_{Total}$ scores in 16 completely returned questionnaires averaged 37.1 $\pm$ 7.8 (= severe motor fatigue), 37.6 $\pm$ 8.2 (= severe cognitive fatigue), and 74.7 $\pm$ 14.9 (= severe total fatigue), respectively.

Performance on the Treadmill

After assessing their ventilatory threshold (VT1), all rehabilitants participated in a motor performance fatigability test for a maximum of 60 min walking on a treadmill. The average walking time was 42.74 $\pm$ 18.6 min, with 14 patients reaching the full duration of 60 min. The shortest walking time was 9 min. Walking speed averaged 5.1 $\pm$ 1 km/h with a range of 1.9 to 6.4 km/h. The walked time (in min) of the fatigability test did not show a statistically significant correlation ($p > 0.05$) to subjective fatigue, operationalized using the FSMC [including only complete datasets ($n = 16$), see Fatigue Scale for Motor and Cognition (FSMC)].

Metabolic Analyses

Compared to an expected steady state of oxygen consumption and ventilation (see **Figure 2**, blue line), the results of the Augmented Dickey-Fuller tests revealed that eight rehabilitants out of 29 (27%) had a non-steady-state behavior (orange line) in both, their oxygen as well as ventilation, data (one example is shown in **Figure 2**). Looking solely at descriptive results, absolute values of oxygen uptake, in participants with a steady state, were 14.17 ($\pm$ 3.2) ml/kg/min, and ventilation was 37.5 ($\pm$ 7.8) L/min. For both, the statistical analyses showed that there is no significant difference ($p = 0.16$) as well as a high correlation ($r = 0.9$, $p < 0.001$) to the values provided in literature (21, 22).

Fatigue Index Kliniken Schmieder (FKS)

The FKS is a sensitive measure to capture motor performance fatigability (17, 39). From all participating patients, attractors of their gait tests were calculated and the respective FKS was determined according to the attractor method algorithm (**Figure 3**). The findings show that all individuals, with only two exceptions (subjects 14 and 18), are well below the cutoff of FKS $\geq$4. Thus, motor performance fatigability cannot be attested in 94% of all cases. The attractor analysis (FKS in m/s^2) during the fatigability test did not show a statistically significant correlation to subjective fatigue, operationalized using the FSMC [including only complete datasets ($n = 16$), see Fatigue Scale for Motor and Cognition (FSMC)].

DISCUSSION

The purpose of the present study was to investigate, whether changes in gait behavior, oxygen consumption, and ventilation during walking on a treadmill, as potential indications of an organic failure, can be observed. In all, except for two, initially non-hospitalized patients with post-COVID-syndrome complaining of serious motor fatigue [$FSMC_{Motor}$ averaged 37.1 $\pm$ 7.8 (= severe Fatigue)], we found no gait abnormalities during walking till exhaustion; suggesting no organic lesion in the central motor system comparable to those found in patients with MS. The latter had also been reported in a previous study for patients with depression (17). Thus, this marks a striking difference from the common finding of motor fatigability in patients with MS (14, 39). We argue that this is an important outcome to begin to disentangle the complex, prominent and frequent phenomenon of fatigue in post-COVID syndrome. We were very much surprised that almost half of our patients were able to walk for 1 h on the treadmill at a fairly good speed around 5 km/h. Patients themselves were surprised that they managed to walk faster and longer than expected. Some of them complained about the backdrop the next day. Others did not experience the backdrop they had expected. From patients with MS, we know that fatigability while walking manifests at a particular localization in the nervous system where a focal infection caused a focal lesion which partially regenerated but left behind a "locus resistenciae minoris" – a weak point in the motor control system. When a patient is brought to his personal limit, such as walking at an unusually high speed, the latent lesion increases the weakness and causes a failure. Our study is in line with the hypothesis that a very careful neurological examination at rest might predict whether the patient will show fatigability during exertion or not. If there is no slight abnormality at rest, there might be no "locus resistance minoris" – no weak point, which gives way to fatigability during exertion. Finding no fatigability like that in MS does not exclude other potential organic causes like endothelial dysfunction, mitochondrial dysfunction, persisting inflammation, autoimmunity, or dysregulation of specific cytokines (6). It is the first step to disentangling the conundrum of post-COVID syndrome (8). Symptoms may also be related to psychosocial stress, trauma,

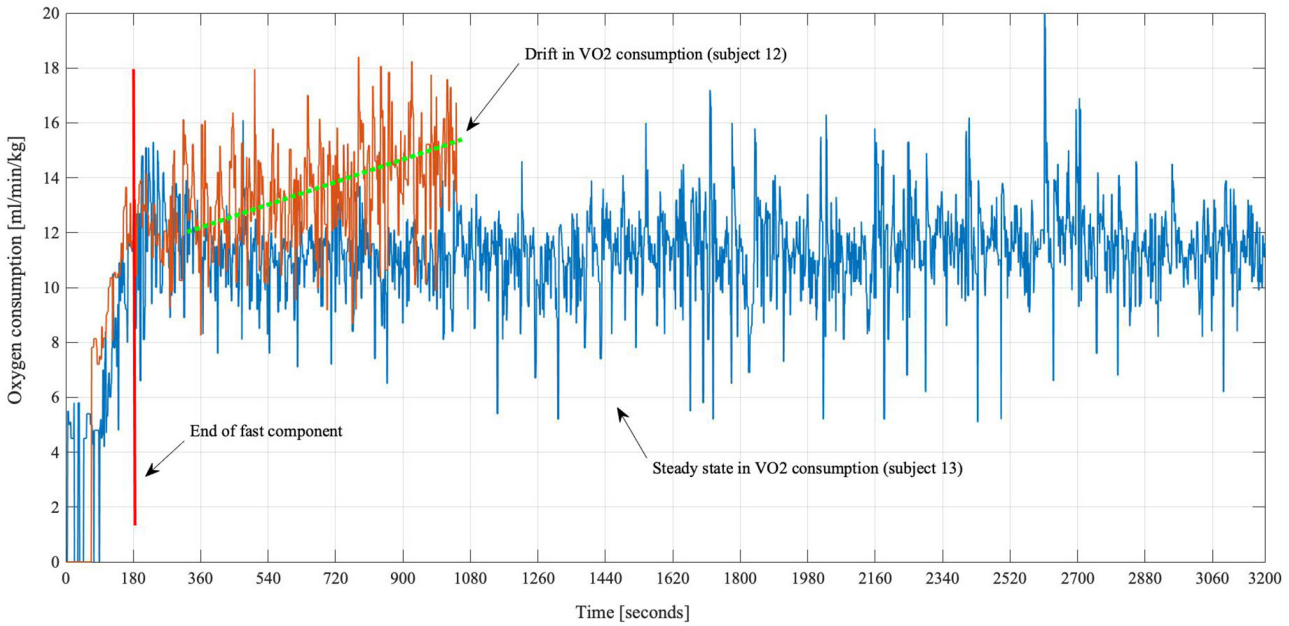


FIGURE 2 | Oxygen consumption curves of two subjects. Subject 13 (blue) showed a normal oxygen kinetic, increasing from rest and leveling off until the walking session was stopped at the maximal time of 60 min. Subject 12 (orange) had to stop after approximately 17 min. It is visible that the VO_2 consumption was steadily rising for subject 12.

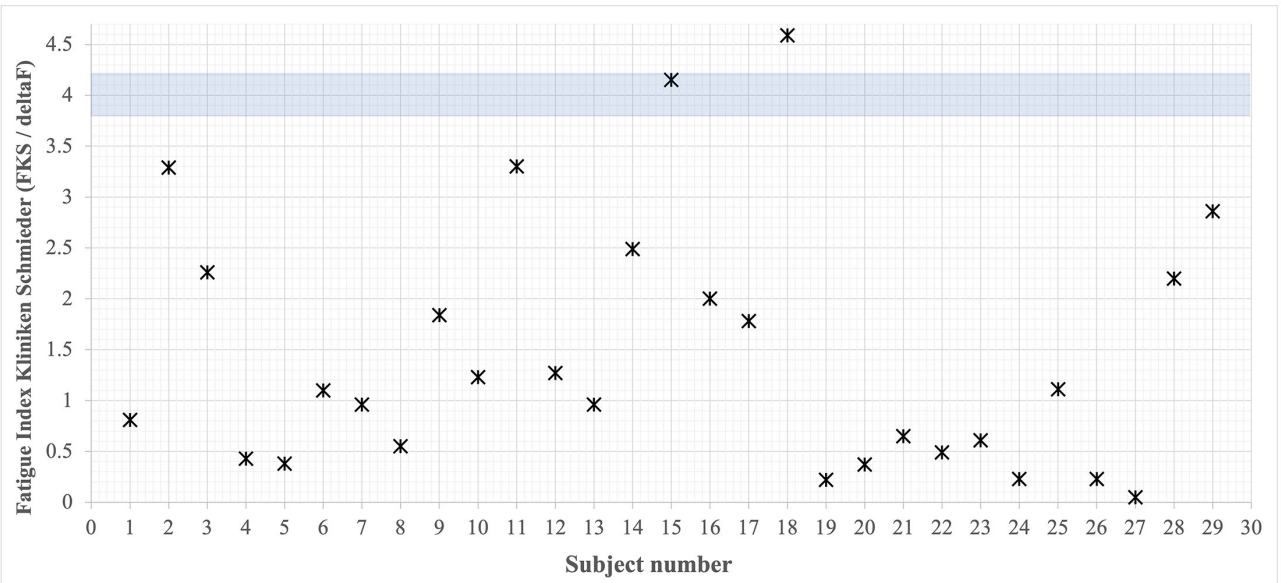


FIGURE 3 | Overview of all rehabilitants' FKS. Cutoff value <4 [based on (42)] defines no present performance fatigability. Only two patients lay above this critical value.

or maladaptive coping style (43, 44). In contrast, common practice identification of an organic cause does not exclude psychological or psychiatric comorbidities and *vice versa*. In many neurological diseases, organic and psychogenic factors are both present and relevant and might exaggerate each other (45).

The described motor-related findings are also underlined by the fact that a normal oxygen and ventilation behavior was established in the majority of the patients. After the initial exercise-induced increase, a steady-state behavior was observed, which showed that these subjects were able to perform the specified exercise without respiratory difficulties. Exceptions

("drift") from this behavior (see **Figure 2**, orange line) were only observed in persons who dropped out of the test markedly prematurely. Even though these data might be associated with the early dropout of the exercise, the absolute values of oxygen uptake and ventilation were within normal limits as it was demonstrated by the statistical tests compared to the literature. Also, based on the emotional expressions of early quitters, stress- or anxiety-related increase must be considered. In future studies, this could be assessed by questionnaires beforehand to determine the predisposition and afterwards to evaluate the actual occurrence of anxiety or negative expectations.

Describing what the phenomenon of fatigue in post-COVID is not like does not tell us what it is. However, it is the first step and the first approach to characterizing the complex phenomenon of fatigue. There is an obvious and broad discrepancy between the very frequent subjective complaints about fatigue and missing objective data of performance fatigability corroborating the complaints of the patients. The discrepancy between subjective complaints of the patients and missing objective deficits made some people claim that fatigue in post-COVID syndrome resembles or equals myeloencephalitis/chronic fatigue syndrome (ME/CFS). The subjective-objective cognitive mismatch in ME/CFS caused even a comparison to the functional cognitive disorder spectrum [(46) cited from (47)]. Fatigue, pain, and excessive interoceptive monitoring in ME/CFS may produce a shift from externally directed attention to subjective complaints, resulting in perceiving cognitive and motor tasks as extremely effortful (46).

Since there does not exist any motor, and only a few metabolic, potentially unspecific, anomalies emerging in our patient sample, we expect that psychosocial factors may be contributing or driving forces in the course of the disease for the patients. However, this can only be confirmed on the basis of future studies. The test design will be extended in order to allow a deeper investigation of the metabolic processes: Here, lactate measurements will be used as a marker for anaerobic energy utilization as well as heart rate data to gain insights into the acute response of the cardiovascular system. Furthermore, cooperative groups from the Schmieder Clinics as well as the University of Konstanz will conduct investigations on cognitive, emotional, and endocrine function or combined effects. Structured clinical interviews and neuroimaging (fMRI) will be used in our group to assess psychosomatic and psychiatric comorbidities.

We are optimistic that patients will not permanently suffer from fatigue when participating in adequate and intense exercise and cognitive behavioral training. Cognitive behavioral therapy will be a central component in our patient management besides individually tailored exercise and training. This might be even helpful in those patients in whom an organic trigger is identified. All of our patients were not initially hospitalized and represent a group of initially "mildly affected" patients. Thus, our observations and conclusions cannot be generalized to all post-COVID-19 patients and certainly not to those who had been ventilated in an intensive care unit. In those patients, one might often expect organic deficits, particularly concerning pulmonary and cardiac function or the central and peripheral nervous system.

CONCLUSION

Initially, non-hospitalized patients with post-COVID syndrome should be examined with a holistic and multidisciplinary approach. After exclusion of cardiac and pulmonary deficits, patients in our sample with prominent fatigue did not show any signs of motor performance fatigability like patients with MS. This implies that we did not find signs of a lesion of the central motor system despite the prominent complaint of motor fatigue. It was not clear whether mild anomalies in ventilation were caused by metabolic or psychogenic alterations. Additional investigation of lactate and heart rate data will be helpful. Nevertheless, the test procedure used here has proven to be very useful for detecting motor and metabolic changes during physical exertion in patients complaining about fatigue. We assume that psychiatric and psychosomatic comorbidities may be involved in many initially non-hospitalized patients with post-COVID-syndrome.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Konstanz Ethics Committee (Ref No 44/2021). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CW introduced important parts of the methodology and particularly the spirometric measurements and the ramp test. He collected and analyzed all data, wrote major parts of the manuscripts, and finalized the final version. CD initiated the study, supervised the treadmill test, and wrote an outline of the manuscript. RS organized and performed the ramp test and the exertional test. LS helped perform the treadmill test and collected data from the electronic patients' sheets. MV executed the attractor-based gait analysis and supervized the methodology of the gait analyses. MJ organized the study acquired patients, helped with the interpretation, and finalized the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

CW and RS were partially supported by the Lurija Institute.

ACKNOWLEDGMENTS

We are very grateful to R.L. Jensen for his thorough language editing and his constructive criticism.

REFERENCES

1. Townsend L, Dyer AH, Jones K, Dunne J, Mooney A, Gaffney F, et al. Persistent fatigue following SARS-CoV-2 infection is common and independent of severity of initial infection. *PLoS ONE.* (2020) 15:e0240784. doi: 10.1371/journal.pone.0240784
2. Bungenberg J, Humkamp K, Hohenfeld C, Rust MI, Ermis U, Dreher M, et al. Long COVID-19: objectifying most self-reported neurological symptoms. *Ann Clin Transl Neurol*. (2022) 9:acn3.51496. doi: 10.1002/acn3.51496
3. Menges D, Ballouz T, Anagnostopoulos A, Aschmann HE, Domenghino A, Fehr JS, et al. Burden of post-COVID-19 syndrome and implications for healthcare service planning: a population-based cohort study. *PLoS ONE.* (2021) 16:e0254523. doi: 10.1371/journal.pone.0254523
4. Callard F, Perego E. How and why patients made long Covid. *Soc Sci Med.* (2021) 268:113426. doi: 10.1016/j.socscimed.2020.113426
5. Rushforth A, Ladds E, Wieringa S, Taylor S, Husain L, Greenhalgh T. Long Covid – the illness narratives. *Soc Sci Med.* (2021) 286:114326. doi: 10.1016/j.socscimed.2021.114326
6. Crook H, Raza S, Nowell J, Young M, Edison P. Long covid—mechanisms, risk factors, and management. *BMJ*. (2021) 374:n1648. doi: 10.1136/bmj.n1648
7. Davis HE, Assaf GS, McCorkell L, Wei H, Low RJ. Re'em Y, Redfield S, Austin JP, Akrami A. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EClinicalMedicine.* (2021) 38:101019. doi: 10.1016/j.eclinm.2021.101019
8. Garg M, Maralakunte M, Garg S, Dhooria S, Sehgal I, Bhalla AS, et al. The conundrum of 'long-COVID-19': a narrative review. *Int J Gen Med*. (2021) 14:2491–506. doi: 10.2147/IJGM.S316708
9. Mehandru S, Merad M. Pathological sequelae of long-haul COVID. *Nat Immunol.* (2022) 23:194–202. doi: 10.1038/s41590-021-01104-y
10. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: Proposal for a unified taxonomy. *Neurology.* (2013) 80:409–16. doi: 10.1212/WNL.0b013e31827f07be
11. Dettmers C, Marchione S, Weimer-Jaekel A, Godde B, Joebges M. Cognitive Fatigability, not Fatigue predicts employment status in patients with MS three months after rehabilitation. *Mult Scler Relat Disord.* (2021) 56:103215. doi: 10.1016/j.msard.2021.103215
12. Penner I, Raselli C, Stöcklin M, Opwis K, Kappos L, Calabrese P. The fatigue scale for motor and cognitive functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler.* (2009) 15:1509–17. doi: 10.1177/1352458509348519
13. Linnhoff, Fiene, Heinze, Zaehle. Cognitive fatigue in multiple sclerosis: an objective approach to diagnosis and treatment by transcranial electrical stimulation. *Brain Sci.* (2019) 9:100. doi: 10.3390/brainsci9050100
14. Sehle A. *Quantifizierung motorischer Fatigue durch Bewegungsanalyse: Entwicklung und Evaluation eines neuen Diagnostikverfahrens bei Patienten mit Multipler Sklerose*. [Dissertation]. Konstanz: University of Konstanz (2015). Available online at: http://nbn-resolving.de/urn:nbn:de:bsz:352-0-308197 (accessed February 10, 2022).
15. Vieten MM, Sehle A, Jensen RL. A novel approach to quantify time series differences of gait data using attractor attributes. *PLoS ONE.* (2013) 8:e71824. doi: 10.1371/journal.pone.0071824
16. Vieten MM, Weich C. The kinematics of cyclic human movement. *PLoS ONE.* (2020) 15:e0225157. doi: 10.1371/journal.pone.0225157
17. Dettmers C, Riegger M, Müller O, Vieten M. Fatigability assessment using the Fatigue Index Kliniken Schmieder (FKS) is not compromised by depression. *Health.* (2016) 08:1485–94. doi: 10.4236/health.2016.814147
18. Vucic S, Burke D, Kiernan MC. Fatigue in multiple sclerosis: mechanisms and management. *Clin Neurophysiol.* (2010) 121:809–17. doi: 10.1016/j.clinph.2009.12.013
19. Burnley M, Jones AM. Oxygen uptake kinetics as a determinant of sports performance. *Eur J Sport Sci.* (2007) 7:63–79. doi: 10.1080/17461390701456148
20. Meyer T, Lucía A, Earnest CP, Kindermann W. A conceptual framework for performance diagnosis and training prescription from submaximal gas exchange parameters - theory and application. *Int J Sports Med.* (2005) 26:S38–48. doi: 10.1055/s-2004-830514
21. Glass S, Dwyer GB, American College of Sports Medicine, editors. *ACSM'S Metabolic Calculations Handbook*. Philadelphia, PA: Lippincott Williams & Wilkins (2007), p. 111.
22. Wilmore JH, Costill DL, Kenney WL. *Physiology of Sport and Exercise,* 4th ed. Champaign, IL: Human Kinetics (2008), p. 574.
23. Kindermann W. Anaerobe schwelle. *Dtsch Z Sportmed.* (2004) 55:161–2.
24. Orr GW, Green HJ, Hughson RL, Bennett GW. A computer linear regression model to determine ventilatory anaerobic threshold. *J Appl Physiol.* (1982) 52:1349–52. doi: 10.1152/jappl.1982.52.5.1349
25. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol.* (1986) 60:2020–7. doi: 10.1152/jappl.1986.60.6.2020
26. Behrens M, Broscheid K-C, Schega L. Taxonomie und determinanten motorischer performance fatigability bei multipler sklerose. *Neurol Rehabil.* (2021) 27:3–12. doi: 10.14624/NR2101001
27. Weich CM, Vieten M. The gaitprint: identifying individuals by their running style. *Sensors*. (2020) 20:3810. doi: 10.3390/s20143810
28. Weich C, Vieten MM, Jensen RL. Transient effect at the onset of human running. *Biosensors*. (2020) 10:117. doi: 10.3390/bios10090117
29. Broscheid K-C, Dettmers C, Vieten M. Is the limit-cycle-attractor an (almost) invariable characteristic in human walking? *Gait Posture.* (2018) 63:242–7. doi: 10.1016/j.gaitpost.2018.05.015
30. Weich C, Jensen RL, Vieten M. Triathlon transition study: quantifying differences in running movement pattern and precision after bike-run transition. *Sports Biomech.* (2019) 18:215–28. doi: 10.1080/14763141.2017.1391324
31. Weich C, Moore S, Fjeldheim, S, Jensen R. The attractor method – a Sensitive tool to highlight subtle differences in cross-country ski skating techniques (V1 vs. V2). In: Karczewska-Lindinger M, Hakkarainen A, Linnamo V, Lindinger S, editors. *Book of the 8th International Congress on Science and Skiing*. Vuokatti: University of Jyväskylä, Finland (2020), p. 161–8.
32. Moore S, Weich C, Torchia I, Jensen R. Influence of movement deviation on metabolic economy of the V1 and V2 cross-country skate techniques. In: Karczewska-Lindinger M, Hakkarainen A, Linnamo V, Lindinger S, editors. *Book of the 8th International Congress on Science and Skiing*. Vuokatti: University of Jyväskylä, Finland (2020), p. 122–9.
33. Broscheid K-C. *Movement Quality of walking in multiple sclerosis patients – Improvement from pre to post rehabilitation*. [Master thesis] Konstanz: University of Konstanz. (2016).
34. Byrnes SK, Nüesch C, Loske S, Leuenberger A, Schären S, Netzer C, Mündermann A. inertial sensor-based gait and attractor analysis as clinical measurement tool: functionality and sensitivity in healthy subjects and patients with symptomatic lumbar spinal stenosis. *Front Physiol*. (2018) 9:1095. doi: 10.3389/fphys.2018.01095
35. Loske S, Nüesch C, Byrnes KS, Fiebig O, Schären S, Mündermann A, et al. Decompression surgery improves gait quality in patients with symptomatic lumbar spinal stenosis. *Spine J.* (2018) 18:2195–204. doi: 10.1016/j.spinee.2018.04.016
36. Sehle A, Mündermann A, Starrost K, Sailer S, Becher I, Dettmers C, et al. Objective assessment of motor fatigue in multiple sclerosis using kinematic gait analysis: a pilot study. *J NeuroEngineering Rehabil.* (2011) 8:59. doi: 10.1186/1743-0003-8-59
37. Sehle A, Vieten M, Mündermann A, Dettmers C. Difference in motor fatigue between patients with stroke and patients with multiple sclerosis: a pilot study. *Front Neurol*. (2014) 5:279. doi: 10.3389/fneur.2014.00279
38. Vieten MM, Jensen RL. The Attractor method - a technique to quantify differences of cyclic processes and their variability. *Proceedings of the 33rd International Conference on Biomechanics in Sports*. Poitiers (2015).
39. Sehle A, Vieten M, Sailer S, Mündermann A, Dettmers C. Objective assessment of motor fatigue in multiple sclerosis: the Fatigue index Kliniken Schmieder (FKS). *J Neurol.* (2014) 261:1752–62. doi: 10.1007/s00415-014-7415-7
40. Broscheid K-C, Behrens M, Dettmers C, Jöbges M, Schega L. Quantifizierung Motorischer Performance Fatigability bei Multipler Sklerose [Quantification of motor performance fatigability in multiple sclerosis]. *Neurol Rehabil.* (2021) 27:13–22. doi: 10.14624/NR2101002

41. Pal A, Prakash P. *Practical Time Series Analysis*. (2017). Available online at: http://app.knovel.com/hotlink/toc/id:kpPTSA0003/practical-time-series?kpromoter=marc (accessed October 27, 2021).
42. Dickey DG. Dickey-fuller tests. In: Lovric M, editor. *International Encyclopedia of Statistical Science*. Berlin, Heidelberg: Springer Berlin Heidelberg (2011), p. 385–8. doi: 10.1007/978-3-642-04898-2_210
43. Pust GEA, Randerath J, Goetzmann L, Weierstall R, Korzinski M, Gold SM, et al. Association of fatigue severity with maladaptive coping in multiple sclerosis: a data-driven psychodynamic perspective. *Front Neurol.* (2021) 12:652177. doi: 10.3389/fneur.2021.652177
44. Pust GEA, Dettmers C, Randerath J, Rahn AC, Heesen C, Schmidt R, et al. Fatigue in multiple sclerosis is associated with childhood adversities. *Front Psychiatry.* (2020) 11:811. doi: 10.3389/fpsyt.2020.00811
45. Schmidt R, Lutgehetmann R, Krauss B, Schorner K. Vom <<entweder-oder<< zum>> sowohl als auch>>: Die integrierte Versorgung komorbider neurologischer und funktionell psychischer Storungen im neurologischen Fach-und Rehabilitationskrankenhaus. *Neurol Rehabil.* (2007) 13:109–18. Available online at: https://www.hippocampus.de/media/316/cms_4a94f41aa85e4.pdf
46. Teodoro T, Edwards MJ, Isaacs JD. A unifying theory for cognitive abnormalities in functional neurological disorders, fibromyalgia and chronic fatigue syndrome: systematic review. *J Neurol Neurosurg Psychiatry.* (2018) 89:1308–19. doi: 10.1136/jnnp-2017-317823
47. Mantovani E, Mariotto S, Gabbiani D, Dorelli G, Bozzetti S, Federico A, et al. Chronic fatigue syndrome: an emerging sequela in COVID-19 survivors? *J Neurovirol.* (2021) 27:631–7. doi: 10.1007/s13365-021-01002-x

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.

frontiers | Frontiers in Neurology

SYSTEMATIC REVIEW
published: 15 July 2022
doi: 10.3389/fneur.2022.887164

A Systematic Review on Neurological Aspects of COVID-19: Exploring the Relationship Between COVID-19-Related Olfactory Dysfunction and Neuroinvasion

Sujata Purja[1†], *SuA Oh*[1†] *and EunYoung Kim*[1,2*†]

[1] *Evidence-Based and Clinical Research Laboratory, Department of Health, Social and Clinical Pharmacy, College of Pharmacy, Chung-Ang University, Seoul, South Korea,* [2] *The Graduate School for Food and Drug Administration, The Graduate School for Pharmaceutical Industry Management, College of Pharmacy, Chung-Ang University, Seoul, South Korea*

OPEN ACCESS

Edited by:
Anastasios Mpotsaris, München Hospital, Germany

Reviewed by:
Jorge Matias-Guiu, Complutense University of Madrid, Spain
Amjad Maher Elmashala, Al-Quds Cognitive Neuroscience Lab, Al-Quds University, Palestine

***Correspondence:**
EunYoung Kim
eykimjcb777@cau.ac.kr

†ORCID:
Sujata Purja
orcid.org/0000-0001-6507-915X
SuA Oh
orcid.org/0000-0001-5682-4066
EunYoung Kim
orcid.org/0000-0003-3525-8805

Specialty section:
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

Received: *01 March 2022*
Accepted: *21 June 2022*
Published: *15 July 2022*

Citation:
Purja S, Oh S and Kim E (2022) A Systematic Review on Neurological Aspects of COVID-19: Exploring the Relationship Between COVID-19-Related Olfactory Dysfunction and Neuroinvasion. Front. Neurol. 13:887164. doi: 10.3389/fneur.2022.887164

Objectives: To identify neurological aspects of Coronavirus disease 2019 (COVID-19) and to investigate COVID-19 infected patients with and without olfactory dysfunction in relation to polymerase chain reaction (PCR) assay results for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in the cerebrospinal fluid (CSF).

Methods: PubMed and EMBASE databases were searched until March 26, 2021, for observational studies with COVID-19 patients that had performed CSF PCR assay due to the neurologic symptom and reported anosmia status.

Results: Initially, 2,387 studies were identified;167 studies performed SARS-CoV-2 CSF PCR assay, of which our review comprised 45 observational studies that conducted CSF PCR assay for SARS-CoV-2 in 101 patients and reported anosmia status in 55 of 101 patients. Central and peripheral neurological manifestations observed in COVID-19 patients were diverse. The most common neurological diagnoses were Guillain-Barré syndrome (GBS) and its variants (24%), followed by encephalopathy (21%). The SARS-CoV-2 PCR assay was positive in only four CSF samples, of which two patients had olfactory dysfunction while the others did not.

Conclusions: The neurological spectrum of COVID-19 is diverse, and direct neuroinvasion of SARS-CoV-2 is rare. The neuroprotection against SARS-CoV-2 in COVID-19 patients with anosmia is controversial, as an equal number of patients with and without olfactory dysfunction had positive CSF PCR results for SARS-CoV-2 in our study, and further studies are required to provide more insight into this topic.

Keywords: COVID-19, SARS-CoV-2, anosmia, cerebrospinal fluid, neuroinvasion

INTRODUCTION

The olfactory nerve connects the nasal cavity to the central nervous system (CNS) and provides a neuroinvasive shortcut to respiratory neurotropic viruses (1). The detection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in the olfactory nerve and CNS of patients with coronavirus disease 2019 (COVID-19) suggests that SARS-CoV-2 has neuroinvasive potential

via the olfactory pathway (2). Although SARS-CoV-2 neuroinvasion is uncommon, CNS viral transmission poses a significant threat to life (3).

Previous animal studies have demonstrated that respiratory neurotropic viral invasion induces apoptosis of olfactory receptor neurons (ORNs), preventing the viral transmission to the olfactory bulb and the CNS (4, 5). Although the exact mechanism underlying COVID-19 related anosmia is unclear, human and animal studies have shown that anosmia is a consequence of a host defense mechanism against viral invasion involving the damage of olfactory epithelium might provide neuroprotection (2, 5–9). Furthermore, anosmia is frequently seen in milder forms of COVID-19 with a lower mortality rate (10, 11). Therefore, neuroprotection is anticipated in COVID-19 patients with anosmia.

Understanding the underlying mechanism and prognostic value of COVID-19-related anosmia will aid better patient management since olfactory dysfunction is often associated with several neurological disorders (12). This systematic review aimed to compile studies involving COVID-19 patients with neurological manifestations who have undergone polymerase chain reaction (PCR) testing for SARS-CoV-2 in cerebrospinal fluid (CSF) and reported the patient's anosmia status for identifying neurological aspects of COVID-19 and exploring the COVID-19 infected patients with or without anosmia in relation to their CSF PCR assay results.

METHODS

Eligibility Criteria

The observational studies related to CSF analysis of COVID-19 patients with neurological symptoms were included. Target patients were COVID-19 patients diagnosed based on either positive SARS-CoV-2 PCR or serologic testing who had a neurological manifestation and have undergone SARS-CoV-2 CSF PCR testing to identify COVID-19-related neurological disorders. Studies that conducted CSF PCR assay for SARS-CoV-2 but did not report information on the status of anosmia were excluded. The study covered primary, retrievable scientific literature available in English. Collected data were each patient's sex and age distribution, SAR-CoV-2 CSF PCR assay, neurological presentation, treatment, and outcome. Therefore, studies that did not report these data properly were also excluded.

Search Strategy

We conducted a broad literature search of databases such as EMBASE and PubMed until March 26, 2021, following preferred reporting items for systematic reviews and meta-analysis (PRISMA) checklist (13) for studies that performed CSF PCR assay for SARS-CoV-2 in COVID-19 patients using population search terms "SARS-CoV-2" or "COVID-19" and intervention search terms "brain" or "cerebrospinal fluid" or "anosmia".

Study Selection

Two independent authors screened studies based on the titles and abstracts. Any studies relevant to the CSF analysis of patients with COVID-19 were advanced to the second stage of the review. Full texts were reviewed using the eligibility criteria mentioned above in the second screening. Any disagreement between the authors was resolved by discussion.

Risk of Bias Assessment

The Joanna Briggs Institute (JBI) critical appraisal checklist was used to assess the risk of bias in each included study (14).

Data Extraction and Analysis

Two authors independently collected the data items included in the study design for each eligible study. For evaluating neurological aspects of COVID-19, individual patient data on neurological presentation, treatment, and outcomes were collected. The data items included individual's age and sex distribution, CSF PCR assay result, anosmia status, COVID-19-related neurological symptoms, neurological diagnosis, treatment, and outcomes. Each COVID-19 patient's data with neurological manifestations who had undergone CSF PCR testing for SARS-CoV-2 to identify COVID-19-related neurological disorders was summarized to characteristics, clinical presentation, SARS-CoV-2 PCR assay results, neurological diagnosis, treatment, and outcomes.

RESULTS

Study Selection

In total, 2,387 studies were identified through a literature search after removing duplicates. After preliminary screening based on the titles and abstracts, a total of 379 studies related to CSF analysis of COVID-19 patients with neurological symptoms were included; among them, 167 studies (44%) that conducted PCR tests for SARS-CoV-2 in CSF were selected for full-text review. A total of 122 studies that conducted CSF PCR assay for SARS-CoV-2 but did not report information on the status of anosmia were excluded. Thus, only 45 articles that met the inclusion criteria were included in our study (15–59). A flow diagram of the study selection process is shown in **Figure 1**.

Risk of Bias

Overall, the risk of bias in the included studies was low except for three studies (15, 21, 57). The summary of JBI critical appraisal results for case reports and case series can be seen in **Supplementary Tables 1**, **2**.

Participants and Characteristics of Studies

The total number of participants was 104, while the SARS-CoV-2 CSF PCR testing was performed on only 101 patients. **Table 1** shows the characteristics of the 101 participants included in the review. More than 63.4% (64/101) were men. The mean age was 57 ± 16.37 years. The number of men and women infected with COVID-19 increased with age. COVID-19 infected patients of both sexes, predominantly in the 60–79 age group (**Figure 2**).

Neurological Aspects of COVID-19

Clinical Presentation

The neurological symptoms observed in COVID-19 individuals were diverse. The most common COVID-19-related neurological

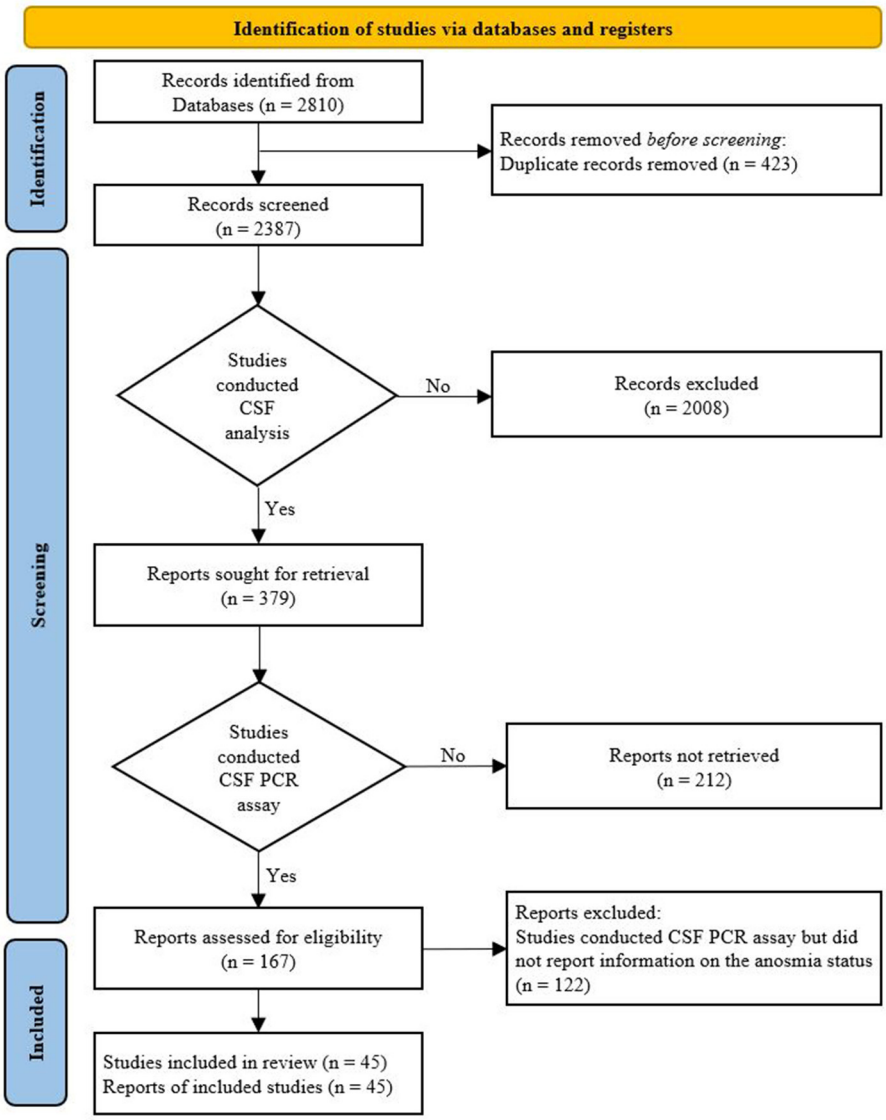


FIGURE 1 | PRISMA flowchart.

symptoms were smell disorder, taste disorder, headache, myalgia, altered consciousness, related paresis, and related cognitive and behavioral disturbances.

Neurological Diagnosis

The neurological diagnosis made after the neurological and radiological examination was localized to CNS (61.39%), peripheral nervous system (32.67%), or both (5.94%). In a study comprising 30 participants, six patients were diagnosed with two neurological disorders (57). The most common neurological diagnosis included Guillain-Barré syndrome and its variant (24%), followed by encephalopathy (21%) (**Table 1**).

Treatment and Outcomes

Information on the therapeutic management of COVID-19 was available for only 69 patients, including one patient who did not require medical treatment. In most cases, therapeutic management of COVID-19 patients involved combinational therapies. Common treatments included steroids administration (n = 32/69), intravenous immunoglobulin infusion (n = 28/69), hydroxychloroquine (n = 18/69), and plasma exchange (n = 11/69). Other medications used in the management of COVID-19 patients are shown in **Table 1**. The administration of medications resulted in neurological improvement in most patients. There were 63 non-fatal cases, five fatal cases, and one

TABLE 1 | Characteristics of studies included in the review.

References	Total case	Age/sex	SARS-CoV-2 diagnostic	CSF PCR	LOS	Neurological diagnosis	Treatment	Outcome
Case report								
Andriuta et al. (15)	2	NR/F	NPS	Neg	Yes	Encephalopathy	NR	NR
		NR/M	NS	Neg	NR	Encephalopathy	NR	Unawaken
Assini et al. (16)	2	55/M	OPS	Neg	Yes	Polyradicoloneuritis	Idrossichlorochine, arbidol, L/R, IVIG	Non-fatal
		60/M	NPS	Neg	NR	Polyradicoloneuritis	HCQ, ART, Tocilizumab, IVIG	Non-fatal
Atakla et al. (17)	1	41/M	NPS	Neg	Yes	GBS	IVIG, AZ, chloroquine	Non-fatal
Bigaut et al. (18)	2	43/M	NPS	Neg	Yes	GBS	IVIG	Non-fatal
		70/F	NPS	Neg	Yes	GBS	IVIG	Non-fatal
Bodro et al. (19)	2	25/M	NPS	Neg	No	Encephalitis	AC, antibiotics	Non-fatal
		49/M	NPS	Neg	No	Encephalitis	AC, antibiotics	Non-fatal
Canavero et al. (20)	2	25/F	NPS	Neg	Yes	Post-infectious demyelinating myelitis	Steroid	Non-fatal
		69/M	NPS	Neg	NR	Encephalomyelitis	CF, AC, L/R, HCQ, steroid, IVIG	Non-fatal
Casez et al. (21)	1	96/F	Serology	Neg	Yes	Encephalitis	NR	NR
Cebrián et al. (22)	1	74/F	NPS	Pos	No	Headache	NSAIDs, CF, HCQ, L/R	Non-fatal
Chakraborty et al. (23)	1	59/F	NPS, OPS	Neg	No	Acute transverse myelitis	Steroids and antipyretics	Fatal
Chan et al. (24)	1	58/M	OPS	Neg	No	GBS	IVIG	Non-fatal
Chauffier et al. (25)	1	47/M	NPS	Neg	No	Encephalopathy	No medical treatment	Non-fatal
Chaumont et al. (26)	1	69/M	BAL	Neg	Yes	Meningoencephalitis	AC, HCQ, AZ	Non-fatal
Chow et al. (27)	1	60/M	NPS	Neg	Yes	Acute transverse myelitis	Steroid	Non-fatal
Civardi et al. (28)	1	72/F	NPS	Neg	Yes	GBS	IVIG, HCQ, Doxycycline	Non-fatal
Cohen et al. (29)	1	45/M	NPS	Neg	Yes	Parkinson's disease	Steroid, biperiden	Non-fatal
Corrêa et al. (30)	1	51/F	NS	Neg	Yes	Enecephalomyeloradiculitis	Steroid, PE, azathioprine	Non-fatal
De Gennaro et al. (31)	2	42/M	NPS	Neg	No	Cranial neuritis	Remdesivir, sedatives, curare, IVIG	Non-fatal
		67/M	NPS	Neg	No	Cranial neuritis	Antibiotics, anesthetic, noradrenalin, IVIG	Non-fatal
Demirci Otluoglu et al. (32)	1	48/M	CSF	Pos	Yes	Encephalomyelitis	HCQ, Favipiravir, P/T,levetiracetam, steroid, AC	Non-fatal
Dijkstra et al. (33)	1	44/M	NPS	Neg	Yes	Myoclonic syndrome	Steroid and IVIG	Non-fatal

(Continued)

TABLE 1 | Continued

References	Total case	Age/sex	SARS-CoV-2 diagnostic	CSF PCR	LOS	Neurological diagnosis	Treatment	Outcome
Fadakar et al. (34)	1	47/M	NPS, OPS	Pos	No	Cerebellitis	L/R	Non-fatal
Grimaldi et al. (35)	1	72/M	NPS	Neg	No	Encephalitis	IVIG, steroid, benzodiazepines	Non-fatal
Gutiérrez-Ortiz et al. (36)	2	50/M	OPS	Neg	Yes	Miller fisher syndrome	IVIG	Non-fatal
		39/M	OPS	Neg	NR	PNC	Acetaminophen	Non-fatal
Helbok et al. (37)	1	68/M	Serology	Neg	Yes	GBS	Steroid, IVIG, PE	Non-fatal
Huber et al. (38)	1	21/F	Serology	Neg	Yes	Myasthenia gravis	IVIG and pyridostigmine	Non-fatal
Le Guennec et al. (39)	1	69/M	TA	Neg	Yes	Status epilepticus	Levetiracetam and IVIG	Non-fatal
Lim et al. (40)	1	55/F	NPS	Neg	Yes	Psychotic disorder	Benzodiazepine, antipsychotic	Non-fatal
Moore et al. (41)	1	28/M	NPS	Neg	Yes	Multiple sclerosis	Steroid	Non-fatal
Muccioli et al. (42)	1	47/F	NPS	Neg	Yes	Encephalopathy	Tocilizumab	Non-fatal
Naddaf et al. (43)	1	58/F	Serology	Neg	No	GBS	HCQ, zinc, steroid, PE	Non-fatal
Novi et al. (44)	1	64/F	CSF	Pos	Yes	ADEM	Steroid with OPT, IVIG	Non-fatal
Oguz-Akarsu et al. (45)	1	53/F	NPS	Neg	No	GBS	PE, HCQ, AZ	Non-fatal
Palao et al. (46)	1	29/F	Serology	Neg	Yes	Multiple sclerosis	Steroid with OPT	Non-fatal
Pascual-Goñi et al. (47)	2	60/F	NPS	Neg	Yes	Encephalopathy	Thiamine, pyridoxine, HCQ, AZ	Non-fatal
		35/F	NPS	Neg	NR	Encephalopathy	Thiamine and pyridoxine	Non-fatal
Riva et al. (48)	1	^{NR}/M	Serology	Neg	Yes	GBS	IVIG	Non-fatal
Umapathi et al. (49)	2	59/M	NPS	Neg	No	ADEM	Low molecular weight heparin, IVIG	Non-fatal
		73/M	NPS	Neg	NR	Encephalopathy	Interferon-beta 1b, L/R, steroid	Fatal
Vandervorst et al. (50)	1	29/M	NPS	Neg	Yes	Encephalitis	HCQ, nebivolol, amlodipine, antipsychotic, benzodiazepines	Non-fatal
Zanin et al. (51)	1	54/F	Pos; swab unclear	Neg	Yes	Brain & spine demyelinating lesions	ART, HCQ, antiepileptics, steroid	Non-fatal
Zhou et al. (52)	1	26/M	NS, OPS	Neg	No	MOG-IgG-MD	Steroid with OPT	Non-fatal
Zoghi et al. (53)	1	21/M	Serology	Neg	No	Central demyelinating brain injury	PE, antibiotics, AC	Non-fatal
Case series								
Cao et al. (54)	5	49/M	NPS/TA	Neg	NR	Encephalitis	Steroid and PE	Non-fatal
		56/M	NPS/TA	Neg	NR	Encephalitis	Steroid and PE	Non-fatal
		61/M	NPS/TA	Neg	NR	Encephalitis	Steroid and PE	Non-fatal

(Continued)

TABLE 1 | Continued

References	Total case	Age/sex	SARS-CoV-2 diagnostic	CSF PCR	LOS	Neurological diagnosis	Treatment	Outcome
		37/M	NPS/TA	Neg	NR	Encephalitis	Steroid and PE	Fatal
		77/F	NPS/TA	Neg	Yes	Encephalitis	Steroid and PE	Fatal
Delorme et al. (55)	4	72/M	NPS	Neg	Yes	Encephalopathy	IVIG	Non-fatal
		66/F	NPS	Neg	NR	Encephalopathy	IVIG and steroid	Non-fatal
		60/F	NPS	Neg	NR	Encephalopathy	Steroid, antidepressants	Non-fatal
		69/M	NPS	Neg	Yes	Encephalopathy	Levetiracetam, sedative, IVIG, steroid	Non-fatal
Manganotti et al. (56)	4	72/M	NPS	Neg	Yes	GBS	HCQ, antivirals, steroid, tocilizumab	Non-fatal
		72/M	NPS	Neg	Yes	GBS	HCQ, L/R, steroid	Non-fatal
		49/F	NPS	Neg	Yes	GBS	HCQ, L/R, steroid	Non-fatal
		76/M	NPS	Neg	Yes	GBS	HCQ, antivirals, steroid, tocilizumab, antibiotics, fluconazole	Non-fatal
Neumann et al. (57)	30	81/M	NPS	Neg	NR	TIA	NR	NR
		25/F	NPS	Neg	NR	CVST	NR	NR
		48/F	BAL	Neg	NR	Encephalitis-HSV-1	NR	NR
		73/F	NPS	Neg	NR	Suspected post-stroke movement disorder	NR	NR
		63/M	BAL	Neg	NR	Miller fisher syndrome	NR	NR
		58/M	BAL	Neg	NR	Encephalopathy with Seizure	NR	NR
		75/F	NPS	Neg	Yes	Encephalopathy DD limbic Encephalitis	NR	NR
		66/M	NPS, BAL	Neg	NR	Intracranial hemorrhage	NR	NR
		56/M	OPS, BAL	Neg	NR	Encephalopathy, CIP	NR	NR
		41/F	OPS	Neg	NR	Osmotic demyelination syndrome	NR	NR
		68/M	BAL	Neg	NR	Seizure	NR	NR
		64/M	OPS, BAL	Neg	NR	Encephalopathy, CIP	NR	NR
		57/M	OPS, BAL	Neg	NR	Status epilepticus	NR	NR
		75/M	OPS, BAL	Neg	NR	Encephalopathy, CIP	NR	NR
		47/M	OPS, BAL	Neg	NR	Encephalopathy, CIP	NR	NR
		50/M	OPS, BAL	Neg	NR	Seizure	NR	NR
		51/M	OPS, BAL	Neg	NR	Encephalopathy	NR	NR
		65/F	OPS	Neg	NR	Encephalopathy	NR	NR
		45/M	OPS	Neg	NR	Unclear headache	NR	NR
		68/F	OPS	Neg	NR	Encephalopathy	NR	NR

(Continued)

TABLE 1 | Continued

References	Total case	Age/sex	SARS-CoV-2 diagnostic	CSF PCR	LOS	Neurological diagnosis	Treatment	Outcome
		81/M	OPS, BAL	Neg	NR	Encephalopathy	NR	NR
		48/M	OPS	Neg	Yes	UVN	NR	NR
		58/F	OPS	Neg	NR	UANP	NR	NR
		80/M	OPS	Neg	Yes	Encephalopathy	NR	NR
		70/M	OPS, BAL	Neg	NR	CIP, Ischemic stroke	NR	NR
		76/F	OPS, BAL	Neg	NR	Prolonged coma	NR	NR
		79/F	OPS, BAL	Neg	NR	GBS and encephalopathy	NR	NR
		28/F	OPS	Neg	NR	Ischemic stroke	NR	NR
		68/M	OPS	Neg	NR	Seizures	NR	NR
		86/F	OPS	Neg	NR	GBS	NR	NR
Perrin et al. (58)	5	71/F	NPS	Neg	NR	Encephalopathy	Levetiracetam and steroid	Fatal
		64/M	NPS	Neg	NR	Encephalopathy	L/R, benzodiazepine, steroid, IVIG	Non-fatal
		53/F	NPS	Neg	NR	Encephalopathy	HCQ	Non-fatal
		51/M	NPS	Neg	NR	Encephalopathy	HCQ	Non-fatal
		67/M	NPS	Neg	Yes	Encephalopathy	HCQ, steroids	Non-fatal
Toscano et al. (59)	5	77/F	NPS	Neg	NR	GBS	IVIG	Non-fatal
		23/M	NPS	Neg	NR	GBS	IVIG	Non-fatal
		55/M	NPS	Neg	NR	GBS	IVIG	Non-fatal
		76/M	NPS	Neg	Yes	GBS	IVIG	Non-fatal
		61/M	Serology	Neg	Yes	GBS	IVIG and PE	Non-fatal

AC, acyclovir; ADEM, Acute disseminating encephalomyelitis; ART, antiretroviral therapy; AZ, azithromycin; BAL, Bronchoalveolar lavage; CF, ceftriaxone; CIP, critical illness polyneuropathy; CSF, cerebrospinal fluid; CVST, Cerebral venous sinus thrombosis; DD, differential diagnosis; GBS, Guillain-Barré syndrome; HCQ, hydroxychloroquine; HSV, herpes simplex virus; IVIG, intravenous immunoglobulin; LOS, loss of smell; L/R, lopinavir and ritonavir; MOG-IgG-MD, myelin oligodendrocyte glycoprotein- antibody-mediated disease; NPS, nasopharyngeal swab; NS, nasal swab; NSAIDs, Non-steroidal anti-inflammatory drug; OPS, oropharyngeal swab; OPT, oral prednisolone tapering; PCR, polymerase chain reaction; PE, plasma exchange; PNC, Polyneuritis cranialis; P/T, piperacillin and tazobactam; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; TA, tracheal aspirate; TIA, Transient ischemic attack; UVN, Unilateral Vestibular Neuritis.

NR denotes data not reported in the studies, F means female, M means male, Pos means positive, and Neg means negative.

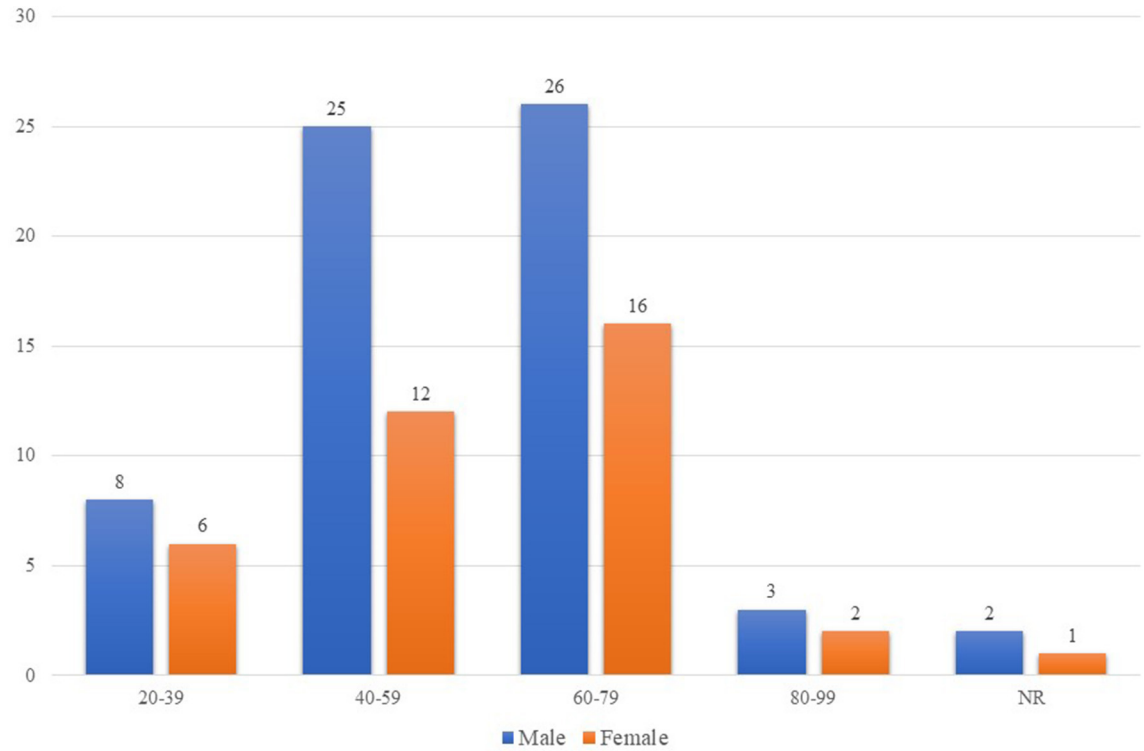


FIGURE 2 | Age and sex distribution of COVID-19 patients who underwent CSF PCR assay for SARS-CoV-2. The number of men and women infected with COVID-19 who developed severe neurological manifestations and underwent CSF PCR assay for SARS-CoV-2 increased as the age of the individuals increased. The impact of COVID-19 was higher in patients aged 60-70 years old of both sexes. In addition, there were more COVID-19 infected men than COVID-19 infected women of all ages. COVID-19, coronavirus disease 2019; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2. NR denotes that the ages of two males and one female were not reported.

patient did not regain consciousness even after sedation was discontinued.

SARS-CoV-2 PCR Assay Results

Patients were confirmed to be COVID-19 positive when tested positive in PCR assay from nasopharyngeal swab or nasal swab (50/101), oropharyngeal swab (14/101), bronchoalveolar lavage (5/101), tracheal aspirate (1/101), or a combination of them (20/101). The PCR assay was positive in one study, but the swab used was not specified (51). Two patients were confirmed COVID-19 positive with the presence of SARS-CoV-2 in CSF (32, 44), and in eight patients with negative PCR test, COVID-19 infection was diagnosed with the presence of anti-SARS-CoV-2 in serum (**Table 1**) (21, 37, 38, 43, 46, 48, 53, 59).

CSF PCR assay for SARS-CoV-2 was positive for only four (3.96%) patients (22, 32, 34, 44) and negative in 97 (96.04%) patients (15–21, 23–31, 33, 35–43, 45–59). Of the 101 patients, information on the status of anosmia was available in 55 patients (51 patients had negative CSF PCR results, while four had positive CSF PCR results). Out of 51 patients with negative CSF PCR results, 38 had smell disorder, while 13 had no nasal symptoms. Meanwhile, two of the four patients with positive CSF PCR results for SARS-CoV-2 had olfactory dysfunction, while the other two did not (**Table 1**).

DISCUSSION

This systematic review identified studies that performed CSF PCR assay for SARS-CoV-2 in COVID-19 positive patients and reported anosmia status to identify the common neurological manifestations associated with COVID-19 and to analyze the interrelation between CSF PCR results and anosmia. The neurological manifestations of COVID-19 are diverse. There was an equal number of patients with and without olfactory disorders who had positive CSF PCR results for SARS-CoV-2.

COVID-19 can trigger other autoimmune neurological complications such as neuromyelitis optica spectrum disorders or multiple sclerosis (30, 41, 46), which should be identified and treated promptly (44). In addition, COVID-19 patients with olfactory disorders and other severe neurological symptoms should be examined for possible neurodegenerative disease when suspected of having one (29).

In our study, ∼4% of the participants had positive CSF PCR assay for SARS-CoV-2, similar to the finding of one study, which showed positive results in 6% of the participants, indicating SARS-CoV-2 neuroinvasion is a rare occurrence (3). However, negative CSF PCR results for SARS-CoV-2 may be due to delayed immune-mediated neurological damage after viral clearance (51, 53). Furthermore, the sensitivity decreases if

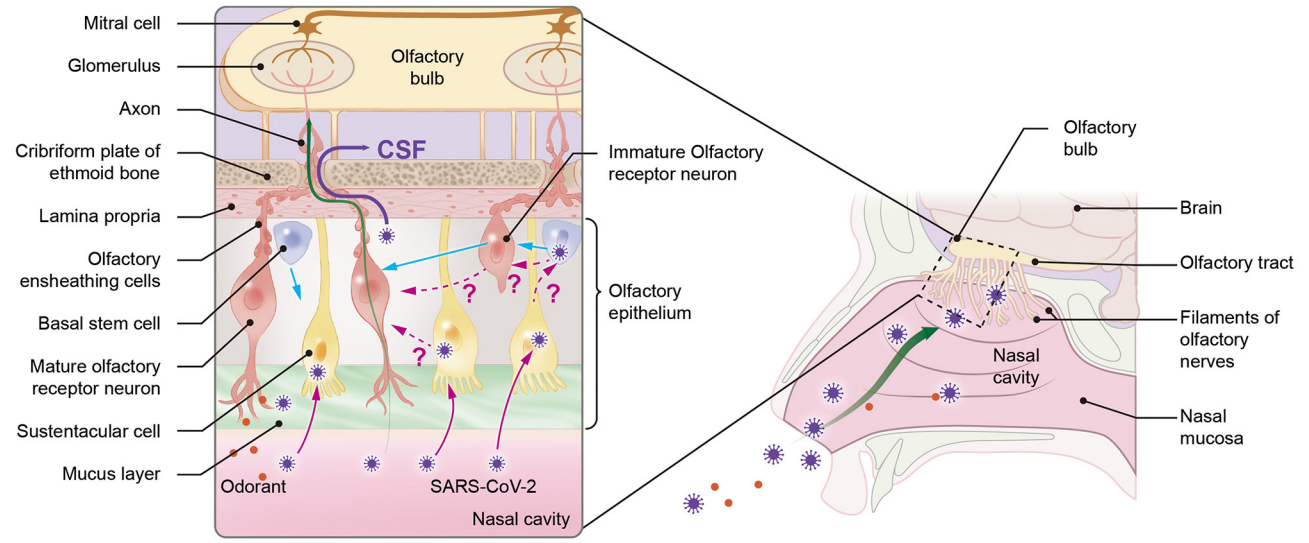


FIGURE 3 | Possible mechanisms of neuroinvasion of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) *via* the olfactory pathway. SARS-CoV-2 can enter the central nervous system (CNS) through the olfactory system in one of two ways: directly through the cerebrospinal fluid (CSF) by crossing the channels created by olfactory ensheathing cells (OECs) (purple straight line) or through olfactory receptor neurons (ORNs). Since ORNs lack angiotensin-converting enzyme-2 (ACE-2), viruses are hypothesized to be transferred from sustentacular (SUS) cells, which contain ACE-2, either directly to mature ORNs (mORNs) or to stem cells (also containing ACE-2), which can then transfer the virus to immature ORNs (iORNs) during ORN regeneration process, where infected iORNs could grow into infected mORNs. The pink dotted line represents the hypotheses. Viruses can directly enter the CNS from ORN through ACE-2-independent mechanisms (green straight line) using factors such as neurolipin-1. The blue straight line represents the regeneration of ORNs and SUS cells from stem cells.

samples are tested after a long period of symptom onset, giving negative results (43, 58). In addition, according to our review, only 44% of the published articles on CSF studies performed CSF PCR assay for SARS-CoV-2 in COVID-19 infected patients who experienced neurological symptoms. Therefore, despite the procedural and logistical complexity, the authors suggest an early collection of CSF samples, performing CSF PCR assay for SARS-CoV-2, detecting anti-neuronal autoantibodies, and using 18 F-fluorodeoxyglucose positron emission tomography in suspected cases could aid in the diagnosis and management of the patients, notably in magnetic resonance imaging negative cases (35, 51). Although the additional financial concern associated with the CSF PCR assay cannot be avoided, there were cases of testing positive in CSF PCR assay despite being negative in a nasal PCR or rapid COVID-19 test (32, 44). In addition, cost-effective studies in other neurotropic viruses have shown that the CSF PCR assay is cost-effective; similar studies in COVID-19 are required (60). Furthermore, a negative CSF PCR assay does not rule out the presence of the virus in the CNS; therefore, further studies of SARS-CoV-2 antibodies are required (57). Moreover, a recent study has shown that SARS-CoV-2 retrograde neuroinvasion *via* the olfactory route causes neuroinflammation (9). The detection of SARS-CoV-2 in the olfactory epithelium and various radiological findings in patients with COVID-19 suggests that despite the rarity of SARS-CoV-2 neuroinvasion *via* the olfactory system, it should not be overlooked (9, 21, 39, 61).

Similar to other respiratory neurotropic viruses, the direct neuroinvasion of SARS-CoV-2 in COVID-19 patients could occur mainly in two ways: damage to the olfactory epithelium or diffusion through the olfactory ensheathing cell (OEC) (1, 2) (**Figure 3**). Although ORNs of humans do not express SARS-CoV-2 entry proteins, factors other than angiotensin-converting enzyme-2 may be involved in a viral entry, such as neurolipin-1, which is highly expressed in ORNs (62–64) or SARS-CoV-2 can have non-neuronal mechanism (6, 9, 63). The neuronal and non-neuronal damage of the olfactory epithelium are responsible for the mechanism of loss of smell observed in COVID-19 patients (6, 7, 9). Nevertheless, viruses that are rapidly transported to the olfactory bulb before being affected by ORN apoptotic actions may invade the CNS (5).

In addition, viruses as small as 100nm can also diffuse *via* the channels formed by OEC gaining direct access to the CSF (1, 65). The size of SARS-CoV-2 ranges from 60 to 140 nm (66). Additionally, direct infection of the OEC can release viruses into these channels and subsequently transport the virus to the olfactory bulb (1). Thus, SARS-CoV-2 with a smaller size can utilize this mode of viral transmission.

Studies analyzing the olfactory mucosa of COVID-19 patients with and without anosmia are required to acknowledge that apoptosis of ORNs is the cause of COVID-19-related anosmia. Future studies with a larger sample size involving nasal brush sampling method and CSF PCR assay can be performed on COVID-19 patients to determine whether apoptosis of ORNs could provide neuroprotection in COVID-19 patients with anosmia (9).

This study has several limitations. Olfactory mucosa biopsy is required to effectively analyze the association between apoptosis of ORNs with anosmia and neuroprotection. However, few

studies were included in this analysis. Because the biopsy is an invasive procedure, it is rarely done in patients with COVID-19 only for research purposes (9), unlike animal studies. Additionally, studies that determine whether apoptosis of ORNs occurs in COVID-19 patients experiencing anosmia and SARS-CoV-2 CSF PCR assays are not available. For these reasons, the study design for analyzing the hypothesis was only feasible with observational studies. Though the risk of bias assessment showed an overall low risk, fundamental bias from the study design cannot be fully excluded. The findings from this review are not directly comparable with the results from other neurotropic viruses till these unanswered issues are solved. The number of patients with positive CSF PCR results did not differ by anosmia status, which may be related to the limited sample size and non-standard CSF PCR assay procedures. The CSF PCR assay is not commonly performed in COVID-19 patients with neurological manifestation. In this study, among COVID-19 patients with neurological manifestation, only 44% of patients underwent PCR assay for SARS-CoV-2 in the CSF to identify COVID-19 related neurological disorders. Though anosmia is common in COVID-19 patients, underreporting issues cannot be ignored, and because of limitation to our methodology, the neurological manifestations observed in individuals with COVID-19 cannot be generalized. Similarly, the possibility of an indirect mechanism of neuroinvasion of SARS-CoV-2 should not be overlooked. We could not investigate the neurological aspects of different strains of SARS-CoV-2 in COVID-19 infected patients and geographical and temporal relationships, particularly those concerning olfactory alteration, because information about the SARS-CoV-2 strain along with geographical and temporal information was not available in the included studies. Future studies with proper sample sizes involving definitive methods such as the nasal mucosa sampling method could provide a clear answer to the association between apoptosis of ORNs with anosmia and neuroprotection.

CONCLUSION

The neurological spectrum of COVID-19 is wide. Direct neuroinvasion of SARS-CoV-2 *via* the olfactory route is uncommon. Although previous experimental models of respiratory neurotropic viruses have demonstrated that apoptosis of the olfactory nerve blocks its neuroinvasive ability, this remains controversial in the case of SARS-CoV-2, since at present, human evidence is too scare limiting any conclusion to be drawn about the protection role of virus' olfactory mucosa invasion toward CNS invasion. More research with definitive methods is required to study the neuroprotective potential of ORN apoptosis in COVID-19 patients.

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/s.

AUTHOR CONTRIBUTIONS

EK had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. SP and EK: conceptualization, writing original draft, and formal analysis. SP, SO, and EK: data acquisition and writing review and editing. EK: funding and supervision. All authors contributed to the article and approved the submitted version.

FUNDING

This work was funded by a grant from the Korean government, South Korea (Ministry of Science and ICT, MICT; NRF-2021R1F1A1062044) and by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, South Korea (Grant Number 2021R1A6A1A03044296). The funder had no role in the trial design, data collection, data interpretation, or report preparation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.887164/full#supplementary-material

REFERENCES

1. Van Riel D, Verdijk R, Kuiken T. The olfactory nerve: a shortcut for influenza other viral diseases into the central nervous system. *J Pathol.* (2015) 235:277–87. doi: 10.1002/path.4461
2. Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat. Neurosci.* (2021) 24:168–175. doi: 10.1101/2020.06.04.135012
3. Lewis A, Frontera J, Placantonakis DG, Lighter J, Galetta S, Balcer L, et al. Cerebrospinal fluid in COVID-19: a systematic review of the literature. *J Neurol Sci.* (2021) 421:117316. doi: 10.1016/j.jns.2021.117316
4. Mori I, Goshima F, Imai Y, Kohsaka S, Sugiyama T, Yoshida T, et al. Olfactory receptor neurons prevent dissemination of neurovirulent influenza A virus into the brain by undergoing virus-induced apoptosis. *J Gen Virol.* (2002) 83:2109–2116. doi: 10.1099/0022-1317-83-9-2109
5. Mori I, Nishiyama Y, Yokochi T, Kimura Y. Virus-induced neuronal apoptosis as pathological and protective responses of the host. *Rev Med Virol.* (2004) 14:209–16. doi: 10.1002/rmv.426
6. Butowt, R. and von Bartheld CS, Anosmia in COVID-19: underlying mechanisms and assessment of an olfactory route to brain infection. *Neuroscientist.* (2020) 2020:1073858420956905. doi: 10.1177/1073858420956905
7. Bryche B, St Albin A, Murri S, Lacôte S, Pulido C, Ar Gouilh M, et al. Massive transient damage of the olfactory epithelium associated with infection of sustentacular cells by SARS-CoV-2 in golden Syrian hamsters. *Brain Behav Immun.* (2020) 89:579–86. doi: 10.1016/j.bbi.2020.06.032

8. Le Bon SD. and Horoi M, Is anosmia the price to pay in an immune-induced scorched-earth policy against COVID-19? *Med Hypotheses.* (2020) 143:109881. doi: 10.1016/j.mehy.2020.109881
9. de Melo GD, Lazarini F, Levallois S, Hautefort C, Michel V, Larrous F, et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. *Sci Transl Med.* (2021) 13:eabf8396. doi: 10.1126/scitranslmed.abf8396
10. Talavera B, García-Azorín D, Martínez-Pías E, Trigo J, Hernández-Pérez I, Valle-Peñacoba G, et al. Anosmia is associated with lower in-hospital mortality in COVID-19. *J Neurol Sci.* (2020) 419:117163. doi: 10.1016/j.jns.2020.117163
11. Purja S, Shin H, Lee JY, Kim E. Is loss of smell an early predictor of COVID-19 severity: a systematic review and meta-analysis. *Arch Pharm Res.* (2021) 44:725–40. doi: 10.1007/s12272-021-01344-4
12. Ciurleo R, De Salvo S, Bonanno L, Marino S, Bramanti P, Caminiti F. Parosmia and neurological disorders: a neglected association. *Front Neurol.* (2020) 11:543275. doi: 10.3389/fneur.2020.543275
13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ.* (2021) 372:n71. doi: 10.1136/bmj.n71
14. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Chapter 7: systematic reviews of etiology and risk. In *JBI Manual for Evidence Synthesis JBI*, editors Aromataris E, Munn Z (2020). Available online at: https://synthesismanual.jbi.global (accessed October 21, 2021).
15. Andriuta D, Roger PA, Thibault W, Toublanc B, Sauzay C, Castelain S, et al. COVID-19 encephalopathy: detection of antibodies against SARS-CoV-2 in CSF. *J Neurol.* (2020) 267:2810–1. doi: 10.1007/s00415-020-09975-1
16. Assini A, Benedetti L, Di Maio S, Schirinzi E, Del Sette M. New clinical manifestation of COVID-19 related Guillain-Barrè syndrome highly responsive to intravenous immunoglobulins: two Italian cases. *Neurol Sci.* (2020) 41:1657–8. doi: 10.1007/s10072-020-04484-5
17. Atakla HG, Noudohounsi M, Sacca H, Tassiou NRA, Noudohounsi WC, Houinato DS. Acute Guillain-Barré polyradiculoneuritis indicative of COVID-19 infection: a case report. *Pan Afr Med J.* (2020) 35:150. doi: 10.11604/pamj.supp.2020.35.2.25745
18. Bigaut K, Mallaret M, Baloglu S, Nemoz B, Morand P, Baicry F, et al. Guillain-Barré syndrome related to SARS-CoV-2 infection. *Neurol Neuroimmunol Neuroinflamm.* (2020) 7:e785. doi: 10.1212/NXI.0000000000000785
19. Bodro M, Compta Y, Llansó L, Esteller D, Doncel-Moriano A, Mesa A, et al. Increased CSF levels of IL-1β, IL-6, and ACE in SARS-CoV-2-associated encephalitis. *Neurol Neuroimmunol Neuroinflamm.* (2020) 7:e821. doi: 10.1212/NXI.0000000000000821
20. Canavero I, Valentino F, Colombo E, Franciotta D, Ferrandi D, Mussa M, et al. Acute myelopathies associated to SARS-CoV-2 infection: viral or immune-mediated damage? *Travel Med Infect Dis.* (2021) 40:102000. doi: 10.1016/j.tmaid.2021.102000
21. Casez O, Willaume G, Grand S, Nemoz B, Lupo J, Kahane P, et al. Teaching neuroimages: SARS-CoV-2–related encephalitis. *MRI Pattern Olfact Tract Involvement Neurol.* (2021) 96:e645–6. doi: 10.1212/WNL.0000000000011150
22. Cebrián J, Gonzalez-Martinez A, García-Blanco MJ, Celdrán-Vivancos D, Palacios EL, Reig-Roselló G, et al. Headache and impaired consciousness level associated with SARS-CoV-2 in CSF: a case report. *Neurology.* (2020) 95:266–8. doi: 10.1212/WNL.0000000000010213
23. Chakraborty U, Chandra A, Ray AK, Biswas P. COVID-19-associated acute transverse myelitis: a rare entity. *BMJ Case Rep.* (2020) 13:e238668. doi: 10.1136/bcr-2020-238668
24. Chan JL, Ebadi H, Sarna JR. Guillain-Barré Syndrome with facial diplegia related to SARS-CoV-2 infection. *Can J Neurol Sci.* (2020) 47:852–4. doi: 10.1017/cjn.2020.106
25. Chauffier J, Poey N, Husain M, de Broucker T, Khalil A, Lariven S, et al. First case of mild encephalopathy with reversible splenial lesion in SARS-CoV-2 infection. *Infect Dis Now.* (2021) 51:99–101. doi: 10.1016/j.medmal.2020.09.018
26. Chaumont H, Etienne P, Roze E, Couratier C, Roger PM, Lannuzel A. Acute meningoencephalitis in a patient with COVID-19. *Rev Neurol.* (2020) 176:519–21. doi: 10.1016/j.neurol.2020.04.014
27. Chow CCN, Magnussen J, Ip J, Su Y. Acute transverse myelitis in COVID-19 infection. *BMJ Case Rep.* (2020) 13:e236720. doi: 10.1136/bcr-2020-236720
28. Civardi C, Collini A, Geda DJ, Geda C. Antiganglioside antibodies in Guillain-Barré syndrome associated with SARS-CoV-2 infection. *J Neurol Neurosurg Psychiatry.* (2020) 91:1361–2. doi: 10.1136/jnnp-2020-324279
29. Cohen ME, Eichel R, Steiner-Birmanns B, Janah A, Ioshpa M, Bar-Shalom R, et al. A case of probable Parkinson's disease after SARS-CoV-2 infection. *Lancet Neurol.* (2020) 19:804–5. doi: 10.1016/S1474-4422(20)30305-7
30. Corrêa DG, de Souza Lima FC, da Cruz Bezerra D, Coutinho AC, Hygino da. Cruz LC. COVID-19 associated with encephalomyeloradiculitis and positive anti-aquaporin-4 antibodies: cause or coincidence? *Mult Scler.* (2021) 27:973–6. doi: 10.1177/1352458520949988
31. De Gennaro R, Gastaldo E, Tamborino C, Baraldo M, Casula N, Pedrali M, et al. Selective cranial multineuritis in severe COVID-19 pneumonia: two cases and literature review. *Neurol Sci.* (2021) 2021:1–6. doi: 10.1007/s10072-021-05087-4
32. Demirci Otluoglu G, Yener U, Demir MK, and Yilmaz B. Encephalomyelitis associated with Covid-19 infection: case report. *Br J Neurosurg.* (2020) 2020:1–3. doi: 10.1080/02688697.2020.1787342
33. Dijkstra F, Van den Bossche T, Willekens B, Cras P, Crosiers D. Myoclonus and cerebellar ataxia following Coronavirus Disease (2019). *(COVID-19) Mov Disord Clin Pract.* (2020) 7:974–6. doi: 10.1002/mdc3.13049
34. Fadakar N, Ghaemmaghami S, Masoompour SM, Shirazi Yeganeh B, Akbari A, Hooshmandi S, et al. A first case of acute cerebellitis associated with coronavirus disease (COVID-19): a case report and literature review. *Cerebellum.* (2020) 19:911–4. doi: 10.1007/s12311-020-01177-9
35. Grimaldi S, Lagarde S, Harlé JR, Boucraut J, Guedj E. Autoimmune Encephalitis Concomitant with SARS-CoV-2 infection: insight from (18)F-FDG PET imaging and neuronal autoantibodies. *J Nucl Med.* (2020) 61:1726–9. doi: 10.2967/jnumed.120.249292
36. Gutiérrez-Ortiz C, Méndez-Guerrero A, Rodrigo-Rey S, San Pedro-Murillo E, Bermejo-Guerrero L, Gordo-Mañas R, et al. Miller fisher syndrome and polyneuritis cranialis in COVID-19. *Neurology.* (2020) 95:e601–5. doi: 10.1212/WNL.0000000000009619
37. Helbok R, Beer R, Löscher W, Boesch S, Reindl M, Hornung R, et al. Guillain-Barré syndrome in a patient with antibodies against SARS-CoV-2. *Eur J Neurol.* (2020) 27:1754–6. doi: 10.1111/ene.14388
38. Huber M, Rogozinski S, Puppe W, Framme C, Höglinger G, Hufendiek K, et al. Postinfectious onset of myasthenia gravis in a COVID-19 patient. *Front Neurol.* (2020) 11:576153. doi: 10.3389/fneur.2020.576153
39. Le Guennec L, Devianne J, Jalin L, Cao A, Galanaud D, Navarro V, et al. Orbitofrontal involvement in a neuroCOVID-19 patient. *Epilepsia.* (2020) 61:e90–4. doi: 10.1111/epi.16612
40. Lim ST, Janaway B, Costello H, Trip A, Price G. Persistent psychotic symptoms following COVID-19 infection. *BJPsych Open.* (2020) 6:e105. doi: 10.1192/bjo.2020.76
41. Moore L, Ghannam M, Manousakis G, A. first presentation of multiple sclerosis with concurrent COVID-19 infection. *eNeurologicalSci.* (2021) 22:100299. doi: 10.1016/j.ensci.2020.100299
42. Muccioli L, Pensato U, Cani I, Guerra L, Provini F, Bordin G, et al. COVID-19-related encephalopathy presenting with aphasia resolving following tocilizumab treatment. *J Neuroimmunol.* (2020) 349:577400. doi: 10.1016/j.jneuroim.2020.577400
43. Naddaf E, Laughlin RS, Klein CJ, Toledano M, Theel ES, Binnicker MJ, et al. Guillain-barré syndrome in a patient with evidence of Recent SARS-CoV-2 Infection. *Mayo Clin Proc.* (2020) 95:1799–801. doi: 10.1016/j.mayocp.2020.05.029
44. Novi G, Rossi T, Pedemonte E, Saitta L, Rolla C, Roccatagliata L, et al. Acute disseminated encephalomyelitis after SARS-CoV-2 infection. *Neurol Neuroimmunol Neuroinflamm.* (2020) 7:e797. doi: 10.1212/NXI.0000000000000797
45. Oguz-Akarsu E, Ozpar R, Mirzayev H, Acet-Ozturk NA, Hakyemez B, Ediger D, et al. Guillain-Barré syndrome in a patient with minimal symptoms of COVID-19 infection. *Muscle Nerve.* (2020) 62:e54–7. doi: 10.1002/mus.26992
46. Palao M, Fernández-Díaz E, Gracia-Gil J, Romero-Sánchez CM, Díaz-Maroto I, Segura T. Multiple sclerosis following SARS-CoV-2 infection. *Mult Scler Relat Disord.* (2020) 45:102377. doi: 10.1016/j.msard.2020.102377
47. Pascual-Goñi E, Fortea J, Martínez-Domeño A, Rabella N, Tecame M, Gómez-Oliva C, et al. COVID-19-associated ophthalmoparesis and

hypothalamic involvement. *Neurol Neuroimmunol Neuroinflamm.* (2020) 7:e823. doi: 10.1212/NXI.0000000000000823
48. Riva N, Russo T, Falzone YM, Strollo M, Amadio S, Del Carro U, et al. Post-infectious Guillain-Barré syndrome related to SARS-CoV-2 infection: a case report. *J Neurol.* (2020) 267:2492–4. doi: 10.1007/s00415-020-09907-z
49. Umapathi T, Quek WMJ, Yen JM, Khin HSW, Mah YY, Chan CYJ, et al. Encephalopathy in COVID-19 patients; viral, parainfectious, or both? *eNeurologicalSci.* (2020) 21:100275. doi: 10.1016/j.ensci.2020.100275
50. Vandervorst F, Guldolf K, Peeters I, Vanderhasselt T, Michiels K, Berends KJ, et al. Encephalitis associated with the SARS-CoV-2 virus: a case report. *Interdiscip Neurosurg.* (2020) 22:100821. doi: 10.1016/j.inat.2020.100821
51. Zanin L, Saraceno G, Panciani PP, Renisi G, Signorini L, Migliorati K, et al. SARS-CoV-2 can induce brain and spine demyelinating lesions. *Acta Neurochir (Wien).* (2020) 162:1491–4. doi: 10.1007/s00701-020-04374-x
52. Zhou S, Jones-Lopez EC, Soneji DJ, Azevedo CJ, Patel VR. Myelin oligodendrocyte glycoprotein antibody-associated optic neuritis and myelitis in COVID-19. *J Neuroophthalmol.* (2020) 40:398–402. doi: 10.1097/WNO.0000000000001049
53. Zoghi A, Ramezani M, Roozbeh M, Darazam IA, Sahraian MA, A. case of possible atypical demyelinating event of the central nervous system following COVID-19. *Mult Scler Relat Disord.* (2020) 44:102324. doi: 10.1016/j.msard.2020.102324
54. Cao A, Rohaut B, Le Guennec L, Saheb S, Marois C, Altmayer V, et al. Severe COVID-19-related encephalitis can respond to immunotherapy. *Brain.* (2020) 143:e102–e102. doi: 10.1093/brain/awaa337
55. Delorme C, Paccoud O, Kas A, Hesters A, Bombois S, Shambrook P, et al. COVID-19-related encephalopathy: a case series with brain FDG-positron-emission tomography/computed tomography findings. *Eur J Neurol.* (2020) 27:2651–7. doi: 10.1111/ene.14478
56. Manganotti P, Bellavita G, D'Acunto L, Tommasini V, Fabris M, Sartori A, et al. Clinical neurophysiology and cerebrospinal liquor analysis to detect Guillain-Barré syndrome and polyneuritis cranialis in COVID-19 patients: a case series. *J Med Virol.* (2021) 93:766–74. doi: 10.1002/jmv.26289
57. Neumann B, Schmidbauer ML, Dimitriadis K, Otto S, Knier B, Niesen WD, et al. Cerebrospinal fluid findings in COVID-19 patients with neurological symptoms. *J Neurol Sci.* (2020) 418:117090. doi: 10.1016/j.jns.2020.117090
58. Perrin P, Collongues N, Baloglu S, Bedo D, Bassand X, Lavaux T, et al. Cytokine release syndrome-associated encephalopathy in patients with COVID-19. *Eur J Neurol.* (2021) 28:248–58. doi: 10.1111/ene.14491
59. Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, et al. Guillain-Barré syndrome associated with SARS-CoV-2. *N Engl J Med.* (2020) 382:2574–6. doi: 10.1056/NEJMc2009191
60. Hauser RG, Campbell SM, Brandt CA, Wang S. Cost-effectiveness study of criteria for screening cerebrospinal fluid to determine the need for herpes simplex virus PCR testing. *J Clin Microbiol.* (2017) 55:1566–75. doi: 10.1128/JCM.00119-17
61. Guedj E, Million M, Dudouet P, Tissot-Dupont H, Bregeon F, Cammilleri S, et al. (18)F-FDG brain PET hypometabolism in post-SARS-CoV-2 infection: substrate for persistent/delayed disorders? *Eur J Nucl Med Mol Imaging.* (2021) 48:592–5. doi: 10.1007/s00259-020-04973-x
62. Daly JL, Simonetti B, Klein K, Chen KE, Williamson MK, Antón-Plágaro C, et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science.* (2020) 370:861–5. doi: 10.1126/science.abd3072
63. Brann DH, Tsukahara T, Weinreb C, Lipovsek M, Van den Berge K, Gong B, et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Sci Adv.* (2020) 6:eabc5801. doi: 10.1126/sciadv.abc5801
64. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science.* (2020) 370:856–60. doi: 10.1126/science.abd2985
65. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of inhaled ultrafine particles to the brain. *Inhal Toxicol.* (2004) 16:437–45. doi: 10.1080/08958370490439597
66. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from patients with pneumonia in China. *N Engl J Med.* (2020) 382:727–733. doi: 10.1056/NEJMoa2001017

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.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 27 September 2022
DOI 10.3389/fneur.2022.988359

OPEN ACCESS

EDITED BY
Beatrice Paradiso,
Dolo Hospital, Italy

REVIEWED BY
Erika Molteni,
King's College London,
United Kingdom
Laurence Ris,
University of Mons, Belgium

*CORRESPONDENCE
Julia Lier
Julia.Lier@medizin.uni-leipzig.de

SPECIALTY SECTION
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

RECEIVED 07 July 2022
ACCEPTED 29 August 2022
PUBLISHED 27 September 2022

CITATION
Lier J, Stoll K, Obrig H, Baum P, Deterding L, Bernsdorff N, Hermsdorf F, Kunis I, Bräsecke A, Herzig S, Schroeter ML, Thöne-Otto A, Riedel-Heller SG, Laufs U, Wirtz H, Classen J and Saur D (2022) Neuropsychiatric phenotype of post COVID-19 syndrome in non-hospitalized patients. *Front. Neurol.* 13:988359. doi: 10.3389/fneur.2022.988359

Neuropsychiatric phenotype of post COVID-19 syndrome in non-hospitalized patients

Julia Lier[1]*, Kristin Stoll[1], Hellmuth Obrig[2], Paul Baum[3], Lea Deterding[4], Nora Bernsdorff[1], Franz Hermsdorf[1], Ines Kunis[1], Andrea Bräsecke[1], Sabine Herzig[2], Matthias L. Schroeter[2], Angelika Thöne-Otto[2], Steffi G. Riedel-Heller[5], Ulrich Laufs[3], Hubert Wirtz[4], Joseph Classen[1] and Dorothee Saur[1]

[1]Department of Neurology, University of Leipzig Medical Center, Leipzig, Germany, [2]Max-Planck-Institute of Human Cognitive and Brain Sciences & Clinic for Cognitive Neurology, University of Leipzig Medical Center, Leipzig, Germany, [3]Department for Cardiology, University of Leipzig Medical Center, Leipzig, Germany, [4]Department of Pneumology, University of Leipzig Medical Center, Leipzig, Germany, [5]Institute of Social Medicine, Occupational Health and Public Health, University of Leipzig Medical Center, Leipzig, Germany

The post COVID-19 syndrome (PCS) is an emerging phenomenon worldwide with enormous socioeconomic impact. While many patients describe neuropsychiatric deficits, the symptoms are yet to be assessed and defined systematically. In this prospective cohort study, we report on the results of a neuropsychiatric consultation implemented in May 2021. A cohort of 105 consecutive patients with merely mild acute course of disease was identified by its high symptom load 6 months post infection using a standardized neurocognitive and psychiatric-psychosomatic assessment. In this cohort, we found a strong correlation between higher scores in questionnaires for fatigue (MFI-20), somatization (PHQ15) and depression (PHQ9) and worse functional outcome as measured by the post COVID functional scale (PCFS). In contrast, neurocognitive scales correlated with age, but not with PCFS. Standard laboratory and cardiopulmonary biomarkers did not differ between the group of patients with predominant neuropsychiatric symptoms and a control group of neuropsychiatrically unaffected PCS patients. Our study delineates a phenotype of PCS dominated by symptoms of fatigue, somatisation and depression. The strong association of psychiatric and psychosomatic symptoms with the PCFS warrants a systematic evaluation of psychosocial side effects of the pandemic itself and psychiatric comorbidities on the long-term outcome of patients with SARS-CoV-2 infection.

KEYWORDS

COVID-19, post COVID-19 syndrome, MFI-20, PCFS, neuropsychiatric disorders

Introduction

According to the British guidelines, the post COVID-19 syndrome (PCS) is defined as a constellation of symptoms which develops following a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and persists for more than 12 weeks, while not being explained by an alternative diagnosis (1). Neurological symptoms affecting patients during the acute course of COVID-19 are common and diverse including neuromuscular, cerebrovascular or inflammatory manifestations (2). In contrast, chronic neurological sequelae are less well defined (3). In the UK, a study analyzing retrospective data from over 200.000 patients reported that 12.8% with COVID-19 received a new neurological or psychiatric diagnosis during the first 6 months after initial infection (4). In hospitalized patients, post COVID-19 sequelae were detected in 80%, with a higher risk associated with treatment in the intensive care unit [ICU, (5–7)]. This observation appears to suggest a relationship between the severity of the COVID-19 manifestation and subsequent neuropsychiatric symptom load. However, even young patients who were not hospitalized for COVID-19 and asymptomatic individuals frequently describe neurological and psychiatric sequelae such as anosmia, fatigue, impaired concentration or memory problems months after the infection (8–10). In a meta-analysis covering 39 studies investigating acute and chronic symptoms following an infection with SARS-CoV-2, fatigue presented as the most common symptom in patients with PCS (44%), while anosmia was reported by 10% of the patients (11).

Since the neurobiological substrates underlying the neuropsychiatric manifestations of PCS are largely unknown, an accurate description of the clinical presentation is essential to better understand this syndrome. While many studies describe the symptoms reported by the patients, a systematic and objective characterization of the neuropsychiatric PCS phenotype is still pending. In this prospective study, we present a cohort of 105 consecutive patients from our neurological post COVID-19 consultation examined by a standardized neuropsychiatric assessment. Our main aim was to better understand which neurological, cognitive, psychiatric and psychosomatic symptoms mostly affect the functional long-term outcome of patients with SARS-CoV2 infection. In addition, a control cohort allowed us to compare clinical data, as well as laboratory and cardiopulmonary biomarker profiles between patients with and without neuropsychiatric symptoms.

Methods

In May 2021, we implemented an interdisciplinary outpatient clinic for patients suffering from health complaints after a documented infection with SARS-CoV-2, proven by PCR testing. These patients were referred by their general practitioner and primarily seen by an internal medicine specialist. During the initial contact, a thorough cardiopulmonary assessment, standard cardiopulmonary biomarkers (Table 1), SARS-CoV-2 PCR testing on nasopharyngeal swab samples, IgG antibody testing against the spike protein (receptor binding domain, RBD) and nucleocapsid (NC) to confirm the immunological response to the SARS-CoV-2 infection, and the Post COVID Functional Scale (PCFS) were performed. Additionally, several self-questionnaires, including the Multidimensional Fatigue Inventory (MFI-20), Patient Health Questionnaires 9 and 15 (PHQ-9, PHQ-15), the Generalized Anxiety Disorder scale 7 (GAD-7) were used as a basic psychiatric-psychosomatic assessment. When scores in the self-questionnaires were above predefined cut-offs (see below) or the patients reported neuropsychiatric symptoms, a neurological consultation was offered to the patients, if the symptoms were not explained by an alternative diagnosis. In order to further assess the reported deficits possibly associated with PCS, a full neurological examination and neurocognitive testing was performed (Figure 1A). The neurocognitive tests were conducted by a trained medical assistant (IK). All individuals gave their written consent for the scientific use of their data.

Post COVID functional scale (PCFS)

The five-point PCFS was introduced to monitor the functional long-term effects of COVID-19 (12). Even though it is currently not validated, several groups have found an association between a high PCFS score and treatment in the intensive care unit or need for oxygen supplementation during the acute course of illness (13). In an observational study, 70.5% of the analyzed COVID-19 patients described a fully recovered functional status six month after the acute infection (14). For our study, we translated the PCFS into German (Supplementary Figure 1). The PCFS was applied twice, at the initial contact and again at the neurological consultation by the neurologist. In case of discrepancies, the value of the second PCFS was used as primary functional outcome measure.

Multidimensional fatigue inventory (MFI-20)

The MFI-20 is a self-questionnaire and consists of five subscales covering different domains of fatigue, i.e., general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue. The subscores in each domain range from 4 to 20, with higher scores indicating higher levels of fatigue. The MFI-20 was validated in various clinical and healthy cohorts (15) and has since been widely used to assess the severity of fatigue. Currently, there are no strict cut-off values (16). For descriptive statistics, we included (i) the exact values of the subscores for

TABLE 1 Descriptive statistics.

	Range	Total cohort	Controls	Study cohort	*p*–value
n		219	55	105	
Female (*n*, %)		142; 64.5	28; 50.9	69; 66	
Age (median, IQR;[years])		49; 36.75–58.25	56; 48–68.5	44.5; 34–55.75	<0.001
Time post infection [months]		7; 5–9	9;6–10	6; 4–8	<0.001
BMI (median, IQR)		26.1; 23.1–30.2, NA 1	27.6; 24.1–29.7; NA 1	25.6; 22.8–30.7	0.52
Psychiatric premorbidities					
Total (*n*, %)		30; 13.6	4; 7.3	16; 15.1	
Depression *(n)*		22	3	14	
Anxiety *(n)*		5	1	1	
PTSD *(n)*		3	0	1	
Cardiopulmonary biomarkers					
RR syst (median, IQR; [mmHg])		140; 129.8–155; NA 4	145; 130.2–157.5; NA 1	140; 128–151; NA 3	0.06
RR dist (median, IQR; [mmHg])		85; 78–93; NA 4	82.5; 79.3–91.5; NA 1	85; 78.5–94.5; NA 3	0.74
LVEF (median, IQR; [%])		62; 58–66; NA 41	63; 59–65; NA 6	62; 59–66; NA 30	0.87
FEV1 (median, IQR; [%])		97.1; 89.85–105.6; NA 101	95.8; 90.2–109.25; NA 36	96.5; 91–105.35; NA 39	0.68
Laboratory biomarkers					
HbA1c (median, IQR; [%])		5.5; 5.2–5.7; NA 2	5.6; 5.3–5.8;NA 1	5.4; 5.2–5.6; NA 1	0.006
GFR (CKDEPI; median, IQR; [ml/min/1.73 m2)		86; 74–98.25	78; 69–90	87.5; 75–100	0.006
IL−6 (median, IQR; [pg/ml])		1.75; 1.75–1.75; NA 2	1.75; 1.75–1.75; NA 1	1.75; 1.75–1.75	0.19
CRP (median, IQR; [mg/l])		1.12; 0.62– 2.39; NA 1	1.1; 0.68–1.6; NA 1	1; 0.52–2.48	0.46
Ferritin (median, IQR; [μg/l])		98.45; 40.65– 200; NA 2	124; 49.3–259.8; NA 1	96.9; 40.7–182.5	0.58
Self–questionnaires					
MFI−20 (median, IQR)	20–100	63; 50–75.25	42; 29.5–51.5	71; 61–81.75	<0.001
PHQ−9 (median, IQR)	0–27	8; 4–12	3; 1–4.5	10.5;8–14	<0.001
PHQ−15 (median, IQR)	0–30	12; 7–16	5; 3–7	14; 10–18	<0.001
GAD−7 (median, IQR)	0–21	6; 3–9	2; 0–4	7; 5–11	<0.001
PCFS	0–4	2;1–2	0;0–1	2;2–3	<0.001
Immune status					
Anti–nucleocapside (median, IQR; [S/CO])		1.4; 1.1–2.3; NA 10	1.4; 1.1–2.325; NA 3	1.4; 1.4–2.6; NA 1	0.36
Anti–RBD (median, IQR; [AU/ml])		3763; 571–12502; NA 7	5256; 2148–14666; NA 2	2250; 376.2–10273; NA 1	0.0046
BAU/ml (median, IQR)		1243; 85.9–1759; NA 15	746.4; 356–1895; NA 6	465; 66–1464; NA 4	0.012

RR syst, systolic blood pressure; RR diast, diastolic blood presure; LVEF, left ventricular ejection fraction, FEV1, forced exspiratory volume; GFR (CKDEPI), Glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration); MFI−20, Multidimensional Fatigue Inventory; PHQ9 and 15, Patient Health Questionnaire 9 (depression module) and 15 (somatisation module); GAD−7, Generalized anxiety disorder scale; anti–RBD, antibodies against receptor binding domain; BAU/ml, binding antibody units per milliliters; IQR, interquartile range; NA, not available.

each patient. (ii) the number of domains, where the result was above the third quartile considering the mean values in the general population (16) and (iii) the total value in the MFI-20.

Patient health questionnaire-9 (PHQ-9)

The PHQ9 is a short and reliable self-questionnaire, scoring each of the nine DSM-IV criteria for depressive disorders. The score ranges between 0 to 27 with higher values indicating more severe depressive symptoms. Scores from 10 had a sensitivity of 88% and a specificity of 88% for major depression (17), making it a sufficient tool in detecting depressive disorders. Accordingly, in our study, scores from 10 were used as indicator for a clinically relevant depression.

Patient health questionnaire-15 (PHQ-15)

The PHQ15 self-questionnaire is the somatisation module of the PHQ and consists of 13 questions regarding somatoform disorders and two questions from the depressive disorders module asking about sleep disorders and lack of energy (18). The score ranges between 0 to 30 with

10.3389/fneur.2022.988359

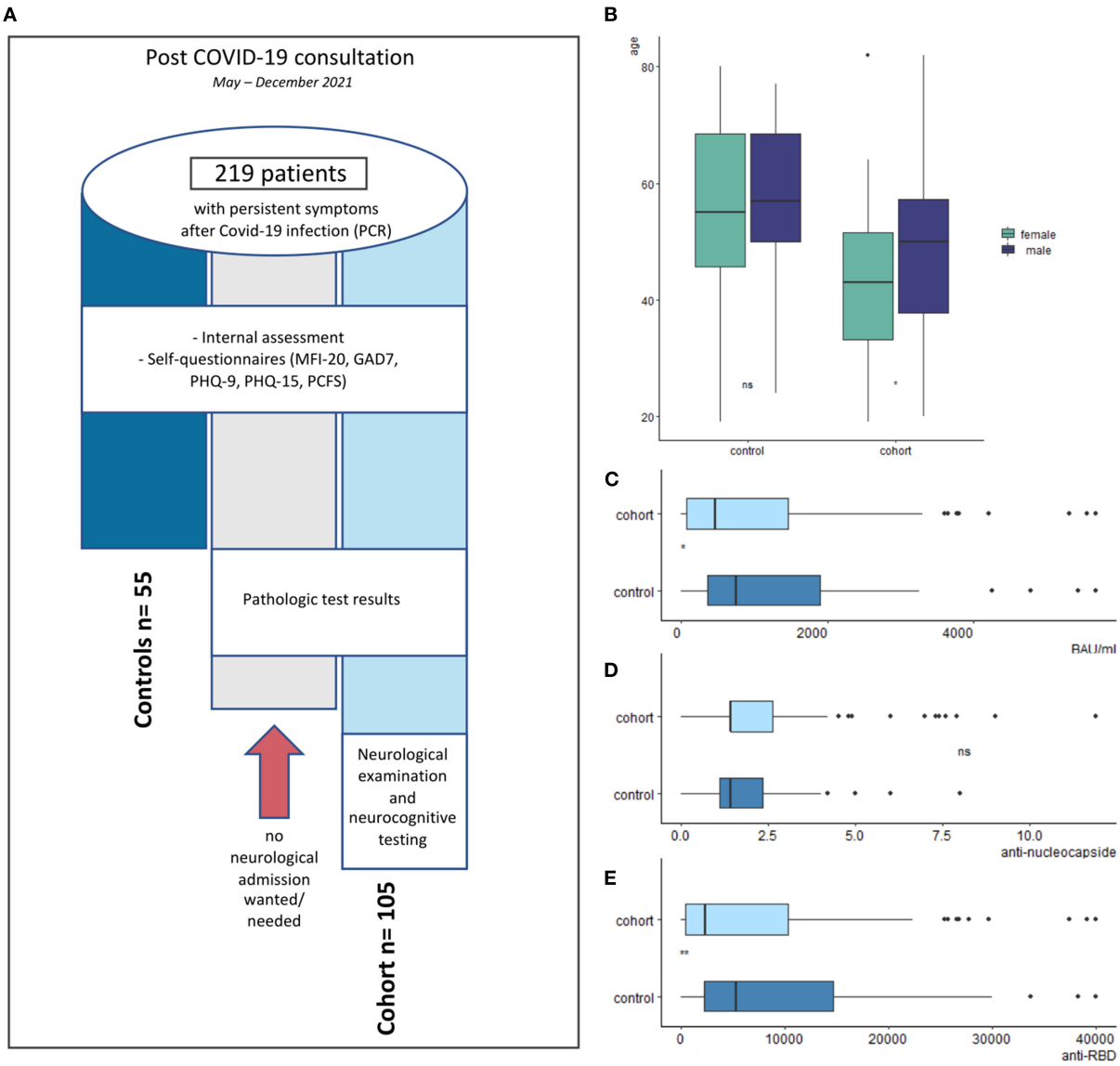


FIGURE 1
Study design and description of study cohort. **(A)** Flowchart of patient distribution. **(B)** Age between cohort and control. **(C–E)** Comparison of antibody levels between cohort and control. **(E)** Concentration of neutralizing antibodies (binding antibody units per milliliter (BAU/ml) tended to be higher in the control ($p = 0.012$). While the concentration of anti–nucleocapside antibodies did not differ, the control group had significantly higher concentrations of antibodies against the receptor binding domain (anti–RBD; $p = 0.0046$), however possible vaccination–associated influences were not examined.

higher values indicating a more severe somatisation. Significant correlations of health anxiety with illness behavior were described (19). The questionnaire was validated in different cohorts with scores of 5, 10 and 15 representing cut-off values for low, medium and high somatic symptom severity (18). In our study, scores of 10 or more were considered as an indicator for a relevant somatisation disorder.

Generalized anxiety disorder scale 7 (GAD-7)

The GAD7 is a self-questionnaire and screening tool for general anxiety disorder (GAD) but also for panic, social anxiety and PTSD. It consists of seven items which describe the most important diagnostic criteria for GAD after the DSM-IV. The score ranges from 0–21 with higher values indicating a more

severe disorder. Using a cut-off score of 10, it had a sensitivity value of 0,89 and a specificity value of 0,82 for diagnosing GAD (20). Accordingly, in our study we used a cut-off score of 10 as an indicator for GAD.

Clinical examination and neurocognitive screening

The clinical examination includes a full neurological status with testing of cranial nerves, motor, sensory and coordination functions. Neurocognitive screening consists of questions to test orientation, memory (number span forward/backward, delayed recall of three words), abstract thinking, language and praxis. The neurocognitive screening was mainly used to obtain a test-independent impression of the cognitive level of the patients.

Sniffin' sticks 12-identification test (SIT-12)

The SIT-12 is a test of nasal chemosensory performance. It consists of a battery of odorant-filled pens. Due to COVID-19-associated hygiene standards, these pens were used to create a line of two centimeters on a neutral fragrance strip. The patients were then asked to smell 3 cm in front of both nostrils and to identify the correct odorant from a list of four descriptors. The odorants are selected to be applicable to the general European population (21). Validated in several countries, a Danish study detected a mean identification score of 11 out of possible 12 among normosmic healthy adult participants (22). In our study, we used a cut-off value < 9 as an indicator for hyposmia.

Montreal cognitive assessment (MoCA)

The MoCA is a brief cognitive screening tool with high sensitivity and specificity to detect a mild cognitive impairment (23). The score ranges between 0 to 30, with higher values indicating better performance. We used the original cut-off score of < 26 as indicator for cognitive impairments. When deficits were detected during testing, elements were repeated during the neurocognitive exploration in order to verify the deficit.

Trailmaking test (TMT) A and B

The TMT consists of two parts, where the participant is instructed to connect a set of 25 dots as quickly as possible while still maintaining accuracy. In TMT A, the dots depict the numbers 1 to 25 and the participant is supposed to connect the numbers in the right order without lifting the pen from the paper. This version is used to examine cognitive processing speed. In TMT B, the participant is asked to alternate between numbers from 1 to 13 and letters from A to L. This part is used to examine executive functioning (24). The time is stopped with a clock in seconds. In our study, we used a modified version for younger populations and applied cut-off values adapted for age and education (25). A percentile ranking <16 was judged as abnormal.

Semantic verbal fluency test

The semantic verbal fluency test is a short test of verbal executive functioning. In the standard versions of the test, participants are given 1 min to produce as many unique words as possible within a semantic category. The participant's score in each task is the number of unique correct words within 1 min. In our study, we used the category "animal" and applied age and education adapted cut-off scores as suggested by Aschenbrenner et al. (26). Again, a percentile ranking <16 was judged as abnormal.

Statistical analyses

Statistical analyses were performed using R (Version 4.1.2, http://www.R-project.org). Parameters were tested for normal distribution using Shapiro-Wilk test. For normally distributed data, parametric tests such as *t*-test and Pearson correlation were used. In case of non-parametric data or extreme outliers, we used non-parametric tests such as Mann-Whitney-U-test or Spearman correlation. To adjust the *p*-value for multiple comparison, *post-hoc* Bonferroni correction was performed if needed. A p-value <0.05 was considered significant.

Results

From May to December 2021, 219 consecutive patients visited our interdisciplinary post COVID outpatient clinic. Of these, 105 individuals (48%, female $n = 69$, 66%) with a median age of 44.5 years were transferred to the neurological consultation based on the scores in the initial self-questionnaires or their complaints. This group formed the principal study cohort. 55 individuals (25%, female $n = 28$, 51%) with a median age of 56 years showing no deficits in the psychiatric-psychosomatic self-questionnaires assessed during the first consultation acted as control cohort for the parameters outside the neuropsychiatric assessment (Table 1). The remaining 59 patients did not want a neurological consultation despite (single) scores in the self-questionnaires were above the predefined cut-offs (Figure 1).

Cardiopulmonary and laboratory biomarkers

While cardiopulmonary and inflammatory markers such as the left ventricular ejection fraction (LVEF), forced exspiratory volume (FEV1) or C-reactive proteine (CRP) did not differ, renal function and HbA1c differed significantly between both groups, a phenomenon which we attributed to the younger median age of the principal cohort (Table 1, Figure 1). All PCR testings for SARS-CoV-2 were negative at the time of admission. In the total post COVID-19 outpatient cohort, RBD-antibodies were positive in 92.2% and NC-antibodies in 56.2%, demonstrating seropositivity in most patients. Interestingly, the study cohort had significantly lower levels of RBD-antibodies and concentrations of neutralizing antibodies (binding antibody units per milliliters, BAU/ml; Table 1, Figures 1C–E). However, since the levels of NC-antibodies decrease with time after infection, whereas the levels of anti-RBD antibodies increase, a vaccination-related effect on anti-RBD must be considered. This, however, was not examined systematically, since the vaccination status was not documented during the whole study period.

Functional outcome in the study cohort

94.3% ($n = 99$) of the study cohort were home-isolated with no or mild symptoms during the acute course of infection. The median time of consultation was 6 months post infection (IQR 4–8). Notably, 89% of the patients were younger than 60 ($n = 93$). Two thirds of the patients referred to the neurological consultation were women, who were significantly younger than the men in our cohort (female median age = 43, IQR 34–52, male median age = 49.5, IQR 38–57; $p = 0.046$). However, none of the tests or questionnaires displayed a significant difference between male and female patients (Supplementary Table 1). The median PCFS in our study cohort was 2, reflecting slight to moderate functional limitations in everyday life. The number of pre-COVID morbidities and the number of medications taken by the patient correlated significantly with the PCFS ($\rho = 0.28$, $p = 0.003$). At the time of consultation, 27.6% of the patients were still out of work due to persisting symptoms after the SARS-CoV-2 infection (Table 2). Furthermore, 60% made use of rehabilitation measures such as neurocognitive training or psychological support or somatic rehabilitation.

Clinical neurological examination

The clinical neurological examination was unremarkable in two thirds of the patients. Mild pallhypaesthesia or hearing deficits were detected in the remainder, with no clear links to the SARS-CoV-2 infection (Table 3). One patient suffered from critical illness neuromyopathy as a direct result of the intensive care medicine during the acute course of the disease. Regarding olfaction, < 9 correctly identified odors in the SIT-12 were detected by 15.6% ($n = 17$) of the patients, indicating mild to more severe olfactory deficits.

TABLE 2 Demographic and clinical data of the study cohort with neurological consultation.

Comorbidities	Mean (Max;NA) = 1.65 (6;1)
Medications	Mean (Max;NA) = 1.72 (10;2)
Education [years]	Median(IQR) = 13;13–16
Acute COVID−19 features	
No/mild symptoms	94.3% ($n = 99$)
Hospitalization (non–ICU)	2.86% ($n = 3$)
ICU care	0.95% ($n = 1$)
Unknown	1.9% ($n = 2$)
Functional outcome	
Sick leave	27.6% ($n = 29$)
Part–time job	9.5% ($n = 10$)
Full–Time Job	39.05% ($n = 41$)
Unemployed	1.9% ($n = 2$)
Retired	5.7% ($n = 6$)
Unknown	16.2% ($n = 17$)
Treatment	
No aftercare	33.3% ($n = 35$)
Neurocognitive training	30.48% ($n = 32$)
Psychosomatic support	25,7% ($n = 27$)
Rehabilitation	9.5% ($n = 10$)
Unknown	6.67% ($n = 7$)

ICU, intensive care unit.

Psychiatric-psychosomatic self-questionnaires

As prespecified by our experimental design, the study cohort revealed significantly higher scores in all psychiatric-psychosomatic self-questionnaires compared to the control cohort (Table 1, Figures 2A–E). A persistent exhaustion since the infection was the most often reported symptom. Eighty four patients (80%) of our study cohort described symptoms in at least four domains of fatigue tested in the MFI-20. Furthermore, there was a strong significant correlation of the overall results in the MFI-20 with the PCFS ($\rho = 0.66$, $p < 0.001$; Figure 2F). In contrast to the existing literature (16), there was no association of fatigue with age or a specific gender (Supplementary Figure 2). A positive correlation with the PCFS was also seen for the scores in the somatisation module PHQ-9 (Figure 2G), the depression module PHQ-15 (Figure 2H) and the anxiety module GAD-7 (Figure 2I). Analyzing the subgroup who did not receive hospitalization ($n = 99$) did not change these results (Supplementary Table 1).

TABLE 3 Neurological examination and neurocognitive testing of the study cohort ($N = 105$).

Test		Median (IQR;NA)	Min–Max	Cut–off	Pathologic tests *n* (%)
Neurostatus					
Non-descript					22 (21)
	Pallhypaesthesia				16 (15.24)
	Critical illness myopathy				1 (0.95)
	Other sensory deficits				5 (4.7)
SIT−12	Hyposmia	10 (9–11;4)	3–12	<9	17 (15.6)
Bed side test					
	Number sequence forward	6 (5–6;42)	3–8	<5	2 (1.9)
	Number sequence backward	4 (4–5;44)	3–6	<4	10 (9.5)
	Delayed recall	3(2–3;42)	0–3	<3	25 (23.8)
MoCA		27 (25–28)	16–30	26	37 (35.2)
	#Visuospatial	4(4–5)	1–5	<5	59 (56.2)
	#Language	5(4–5)	2–5	<4	7 (6.7)
	#Phonemic fluency			<1	56 (53.3)
	#Alertness	6(5–6)	3–6	<5	7 (6.7)
	#Abstraction	2(2–2)	0–2	<2	21 (20)
	#Memory	4(3–5)	0–5	<4	48 (45.7)
	#Orientation	6(6–6)	5–6	<5	0 (0)
Semantic fluency [words]		24.5 (20–29;3)	10–43	age– and education adapted	15 (14.3)
Trailmaking					
	TMT A [seconds]	31 (23–39.75;3)	14–89	age– and education adapted	32 (30.5)
	TMT B [seconds]	54 (44–74;4)	22–160	age– and education adapted	29 (27.2)
MFI−20		72 (61–82)	40–97	no validated cut–off	
	#1 General fatigue	16 (14–19; 4)	9–20	no validated cut–off	
	#2 Physical fatigue	16 (13–17; 4)	5–20	no validated cut–off	
	#3 Reduced activity	15 (12–17; 4)	6–20	no validated cut–off	
	#4 Reduced motivation	14 (12–17; 4)	6–20	no validated cut–off	
	#5 Mental fatigue	11 (8–14; 4)	4–19	no validated cut–off	

SIT−12, Sniffin' Sticks−12 identification test; MoCA, Montreal cognitive assessment; in the subtest of phonemic fluency, a number of words <11 was used as cut–off. MFI−20, Multidimensional Fatigue Inventory; IQR, interquartile range; NA, not available.

Neurocognitive testing

35.2% of the patients of our study cohort showed slight impairments in the MoCA when applying our predefined cut-off value (Table 3). Deficits were detected for memory, letter fluency and visuospatial functions. However, we frequently noted that similar tasks could often be performed flawlessly and with greater ease during the clinical neurocognitive exploration. While 56 patients failed the letter fluency test in the MoCA, only 11 of them showed relevant deficits in the additional semantic verbal fluency test. Furthermore, 17 patients failed the MoCA memory task, while demonstrating an error-free delayed recall on clinical examination. Errors in orientation, abstraction, alertness and language were rarely relevant (Table 3). While results in the neurocognitive testing correlated with age (Figures 3A–D), they did not correlate with the PCFS (Figures 3E–H).

Discussion

In this study, we describe the neuropsychiatric phenotype of the PCS in a prospective cohort of patients 6 months after an acute SARS-CoV-2 infection that did not require hospitalization. Despite favorable cardiopulmonary recovery, most patients still suffered from slight to moderate functional limitations in everyday life. Functional outcome highly correlated with the symptoms of fatigue, depression and somatisation, while no correlation was found with the neurocognitive scores.

All patients of our study cohort underwent a systematic neuropsychiatric assessment. Except for hyposmia in about 15% of the patients, the clinical neurological examination remained unremarkable for COVID-19 associated deficits. However, many patients reported difficulties in memory or attention. Neurocognitive testing detected slight neurocognitive impairments in about one third of the patients. However,

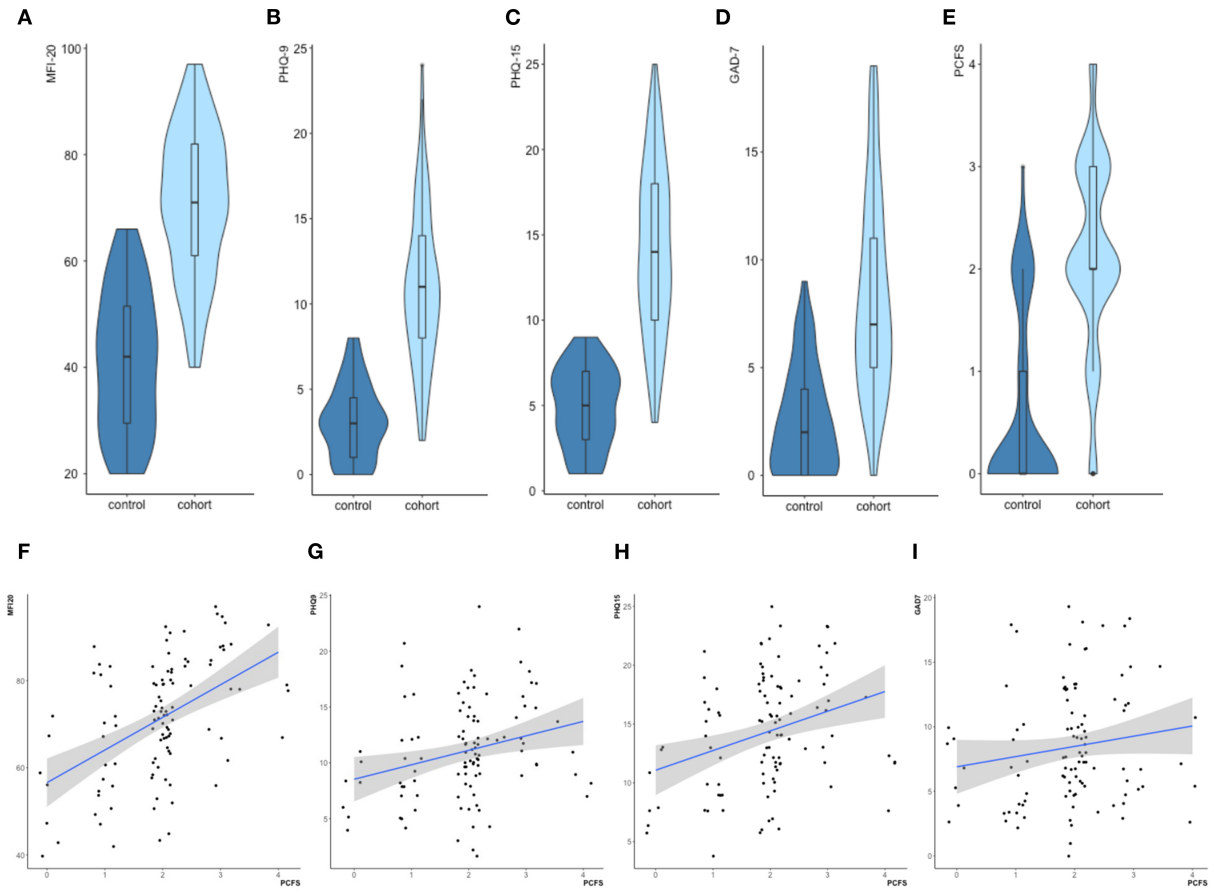


FIGURE 2
Psychiatric–psychosomatic assessment. **(A–E)** The study cohort revealed significantly higher test results in all psychiatric–psychosomatic self–questionnaires when compared to the neuropsychiatrically unaffected control cohort (all p-values < 0.001). **(F–I)** Significant correlations of the Post COVID Functional Scale (PCFS) with the total scores of the MFI–20 (**F**$\rho = 0.66$, $p < 0.001$), PHQ–9 (**G**$\rho = 0.59$, $p < 0.001$) and PHQ–15 (**H**$\rho = 0.56$, $p < 0.001$) and GAD–7 (**I**$\rho = 0.4$, p < 0.001) in the total cohort (N = 219).

discrepant results between the neurocognitive testing and the clinical examination were frequent, suggesting some degree of invalidity in our testing (e.g., low sensitivity for cognitive impairments only affecting high-level performance) and/or functional symptom load in the patients. Our results are in line with a recent study of home-isolated patients with neuropsychiatric complaints in which slight cognitive impairments in the MoCA were also found in one third of the patients about 6 months after the infection (27). Using the Mini-Mental State Examination, a large-scale study on multi-organ assessment in non-hospitalized individuals showed no differences compared to a matched control cohort (28). In addition, neuroimaging biomarkers for vascular brain damage and atrophy in that study did not differ between the groups. In contrast to the prominent complaints, formal neurocognitive testing in our study and others has not clearly revealed severe persistent neurocognitive deficits as part of the PCS. Rather, the mild severity of neurocognitive impairments was contrasted with the observation of severe symptoms of fatigue, depression and somatisation which correlated with functional outcome in the PCFS. This suggests that mainly psychiatric and psychosomatic symptoms influence the long-term outcome after a SARS-CoV-2 infection. However, one needs to emphasize, that especially the PHQ-15 covers multiple physical complaints, which might not be detectable by the internal assessment. Hence, it does not necessarily explain a psychiatric cause for these symptoms.

Regarding the pathogenesis of neuropsychiatric manifestations of COVID-19, several studies point to a potential neurotropism of SARS-CoV-2 (29). The virus enters human cells *via* the angiotensin-converting enzyme 2 receptor which is widely expressed throughout the central nervous system (CNS). However, in autopsy samples with a short post mortem interval, SARS-CoV-2 was only detected in the olfactory mucosa, but not in the olfactory sensory neurons or the parenchyma of the olfactory bulb (30), suggesting an

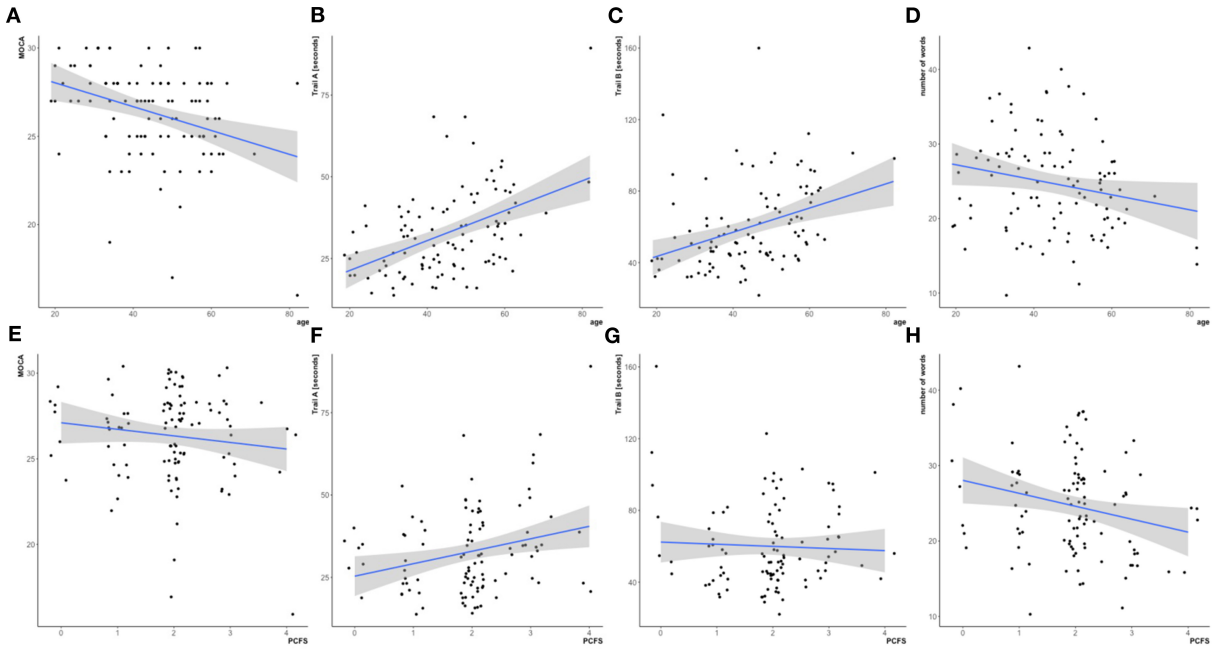


FIGURE 3
Neurocognitive assessment in the study cohort ($N = 105$). Upper row, correlation with age: Significant correlations of the MoCA (**A** $\rho = -0.34$, $p < 0.05$), time [in seconds] in the Trail making test A (**B** $\rho = 0.44$, $p < 0.05$) and Trail making test B (**C** $\rho = 0.44$, $p < 0.05$) with age. Correlation of number of correct words in the semantic fluency task with age did not stay significant after adjustment for multiple comparisons (**D** $\rho = -0.21$, $p = 0.34$). Lower row, correlation with post COVID functional scale (PCFS): Not significant correlations of the MoCA (**E** $\rho = -0.06$, $p = 1$), time [in seconds] in the Trail making A (**F** $\rho = 0.2$, $p = 0.33$) and Trail making B (**G** $\rho = 0.08$, $p = 1$) and the number of correct words in the semantic fluency task (**H**, $\rho = -0.2$, $p = 0.2$) with the PCFS.

effective barrier preventing the entry into the CNS. In this regard, analyses of the cerebrospinal fluid (CSF) of patients with COVID-19 and neurological symptoms suggest that direct CNS infection seems to be rare, given that classical signs of intrathecal CSF inflammation are typically missing and SARS-CoV-2 PCR testing usually remains negative (31). Against this background, persistence of the virus in the CNS therefore seems to be an unlikely explanation for the long-term neuropsychiatric symptoms. Alternative hypotheses include a persistent disruption of the blood-cerebrospinal fluid barrier (31), an ongoing immune-mediated inflammation (32–34) or a disrupted microcirculation (35). However, most studies were performed in *ex vivo* experimental settings or in autopsy samples of patients with SARS-CoV-2 infection, making assumptions on the potential long-term effects in the living brain difficult. Considering the absence of elevated inflammatory biomarkers and missing evidence for persistent virus or viral antigens due to the negative SARS-CoV-2 PCR testing in our cohort, a chronic inflammation driven by the virus itself seems unlikely. While we detected differences in RBD-antibody levels between the neuropsychiatric and the control cohort, the significance of this finding remains unclear. This is because RBD-antibody levels are also induced by vaccination. This conclusion was supported by the fact that the levels of IgG-antibodies against the nucleocapsid did not differ between the neuropsychiatric and the control group. Therefore, we did not find evidence for an enhanced or diminished infection-associated immune response in patients with neuropsychiatric symptoms. In line with that, other studies found no difference of antibody levels in individuals with confirmed COVID-19 with and without PCS (32, 36).

In the light of a missing distinct neurobiological substrate of the neuropsychiatric PCS, psychiatric and psychosocial factors need to be considered. Whiteside et al. (37) examined 54 outpatient patients 6 months after the acute SARS-CoV-2 infection. They found that formal cognitive performance correlated with mood and anxiety, but neither with the severity of the acute disease nor with the cognitive complaints, pointing to the importance of psychological distress for cognitive performance. This is also in line with a meta-analysis examining psychiatric symptoms after infections with other coronaviruses (SARS and MERS). Fifteen percent of the recovered patients described sleep disorders, emotional lability, impaired concentration and fatigue. However, it was not possible to distinguish between an actual pathophysiologic response to the virus infection and the general effects of the pandemic (38). Even a remarkable number of patients who, contrary to their

belief, had not even had contracted a SARS-CoV-2 infection, suffered from symptoms of PCS (39). This finding suggests that PCS could be attributed to the negative effects of the pandemic itself, i.e., the increased psychosocial burden, social isolation and existential fears. Most of the patients who came to the neuropsychiatric consultation described their concerns about limitations at work, social anxiety and worries about long-term consequences of the infection. For some of them, psychological distress seems to be exacerbated by public and social media coverage of post-COVID symptoms. Interestingly, our principal study cohort was significantly younger than the control group. While we scientifically cannot explain the age difference based on our data, socioeconomic factors as discussed above could be a reason for the higher sensitivity for complaints after a Covid-19 infection. The overrepresentation of women in our cohort is consistent with results found in multiple studies where female sex was associated with an increased risk of developing symptoms of PCS such as fatigue and cognitive impairments (5, 40). That women may have a higher risk of developing PCS may correspond to the fact that women tend to carry a larger share of the burden of the pandemic than men (41). One needs to discuss the relation of the symptoms of PCS to the psychosocial environment and a weakened psychosocial resilience due to pre-existing psychiatric comorbidities or long-term psychological stress factors, such as single parenting, fear of job loss, and financial difficulties which may affect more women than men. In line with a predominantly psychosocial origin of PCS, in our cohort, premorbid depression was more frequent in the study than in the control cohort. Future studies will need to evaluate the role of psychosocial factors in the pathogenesis of PCS more systematically and in more detail.

Irrespective of the underlying cause of PCS, it is evident that the large number of patients who are still unable to return to their work or activity level before the pandemic poses a severe socioeconomic problem. While reliable numbers of post COVID-19 cases recognized as occupational diseases are still lacking, insurance companies report record numbers in requests (42, 43). Therefore, long-term programs are needed to provide support independently of the underlying cause of persisting symptoms after COVID-19. It seems likely that symptom management will be less successful when based solely on biological rather than incorporating psychosocial concepts of illness. Fortunately, first studies show that the reported cognitive deficits may regress over time (44) and are less likely to appear in vaccinated patients (45).

The rapidly increasing case numbers around the world due to the predominance of the omicron variant might be both, a challenge and a chance. While higher case numbers could mean even more patients suffering from long-term symptoms, the social significance of an infection may decrease, as it becomes more common to become infected by SARS-CoV-2.

Limitations

There are certain limitations to our study, which we would like to address. First, since our control group also suffered from symptoms due to the SARS-CoV-2 infection, we did not test a healthy control group. Therefore, strict conclusions on the influence of the pandemic itself on neuropsychiatric symptoms remain hypothetical. Secondly, our neurocognitive tests did not allow for the detection of subtler cognitive impairments, in particular those only affecting high-level performance in daily life. Therefore, the contribution of slight cognitive impairments to PCS might be underestimated in our study and future studies should put a particular emphasis on the detection of subtle, but still functionally relevant neurocognitive deficits. This consideration must not neglect the discrepancy between the findings in the clinical neurocognitive testing and the psychiatric-psychosomatic assessment. Thirdly, we did not examine biomarkers for neurodegeneration and brain injury in blood or cerebrospinal fluid. However, although we cannot rule out permanent neuronal injury in individual cases, the results of our neurological and neurocognitive examinations do not indicate persistent organic brain dysfunction.

Conclusions

In this article, we present a prospective cohort of mainly non-hospitalized patients about 6 months after the acute SARS-CoV-2 infection who present with a clinical phenotype dominated by symptoms of depression, somatisation and fatigue. The strong association of the severity of these symptoms with the PCFS underlines the functional importance of these symptoms for long-term outcome after an infection with SARS-CoV-2. Although we did not focus on the mechanisms underlying the neuropsychiatric manifestations of PCS, our findings provide indirect evidence to suggest that PCS is strongly influenced by psychosocial consequences of the pandemic itself and by premorbid psychiatric and psychosomatic comorbidities.

Data availability statement

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

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

JL, FH, NB, SH, HO, AT-O, and MS carried out the neurological consultation. KS assisted in data collection and preparation. IK and AB carried out the cognitive and neuropsychiatric testing. PB, LD, HW, and UL carried out the internal consultation and contributed the laboratory data. SH, HO, AT-O, MS, PB, SR-H, and DS were involved in planning the prospective study procedures. JL processed the experimental data, performed the analysis, drafted the manuscript, and designed the figures. JC contributed to the implementation of the research and revised the manuscript thoroughly. DS supervised the project. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Acknowledgments

We thank all nursing stuff of the outpatient clinic of the University of Leipzig Medical Center for their personnel support in the post COVID consultation.

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.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2022.988359/full#supplementary-material

SUPPLEMENTARY TABLE 1
Statistics of cognitive and neuropsychiatric tests.

SUPPLEMENTARY FIGURE 1
German translation of post COVID functional scale. **(A)** Flowchart. **(B)** Patient questionnaire. Following the instructions given by Klok et al., (12), the PFCS is used to assess recovery after the SARS−CoV−2 infection.

SUPPLEMENTARY FIGURE 2
Analysis of the MFI subscales in the study cohort ($N = 105$) showed no differences between male and female patients **(A–E)**. An analysis of the MFI−20 total score did not show a significant correlation with age **(F)**, ($\rho = 0.17$, p.adj. $= 0.58$).

References

1. Shah W, Hillman T, Playford ED, Hishmeh L. Managing the long-term effects of covid-19: summary of NICE, SIGN, and RCGP rapid guideline. *BMJ.* (2021) 372:n136. doi: 10.1136/bmj.n136

2. Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. *Nat Rev Neurol.* (2020) 16:636–44. doi: 10.1038/s41582-020-0398-3

3. Balcom EF, Nath A, Power C. Acute and chronic neurological disorders in COVID-19: potential mechanisms of disease. *Brain.* (2021) 144:3576–88. doi: 10.1093/brain/awab302

4. Taquet M, Geddes JR, Husain M, Luciano S, Harrison PJ. 6-month neurological and psychiatric outcomes in 236 379 survivors of COVID-19: a retrospective cohort study using electronic health records. *The Lancet Psychiatry.* (2021) 8:416–27. doi: 10.1016/S2215-0366(21)00084-5

5. Halpin SJ, McIvor C, Whyatt G, Adams A, Harvey O, McLean L et al. Postdischarge symptoms and rehabilitation needs in survivors of COVID-19 infection: A cross-sectional evaluation. *J Med Virol.* (2021) 93:1013–22. doi: 10.1002/jmv.26368

6. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet.* (2021) 397:220–32. doi: 10.1016/S0140-6736(20)32656-8

7. Raman B, Cassar MP, Tunnicliffe EM, Filippini N, Griffanti L, Alfaro-Almagro F et al. Medium-term effects of SARS-CoV-2 infection on multiple vital organs, exercise capacity, cognition, quality of life and mental health, post-hospital discharge. *EClinicalMedicine.* (2021) 31:100683. doi: 10.1016/j.eclinm.2020.100683

8. Blomberg B, Mohn KG-I, Brokstad KA, Zhou F, Linchausen DW, Hansen B-A et al. Long COVID in a prospective cohort of home-isolated patients. *Nat Med.* (2021) 27:1607–13. doi: 10.1038/s41591-021-01433-3

9. Graham EL, Clark JR, Orban ZS, Lim PH, Szymanski AL, Taylor C et al. Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers". *Ann Clin Transl Neurol.* (2021) 8:1073–85. doi: 10.1002/acn3.51350

10. Tenforde MW, Kim SS, Lindsell CJ, Billig Rose E, Shapiro NI, Files DC et al. Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a Multistate Health Care Systems Network - United States, March-June 2020. *MMWR Morb Mortal Wkly Rep.* (2020) 69:993–8. doi: 10.15585/mmwr.mm6930e1

11. Jennings G, Monaghan A, Xue F, Mockler D, Romero-Ortuño R. A systematic review of persistent symptoms and residual abnormal functioning following acute COVID-19: ongoing symptomatic phase vs. Post-COVID-19 syndrome. *J Clin Med.* (2021)10:45913. doi: 10.3390/jcm10245913

12. Klok FA, Boon GJAM, Barco S, Endres M, Geelhoed JJM, Knauss S, et al. The Post-COVID-19 Functional Status scale: a tool to measure functional status over time after COVID-19. *Eur Respir J.* (2020) 56:2020. doi: 10.1183/13993003.01494-2020

13. Mohamed Hussein AA, Saad M, Zayan HE, Abdelsayed M, Moustafa M, Ezzat AR et al. Post-COVID-19 functional status: relation to age, smoking, hospitalization, and previous comorbidities. *Ann Thorac Med.* (2021) 16:260–5. doi: 10.4103/atm.atm_606_20

14. Du H-W, Fang S-F, Wu S-R, Chen X-L, Chen J-N, Zhang Y-X et al. Six-month follow-up of functional status in discharged patients with coronavirus disease 2019. *BMC Infect Dis.* (2021) 21:1271. doi: 10.1186/s12879-021-06970-3

15. Smets EMA, Garssen B, Bonke B, De Haes JCJM. The Multidimensional fatigue Inventory (MFI) - psychometric qualities of an instrument to assess fatigue. *J Psychosom Res.* (1995) 39:315–25. doi: 10.1016/0022-3999(94)00125-O

16. Schwarz R, Krauss O, Hinz A. Fatigue in the general population. *Onkologie.* (2003) 26:140–4. doi: 10.1159/000069834

17. Kroenke K, Spitzer RL, Williams JBW. The PHQ-9. Validity of a brief depression severity measure. *J General Int Med.* (2001) (16):606–13. doi: 10.1046/j.1525-1497.2001.016009606.x

18. Kroenke K, Spitzer RL, Williams JBW. The PHQ-15: validity of a new measure for evaluating the severity of somatic symptoms. *Psychosom Med.* (2002) (64):258–66. doi: 10.1097/00006842-200203000-00008

19. Zijlema WL, Stolk RP, Löwe B, Rief W, White PD, Rosmalen JGM. How to assess common somatic symptoms in large-scale studies: a systematic review of questionnaires. *J Psychosom Res.* (2013) 74:459–68. doi: 10.1016/j.jpsychores.2013.03.093

20. Spitzer RL, Kroenke K, Williams JBW, Löwe B, A. brief measure for assessing generalized anxiety disorder: the GAD7. *Arch Intern Med.* (2006) 166:1092–7. doi: 10.1001/archinte.166.10.1092

21. Kobal G, Hummel T, Sekinger B, Barz S, Roscher S, Wolf S. "Sniffin' sticks": screening of olfactory performance. *Rhinology.* (1996) 34:222–6. doi: 10.1037/t58174-000

22. Fjaeldstad A, Kjaergaard T, van Hartevelt TJ, Moeller A, Kringelbach ML, Ovesen T. Olfactory screening: validation of Sniffin' Sticks in Denmark. *Clin Otolaryngol.* (2015) 40:545–50. doi: 10.1111/coa.12405

23. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc.* (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x

24. Kopp B, Rösser N, Tabeling S, Stürenburg HJ, Haan B de, Karnath H-O et al. Errors on the trail making test are associated with right hemispheric frontal lobe damage in stroke patients. *Behav Neurol.* (2015) 2015:309235. doi: 10.1155/2015/309235

25. Rodewald K, Bartolovic M, Debelak R, Aschenbrenner S, Weisbrod M, Roesch-Ely D. A normative study of a modified trail making test in a German speaking population. *Zeitschrift für Neuropsychologie.* (2012) 23:60. doi: 10.1024/1016-264X/a000060

26. Aschenbrenner S, Tucha O, Lange KW. *Regensburger Wortflüssigkeits-Test: RWT. Hogrefe, Verlag für Psychologie.* (2000).

27. Dressing A, Bormann T, Blazhenets G, Schroeter N, Walter LI, Thurow J et al. Neuropsychologic profiles and cerebral glucose metabolism in neurocognitive long COVID syndrome. *J Nucl Med.* (2022) 63:1058–63. doi: 10.2967/jnumed.121.262677

28. Petersen EL, Goßling A, Adam G, Aepfelbacher M, Behrendt C-A, Cavus E et al. Multi-organ assessment in mainly non-hospitalized individuals after SARS-CoV-2 infection: the Hamburg City Health Study COVID programme. *Eur Heart J.* (2022) 43:1124–37. doi: 10.1093/eurheartj/ehab914

29. Zubair AS, McAlpine LS, Gardin T, Farhadian S, Kuruvilla DE, Spudich S. Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of Coronavirus Disease 2019: a review. *JAMA Neurol.* (2020) 77:1018–27. doi: 10.1001/jamaneurol.2020.2065

30. Khan M, Yoo S-J, Clijsters M, Backaert W, Vanstapel A, Speleman K et al. Visualizing in deceased COVID-19 patients how SARS-CoV-2 attacks the respiratory and olfactory mucosae but spares the olfactory bulb. *Cell.* (2021) 184:5932–49.e15. doi: 10.1016/j.cell.2021.10.027

31. Jarius S, Pache F, Körtvelyessy P, Jelčić I, Stettner M, Franciotta D et al. Cerebrospinal fluid findings in COVID-19: a multicenter study of 150 lumbar punctures in 127 patients. *J Neuroinflammation.* (2022) 19:19. doi: 10.1186/s12974-021-02339-0

32. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. *Science.* (2022) 375:1122–7. doi: 10.1126/science.abm8108

33. Ryan FJ, Hope CM, Masavuli MG, Lynn MA, Mekonnen ZA, Yeow AEL et al. Long-term perturbation of the peripheral immune system months after SARS-CoV-2 infection. *BMC Med.* (2022) 20:26. doi: 10.1186/s12916-021-02228-6

34. Schwabenland M, Salié H, Tanevski J, Killmer S, Lago MS, Schlaak AE et al. Deep spatial profiling of human COVID-19 brains reveals neuroinflammation with distinct microanatomical microglia-T-cell interactions. *Immunity.* (2021) 54:1594–1610.e11. doi: 10.1016/j.immuni.2021.06.002

35. Lee M-H, Perl DP, Nair G, Li W, Maric D, Murray H et al. Microvascular injury in the brains of patients with Covid-19. *N Engl J Med.* (2021) 384:481–3. doi: 10.1056/NEJMc2033369

36. Pereira C, Harris BHL, Di Giovannantonio M, Rosadas C, Short C-E, Quinlan R et al. The association between antibody response to severe acute respiratory syndrome coronavirus 2 infection and post-COVID-19 syndrome in healthcare workers. *J Infect Dis.* (2021) 223:1671–6. doi: 10.1093/infdis/jiab120

37. Whiteside DM, Basso MR, Naini SM, Porter J, Holker E, Waldron EJ et al. Outcomes in post-acute sequelae of COVID-19 (PASC) at 6 months post-infection Part 1: cognitive functioning. *Clin Neuropsychol.* (2022) 36:806–28. doi: 10.1080/13854046.2022.2030412

38. Rogers JP, Chesney E, Oliver D, Pollak TA, McGuire P, Fusar-Poli P et al. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. *Lancet Psychiatr.* (2020) 7:611–27. doi: 10.1016/S2215-0366(20)30203-0

39. Matta J, Wiernik E, Robineau O, Carrat F, Touvier M, Severi G et al. Association of self-reported COVID-19 infection and SARS-CoV-2 serology test results with persistent physical symptoms among french adults during the COVID-19 pandemic. *JAMA Intern Med.* (2022) 182:19–25. doi: 10.1001/jamainternmed.2021.6454

40. Ceban F, Ling S, Lui LMW, Lee Y, Gill H, Teopiz KM et al. Fatigue and cognitive impairment in Post-COVID-19 syndrome: a systematic review and meta-analysis. *Brain Behav Immun.* (2022) 101:93–135. doi: 10.1016/j.bbi.2021.12.020

41. O'Donnell M, Bourgault S, McDougal L, Dehingia N, Cheung WW, Raj A. *The Impacts of COVID-19 on Women's Social and Economic Outcomes: An Updated Review of the Evidence. CGD Policy Paper 225.* (2021). Available online at: https://cnxus.org/wp-content/uploads/2021/10/impacts-covid-19-womens-social-and-economic-outcomes-updated-review-evidence.pdf (accessed September 16, 2022).

42. Deutsche Gesetzliche Unfallversicherung e.V. *(DGUV), Glinkastraße 40, 10117 Berlin.* Available online at: https://www.dguv.de/medien/inhalt/mediencenter/hintergrund/covid/dguv_zahlen_covid.pdf (accessed September 16, 2022).

43. Berufsgenossenschaft für Gesundheitsdienst und Wohlfahrtspflege (BGW). *Pappelallee 33/35/37, 22089 Hamburg.* Available online at: https://www.bgw-online.de/bgw-online-de/presse/corona-berufskrankheit-unterstuetzung-post-covid-betroffene-64146 (accessed September 16, 2022).

44. Del Brutto OH, Rumbea DA, Recalde BY, Mera RM. Cognitive sequelae of long COVID may not be permanent: a prospective study. *Eur J Neurol.* (2021). doi: 10.1111/ene.15215

45. Kuodi P, Gorelik Y, Zayyad H, Wertheim O, Wiegler KB, Jabal KA, et al. Association between BNT162b2 vaccination and reported incidence of post-COVID-19 symptoms: Cross-sectional study 2020–21, Israel. *NPJ Vaccines.* (2022) 7:101. doi: 10.1038/s41541-022-00526-5

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 14 October 2022
DOI 10.3389/fneur.2022.979152

OPEN ACCESS

EDITED BY
Beatrice Paradiso,
Dolo Hospital, Italy

REVIEWED BY
Jordi A. Matias-Guiu,
Hospital Clínico San Carlos, Spain
Clara Limback,
Imperial College London,
United Kingdom

*CORRESPONDENCE
Simon Faissner
simon.faissner@rub.de

SPECIALTY SECTION
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

RECEIVED 27 June 2022
ACCEPTED 23 September 2022
PUBLISHED 14 October 2022

CITATION
Schulze H, Charles James J, Trampe N, Richter D, Pakeerathan T, Siems N, Ayzenberg I, Gold R and Faissner S (2022) Cross-sectional analysis of clinical aspects in patients with long-COVID and post-COVID syndrome. *Front. Neurol.* 13:979152. doi: 10.3389/fneur.2022.979152

Cross-sectional analysis of clinical aspects in patients with long-COVID and post-COVID syndrome

Hannah Schulze, Jeyanthan Charles James, Nadine Trampe, Daniel Richter, Thivya Pakeerathan, Nadine Siems, Ilya Ayzenberg, Ralf Gold and Simon Faissner*

Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany

Objective: Regarding pathogenesis, clinical manifestations, at-risk individuals, and diagnostic methods for stratifying patients for therapeutic approaches, our understanding of post-COVID syndrome is limited. Here, we set out to assess sociodemographic and clinical aspects in patients with the long-COVID and post-COVID syndrome.

Methods: We performed a cross-sectional analysis of patients presenting at our specialized university hospital outpatient clinic. We assessed patients' clinical presentation, fatigue, symptoms of depression and anxiety, and impairment of smell.

Results: A total of 101 patients were included (73.3% female), of whom 78.2% had a mild course of COVID-19. At presentation, 93.1% suffered from fatigue, 82.2% from impaired concentration, and 79.2% from impaired memory, 53.5% had impaired sleep. The most common secondary diagnosis found in our cohort was thyroid disease. Fatigue analysis showed that 81.3% of female and 58.8% of male patients had severe combined fatigue. Female gender was an independent risk factor for severe fatigue (severe cognitive fatigue OR = 8.045, $p = 0.010$; severe motor fatigue OR = 7.698, $p = 0.013$). Males suffered from more depressive symptoms, which correlated positively with the duration of symptom onset. 70.3% of patients with anamnestic smell impairment had hyposmia, and 18.9% were anosmic.

Interpretation: Most long-COVID patients suffered from severe fatigue, with the female sex as an independent risk factor. Fatigue was not associated with symptoms of depression or anxiety. Patients with long-COVID symptoms should receive an interdisciplinary diagnostic and therapeutic approach depending on the clinical presentation.

KEYWORDS

COVID-19, post-COVID syndrome, fatigue, smell disorder, depression, anxiety, SARS-CoV-2

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has led to a global pandemic. A "standard course" with specific disease phases has not yet been identified. The SARS-CoV-2 infection can be divided into three phases (1): During the acute phase, active viral replication and the initial host response occur. This phase can be accompanied by clinical manifestation but can also be asymptomatic or clinically inapparent in 67% (2). The acute phase can last days to weeks (3). Two to five weeks after the host's acute infection and virus elimination, hyperinflammation may occur in some patients, even in organ systems unaffected by the virus. This disease entity is subsumed as a multisystemic inflammatory syndrome and may present with gastrointestinal, cardiovascular, dermatologic, pulmonological, and neurologic symptoms (4). Up to 87.4% of patients having recovered from Coronavirus Disease 2019 (COVID-19) experience symptoms that persist for a protracted period from the fourth week after primary infection (1, 5), summarized as long-COVID (after the fourth week) or post-COVID syndrome (after the twelfth week). There is growing evidence that a substantial number of patients still suffer from persisting symptoms months after the acute phase of the disease. The spectrum of symptoms of long-COVID and the post-COVID syndrome is wide, ranging from fatigue, depression, and neuropsychological deficits such as memory or word-finding disorders to myopathies, muscle weakness, or sleep disturbances, amongst others (5–7). Until now, it remains incompletely understood which factors might predispose to the development of the post-COVID syndrome and whether it indeed is a new unique entity.

Mechanisms of the post-COVID syndrome are still under investigation. Causal factors are thought to include an immune system imbalance that persists long after the disease, leading to the release of pro-inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α), Interferon-1β (IFN-1β), and nitrogen metabolites such as inducible Nitric Oxide Synthase (iNOS), driving the expression of pro-inflammatory microglia in the central nervous system, among others (8). Pathophysiological considerations also include the formation of a subset of exhausted T cells and dedifferentiated monocytes observed in patients with neurological manifestations of COVID-19 (9) or anti-idiotypic antibodies (10).

Initial cross-sectional studies of patients who have experienced COVID-19 disease and have persistent symptoms show that patients who develop post-COVID syndrome usually have a mild course of the disease and suffer primarily from mood swings, fatigue, and perceived cognitive impairment (11). Until now, it remains elusive whether there might be sociodemographic characteristics or certain comorbidities driving the risk of developing the post-COVID syndrome.

In this cross-sectional non-interventional study of patients with the long-COVID and post-COVID syndrome, we characterized patients with COVID-19 disease who presented to our specialized university hospital neurological outpatient clinic regarding sociodemographic variables and clinical phenotype with a focus on fatigue, symptoms of depression, anxiety, and impairment of smell. First, we wanted to characterize patients with long-COVID regarding sociodemographic variables, secondary diseases, symptoms following COVID-19, and their duration. Second, we performed a psychometric quantification of fatigue and depressive symptoms in patients with long-COVID and post-COVID syndromes to assess whether fatigue and affective symptoms might be associated with the severity of COVID, age, sex, and prior psychiatric disorders. Those data may help to better understand neurological manifestations of long-COVID syndrome and to guide directions for therapeutic approaches.

Methods

Patients were included after having written informed consent to participate. The study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the local ethics committee of Ruhr-University Bochum (20–6827; 21–7423). Patients have been recruited at our specialized neurology long-COVID clinic at the Ruhr-University Bochum, St. Josef Hospital. Patients were recruited from January 2021 onwards. All patients were examined by an experienced, board-certified senior neurology consultant. Patients presented after a referral from a resident specialist or general practitioner. Only patients with long-COVID or post-COVID syndrome were recruited for the study. We included $n = 101$ patients older than 18 years. The COVID-19 infection was at least 2 months before the presentation. The majority of patients presented more than 3 months after infection (87/98 patients, 88.8%). To understand the long-term effects of a mild or moderate disease, only patients with a moderate disease corresponding to less than six points in the World Health Organization (WHO) clinical progression scale were included (12). Demographic data, disease duration,

Abbreviations: ACE-2-Receptor, Angiotensin-Converting Enzyme 2 Receptor; ART, Automatic Real-Time; CDC, U.S. Centers for Disease Control and Prevention; CFS, Chronic Fatigue Syndrome; CI, Confidence Interval; COPD, Chronic Obstructive Pulmonary Disease; COVID-19, Coronavirus Disease 2019; DNA, Deoxyribonucleic Acid; FSMC, Fatigue Scale for Motor Function and Cognition; GPCR-A, G Protein-Coupled Receptor A; HADS, Hospital Anxiety and Depression Scale; ICU, Intensive Care Unit; IFN-1β, Interferon 1 β; iNOS, Inducible Nitric Oxide Synthase; ME, Myalgic Encephalomyelitis; OR, Odds ratio; PEM, Post-Exertional Malaise; pRNFL, peripapillary Retinal Nerve Fiber Layer; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; SD-OCT, Spectral-Domain Optical Coherence Tomography; SD, Standard Deviation; TNF-α, Tumor Necrosis Factor α; WHO, World Health Organization.

symptoms, family history, medication history, and previous therapies were recorded.

Assessment of cognitive and motor fatigue

We performed the Fatigue Scale for Motor Function and Cognition (FSMC) to objectively measure fatigue symptoms. The FSMC is a self-report measure developed to characterize motor and cognitive fatigue symptoms into mild, moderate, and severe fatigue in patients with Multiple Sclerosis. It is also available in German (13). Patients who did not fill out all the items of the FSMC were excluded from the analysis. Accordingly, 36 patients were excluded from the evaluation, and the questionnaires of 65 patients were analyzed.

Hospital anxiety and depression scale

Psychometrics of affective symptoms were performed using the German version of the self-reported *Hospital Anxiety and Depression Scale* (HADS-D), consisting of a questionnaire with 14 items (14). Depression and anxiety symptoms are measured by seven items each, and the two subscales are interpreted independently. Twelve incomplete questionnaires had to be removed ($n = 89$ analyzed).

Odor testing

The odor testing was performed using Sniffin' Sticks (15, 16). An experienced examiner performed the test. The correct identification of 13 odors or more was considered normosmia, 8 to 12 odors indicated hyposmia, and seven or fewer were considered anosmia (17).

Statistical analysis

Data were presented as shown in the respective figure legends. We compared demographics, clinical characteristics, and outcomes between patients with univariate analysis using appropriate nonparametric tests (Mann-Whitney–*U* test, Chi-squared test). Furthermore, we applied two multivariable logistic regression models to calculate odds ratios (OR) and the corresponding 95% confidence intervals (CI) for each outcome of severe motor or cognitive fatigue. A value of $\geq$32 points in the motor subscale of FSMC and $\geq$34 points in the cognitive subscale of FSMC was used as a cut-off to indicate severe motor or cognitive fatigue, respectively. Statistical analysis was performed with GraphPad Prism (version 9.2.0) and SPSS 27.0 for Mac. $P < 0.05$ was defined as the level of statistical significance.

Results

Sociodemographic characteristics and comorbidities

We included 101 patients, and the proportion of female patients was 73.3% ($n = 74$). The mean age of the total cohort was 50.2 years (range 19–84; Supplementary Figure 1). The time from the onset of COVID symptoms to presentation to our outpatient clinic averaged 220.1 days (SD: 118.26, Range: 60–554, $n = 98$). At the presentation time, 83.0% of patients lived in a partnership. Only 2.7% of patients were in education or had no vocational degree at the presentation time. 58.8% were on sick leave and unable to work (Table 1).

The most common secondary diagnosis was thyroid disease (29.7%), with a proportion of 33.8% among females compared to a proportion of 18.5% in males (Figure 1). Thyroid disorders were followed by psychiatric or psychosomatic secondary diagnoses with a total proportion of 17.8% (females 20.3%, males 11.1%). Among the psychiatric or psychological history of patients included, depression was the most common pre-existing condition (16/101, 15.8%), followed by adjustment disorder (2/101, 2,0%), condition after borderline personality disorder (1/101, 1.0%), condition after narcotic abuse (1/101, 1,0%), anxiety disorder (1/101, 1,0%), post-traumatic stress disorder (1/101, 1,0%) and panic disorder (1/101, 1.0%). Some patients had more than one diagnosis. 14.9% of patients reported memory impairment, and 13.9% reported concentration impairment before the onset of COVID infection. Rheumatologic/autoimmune secondary diagnoses were reported by 11.9%. Pre-existing rheumatologic or autoimmunologic conditions included psoriasis (2/101; 1.0%), Bechterew's disease (1/101, 1.0%), Hashimoto's thyroiditis (4/101, 4.0%), psoriatic arthritis (1/101, 1.0%), rheumatoid arthritis (1/101, 1.0%), multiple sclerosis (2/101, 2.0%), fibromyalgia syndrome (1/101, 1.0%), and unspecified rheumatological disease requiring treatment (1/101, 1.0%). Some patients had more than one preexisting condition from the rheumatological or autoimmunological spectrum. The leading cardiovascular risk factors were arterial hypertension, with a proportion of 32.7%, and obesity (12.9%).

The majority of patients had a mild course of COVID-19

To understand whether the initial severity of the course of COVID-19 might have influenced the risk of developing long-COVID, we assessed the severity of COVID-19 using

TABLE 1 Sociodemographic data.

	Total	Male	Female
Number of patients	101	27 (26.7%)	74 (73.3%)
Age (*years*)			
Median	51	54	50.5
Mean	50.2	52.7	49.3
SD	12.9	14.2	12.4
Range	19–84	19–77	24–84
The time between symptom onset and presentation to the outpatient clinic (*days*)	$n = 98$	$n = 25$	$n = 73$
Median	201	214	184
Mean	220.1	225.4	218.3
SD	118.3	101.4	124.1
Range	60–554	72–492	60–554
Marital status			
Cohabitation/permanent partnership/married	73/88 (83.0%)	22/25 (88.0%)	51/63 (81.0%)
Single/divorced/widowed	15/88 (17.0%)	3/25 (12.0%)	12/63 (19.0%)
Highest degree			
Completion of compulsory basic secondary schooling	5/46 (10.9%)	2/13 (15.4%)	3/33 (9.1%)
General certificate of secondary education	13/46 (28.3%)	3/13 (23.1%)	10/33 (30.3%)
Technical college entrance qualification	9/46 (19.6%)	1/13 (7.7%)	8/33 (24.2%)
General qualification for university entrance	19/46 (41.3%)	7/13 (53.8%)	12/33 (36.4%)
Highest professional qualification			
No training completed/still in training	2/75 (2.7%)	1/19 (5.3%)	1/56 (1.8%)
Completed vocational training	51/75 (68.0%)	9/19 (47.4%)	42/56 (75.0%)
Completed university education	22/75 (29.3%)	9/19 (47.4%)	13/56 (23.2%)
Professional *situ*ation prior to *COVID*-19 infection			
Training/further education/retraining	1/82 (1.2%)	–	1/61 (1.6%)
Employment (full-time)	56/82 (68.3%)	16/21 (76.2%)	40/61 (65.6%)
Employment (part-time)	11/82 (13.4%)	–	11/61 (18.0%)
Early retirement	2/82 (2.4%)	1/21 (4.8%)	1/61 (1.6%)
Jobseeker	–	–	–
Housewife/houseman	2/82 (2.4%)	–	2/61 (3.3%)
Retirement	9/82 (11.0%)	4/21 (19.0%)	5/61 (8.2%)
Incapacitated	1/82 (1.2%)	–	1/61 (1.6%)
The professional *situ*ation at the time of the presentation			
Training/further education/retraining	1/52 (1.9%)	–	1/37 (2.7%)
Employment (full-time)	21/52 (40.4%)	8/15 (53.3%)	13/37 (35.1%)
Employment (part-time)	11/52 (21.2%)	–	11/37 (29.7%)
Early retirement	1/52 (1.9%)	–	1/37 (2.7%)
Jobseeker	3/52 (5.8%)	1/15 (6.7%)	2/37 (5.4%)
Housewife/houseman	3/52 (5.8%)	–	3/37 (8.1%)
Retirement	10/52 (19.2%)	5/15 (33.3%)	5/37 (13.5%)
Incapacitated	2/52 (3.8%)	1/15 (6.7%)	1/37 (2.7%)
Sick leave at the time of presentation	20/34 (58.8%)	5/10 (50.0%)	15/24 (62.5%)

the WHO clinical progression scale. The WHO progression scale ranges from 0 to 10, with a score of 1–3 representing a mild course, 4–5 a moderate disease, 6–9 a hospitalized severe disease [Intensive Care Unit (ICU) treatment], and ten representing death due to COVID-19 (12). During the acute phase of infection, 86 had a WHO clinical progression scale score ≤ 3, with the majority having a score of 2, indicating that patients were symptomatic and independent

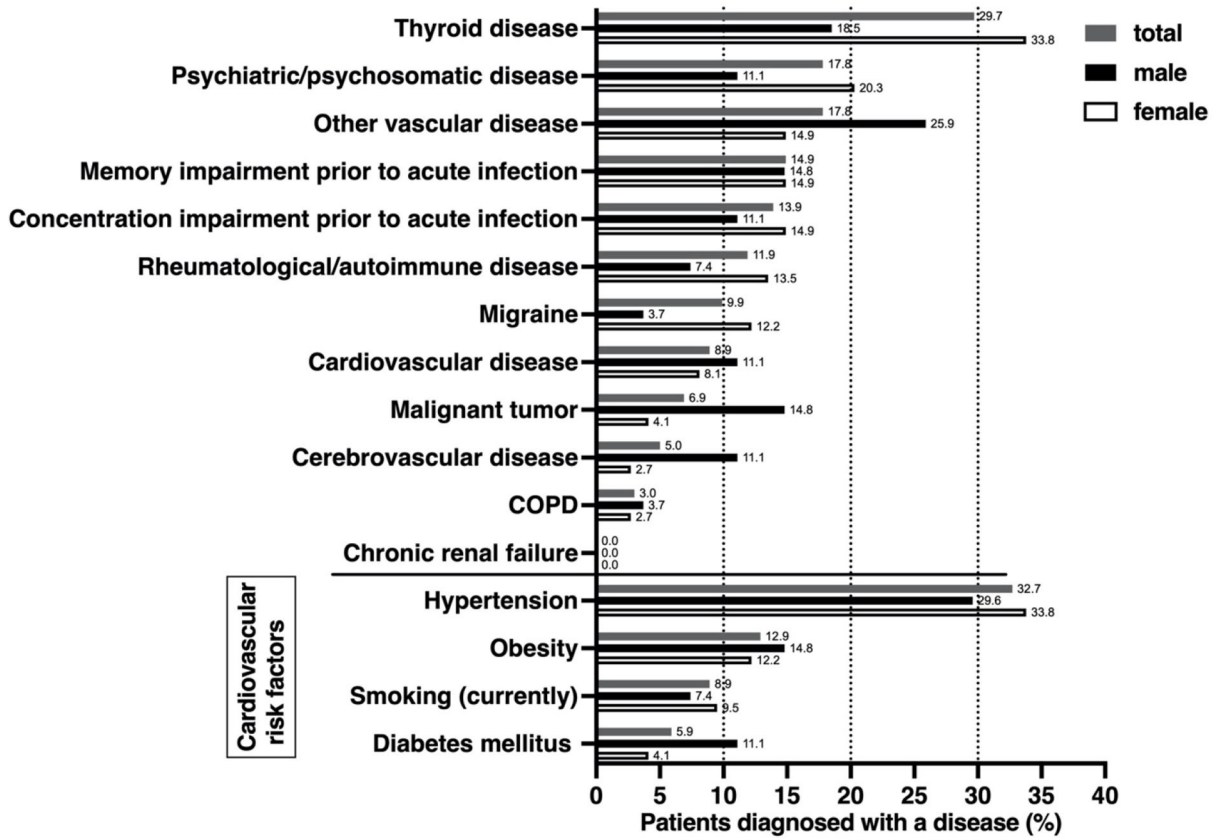


FIGURE 1
Comorbidities, self-reported cognitive impairment, and cardiovascular risk factors prior to acute COVID-19 infection. Data were derived from a self-questionnaire, covering previous comorbidities from various organ systems as well as the most important cardiovascular risk factors. Impairment of cognition or concentration was self-assessed by patients. Differences between females and males were analyzed using a nonparametric two-tailed Mann-Whitney test, which showed no differences.

(78.2%; Supplementary Table 1). 14.9% of the patients had a score >3 and were thus hospitalized. 4.0% were treated in the ICU. There was no difference in the severity of COVID-19 disease depending on gender (Supplementary Figure 2A). We also found no association between age and COVID severity ($r = 0.08$, $p = 0.43$; Supplementary Figure 2B).

Symptoms during acute infection and at the time of presentation

The most common symptoms reported during acute infection were taste disorders (67.3% of patients) and odor disturbances (65.3%). This was followed by exhaustion/fatigue (63.4%), cephalgias (51.2%), arthralgias (49.5%), and myalgias (46.5%) (Table 2). The frequency of reported symptoms changed until the time of presentation. Now, more than 90% of patients reported that they suffered from fatigue (93.1%). Moreover, a large proportion of patients reported impaired concentration (82.2%) and memory (79.2%). Sleep disturbances, only present in 28.7% of patients during the acute phase of COVID-19, increased to 53.5% at the presentation time. Impairment of smell and taste decreased to 34.7 and 27.7%, respectively.

Women are more severely affected by motor fatigue

Fatigue is one of the most prominent symptoms reported by patients following COVID-19. To differentiate between cognitive and motor fatigue, we took advantage of the FSMC. Incomplete questionnaires of individuals in our cohort of 101 patients were excluded. A total of 56 of 65 patients (86.2%) whose questionnaires could be evaluated had an FSMC sum score of ≥43 and, thus, at least mild fatigue symptoms (Figure 2A). Women had significantly higher total FSMC scores than men ($p < 0.05$; $n = 48$). This was reflected in a higher

TABLE 2 Symptoms during acute COVID-19 infection and after recovery/at time of presentation.

Symptom	Acute COVID-19 infection			At the time of the presentation		
	Total $n = 101$	Male $n = 27$	Female $n = 74$	Total $n = 101$	Male $n = 27$	Female $n = 74$
Exhaustion	64 (63.4%)	19 (70.4%)	45 (60.8%)	94 (93.1%)	23 (85.2%)	71 (96.0%)
Concentration impairment	na	na	na	83 (82.2%)	19 (70.4%)	64 (86.5%)
Memory impairment	na	na	na	80 (79.2%)	17 (63.0%)	63 (85.1%)
Sleep disorders	29 (28.7%)	7 (25.9%)	22 (29.7%)	54 (53.5%)	10 (37.0%)	44 (59.5%)
Odor disturbance	66 (65.3%)	11 (40.7%)	55 (74.3%)	35 (34.7%)	4 (14.8%)	31 (41.9%)
Myalgia	47 (46.5%)	8 (29.6%)	39 (52.7%)	33 (32.7%)	4 (14.8%)	29 (39.2%)
Headache	52 (51.2%)	11 (40.7%)	41 (55.4%)	31 (30.7%)	6 (22.2%)	25 (33.8%)
Arthralgia	50 (49.5%)	12 (44.4%)	38 (51.4%)	28 (27.7%)	5 (18.5%)	23 (31.1%)
Taste disorders	68 (67.3%)	14 (51.9%)	54 (73.0%)	28 (27.7%)	4 (14.8%)	24 (32.4%)
Muscle weakness	26 (25.7%)	11 (40.7%)	15 (20.3%)	23 (22.8%)	7 (25.9%)	16 (21.6%)
Paraesthesia	10 (9.9%)	3 (11.1%)	7 (9.5%)	18 (17.8%)	4 (14.8%)	14 (18.9%)
Vertigo	13 (12.9%)	2 (7.4%)	11 (14.9%)	18 (17.8%)	2 (7.4%)	16 (21.6%)
Racing heart	20 (19.8%)	4 (14.8%)	16 (21.6%)	17 (16.8%)	3 (11.1%)	14 (18.9%)
Alopecia	10 (9.9%)	1 (3.7%)	9 (12.2%)	12 (11.9%)	2 (7.4%)	10 (13.5%)
Chest pain	23 (22.8%)	5 (18.5%)	18 (24.3%)	13 (12.9%)	5 (18.5%)	8 (10.8%)
Diarrhea	25 (24.8%)	4 (14.8%)	21 (28.4%)	8 (7.9%)	1 (3.7%)	7 (9.5%)
Nausea	20 (19.8%)	4 (14.8%)	16 (21.6%)	7 (6.9%)	-	7 (9.5%)
Loss of appetite	22 (21.8%)	6 (22.2%)	16 (21.6%)	5 (5.0%)	-	5 (6.8%)
Skin rash	9 (8.9%)	3 (11.1%)	6 (8.1%)	5 (5.0%)	2 (7.4%)	3 (4.1%)
Dysphagia	9 (8.9%)	1 (3.7%)	8 (10.8%)	4 (4.0%)	-	4 (5.4%)
Vomiting	4 (4.0%)	1 (3.7%)	3 (4.1%)	1 (1.0%)	-	1 (1.4%)

Impairment of concentration and memory were not evaluated for the acute phase.
na, not available.

proportion of 81.3% of female patients with severe total fatigue compared to 58.8% of males ($n = 17$). The differentiation between cognitive and motor fatigue showed that cognitive fatigue did not depend on sex ($p = 0.12$, Figure 2B), whereas motor fatigue was significantly more pronounced in females than in males ($p < 0.05$; Figure 2C).

We then applied two logistic regression models to calculate odds ratios (OR) and the corresponding 95% confidence intervals (CI) for both fatigue sub-scores (cognitive and motor fatigue), with gender as the predictor. A value of 34 points for the subscale of cognitive fatigue and a value of 32 points for the subscale of motor fatigue was used as a cut-off to indicate severe fatigue, respectively. We adjusted for demographics (age, sex), preexisting conditions, and COVID-19 severity score according to WHO. For both outcomes (severe motor/ severe cognitive fatigue), the female sex was the only significant and independent predictor in this model. Women in our analysis had an 8-fold increased risk of suffering from severe fatigue (OR = 8.045, $p = 0.010$ for severe cognitive fatigue; OR = 7.698, $p = 0.013$ for severe motor fatigue; Table 3).

To understand whether age might influence fatigue, we correlated it with age and fatigue, which showed no correlation ($r = 0.15$, $p = 0.22$; Figure 2D). We hypothesized that the duration of symptom onset to the time of presentation might be associated with reduced fatigue. However, the severity of fatigue was not influenced by the period between acute infection and time of presentation in women (FSMC total $r = 0.07$; $p = 0.66$) or men ($r = -0.14$; $p = 0.62$; Figures 2E,F).

Depressive symptoms in males correlate with duration since symptom onset

To detect symptoms associated with depression or anxiety, we performed the HADS-D (89 patients with fully completed questionnaires included). Scores ≤7 correspond to normal findings, 8–10 to suspicious findings, and scores >10 to pathological findings. 17/89 patients (19.1%, 14.9% female, 31.8% male) had pathological HADS regarding depressive symptoms. 21/89 (23.6%, 22.4% female, 27.3% male) of the patients had pathological HADS regarding anxiety symptoms (Figure 3A). There was no gender-specific difference. Likewise, there was no significant correlation with age ($r = 0.03$, $p = 0.78$

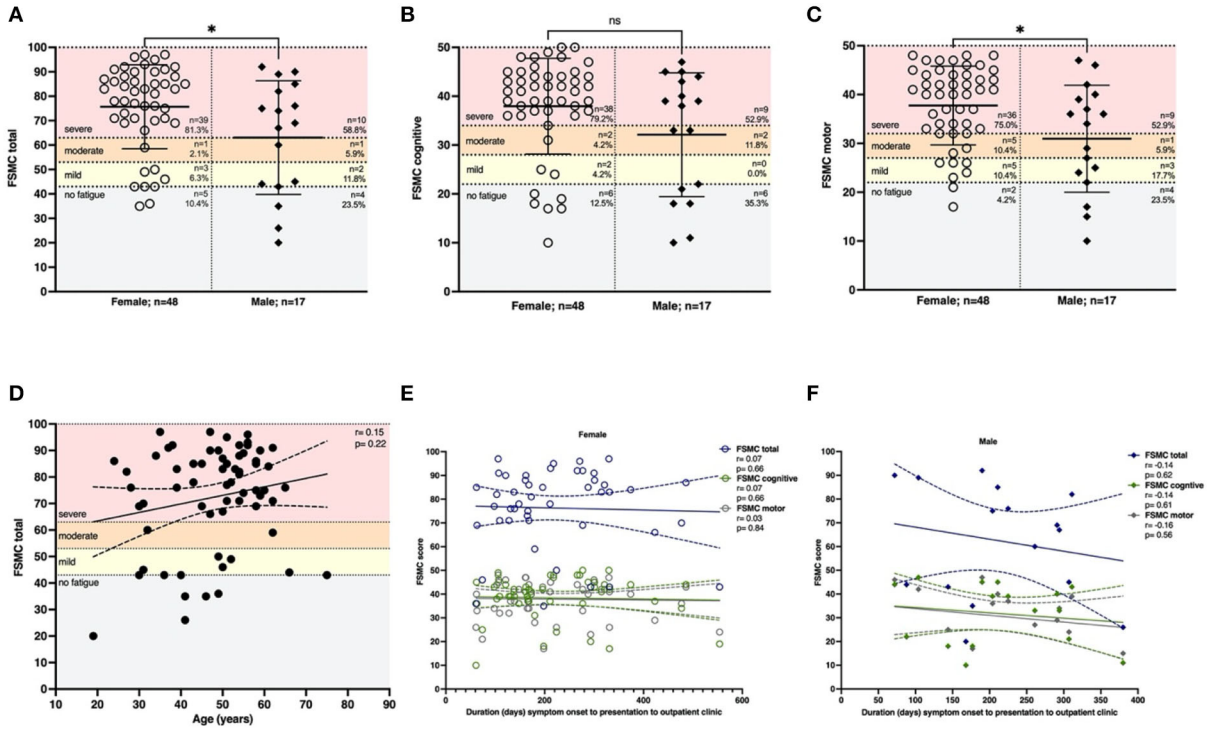


FIGURE 2
Females are affected by more severe motor fatigue. Fatigue was assessed using the FSMC score. **(A)** The majority of patients had severe fatigue (81.3% females, 58.8% males). Females were affected more severely compared to males ($p < 0.05$). This effect was driven by motor fatigue since **(B)** cognitive fatigue was not dependent on sex ($p = 0.12$). **(C)** Females were affected by more severe motor fatigue ($p = 0.02$). **(D)** Total fatigue using the FSMC was not depending on age (r = 0.15, $p = 0.22$; $n = 65$). **(E)** The latency between onset of COVID-19 and presentation did not affect the severity of total fatigue, cognitive fatigue or motor fatigue in females (total: r=0.07, $p = 0.66$; cognitive: r=0.07, $p = 0.66$; motor: $r = -0.03$, $p = 0.84$) or f) males (total: r = −0.14, $p = 0.62$; cognitive: $r = -0.14$, $p = 0.61$; motor: $r = -0.16$, $p = 0.56$). $n = 65$ total, $n = 48$ females, $n = 17$ males. Data are shown as mean ± standard deviation (SD) **(A–C)** or mean with a 95% confidence interval **(D–F)**. Data were analyzed using a two-tailed Mann–Whitney U-test **(A–C)** and nonparametric two-tailed Spearman correlation **(D–F)**. * $p < 0.05$.

for depression, $r = -0.09$, $p = 0.41$ for anxiety). To understand whether a preexisting psychiatric or psychosomatic comorbidity might be a driving factor in the severity of depressive or anxious symptoms, we stratified patients according to comorbidities. In both females and males, the proportion of patients with preexisting psychiatric or psychosomatic comorbidity was lower compared to unaffected patients. Preexisting psychiatric or psychosomatic comorbidity did not affect depressive or anxious symptoms in females or males (Figures 3B,C). We then analyzed whether latency might be a driving factor in developing depressive or anxious symptoms. In females, both the severity of depressive symptoms (r = 0.16, $p = 0.21$) and anxious symptoms ($r = 0.18$, $p = 0.14$) did not depend on the latency from symptom onset to presentation (Supplementary Figures 3A,C). In males, however, we found a significant positive correlation of depressive symptoms with a duration from symptom onset ($r = 0.47$; $p = 0.03$; Supplementary Figure 3B), while anxious symptoms were not affected by latency ($r = 0.20$, $p = 0.38$).

Impaired smell persists over time and is independent of age

Impairment of smell is one of the major symptoms of COVID-19 and was reported in 34.7% of our cohort at the time of presentation. To understand the severity of smell impairment, we performed Sniffin' Sticks on those individuals in our cohort who had anamnestic indications of an olfactory disorder ($n = 37$; female $n = 28$, male $n = 9$). The majority of patients presented with a certain degree of impaired smell, while most patients (70.3%) were categorized as being hyposmic (8–12 recognized odors) and a smaller proportion of 18.9% as being anosmic (Figure 4B). There was no gender-specific difference (Figure 4A). Age had no effect on smell ($r = -0.09$, $p = 0.59$). Moreover, we found no effect on the latency from the acute phase of COVID-19 regarding smell ($r = -0.12$, $p = 0.46$; Figure 4C). We also correlated the severity of COVID-19 according to the WHO clinical progression scale with smell, showing a modest trend that more severely affected patients

TABLE 3A Odds ratios calculated by multivariable logistic regression model for the outcome of severe motor fatigue.

Variable	OR	95% CI	*P*-value
Age	1.030	0.963–1.101	0.396
Female sex	**7.698**	**1.547–38.295**	**0.013**
Smoker	4.377	0.417–45.980	0.219
Hypertension	2.017	0.203–20.050	0.549
Diabetes	0.129	0.001–32.559	0.468
Cardiovascular disease	1.047	0.040–27.428	0.978
Cerebrovascular disease	5,013,837.486	NA	0.999
History of malignancy	966,938,006.822	NA	0.999
COPD	5,720,968.178	NA	0.999
Psychiatric or psychosomatic disease	0.828	0.106–6.464	0.857
Obesity	7.275	0.037–1,428.730	0.461
Thyroid disease	36,666,183.950	NA	0.999
Autoimmune disease	0.607	0.084–4.401	0.621
Any other disease	3.705	0.231–59.509	0.355
COVID-19 severity (WHO)	1.445	0.624–3.345	0.390
Hyperreflective lesions	471,385,404.061	NA	0.999

Significant findings are highlighted in bold.

TABLE 3B Odds ratios calculated by multivariable logistic regression model for the outcome of severe cognitive fatigue.

Variable	OR	95% CI	*P*-value
Age	1.055	0.987–1.128	0.113
Female sex	**8.045**	**1.641–39.445**	**0.010**
Smoker	2.840	0.273–29.521	0.382
Hypertension	0.712	0.095–5.353	0.742
Diabetes	0.127	0.000–113.610	0.552
Cardiovascular disease	2.015	0.104–39.008	0.643
Cerebrovascular disease	4,280,764.102	NA	0.999
History of malignancy	1.900	0.113–31.999	0.656
COPD	16,884,340.377	NA	0.999
Psychiatric or psychosomatic disease	0.717	0.092–5.579	0.751
Obesity	7.935	0.010–6,195.442	0.542
Thyroid disease	47372545.524	NA	0.999
Autoimmune disease	0.479	0.068–3.372	0.460
Any other disease	4.430	0.297–66.023	0.280
COVID-19 severity (WHO)	1.193	0.507–2.810	0.686
Hyperreflective lesions	447,271,914.738	NA	0.999

Significant findings are highlighted in bold.

might be more impaired regarding smell compared to mildly affected patients ($r = -0.21$, $p = 0.20$; Supplementary Figure 4).

Discussion

Long-COVID or post-COVID syndrome is discussed both from a scientific point of view and increasingly in the lay media due to potential effects on many patients with multidimensional implications. Here we show that leading symptoms in the post-acute phase were fatigue, impaired concentration, and subjectively impaired memory. The most common secondary diagnosis found in our cohort was thyroid disease. Most patients with long-COVID initially had a mild disease course of COVID-19; nevertheless, most patients were affected by severe fatigue, not influenced by depressive symptoms or symptoms of anxiety. Females had an 8-fold increased risk of both cognitive and motor fatigue.

Fatigue is one of the most important symptoms found in patients with long COVID, with a prevalence of 44% in a systematic review of 39 studies (18). Other symptoms include sleep disorder (33%), dyspnea (40%), cough (22%), as well as cognitive impairment (15%), anxiety (34%), and depression (32%). Those alterations have a significant personal impact since 57% of patients reported a decreased quality of life (18). Fatigue is seen in several conditions and can also be chronic. According to the *U.S. Centers for Disease Control and Prevention* (CDC), the following mandatory and optional criteria must be met to establish a diagnosis of chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME) (19): 1. Patients are limited by fatigue for more than six months. They cannot carry out their usual daily activities due to abnormal fatigue. 2. There is a so-called stress intolerance, also called post-exertional malaise (PEM). This means that physical, cognitive, or emotional stress leads to a decomposition of the initial symptoms. 3. patients with CFS do not have a restful sleep. Optional diagnostic criteria include difficulty concentrating, memory impairment, and orthostatic intolerance. The etiology and pathogenesis of CFS have not been conclusively clarified (20). Among others, neuroinfectious and consecutive neuroimmunological processes are discussed as potential origins of CFS. Epstein-Barr virus, human herpesvirus 6, enteroviruses, Borna disease virus, Borrelia burgdorferi, Coxiella burnetii, Candida albicans, Mycoplasma pneumonia, and retroviruses are considered possible causative agents (21). Cytokine levels might induce CFS, particularly IL-1β (22), oxidative stress, and mitochondrial dysfunction (23). Moreover, pathophysiological alterations in Long-COVID include cerebrovascular dysregulation with persistent cerebral arteriolar vasoconstriction, small fiber neuropathy and related dysautonomia, respiratory dysregulation, and chronic inflammation (24). Also discussed is autoimmune pathophysiology with the formation of autoantibodies against vasoregulatory G protein-coupled receptors in patients with

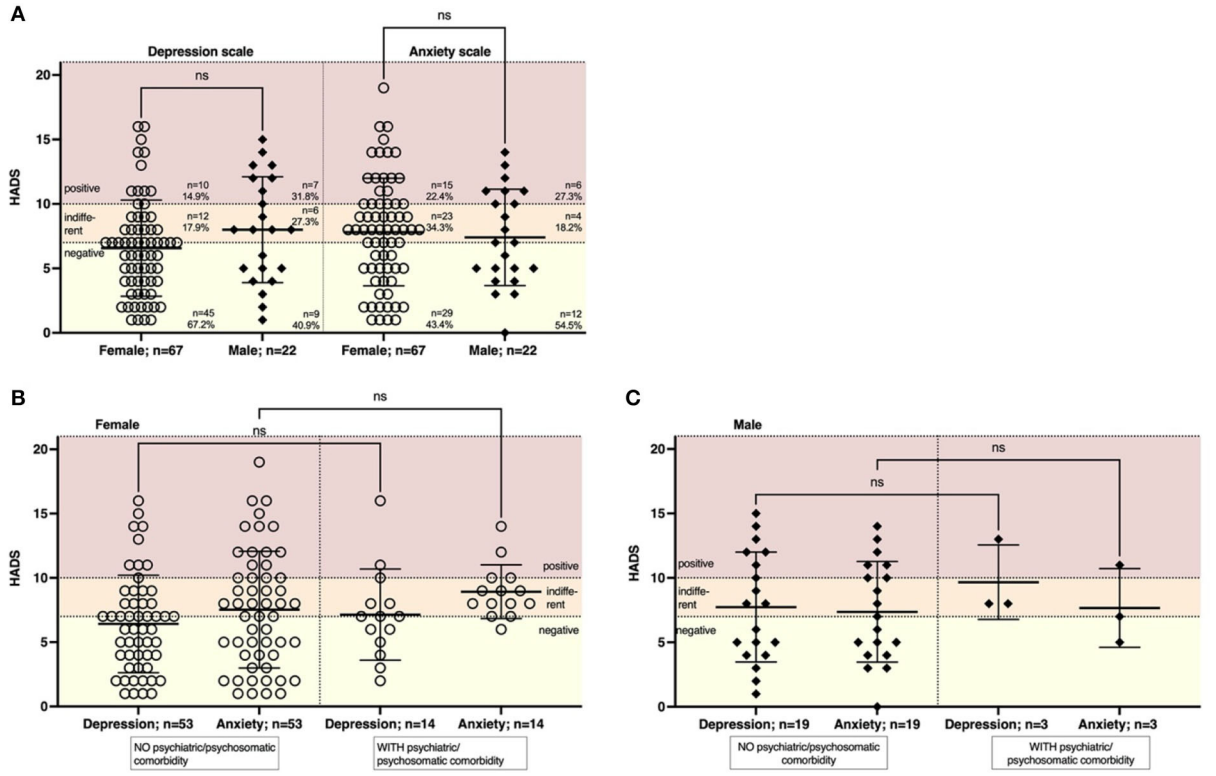


FIGURE 3
The severity of symptoms of depression or anxiety was not dependent on age or psychiatric comorbidity. We performed a HADS to assess symptoms of depression and anxiety. Scores were regarded as: 0–7 = negative, 8–10 = indifferent, >10 = positive. **(A)** The majority of patients had a negative or indifferent HADS regarding depressive symptoms (85.1% females, 68.2% males) or symptoms of anxiety (77.7% females, 72.7% males). **(B)** The severity of depressive symptoms was not affected by a previous psychiatric or psychosomatic disease in females ($n = 14$ with comorbidity, $n = 53$ women without comorbidity) or **(C)** males ($n = 3$ men with comorbidity, $n = 19$ men without comorbidity). Data are shown as mean ± standard deviation (SD). Data were analyzed using a nonparametric two-tailed Kruskal-Wallis test.

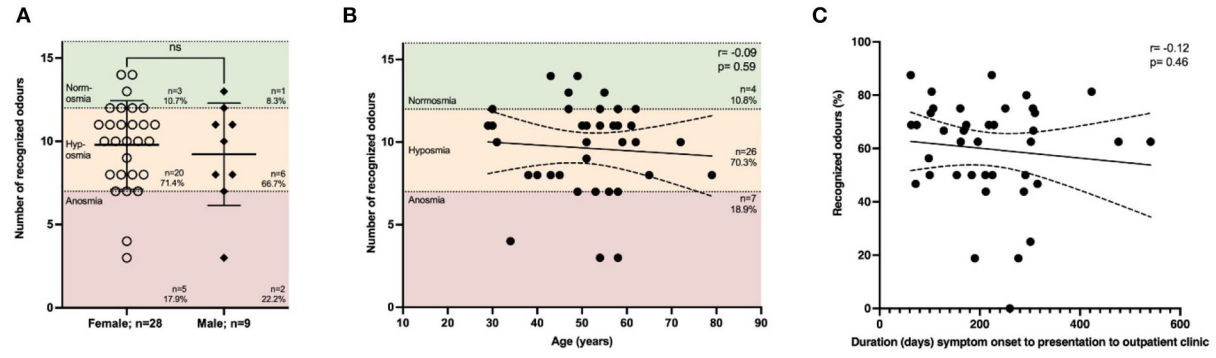


FIGURE 4
Impairment of smell is not affected by duration from symptom onset. The smell was assessed using Sniffin' Sticks®. The following thresholds were used: 0–7 = anosmia, 8–12 = hyposmia, >12 = normosmia. **(A)** Most patients suffered from hyposmia (71.4% females, 66.7% males), while there was no difference depending on gender. **(B)** Impaired smelling was not associated with age ($r = -0.09$, $p = 0.59$; $n = 37$ with all 16 odors tested. None of the patients recognized all 16 odors, while the maximum number of odors detected was 14 out of 16. **(C)** Duration (days) from symptom onset of acute COVID-19 infection to presentation to our clinic did not correlate ($r = -0.12$, $p = 0.46$, $n = 40$; the result of the test in percent because of varying total number of odors tested). **(A)** Data are shown as mean ± standard deviation (SD) and **(B,C)** mean with 95% confidence interval. Data were analyzed using **(A)** nonparametric two-tailed Mann-Whitney *U* test or **(B,C)** nonparametric two-tailed Spearman correlation.

CFS (25). Recently, the first report of BC007, a DNA aptamer drug with high affinity to G-protein-coupled receptors (GPCR-AAbs), showed the functional inactivation of GPCR-antibodies within 48 h after administration, leading to improvement of fatigue, taste, and retinal capillary microcirculation over four weeks in one patient (26).

One possible explanation for the development of fatigue is that patients might be more affected by affective disorders. In line with our findings, Calabria et al. found that in a group of 136 patients with cognitive complaints following COVID-19 infection, 82.3% reported fatigue, especially severe motor fatigue. Interestingly, elevated levels of apathy, anxiety, and executive dysfunction in neuropsychiatric measures and executive and attentional difficulties on cognitive tests were predictors of fatigue (27). Using the HADS, we found evidence of depressive symptoms in 19.1% of the patients, although we did not find any correlation with psychiatric or psychosomatic comorbidities, gender, or age. However, males were more likely to suffer from depressive symptoms the longer the latency was between acute infection and presentation in our clinic. One explanation might be that the persistence of long-COVID symptoms might have induced a depressive state. These data support the notion that patients with persistent long-COVID symptoms should receive diagnostic and therapeutic help to reduce the risk of developing an affective disorder. Using transcranial sonography, we found that patients with a hypoechogenic brainstem raphe structure have a 3.9-fold (95% CI 1.2–12.1) increased risk of depressive symptoms, presumably arguing for increased susceptibility to developing depressive symptoms following a stressful event such as COVID (28). Transcranial sonography could, therefore, be used to identify patients at risk of developing depressive symptoms following COVID. Moreover, data about neuropsychological deficits and fatigue are controversial. Using a comprehensive neuropsychological battery, including standardized and computerized cognitive tests and the MFIS scores (total score and cognitive fatigue score), another group failed to detect reliable neuropsychological predictors of cognitive fatigue in post-COVID patients (29).

Hyposmia and anosmia are often reported in patients with acute COVID-19 and during follow-up. While 65.3% of patients in our cohort reported impaired smell during the acute infection, the frequency dropped to 34.7% at the presentation time. In the majority of patients tested, we found some degree of hyposmia. Moreover, 70.2% (33/47) of patients who did not report having persistent hyposmia were tested to have impaired smell (hyposmia or anosmia). Interestingly, smell impairment did not correlate with age, which could have been explained by unknown preceding neurodegeneration or be a sign of reduced regeneration in older individuals. Qualitative changes in smell can persist for several months and even occur as late-onset symptoms months after full recovery (30). However, smell recovers in >90% of patients after six months (31).

There are several *limitations* to our study which need to be addressed. First, we recruited patients from our outpatient clinic. This might have induced a referral and selection bias, e.g., since patients with more severe depressive symptoms might not have been able to make an appointment, potentially underestimating the risk of COVID-associated affective disorders. On the other hand, patients with only mild long-COVID symptoms might have been missed because they did not see the need for a consultation. Another drawback is the missing control group, which should be recruited from patients without sequelae following COVID-19 and healthy, age-matched subjects.

Moreover, we assessed patients only at one time point; hence, follow-up studies are needed to understand the dynamics of alterations over time. Age-related neurodegeneration, which might have influenced, for example, hyposmia, was not taken into account. Moreover, we did not evaluate cognitive function systematically in this cohort, which should be addressed in further studies. The strength of the data presented here is the sufficiently large number of patients investigated and the conclusive acquisition of data, including socioeconomic and clinical data.

Whether long-COVID is a distinct disease entity with unclear pathophysiology or a spectrum of prolonged viral infection remains unclear. The scientific and media attention induced by COVID-19 almost pushes us to consider symptoms in the post-acute phase as a separate disease entity. However, a critical appraisal of the literature also implies that at least part of the symptoms in the post-acute phase of SARS-CoV-2 infection, namely fatigue, might be a spectrum of CFS with SARS-CoV-2 virus as another virogenic etiology. Persisting alteration of smell, however, seem to be rather specific to SARS-CoV-2 infection.

In summary, we provide a holistic picture of patients with long-COVID presenting in a specialized neurology university hospital setting and show that patients with long-COVID syndrome and mild disease are affected by severe fatigue, with an 8-fold increased risk in women. Further studies, including larger sample sizes, control groups, and longitudinal designs, are needed to better understand the dynamics of long-COVID over time.

Data availability statement

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

Ethics statement

The study was conducted in accordance with the Helsinki Declaration of 1975 and was approved by the Local Ethics

Committee of Ruhr-University Bochum (20-6827 and 21-7423). The patients/participants provided their written informed consent to participate in this study.

Author contributions

HS, JCJ, NT, DR, TP, NS, and IA: revising the manuscript and acquisition of data, analysis, and interpretation of data. RG: revising the manuscript, analysis, and interpretation of data. SF: writing the manuscript, acquisition of data, analysis, and interpretation of data, study concept and design, and study supervision. All authors read and approved the final version of the manuscript.

Acknowledgments

We thank the patients for allowing them to analyze their data and participate in the study. We acknowledge support by the Open Access Publication Funds of the Ruhr-Universität Bochum.

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/fneur.2022.979152/full#supplementary-material

References

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

2. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, Hossmann S, Imeri H, Ipekci AM, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: a living systematic review and meta-analysis. *PLoS Med.* (2020) 17:e1003346. doi: 10.1371/journal.pmed.1003346

3. Young BE, Ong SWX, Ng LFP, Anderson DE, Chia WN, Chia PY, et al. Viral dynamics and immune correlates of coronavirus disease 2019 (COVID-19) severity. *Clin Infect Dis.* (2021) 73:e2932–42. doi: 10.1093/cid/ciaa1280

4. Solomon T. Neurological infection with SARS-CoV-2 - the story so far. *Nat Rev Neurol.* (2021) 17:65–6. doi: 10.1038/s41582-020-00453-w

5. Carfì A, Bernabei R, Landi F. Persistent Symptoms in Patients After Acute COVID-19. *JAMA*. (2020) 324:603–5. doi: 10.1001/jama.2020.12603

6. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, Mcgroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. *Nat Med.* (2021) 27:601–15. doi: 10.1038/s41591-021-01283-z

7. Sigfrid L, Drake TM, Pauley E, Jesudason EC, Olliaro P, Lim WS, et al. Long Covid in adults discharged from UK hospitals after Covid-19: a prospective, multicentre cohort study using the ISARIC WHO clinical characterisation protocol. *Lancet Reg Health Eur.* (2021) 8:100186. doi: 10.1016/j.lanepe.2021.100186

8. Crook H, Raza S, Nowell J, Young M, Edison P. Long covid-mechanisms, risk factors, and management. *BMJ.* (2021) 374:n1648. doi: 10.1136/bmj.n1648

9. Heming M, Li X, Räuber S, Mausberg AK, Börsch AL, Hartlehnert M, et al. Neurological manifestations of COVID-19 feature t cell exhaustion and dedifferentiated monocytes in cerebrospinal fluid. *Immunity.* (2021) 54:164–75. doi: 10.1016/j.immuni.2020.12.011

10. Murphy WJ, Longo DL. A possible role for anti-idiotype antibodies in SARS-CoV-2 infection and vaccination. *N Engl J Med.* (2021) 386:394–6. doi: 10.1056/NEJMcibr2113694

11. Vanichkachorn G, Newcomb R, Cowl CT, Murad MH, Breeher L, Miller S, et al. 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.* (2021) 96:1782–91. doi: 10.1016/j.mayocp.2021.04.024

12. WHO Working Group on the Clinical Characterisation and Management of COVID-19 infection. A minimal common outcome measure is set for COVID-19 clinical research. *Lancet Infect Dis.* (2020) 20:e192–e197. doi: 10.1016/S1473-3099(20)30483-7

13. Penner IK, Raselli C, Stöcklin M, Opwis K, Kappos L, Calabrese P. The fatigue scale for motor and cognitive functions (FSMC): validation of a new instrument to assess multiple sclerosis-related fatigue. *Mult Scler.* (2009) 15:1509–17. doi: 10.1177/1352458509348519

14. Snaith RP. The hospital anxiety and depression scale. *Health Qual Life Outcomes.* (2003) 1:29. doi: 10.1186/1477-7525-1-29

15. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination, and olfactory threshold. *Chem Senses.* (1997) 22:39–52. doi: 10.1093/chemse/22.1.39

16. Hummel T, Hähner A, Witt M, Landis BN. Examination of the sense of smell. *HNO.* (2007) 55:827–37. doi: 10.1007/s00106-007-1593-x

17. Wolfensberger M, Schnieper I. Sniffin'Sticks: a new system for olfactory assessment in routine clinical practice. *HNO.* (1999) 47:629–36. doi: 10.1007/s001060050436

18. Jennings G, Monaghan A, Xue F, Mockler D, Romero-Ortuño R. A systematic review of persistent symptoms and residual abnormal functioning following acute COVID-19: ongoing symptomatic phase vs. post-COVID-19 syndrome. *J Clin Med.* (2021) 10. doi: 10.1101/2021.06.25.21259372

19. Centers for Disease Control and Prevention, N.C.F.E.a.Z.I.D.N., Division of High-Consequence Pathogens and Pathology. *What is ME/CFS*? (2021). Available online at: https://www.cdc.gov/me-cfs/about/index.html (accessed January 27, 2021).

20. Rollnik JD. Chronic fatigue syndrome: a critical review. *Fortschr Neurol Psychiatr.* (2017) 85:79–85. doi: 10.1055/s-0042-121259

21. Alter HJ, Mikovits JA, Switzer WM, Ruscetti FW, Lo SC, Klimas N, et al. A multicenter blinded analysis indicates no association between chronic fatigue syndrome/myalgic encephalomyelitis and either xenotropic murine leukemia

virus-related virus or polytropic murine leukemia virus. *MBio.* (2012) 3:e00266–12. doi: 10.1128/mBio.00266-12

22. Arnett SV, Clark IA. Inflammatory fatigue and sickness behaviour - lessons for diagnosing and managing chronic fatigue syndrome. *J Affect Disord.* (2012) 141:130–42. doi: 10.1016/j.jad.2012.04.004

23. Rutherford G, Manning P, Newton JL. Understanding muscle dysfunction in chronic fatigue syndrome. *J Aging Res.* (2016) 2016:2497348. doi: 10.1155/2016/2497348

24. Novak P, Mukerji SS, Alabsi HS, Systrom D, Marciano SP, Felsenstein D, et al. Multisystem Involvement in Post-Acute Sequelae of Coronavirus Disease 19. *Ann Neurol.* (2022) 91:367–79. doi: 10.1002/ana.26286

25. Freitag H, Szklarski M, Lorenz S, Sotzny F, Bauer S, Philippe A, et al. Autoantibodies to vasoregulative g-protein-coupled receptors correlate with symptom severity, autonomic dysfunction and disability in myalgic encephalomyelitis/chronic fatigue syndrome. *J Clin Med.* (2021) 10:3675. doi: 10.3390/jcm10163675

26. Hohberger B, Harrer T, Mardin C, Kruse F, Hoffmanns J, Rogge L, et al. Case report: neutralization of autoantibodies targeting G-protein-coupled receptors improves capillary impairment and fatigue symptoms after COVID-19 infection. *Front Med (Lausanne).* (2021) 8:754667. doi: 10.3389/fmed.2021.754667

27. Calabria M, García-Sánchez C, Grunden N, Pons C, Arroyo JA, Gómez-Anson B, et al. Post-COVID-19 fatigue: the contribution of cognitive and neuropsychiatric symptoms. *J Neurol.* (2022) 269:3990–9. doi: 10.1007/s00415-022-11141-8

28. Richter, D., Schulze, H., James, J. C., Siems, N., Trampe, N., Gold, R., et al. (2022). Hypoechogenicity of brainstem raphe in long-COVID syndrome-less common but independently associated with depressive symptoms: a cross-sectional study. *J Neurol* 1–7. doi: 10.1007/s00415-022-11154-3

29. Matias-Guiu JA, Delgado-Alonso C, Díez-Cirarda M, Martínez-Petit Á, Oliver-Mas S, Delgado-Álvarez A, et al. Neuropsychological predictors of fatigue in post-COVID syndrome. *J Clin Med.* (2022) 11. doi: 10.3390/jcm11133886

30. Schambeck SE, Crowell CS, Wagner KI, D'ippolito E, Burrell T, Mijočević H, et al. Phantosmia, parosmia, and dysgeusia are prolonged and late-onset symptoms of COVID-19. *J Clin Med.* (2021) 10:5266. doi: 10.3390/jcm10225266

31. Huart C, Philpott CM, Altundag A, Fjaeldstad AW, Frasnelli J, Gane S, et al. Systemic corticosteroids in coronavirus disease 2019 (COVID-19)-related smell dysfunction: an international view. *Int Forum Allergy Rhinol.* (2021) 11:1041–6. doi: 10.1002/alr.22788

frontiers | Frontiers in Neurology

TYPE Mini Review
PUBLISHED 07 December 2022
DOI 10.3389/fneur.2022.1063298

OPEN ACCESS

EDITED BY
Beatrice Paradiso,
Dolo Hospital, Venice, Italy

REVIEWED BY
Eleonora Aricò,
National Institute of Health (ISS), Italy

*CORRESPONDENCE
George D. Vavougios
vavougyios.georgios@ucy.ac.cy;
dantevavougios@hotmail.com

SPECIALTY SECTION
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

RECEIVED 06 October 2022
ACCEPTED 09 November 2022
PUBLISHED 07 December 2022

CITATION
Vavougios GD, Erausquin GAd and Snyder HM (2022) Type I interferon signaling in SARS-CoV-2 associated neurocognitive disorder (SAND): Mapping host-virus interactions to an etiopathogenesis.
Front. Neurol. 13:1063298.
doi: 10.3389/fneur.2022.1063298

Type I interferon signaling in SARS-CoV-2 associated neurocognitive disorder (SAND): Mapping host-virus interactions to an etiopathogenesis

George D. Vavougios[1,2]*, Gabriel A. de Erausquin[3] and Heather M. Snyder[4]

[1]Department of Neurology, University of Cyprus, Lefkosia, Cyprus, [2]Department of Respiratory Medicine, University of Thessaly, Larisa, Greece, [3]The Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, UTHSA, San Antonio, TX, United States, [4]Division of Medical and Scientific Relations, Alzheimer's Association, Chicago, IL, United States

Epidemiological, clinical, and radiological studies have provided insights into the phenomenology and biological basis of cognitive impairment in COVID-19 survivors. Furthermore, its association with biomarkers associated with neuroinflammation and neurodegeneration supports the notion that it is a distinct aspect of LongCOVID syndrome with specific underlying biology. Accounting for the latter, translational studies on SARS-CoV-2's interactions with its hosts have provided evidence on type I interferon dysregulation, which is seen in neuroinflammatory and neurodegenerative diseases. To date, studies attempting to describe this overlap have only described common mechanisms. In this manuscript, we attempt to propose a mechanistic model based on the host-virus interaction hypothesis. We discuss the molecular basis for a SARS-CoV-2-associated neurocognitive disorder (SAND) focusing on specific genes and pathways with potential mechanistic implications, several of which have been predicted by Vavougios and their research group. Furthermore, our hypothesis links translational evidence on interferon-responsive gene perturbations introduced by SARS-CoV-2 and known dysregulated pathways in dementia. Discussion emphasizes the crosstalk between central and peripheral immunity *via* danger-associated molecular patterns in inducing SAND's emergence in the absence of neuroinfection. Finally, we outline approaches to identifying targets that are both testable and druggable, and could serve in the design of future clinical and translational studies.

KEYWORDS

SARS-CoV-2, cognitive impairment, tauopathy, type I interferon signaling, host-virus interaction, dementia

Introduction

Cognitive impairment secondary to COVID-19 is now a recognized, health concern. It emerges as part of the LongCOVID spectrum, without a clearly defined cause (1). Clinical, pathological and radiological manifestations of this SARS-CoV-2 associated neurocognitive disorder (SAND) have outlined its significant overlap with neurodegenerative dementia (2), which extends to biomarkers in some individuals to include biomarkers consistent with neurodegenerative diseases such as Alzheimer's disease (AD), including beta amyloid oligomers (Aβ), tau, neurofilament light chain (Nfl) and others (3, 4). Towards this end, several recent translational studies have confirmed overlap on the molecular level of contributing biology between COVID-19 and AD disease, with innate immunity at its epicenter (5–12). Collectively, these studies point toward type I interferon signaling, a pathway contested by SARS-CoV-2 (13), as the potential culprit. Furthermore, interferon responsive genes such as those in the ISG, OAS, and IFITM families, dysregulated by SARS-CoV-2, have recently and independently emerged as key players in AD (9, 14, 15). To date, studies attempting to summarize the evidence on this overlap have not attempted to explore their synthesis towards an etiopathogenic mechanism emerging from host-virus interactions.

This review aims to outline emerging evidence on the genes and pathways that could define SAND on the molecular level. We aim to go beyond a presentation of potential mechanisms, presenting them instead through the evolution of host-virus interactions, the mobilization of innate immunity, and the consequences of both.

The viral lifecycle: Kinase recruitment and tauopathy

The first specific mechanistic indication that the intracellular lifecycle of SARS-CoV-2 may be linked with neurodegeneration, and specifically with tauopathies, came from a brain organoid infection model; SARS-CoV-2 neuroinfection was quiescent, causing neuronal apoptosis with hyperphosphorylated tau as its hallmark (16). A possible explanation for these findings is that SARS-CoV-2-dependent perturbations in kinases such as FYN (10) and GSK3 (17, 18) during their recruitment as part of the virus' lifecycle could escalate to increasing downstream tau hyperphosphorylation and oligomerization, as seen in other RNA viruses, i.e. DENV (19) and HIV-1 (20). In the setting of the human central nervous system (CNS), the mechanism of tau hyperphosphorylation and oligomerization, however, may not require subsequent *de novo* infection. Rather, increasing evidence suggests that transsynaptic spread of tau (21, 22), amyloid oligomers (8), and viral particles *via* extracellular vesicles (5) may sustain a neuroinflammatory process from an infected hub and this may evolve to or enhance pre-existing neurodegeneration in its connected network (23, 24).

The combination of anosmia, cognitive impairment, and limbic degeneration in some individuals suggests a link between SAND and neurodegenerative dementia (25) and with tau pathology specifically (26). In humans, significant differences in peripheral markers of age-related neurodegeneration, including specific forms of phosphorylated tau or p-tau have been identified both in COVID-19 patients (27) and survivors in the post-COVID-19 setting over 6 months follow up (28). Notably, these changes appear linked with proinflammatory cytokines, however not all data show that these are persistent (3) and there is still much to learn about the biological underpinnings that may continue to contribute.

Taken together, both phenomenology, biomarkers, and underlying genes potentially recruited by SARS-CoV-2 indicate that tauopathy may be a plausible mechanism by which the CNS is affected. Notably, the transmission of tau seeds *via* peripheral sites to the CNS *via* exosomes and their neurotoxicity has been previously observed in *P. Aeruginosa* pneumonia (29), furthermore indicating that systemic infection may affect the CNS even in the absence of neuroinfection. Considering that tau can activate type I interferon signaling as seen in neurodegenerative disease in the absence of infection (30), tau transmission during SARS-CoV-2 infection could be seen as a canonical alarmin/pathogen-associated molecular pattern (PAMP) (31–33), which can readily lead to a detriment for the recipient cell.

The host response: Type I interferon response, amyloid beta, and cognitive impairment

Type I interferon (IFN-1) perturbations are an established hallmark of Alzheimer's disease, mediating neuroinflammatory synapse loss (14, 34). During SARS-CoV-2 infection, IFN-I pathways are among the first activated pathways between host and pathogen, a finding confirmed by multiple translational studies (13, 35). From then on, the interaction between IFN-I signaling, a canonical response to infection (36), and SARS-CoV-2's immunoevasion stratagems are highly complex (37). As a primary event, SARS-CoV-2's lifecycle may be effectively disrupted by a pre-established IFN-I cellular milieu (38). On the contrary, delayed type I responses in the nasal epithelial have been shown to enhance SARS-CoV-2 permissiveness (39). Correspondingly, inborn errors in IFN-I may render carriers specifically vulnerable to SARS-CoV-2, as they correspond to differentially perturbed IFN-I responses (40, 41). Adding to the complexity of this interaction is SARS-CoV-2's armamentarium of proteins that target IFN-I responses (42). Notably, these same targets of virus-host protein interactions also play a central

role in neuroinflammation and neurodegeneration, for example in TBK1 (42, 43), KPNA2/Karyopherin (44, 45), and alpha-synuclein, among others.

Further dissection of the IFN-I signalosome reveals specific genes that are key players in both innate immune responses and AD. In recently published work by Vavougios and colleagues (46), disrupted proteostasis and trained immunity pathways were among overlapping molecular pathways that are common across different tissues in AD. This work suggested that IFN-1 has a specific relationship with unique signaling cascades, focusing on the IFN response to antiviral effectors, such as the interferon-inducible transmembrane (IFITM) and the 2′-5′-oligoadenylate synthase (OAS) family genes in both AD and COVID-19 (15). The specific relationship of perturbed IFN-I signaling to both AD and COVID-19, focuses on interferon responsive antiviral effectors, such as IFITMs and OASs, which are interferon stimulated (ISGs) gene families that provide cellular-level defense against intracellular pathogens. Dysfunctions of IFITMs and OASs on a pathway level not only have the potential to abrogate antiviral activity, but several studies suggest this dysfunction enables these factors to act as pro-viral factors (47–49). Vavougios and colleagues found that IFN-I signatures containing members of these ISG families are common in neurons, peripheral immune cells, and microglia affected by COVID-19 or by AD (10, 15, 33, 50, 51).

The relationship of both gene families, as well as other ISGs such as MX and IFITs (10, 15), and IFN-I signaling as a nexus for both COVID-19 and AD has been corroborated by others in various experimental and model system settings (6, 7, 9, 11–13, 52–54). Gamma secretase activity in response to viral infection has also been shown to be functionally linked with type I and type II interferon responses in peripheral immune cells; gamma secretase is involved in the production of the beta-amyloid protein (55). Lastly, IFN1 signaling in AD-related microglia was shown to upregulate IFITM3, which in turn modulates gamma secretase processing. The antigenic stimulus for this cascade of molecular events was nucleic acid (NA)-enriched neuritic plaques, and notably, microglia may not then distinguish viral from endogenous NAs (14, 49). This suggests that as an innate immunity protein, IFITM3 may canonically intercept SARS-CoV-2 (56), with its upregulation concomitantly building up to both increased beta amyloid production (14) and fed-forward IFN-I upregulation (34). Notably, such interactions have also been observed with the structurally similar IFITM2 in modulating the host's type I interferon signaling.

Taken together, these events show that IFN-I signaling dysregulation secondary to SARS-CoV-2 infection may be relayed by endothelial cells (7, 11, 54) to microglia, priming them (57) and may potentially result in upregulation of IFITMs and increased presence of beta amyloid production (57). If this priming is successful in restricting SARS-CoV-2, as heralded by S1 – $A\beta_{1-42}$ interactions (58), neuroinflammation but not neuroinvasion would be expected to predominate. Notably, S1 itself has been shown to function as a danger-associated molecular pattern (DAMP) for microglia, furthermore inducing neuroinflammatory phenotypes (32, 57), indicating that Roy et al.'s (49). HSV-1 model of AD pathogenesis may also provide some context to consider for SARS-CoV-2 (59). Furthermore, in the same model, the transmission of tau seeds as observed elsewhere (29) would also fit our current understanding of tau and Aβ as Type I interferon stimulants, as observed in neurodegenerative disease (30).

SARS-CoV-2 associated neurocognitive disorder as innate immunity's pyrrhic victory

Regardless of the specific pathogen or PAMP (33) involved, IFN1 signaling canonically induced as an innate immunity response is a firmly recognized inducer of cognitive impairment (34, 55, 60). Specific molecular events that may account for this relationship involve increased beta amyloid production, proinflammatory microglial activation, and impaired neuronal homeostasis (14, 34, 49, 60). SARS-CoV-2 introduction to this system is an immunogenic challenge with potential advanced capabilities to modulate IFN-I signaling, subverting it to its favor processes that enable evasion of the immune system (13). An example of this proposed mechanism can be found in the amyloidogenic interaction between N and alpha-synuclein (aSyn), where N functions as a scaffold for aSyn aggregation (61). The abrogation of aSyn would arrest its function as a canonical, neuron-specific IFN-I modulator (62); the aggregation of aSyn however would in turn activate IFN-I by a (presumably) non-canonical pathway, observed in neurodegenerative disease (63). This sterile proinflammatory signal could be relayed centrally from infected microvascular endothelia or olfactory epithelial cells, to be intercepted primarily by microglia (7, 11, 57). Aside from aSyn specifically, interactions between SARS-CoV-2 proteins and other proteopathic seeds. Notably, as per a previous model proposed by Vavougios et al. (15), the neuroanatomical premise of this concept is supported by imaging data indicating tandem degeneration of entorhinal cortex and hippocampi (25) and murine models of intranasal administration of SARS-CoV-2 that develop late onset proteinopathy, even after viral clearance (57, 59). Furthermore, our model's main premise, i.e. the capability of SARS-CoV-2 protein fragments to induce amyloidogenesis and subsequent neuroinflammation is confirmed in at least one *in vitro* model (64).

Lytic replication or multiple infected sites may not be required for cognitive impairment to manifest, along with molecular events similar to those of neurodegenerative dementias. Successful restriction *via* IFN-I and feed-forward signaling is still impacting the CNS, fully capable of establishing neuroinflammation, proteinopathy, and microgliosis in the absence of a pathogen (57, 59, 64) building up to synapse loss (34, 49). From an immune perspective, however, this

destruction proximal to an infected site successfully walls off an invading pathogen, being informed by both IFN-I and exosomal tau, here functioning as evidence of viral latency (16, 20, 21). Of note, once initiated, the overproduction of beta amyloid was shown to enhance the capability of native molecules to activate microglia and initiate IFN-I cascades (49). This notion indicates that both different pathogens targeting IFN-I (55), Danger-associated molecular signals (DAMPs) (32) and self-DAMPs (34, 49), accumulated by failing organelles and defects in proteostasis and mitochondrial homeostasis, may readily activate this pathogenetic mechanism in the absence of an exogenous immune challenge. Considering that IFN-I may be targeted by the viral lifecycle and successfully suppressed, second-order or non-canonical as described herein activation of IFN-I by the very same "captured" molecules (i.e. aSyn, tau, Aβ) would serve as a failsafe. Notably, the sterile enhancement of microglial IFN-I cascades has been previously shown (34, 49, 62, 63) indicating that their enhancement in the setting of SARS-CoV-2 (61, 65) infections may require proteins or DAMPs rather than a complete virion—a concept that would account for the persistence of neuroinflammation past virus clearance (59).

Conclusions

The SARS-CoV-2 pandemic has provided a forum to better understand the contributions of recurrent and agnostic immunity in response to some pathogen exposure rather than specific exposure and its relationship to AD-specific biology (22). AD is a complex disease, and likely has a number of factors that contribute to later life risk. There are many outstanding questions and in future studies, SAND-related contributions should be considered within the potential limitations.

As a standalone syndrome, the SARS-CoV-2 associated neurocognitive disorder (SAND) poses an interesting question: is the salience of COVID-19, increased population exposure, and potent induction of IFN-I the true culprit? Prior to SARS-CoV-2, HIV-1 and its Tat protein had been shown to intersect with both tau and beta-amyloid and potentially engage with the AD molecular pathology (20), and a corresponding HIV-1 associated neurocognitive dysfunction (HAND) associated with infection. SAND, much like HAND before it, indicates the long-standing impact of a pathogen may be as impactful for the individual as the native infection, when inflammation is either unmitigated, self-propagating, or both.

While these emerging links between neuroinflammation, neurodegeneration, and COVID-19 represent a growing body of literature, it is important to underscore that the natural history of cognitive, functional, and behavioral defects in individuals experiencing long-term neurological sequelae is unknown. There are many unanswered questions about the linkage, and it is important to understand whether translational models and clinical radiological entities represent a clear, mechanistic continuum. Furthermore, it is not yet known if COVID-19's effects on cognition represent lasting or transient impairments. It is also not known why some individuals experience long-term impact on their cognition, function, and behavior, while others do not. COVID-19's effect on cognition should also be considered multifactorial, considering its implication in vascular damage to the brain and sleep-related complaints affecting survivors (66). Furthermore, the introduction of vaccines may provide information on how these biological underpinnings interact with one another.

In this review, we offer a potential model for SAND following the trail of host-virus interactions and combining it with the dual roles of proteopathic seeds as DAMPs/PAMPs and IFN-I signaling and propose a framework to further extend these findings to linkages with neurodegenerative disease. Building upon previous works from Vavougios et al. and others, this manuscript outlines a potential opportunity to formulate a working, testable hypothesis on SAND with implications on cognitive impairment and other dementias. Furthermore, as we have previously indicated, we outline targets that are both testable and druggable (51), and could serve in the design of future clinical and translational studies.

The global research and clinical communities must continue to work together to uncover the answers to these, as well as other, questions on the intersection of COVID-19, the brain, and neurodegeneration.

Author's note

The authors are all participants in the Alzheimer's Association SARS-CoV-2 Consortium on Neurological Sequelae and continue to collaborate to better understand the longterm neurological implications of COVID-19.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

Partial funding to oversee and convene the consortium, including the OA fee for this publication, was paid by the Alzheimer's Association.

Acknowledgments

GV would like to thank Karen A. Krogfelt, Konstantinos I. Gourgoulianis, Georgios Hadjigeorgiou, Artemios Artemiadis, Triantafyllos Doskas, Pelagia Foka, Dimitrios Mysiris, Georgia

Xiromerisiou, Sotirios Zarogiannis, Theodore Mavridis, and Vasilios Stavrou for their continuous support and scientific contribution in various fields of LongCOVID research. GV would also like to thank medical, nursing, and support staff in Thessaly, Athens, and Nicosia for their tireless work during the pandemic. In addition, the authors wish to thank all participants who have actively took part in the studies described here or those that will be essential to expanding our understanding in the future.

Conflict of interest

Author HS is a full time employee of the Alzheimer's Association; the organization's disclosures are present at alz.org/transparency.

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

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. Deer RR, Rock MA, Vasilevsky N, Carmody L, Rando H, Anzalone AJ, et al. Characterizing Long COVID: Deep Phenotype of a Complex Condition. *EBioMedicine*. (2021) 74:103722. doi: 10.1016/j.ebiom.2021.103722

2. de Erausquin GA, Snyder H, Carrillo M, Hosseini AA, Brugha TS, Seshadri S. The chronic neuropsychiatric sequelae of COVID-19: The need for a prospective study of viral impact on brain functioning. *Alzheimers Dement.* (2021) 17:1056–65. doi: 10.1002/alz.12255

3. Laudanski K, Hajj J, Restrepo M, Siddiq K, Okeke T, Rader DJ. Dynamic changes in central and peripheral neuro-injury vs. neuroprotective serum markers in COVID-19 are modulated by different types of anti-viral treatments but do not affect the incidence of late and early strokes. *Biomedicines*. (2021) 9:12. doi: 10.3390/biomedicines9121791

4. Choe K, Park HY, Ikram M, Lee HJ, Park TJ, Ullah R, et al. Systematic review of the common pathophysiological mechanisms in COVID-19 and neurodegeneration: the role of bioactive compounds and natural antioxidants. *Cells*. (2022) 11:32. doi: 10.3390/cells11081298

5. Ahmed SSSJ, Paramasivam P, Kamath M, Sharma A, Rome S, Murugesan R. Genetic exchange of lung-derived exosome to brain causing neuronal changes on COVID-19 infection. *Mol Neurobiol.* (2021) 58:5356–68. doi: 10.1007/s12035-021-02485-9

6. Alexander MR, Brice AM, van Vuren PJ, Rootes CL, Tribolet L, Cowled C, et al. Ribosome-profiling reveals restricted post transcriptional expression of antiviral cytokines and transcription factors during SARS-CoV-2 infection. *Int J Molec Sci*. (2021) 2:22. doi: 10.1101/2021.03.03.433675

7. Krasemann S, Haferkamp U, Pfefferle S, Woo MS, Heinrich F, Schweizer M, et al. The blood-brain barrier is dysregulated in COVID-19 and serves as a CNS entry route for SARS-CoV-2. *Stem Cell Reports*. (2022) 17:307–20. doi: 10.1016/j.stemcr.2021.12.011

8. Lam SM, Zhang C, Wang Z, Ni Z, Zhang S, Yang S, et al. A multi-omics investigation of the composition and function of extracellular vesicles along the temporal trajectory of COVID-19. *Nat Metabolism*. (2021) 3:909–22. doi: 10.1038/s42255-021-00425-4

9. Magusali N, Graham AC, Piers TM, Panichnantakul P, Yaman U, Shoai M, et al. A genetic link between risk for Alzheimer's disease and severe COVID-19 outcomes *via* the OAS1 gene. *Brain*. (2021) 144:3727–41. doi: 10.1093/brain/awab337

10. Vavougios GD, Breza M, Mavridis T, Krogfelt KA, FYN. SARS-CoV-2, and IFITM3 in the neurobiology of Alzheimer's disease. *Brain Disorders*. (2021) 3:100022. doi: 10.1016/j.dscb.2021.100022

11. Yang AC, Kern F, Losada PM, Agam MR, Maat CA, Schmartz GP, et al. Dysregulation of brain and choroid plexus cell types in severe COVID-19. *Nature*. (2021) 595:565–71. doi: 10.1038/s41586-021-03710-0

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

13. Winstone H, Lista MJ, Reid AC, Bouton C, Pickering S, Galao RP, et al. The polybasic cleavage site in SARS-CoV-2 spike modulates viral sensitivity to type I interferon and IFITM2. *J Virol*. (2021)3: 95. doi: 10.1128/JVI.02422-20

14. Hur J-Y, Frost GR, Wu X, Crump C, Pan SJ, Wong E, et al. The innate immunity protein IFITM3 modulates γ-secretase in Alzheimer's disease. *Nature*. (2020) 586:735–40. doi: 10.1038/s41586-020-2681-2

15. Vavougios GD, Nday C, Pelidou S-H, Gourgoulianis KI, Stamoulis G, Doskas T, et al. Outside-in induction of the IFITM3 trafficking system by infections, including SARS-CoV-2, in the pathobiology of Alzheimer's disease. *Brain Behav Immun Health*. (2021) 14:100243. doi: 10.1016/j.bbih.2021.100243

16. Ramani A, Müller L, Ostermann PN, Gabriel E, Abida-Islam P, Müller-Schiffmann A, et al. SARS-CoV-2 targets neurons of 3D human brain organoids. *EMBO J*. (2020) 39:e106230. doi: 10.15252/embj.2020106230

17. Liu X, Verma A, Garcia G, Ramage H, Myers RL, Lucas A, et al. Targeting the coronavirus nucleocapsid protein through GSK-3 inhibition. *Proc Natl Acad Sci USA*. (2021) 11:118. doi: 10.1073/pnas.2113401118

18. Rana AK, Rahmatkar SN, Kumar A, Singh D. Glycogen synthase kinase-3: a putative target to combat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. *Cytokine Growth Factor Rev*. (2021) 58:92–101. doi: 10.1016/j.cytogfr.2020.08.002

19. Petitdemange C, Maucourant C, Tarantino N, Rey J, Vieillard V. Glycogen synthetase kinase 3 inhibition drives MIC-A/B to promote cytokine production by human natural killer cells in Dengue virus type 2 infection. *Eur J Immunol*. (2020) 50:342–52. doi: 10.1002/eji.201948284

20. Hategan A, Masliah E, Nath A, HIV. and Alzheimer's disease: complex interactions of HIV-Tat with amyloid β peptide and Tau protein. *J Neurovirol*. (2019) 25:648–60. doi: 10.1007/s13365-019-00736-z

21. Wang Y, Balaji V, Kaniyappan S, Krüger L, Irsen S, Tepper K, et al. The release and trans-synaptic transmission of Tau via exosomes. *Mol Neurodegener*. (2017) 12:5. doi: 10.1186/s13024-016-0143-y

22. Liu S, Hossinger A, Heumüller S-E, Hornberger A, Buravlova O, Konstantoulea K, et al. Highly efficient intercellular spreading of protein misfolding mediated by viral ligand-receptor interactions. *Nat Commun*. (2021) 12:5739. doi: 10.1038/s41467-021-25855-2

23. Chiricosta L, Gugliandolo A, Mazzon E. SARS-CoV-2 exacerbates beta-amyloid neurotoxicity, inflammation and oxidative stress in Alzheimer's disease patients. *Int J Mol Sci*. (2021)5: 22. doi: 10.3390/ijms222413603

24. Polanco JC, Hand GR, Briner A, Li C, Götz J. Exosomes induce endolysosomal permeabilization as a gateway by which exosomal tau seeds escape into the cytosol. *Acta Neuropathol*. (2021) 141:235–56. doi: 10.1007/s00401-020-02254-3

25. Douaud G, Lee S, Alfaro-Almagro F, Arthofer C, Wang C, McCarthy P, et al. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature*. (2022) 3:45. doi: 10.1101/2021.06.11.21258690

26. Klein J, Yan X, Johnson A, Tomljanovic Z, Zou J, Polly K, et al. Olfactory impairment is related to tau pathology and neuroinflammation in Alzheimer's disease. *J Alzheimers Dis.* (2021) 80:1051–65. doi: 10.3233/JAD-201149

27. Frontera JA, Boutajangout A, Masurkar AV, Betensky RA, Ge Y, Vedvyas A, et al. Comparison of serum neurodegenerative biomarkers among hospitalized COVID-19 patients vs. non-COVID subjects with normal cognition, mild cognitive impairment, or Alzheimer's dementia. *Alzheimers Dement.* (2022). doi: 10.1002/alz.12556

28. Sun B, Tang N, Peluso MJ, Iyer NS, Torres L, Donatelli JL, et al. Characterization and biomarker analyses of post-COVID-19 complications and neurological manifestations. *Cells.* (2021) 2:10. doi: 10.3390/cells10020386

29. Morrow KA, Ochoa CD, Balczon R, Zhou C, Cauthen L, Alexeyev M, et al. Pseudomonas aeruginosa exoenzymes U and Y induce a transmissible endothelial proteinopathy. *Am J Physiol Lung Cell Mol Physiol.* (2016) 310:L337–353. doi: 10.1152/ajplung.00103.2015

30. Sanford SAI, McEwan WA. Type-I interferons in Alzheimer's disease and other tauopathies. *Front Cell Neurosci.* (2022) 8:16. doi: 10.3389/fncel.2022.949340

31. Hasegawa T, Oka T, Demehri S. Alarmin cytokines as central regulators of cutaneous immunity. *Front Immunol.* (2022) 13:876515. doi: 10.3389/fimmu.2022.876515

32. Frank MG, Nguyen KH, Ball JB, Hopkins S, Kelley T, Baratta MV, et al. SARS-CoV-2 spike S1 subunit induces neuroinflammatory, microglial and behavioral sickness responses: evidence of PAMP-like properties. *Brain Behav Immun.* (2022) 100:267–77. doi: 10.1016/j.bbi.2021.12.007

33. Jung HW, Panigrahi GK, Jung GY, Lee YJ, Shin KH, Sahoo A, et al. Pathogen-associated molecular pattern-triggered immunity involves proteolytic degradation of core nonsense-mediated mRNA decay factors during the early defense response[OPEN]. *Plant Cell.* (2020) 32:1081–101. doi: 10.1105/tpc.19.00631

34. Roy ER, Chiu G, Li S, Propson NE, Kanchi R, Wang B, et al. Concerted type I interferon signaling in microglia and neural cells promotes memory impairment associated with amyloid β plaques. *Immunity.* (2022) 55:879–94.e876. doi: 10.1016/j.immuni.2022.03.018

35. Francis ME, Goncin U, Kroeker A, Swan C, Ralph R, Lu Y, et al. SARS-CoV-2 infection in the Syrian hamster model causes inflammation as well as type I interferon dysregulation in both respiratory and non-respiratory tissues including the heart and kidney. *PLoS Pathog.* (2021) 17:e1009705. doi: 10.1371/journal.ppat.1009705

36. Meyts I, Casanova JL. Viral infections in humans and mice with genetic deficiencies of the type I IFN response pathway. *Eur J Immunol.* (2021) 51:1039–61. doi: 10.1002/eji.202048793

37. Lei X, Dong X, Ma R, Wang W, Xiao X, Tian Z, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nat Commun.* (2020) 11:3810. doi: 10.1038/s41467-020-17665-9

38. Lokugamage KG, Hage A, de Vries M, et al. Type I interferon susceptibility distinguishes SARS-CoV-2 from SARS-CoV. *J Virol.* (2020)12:94. doi: 10.1128/JVI.01410-20

39. Hatton CF, Botting RA, Dueñas ME, Haq IJ, Verdon B, Thompson BJ, et al. Delayed induction of type I and III interferons mediates nasal epithelial cell permissiveness to SARS-CoV-2. *Nat Commun.* (2021) 12:7092. doi: 10.1038/s41467-021-27318-0

40. Zhang Q, Bastard P, Liu Z, Pen JL, Moncada-Velez M, Chen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science.* (2020)4:370.

41. Zhang SY, Zhang Q, Casanova JL, Su HC. Severe COVID-19 in the young and healthy: monogenic inborn errors of immunity? *Nat Rev Immunol.* (2020) 20:455–6. doi: 10.1038/s41577-020-0373-7

42. Galani IE, Andreakos E. Impaired innate antiviral defenses in COVID-19: Causes, consequences and therapeutic opportunities. *Semin Immunol.* (2021) 55:101522. doi: 10.1016/j.smim.2021.101522

43. Ahmad L, Zhang SY, Casanova JL, Sancho-Shimizu V. Human TBK1: a gatekeeper of neuroinflammation. *Trends Mol Med.* (2016) 22:511–27. doi: 10.1016/j.molmed.2016.04.006

44. Pasha T, Zatorska A, Sharipov D, Rogelj B, Hortobágyi T, Hirth F. Karyopherin abnormalities in neurodegenerative proteinopathies. *Brain.* (2021) 144:2915–32. doi: 10.1093/brain/awab201

45. Xia H, Cao Z, Xie X, Zhang X, Chen JY-C, Wang H, et al. Evasion of type I interferon by SARS-CoV-2. *Cell Rep.* (2020) 33:108234. doi: 10.1016/j.celrep.2020.108234

46. Vavougios GD, Nday C, Pelidou S-H, Zarogiannis SG, Gourgoulianis KI, Stamoulis G, et al. Double hit viral parasitism, polymicrobial CNS residency and perturbed proteostasis in Alzheimer's disease: a data driven, in silico analysis of gene expression data. *Mol Immunol.* (2020) 127:124–35. doi: 10.1016/j.molimm.2020.08.021

47. Shi G, Kenney AD, Kudryashova E, et al. Opposing activities of IFITM proteins in SARS-CoV-2 infection. *EMBO J.* (2021) 40:e106501. doi: 10.15252/embj.2020106501

48. Choi UY, Kang J-S, Hwang YS, Kim Y-J. Oligoadenylate synthase-like (OASL) proteins: dual functions and associations with diseases. *Exp Mol Med.* (2015) 47:e144. doi: 10.1038/emm.2014.110

49. Roy ER, Wang B, Wan Y-W, Chiu G, Cole A, Yin Z, et al. Type I interferon response drives neuroinflammation and synapse loss in Alzheimer disease. *J Clin Invest.* (2020) 130:1912–30. doi: 10.1172/JCI133737

50. Vavougios GD, Zarogiannis SG, Hadjigeorgiou G, Krogfelt KA, Gourgoulianis KI. SARS-CoV-2 and type I interferon signaling in brain endothelial cells: blurring the lines between friend or foe. *Stem Cell Reports.* (2022) 17:1012–3. doi: 10.1016/j.stemcr.2022.04.011

51. Vavougios GD, Mavridis T, Artemiadis A, Krogfelt KA, Hadjigeorgiou G. Trained immunity in viral infections, Alzheimer's disease and multiple sclerosis: a convergence in type I interferon signalling and IFNβ-1a. *Biochimica et Biophysica Acta (BBA) Mol Basis Dis.* (2022) 1868:166430. doi: 10.1016/j.bbadis.2022.166430

52. Finkel Y, Gluck A, Nachshon A, Winkler R, Fisher T, Rozman B, et al. SARS-CoV-2 uses a multipronged strategy to impede host protein synthesis. *Nature.* (2021) 594:240–5. doi: 10.1038/s41586-021-03610-3

53. Yao AY, Yan R. Activity of Alzheimer's γ-secretase is linked to changes of interferon-induced transmembrane proteins (IFITM) in innate immunity. *Mol Neurodegener.* (2020) 15:69. doi: 10.1186/s13024-020-00417-0

54. Zhang L, Zhou L, Bao L, Liu J, Zhu H, Lv Q, et al. SARS-CoV-2 crosses the blood–brain barrier accompanied with basement membrane disruption without tight junctions alteration. *Sign Transduc Target Ther.* (2021) 6:337. doi: 10.1038/s41392-021-00719-9

55. Svensson A, Jäkärä E, Shestakov A, Eriksson K. Inhibition of γ-secretase cleavage in the notch signaling pathway blocks HSV-2-induced type I and type II interferon production. *Viral Immunol.* (2010) 23:647–51. doi: 10.1089/vim.2010.0013

56. Bozzo CP, Nchioua R, Volcic M, Koepke L, Krüger J, Schütz D, et al. IFITM proteins promote SARS-CoV-2 infection and are targets for virus inhibition *in vitro*. *Nat Commun.* (2021) 12:4584. doi: 10.1038/s41467-021-24817-y

57. Jeong GU Lyu J, Kim K-D, Chung YC, Yoon GY, Lee S, et al. SARS-CoV-2 infection of microglia elicits proinflammatory activation and apoptotic cell death. *Microbiol Spectr.* (2022) 10:e0109122. doi: 10.1128/spectrum.01091-22

58. Hsu JT-A, Tien C-F, Yu G-Y, Shen S, Lee Y-H, Hsu P-C, et al. The effects of Aβ(1-42) binding to the SARS-CoV-2 spike protein s1 subunit and angiotensin-converting enzyme 2. *Int J Mol Sci.* (2021)7:22. doi: 10.3390/ijms22158226

59. Käufer C, Schreiber CS, Hartke A-S, Denden I, Stanelle-Bertram S, Beck S, et al. Microgliosis and neuronal proteinopathy in brain persist beyond viral clearance in SARS-CoV-2 hamster model. *EBioMed.* (2022) 79:103999. doi: 10.1016/j.ebiom.2022.103999

60. Blank T, Detje CN, Spieß A, Hagemeyer N, Brendecke SM, Wolfart J, et al. Brain endothelial- and epithelial-specific interferon receptor chain 1 drives virus-induced sickness behavior and cognitive impairment. *Immunity.* (2016) 44:901–12. doi: 10.1016/j.immuni.2016.04.005

61. Semerdzhiev SA, Fakhree MAA, Segers-Nolten I, Blum C, Claessens M. Interactions between SARS-CoV-2 N-Protein and α-Synuclein Accelerate Amyloid Formation. *ACS Chem Neurosci.* (2022) 13:143–50. doi: 10.1021/acschemneuro.1c00666

62. Monogue B, Chen Y, Sparks H, Behbehani R, Chai A, Rajic AJ, et al. Alpha-synuclein supports type 1 interferon signalling in neurons and brain tissue. *Brain.* (2022) 145:3622–36. doi: 10.1093/brain/awac192

63. Hinkle JT, Patel J, Panicker N, Karuppagounder SS, Biswas D, Belingon B, et al. STING mediates neurodegeneration and neuroinflammation in nigrostriatal α-synucleinopathy. *Proc Natl Acad Sci USA.* (2022) 119:e2118819119. doi: 10.1073/pnas.2118819119

64. Charnley M, Islam S, Bindra GK, Engwirda J, Ratcliffe J, Zhou J, et al. Neurotoxic amyloidogenic peptides in the proteome of SARS-COV2: potential implications for neurological symptoms in COVID-19. *Nat Commun.* (2022) 13:3387. doi: 10.1038/s41467-022-30932-1

65. Idrees D, Kumar V. SARS-CoV-2 spike protein interactions with amyloidogenic proteins: Potential clues to neurodegeneration. *Biochem Biophys Res Commun.* (2021) 554:94–8. doi: 10.1016/j.bbrc.2021.03.100

66. Vavougios GD, Stavrou V, Gourgoulianis KI. Cerebrovascular disease and sleep-disordered breathing need to be accounted for in cognitive impairment following COVID-19. *JAMA Psychiatry.* (2022) 2:9. doi: 10.1001/jamapsychiatry.2022.1773

frontiers | Frontiers in Neurology

TYPE Perspective
PUBLISHED 06 March 2023
DOI 10.3389/fneur.2023.1052811

OPEN ACCESS

EDITED BY
Beatrice Paradiso,
Dolo Hospital, Italy

REVIEWED BY
Kiarash Saleki,
Shahid Beheshti University of Medical Sciences, Iran

*CORRESPONDENCE
Riffat Mehboob
✉ mehboob.riffat@gmail.com
Gerhard Pfaff
✉ pfaff.pigmente@gmx.de

SPECIALTY SECTION
This article was submitted to Neuroinfectious Diseases, a section of the journal Frontiers in Neurology

RECEIVED 24 September 2022
ACCEPTED 07 February 2023
PUBLISHED 06 March 2023

CITATION
Mehboob R, Oehme P and Pfaff G (2023) The role of Substance P in the defense line of the respiratory tract and neurological manifestations post COVID-19 infection. *Front. Neurol.* 14:1052811. doi: 10.3389/fneur.2023.1052811

The role of Substance P in the defense line of the respiratory tract and neurological manifestations post COVID-19 infection

Riffat Mehboob[1]*, Peter Oehme[2] and Gerhard Pfaff[3]*

[1]Lahore Medical Research Center and LMRC Laboratories, LLP, Lahore, Pakistan, [2]Retired, Berlin, Germany, [3]Department of Chemistry, Technical University Darmstadt, Darmstadt, Germany

Substance P (SP) has been a great interest for scientists due to its unique properties and involvement in various physiological and pathological phenomenon. It took almost a century for the current understanding of this peptide so far. Its role in brain and gut were initially discussed and later on it was widely studied and observed in cardiovascular system, asthma, traumatic brain injury, immune response, vasodilation, behavior, inflammation, arthritis, cancer, airway hyper responsiveness and respiratory disorders. Involvement of SP in sudden perinatal death and COVID-19 has also been discussed which shed light on its vital role in respiratory rhythm regulation and initiation of cytokine storming in COVID-19. This article will provide a comprehensive overview of the researches done to understand the basic functions and involvement of SP in different processes of cell and its association with various diseases. This article describes the historical and scientific journey of SP from its discovery until today, including its future perspectives.

KEYWORDS

Substance P, COVID-19, lung inflammation, respiratory disorders, cytokine storming

1. Historical background

In the years 1930/31, the Swedish postgraduate student Ulf Svante von Euler (1905–1983, Nobel Prize for Physiology or Medicine 1970) isolated in the laboratory of Henry Hallett Dale (1875–1968, Nobel Prize for Physiology or Medicine 1936) in London a biological active extract from the intestine of animals (1, 2). For pharmacological studies, this extract was available as a "stable dry powder." The P from the word "powder" was used to identify the substance and has remained part of the name "Substance P" (SP) until today. The peptide chemical group of Susan E. Leeman isolated the substance from the hypothalamus and in 1971 determined the structure to be an undecapeptide with the sequence Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH2 (3). The total synthesis was also performed by the Leeman group (4).

In 1976 was a special year for SP researchers. The meanwhile world-famous physiologist and pharmacologist U. S. von Euler had invited to a Nobel Symposium in Stockholm. The symposium covered the state of knowledge of SP at that time: history, chemistry, mechanisms, distribution, and pharmacology. One focus was on the effect of SP on sensory nerve endings and pain. Pioneering work on this had been done by Fred Lembeck

(1922–2014), who investigated the effect of SP on afferent systems as early as 1953. His paper in Stockholm (5), and other papers, confirmed the hypothesis that SP is a transmitter in primarily sensory afferent neurons and plays an important role in the pain process. Peter Oehme, one of the authors of this contribution, together with Ulf Svante von Euler at the Nobel Symposium 1976.

Two papers at the symposium concerned the effect of SP on tracheobronchial tissue (6, 7). In these, the presence of SP was demonstrated in both nerve fibers and endocrine cells of guinea pig tracheobronchial tissue. At the same time, a strong effect on bronchial tone was found for SP, *in vivo* as well as *in vitro*. The effect observed was 45 times stronger than the effect of histamine. In his paper, Peter Oehme hypothesized that different information is encoded in the SP molecule (8): a direct effect on smooth muscle, sensory nerves, *etc.,* and an indirect effect through modulation of other transmitter systems, e.g., acetylcholine. For both effects, different parts of the SP sequence were discussed by Oehme (Figure 1).

2. Pharmacological actions of Substance P

2.1. Pain threshold

After returning from Stockholm, Peter Oehme started investigations on the action of SP on pain threshold in the Institute for Drug Research (IWF) of the Academy of Sciences in Berlin-Friedrichsfelde, which he founded in 1976. His investigations using the hot plate technique on mice yielded surprising results (9). It was shown that the SP effect depends on the initial condition of the test animals. It was found that SP has an analgesic effect on mice with a short reaction time to pain stimuli. On mice with a long reaction time, SP has a hyperalgesic effect. Both lead to a normalization of reaction time. Subsequent studies from the Oehme group revealed that the analgesic effect component is assigned to the C-terminal SP domain, whereas the hyperalgesic effect component is assigned to the N-terminal SP domain (10). This dual effect of SP was in accordance with the model presented by Oehme at the Stockholm SP Congress in 1976.

2.2. Stress reactions

Interesting findings also followed from the studies on "SP-action on behavior" carried out jointly by Oehme and Karl Hecht's group. In a series of stress models (immobilization, noise, electric footshocks, etc.), it was found in rats that SP is able to normalize the disturbances such as "decrease in learning," "loss of deep sleep and REM sleep," "increase in blood pressure and heart rate" (11, 12). Clinical studies conducted by Karl Hecht's group on patients with stress-induced sleep disorders with nasal SP application also showed positive results. Overall, it appeared that the N-terminus was relevant for the anti-stress effect, whereas the C-terminus was relevant for the acute effects, such as spasmogenic effect. Therefore, the term "regulatory peptide" (Regulide) was proposed for SP by Oehme and Hecht (12).

2.3. Pharmacological effects on chromaffin cells

Since there is an increase of catecholamines in plasma under stress, the interaction of SP with the aminergic system was investigated by the Oehme group. In adrenal slices, which contain chromaffin cells as well as endings of the splanchnic nerve, the electrically stimulated release of acetylcholine was investigated in addition to the release of noradrenaline. SP inhibited both electrically stimulated acetylcholine release and nicotinic release of norepinephrine (13). SP thus has both a presynaptic and a postsynaptic target.

For a more in-depth investigation of postsynaptic attack, studies were performed on isolated chromaffin cells with Bruce Livett (Melbourne) (14) to understand the modulation of synaptic transmission in these cells. This showed that SP has two effects. At first, it inhibits cholinergically induced catecholamine release, and second, it counteracts nicotine-induced desensitization of catecholamine release. Thus, SP can both inhibit excessive release and counteract too rapid depletion of release. Therefore, two separate points of attack are provided. Overall, SP is thus able to modulate synaptic transmission and act in the sense of the above-mentioned "Regulide" (15). It is the N-terminal tetrapeptide that inhibits both presynaptic acetylcholine release and postsynaptic norepinephrine release, thus modulating synaptic transmission in multiple ways. This is consistent with its role as an essential nucleus for the "anti-stress effect" of SP. These effects are independent of the NK 1 receptor. Apparently, the target for this modulation are the polyphosphoinositides (16).

2.4. Pharmacological effects on mast cells

Since it was known from the literature that SP can release histamine from peritoneal mast cells and that SP is released from sensory nerves upon antidromic stimulation, the Oehme group, in cooperation with the Pharmacological Institute of University College in London (UCL), began studies on modulation of synaptic transmission in mast cells. At first, SP, SP fragments and analogs were injected into the forearms in self-experiments. Later, volunteers from UCL joint the experiments. As expected, there was a dose-dependent redness and swelling on the forearms in these experiments. This was to be suppressed by antihistamines. N- and C-terminal SP fragments were ineffective. This implied that the entire SP molecule was necessary for histamine release from mast cells (17). Identical structure-activity relationships were shown on isolated peritoneal mast cells (18). These findings were considered significant for understanding the role of SP in the pathophysiology of inflammatory processes in various tissues, particularly in the bronchial tract.

Another study by Theoharides TC discusses the impact of the coronavirus (SARS-CoV-2) on the body, specifically focusing on the role of mast cells in the development of pulmonary symptoms and long-term complications in patients with COVID-19. The study suggested that activating mast cells can lead to the release of multiple proinflammatory cytokines, which can damage the lungs and contribute to pulmonary edema, inflammation,

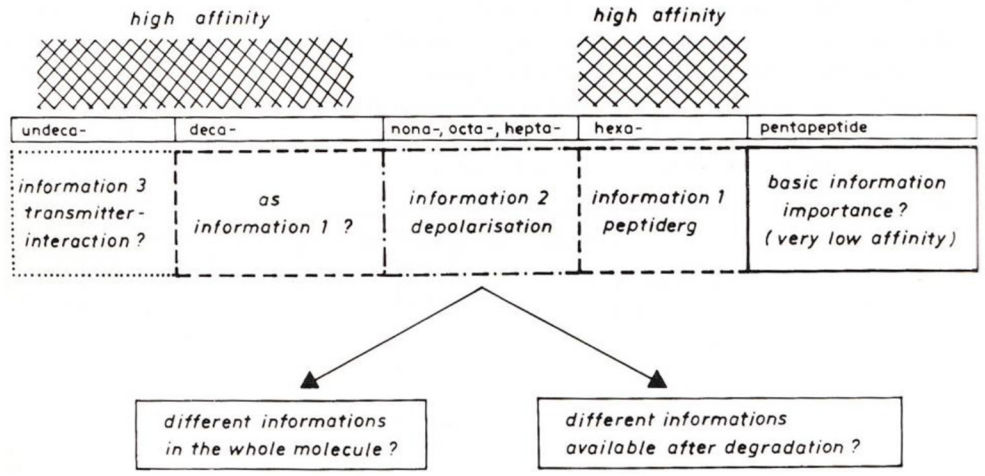


FIGURE 1
Model for different information in Substance P sequences (8).

and thromboses. Additionally, it suggested that many patients who have recovered from or had mild symptoms of COVID-19 may experience diffuse, multiorgan symptoms months after the infection, similar to those presented by patients diagnosed with mast cell activation syndrome (MCAS). The study concluded that it is important to suspect, evaluate, and address MCAS in any patient with COVID-19 who experiences chronic multiorgan symptoms and suggests that blocking mast cells and their mediators, such as the natural flavonoid luteolin, could be useful in preventing and managing symptoms during the COVID-19 pandemic (19).

3. SP-actions in the respiratory tract

The action of SP in the respiratory tract played only a minor role at the SP symposia following the Stockholm SP conference. At the 1983 SP conference hosted by David Powell in Dublin, local release of SP in the bronchial tract of guinea pigs by various chemical irritants was reported (20). This SP release was associated with mucosal edema and bronchospasm. In 1984, the symposium on "Substance P—metabolism and biological actions," initiated by Chris Jordan and Peter Oehme, in conjunction with the 9th IUPHAR International Congress of Pharmacology, was held in Maidstone (UK). In the review lecture by Bengt Pernow on "Substance P: present status and future prospects," the function of SP in sensory nerves was discussed in detail. However, a crucial statement by Bengt Pernow was: "Although there is now strong evidence that SP is an important factor in the development of neurogenic inflammation, the mechanism by which SP exerts its biological effects is not clear" (21).

Starting in 1987, Peter Oehme focused his group's work in this area and formed a joint working group with the Research Institute of Lung Disease and Tuberculosis in Berlin Buch. First of interest was the known bronchospastic effect of SP. As expected, SP1–11 showed a pronounced dose-dependent constrictor effect at the basal tone of the isolated guinea pig trachea (22). The C-terminal heptapeptide SP5–11 also caused a dose-dependent contraction

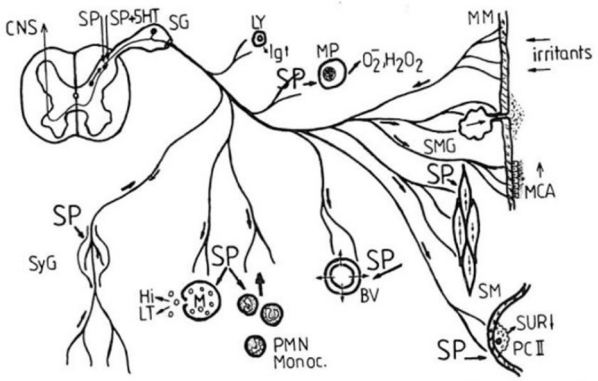


FIGURE 2
Proposed mechanism of Substance P in the respiratory tract (SP, spinal ganglion; Ly, lymphocyte; MP, macrophage; MM, mucous membrane; MCA, mucociliary activity; BV, blood vessels; SM, smooth musculature; SMG, mucous gland; PC II, pneumocyte type II; SUR, surfactant; SyG, sympathetic ganglion; Hi, histamine; LT, leukotrienes; Monoc., monocytes; PMN, polymorphonuclear neutrophils; CNS, central nervous system; 5-HT serotonin) (22).

of the isolated tracheal preparation. In contrast, the N-terminal tetrapeptide SP1–4 showed no constrictor effect. The contraction elicited by acetylcholine was significantly attenuated. Thus, the same picture emerged as in other pharmacological studies. The C-terminal has a direct effect; mediated *via* the NK 1 receptor. The N-terminal tetrapeptide has an indirect protective effect against the acetylcholine effect. This is mediated *via* a different target. Phosphatidylinositols have been discussed in this context. Figure 2 shows the mechanism of SP in the respiratory tract (22).

Since SP also acts on immunocompetent cells in the bronchial tract, it was of interest to determine whether differences also exist between the N- and C-terminal SP fragments. The studies were performed on spleen cell cultures from mice and mononuclear cells from rat lymph nodes (23). SP and the N-terminal sequences SP1–4

and SP1–7 were capable of secreting lymphokines with chemotactic properties for granulocytes and lymphocytes. The maximum of the dose-response curves was between 10–10 and 10–11, but the C-terminal fragments SP6–11, SP7–11, SP8–11, and SP9–11 were unable to induce lymphokines to be expressed.

Therefore, Oehme's group had planned to investigate both antagonists for the NK-1 receptor and N-terminal SP sequences for their therapeutic or preventive utility, primarily for the respiratory tract. In addition, capsaicin was of interest because of its influence on bronchial hyperreactivity (24). However, things were to turn out differently. With German unification, there were serious changes for both the Institute for Drug Research of the Academy of Sciences and the Research Institute for Lung Diseases and Tuberculosis. This led to the end of the research work on SP oriented on the bronchial tract in both institutes (25). A summary on pharmacological effects of SP can be found in the Sitzungsberichte der Akademie der Wissenschaften der DDR, newly published by de Gryuter Verlag (26), and in Reflections on Substance P-Research (27).

4. Role of SP in the first defense line of the respiratory tract

In 2021 saw the first contact between Peter Oehme and Riffat Mehboob. This was triggered by an event of the Leibniz Society of Sciences in Berlin with the chairman of the Drug Commission of the German Medical Association, Wolf-Dieter Ludwig, on the topic "What is the status of COVID-19" (28). An important statement of this meeting was that the next battle against Corona is to be fought in the respiratory tract. This statement prompted Peter Oehme to survey the literature in this direction. In doing so, Peter Oehme came across a paper by Riffat Mehboob on the importance of the NK 1 receptor in the therapy of COVID-19 (29). In particular, this work reported on the use of the NK-1 antagonist Aprepitant, in combination with dexamethasone, for the therapy of severe COVID courses. Riffat Mehboob proposed SP as a possible factor responsible for initiation of cytokine storming after getting infected with any foreign agent such as corona virus. Neurokinin-1 Receptor (NK-1R) antagonist, Aprepitant, was suggested as a potential drug for the treatment by inhibiting the receptor. Some evidences and commonalities were provided by her is support of this theory of SP involvement in respiratory tract infections including COVID-19 e.g., symptoms in COVID-19 infection and SP nociception, airway hypersensitivity/asthma in both phenomenon, variable patterns of COVID-19 disease severity in different age groups which is also addressed by SP theory, high death rate in COVID-19 patients having co-morbidities of diabetes, hypertension and cardiac disorders, viral load correlates with SP secretion and hence, its proposed that SP may be the trigger for cytokine storming during such inflammation. Aprepitant is NK-1R antagonist that has been approved for the treatment of chemotherapy-induced vomiting for a number of years (29).

A review by Karamyan VT suggested that inflammation was a major cause of complications from COVID-19, and studies had focused on pro-inflammatory cytokines and the "cytokine storm" as a mechanism to explain the severity of the disease. More recently in 2021, the article suggested that peptide bradykinin, its dysregulated signaling, or "bradykinin storm," had emerged as a primary mechanism to explain COVID-19-related complications. The article also suggested that two closely related vasoactive peptides, SP and neurotensin, were also likely to have driven microvascular permeability and inflammation and been responsible for the development of COVID-19 pathology. It also postulated that in addition to ACE and neprilysin, peptidase neurolysin (Nln) was also likely to have contributed to accumulation of bradykinin, SP and neurotensin, and progression of the disease. In conclusion, it was proposed that "vasoactive peptide storm" may have underlain the severity of COVID-19 and that simultaneous inhibition of all three peptidic systems could have been therapeutically more advantageous than modulation of any single mechanism alone (30).

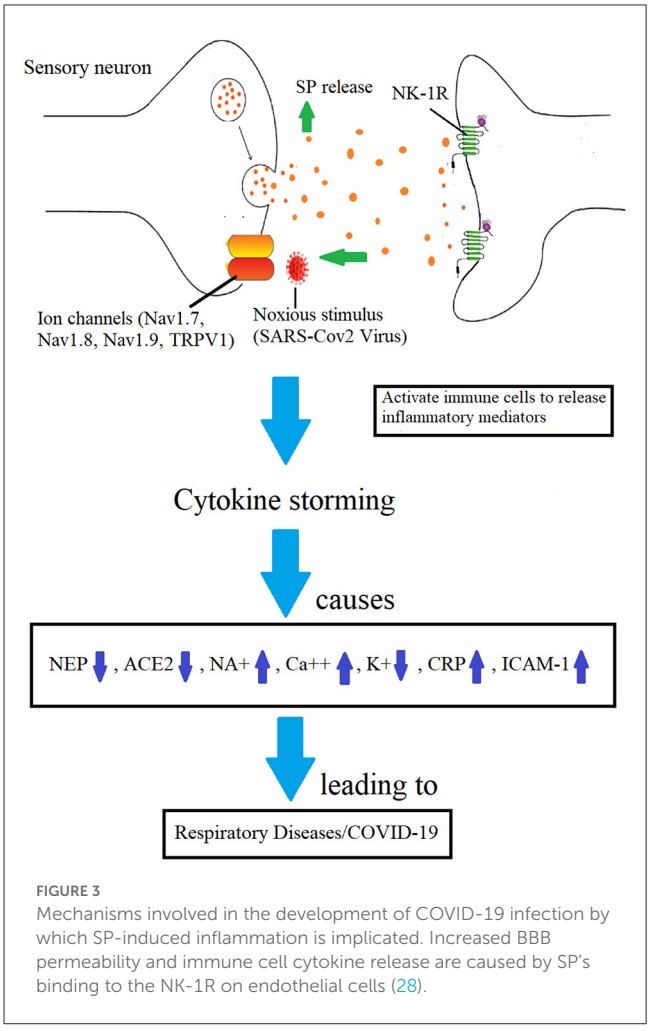


FIGURE 3
Mechanisms involved in the development of COVID-19 infection by which SP-induced inflammation is implicated. Increased BBB permeability and immune cell cytokine release are caused by SP's binding to the NK-1R on endothelial cells (28).

The immune reaction kills the virus to protect the host cells, but if it continues unchecked, it is known as cytokine storming, which may be lethal (Figures 1–3). Patients with COVID-19 infection may develop acute respiratory distress syndrome (ARDS) because immune cells continuously release inflammatory mediators (Figure 3). Therefore, the pathogen itself is not doing much damage, but cytokine storming is the main offender. Additionally, if restricted, illness severity could be reduced (31, 32).

The immune system-stimulating effects of SP could cause a cytokine storm. The inflammatory pathways and, hence, the cytokine storming may both be stopped if its receptor is suppressed by aprepitant. When exposed to a toxic stimulus, SP is the first to react and acts as a quick defense mechanism to ensure survival. Comparing them to controls, NK-1R defective mice were shown to exhibit less pulmonary inflammation (33). Immune cells secrete SP, which has endocrine, paracrine, and autocrine effects (34). It can activate cells that are far away, such as smooth muscle cells, endothelium cells, lymphatics, white blood cells and fibroblasts. It interacts with NK-1R, stimulates the immunological and endocrine systems to produce inflammatory mediators in the airway tracts (35). It is also found on the cardio-ventilatory regulatory centers and phrenic nuclei, which regulate the diaphragm and respiration. It is concentrated in the brainstem nuclei that mediate respiratory regulation (36). Once formed, the SP/NK1-R complex starts a signaling chain that results in the production of IP3 and diacylglycerol (DAG) (37). The activation of NF-kB by macrophages and other immune cells results in the production of inflammatory mediators and the release of pro-inflammatory cytokines (38).

The study by Bellis et al., studied Neprilysin receptors in treating COVID-19. The study explained that SARS-CoV-2 disease causes ACE2 down-regulation and related decrease in angiotensin II degradation, which can lead to a "cytokine storm" and acute lung and cardiovascular injury. The researchers observed that current treatments, such as remdesivir and renin angiotensin system antagonists, have not been shown to be effective in reducing inflammation related to COVID-19. They suggested that neprilysin (NEP) may be an interesting target for preventing organ injury in COVID-19 patients, as it is involved in the degradation of molecules that prevent organ injury (39). NEP is involved in downregulation of SP and reduces inflammation (28) and supports the hypothesis discussed in this study.

The main symptom of COVID-19 is respiratory disease, but it is also becoming apparent that the disease affects multiple systems in the body, including the central nervous system (CNS) through the olfactory nerve and/or enteric nervous system. Neurological symptoms have been linked to a proinflammatory response in the CNS, caused by the ACE2 receptor being expressed in the brain, which ultimately leads to neuroinflammation. A study have shown that increased expression of TRPV1, a nonselective cation channel, leads to an increase in proinflammatory molecules such as substance P and IL-6, which are associated causing "cytokine storm" with more severe disease (40).

5. SP in ventilatory responses

SP has a major role in cardio-respiratory rhythm generation and control evidenced through previous study (35, 38) including ours conducted in University of Milan, Milan, Italy (41–44). They have an impact on how people react to ventilation since they are expressed in several brainstem regions. In a previous study, Riffat Mehboob and Anna Maria Lavezzi at the Lino Rossi Research Center, University of Milan, Italy, found that the increased expression of SP in the brainstem tissues of control infants as compared to infants who had experienced sudden infant death syndrome (SIDS), suggested that SP/NK-1R may be regulating the ventilatory regulation in newborns (41). In a related investigation, the brainstem nuclei of victims showed a marked reduction in SP and NK-1R binding. Due to a failure in cardiorespiratory regulation brought on by this altered SP expression, SIDS may result (45). In unexpected fetal fatalities, SP expression was increased (41, 43) and sudden adult death (46).

These findings may be correlated with mortalities in COVID-19 patients due to respiratory complications. SP also serves as a neuromodulator and vasodilator, contractions of smooth muscles in upper airways, increased excitatory potential by neurons, enhanced saliva production and a higher vascular permeability (38, 47). It may also lead to bronchoconstriction in pathological conditions (28, 47). Another study of Riffat Mehboob has discussed the fact that the gene encoding SP, TAC-1 has un-conventional networking properties such as it is singleton gene, has small protein interaction network and the members of tachykinin family have conserved aminoacyl sequences. These properties are responsible for vulnerability of TAC-1 gene and shows that it is a very important gene, any mutation in this gene may lead to fatal consequences as there will be no other gene copy to compensate its functions. These fatal outcomes may be sudden death due to respiratory failures. The other members of these gene pathway should also be explored (44).

SP and serotonin innervate the medullary motoneurons involved in upper respiratory tract (48) and laryngeal afferent system (38). In the bronchopulmonary C fibers of the respiratory tracts, SP, the most prevalent neuropeptide, and neurotransmitter, is found. It guards the lungs against any harm from irritating substances that are inhaled. The central nervous system (CNS) reacts to nociceptive stimuli by releasing nitric oxide, prostaglandins, and SP from the respiratory epithelium, as well as bronchoconstriction, cough, hypotension, sleep apnea, and mucus secretions in the lungs (48). NK-1R mRNA was found to be raised in broncho-alveolar lavage fluid (25), sputum samples (49) and lung tissue (50), in a study conducted on asthmatic patients. SP/NK-1R binding and the resulting interactions are also vital for the regulation of airway hyper responsiveness (AHR) (51).

An example of extreme hypersensitivity of the bronchial tract is SIDS when exposed to irritants, e.g., the cigarette smoke of the mother (41, 52). In this regard, immunohistochemical studies were published by Lavezzi et al. (41) and Mehboob et al. (42) in 2011 and 2017. These showed downregulated SP expression for SIDS-risk newborns in such brainstem areas that are important for respiratory regulation. This confirms earlier research by Oehme's group on infants at increased risk of SIDS, where a correlation between mean respiratory failure and low SP plasma levels was shown in the first 5 months of life. This has been discussed as an indication of delayed maturation of respiratory control mechanisms (45, 53). Vice versa, Mehboob and Lavezzi (43) questioned whether the minimal probability of healthy neonates and infants to become ill after corona infection is also related to the SP system. Fiona Bright from Australia discussed the abnormalities in the brainstem nuclei may be responsible for cardiorespiratory failure and hence SIDS (52, 54, 55). According to Mehboob et al., a fetus's brainstems exhibit very little SP expression. On the other hand, it is increased for newborns and lowered for kids and adults in controls. While the opposite results in unexpected fatalities (42).

6. SP/NK-1R, its relation to trigeminal ganglion, latency during corona virus infection

Another innovative idea for coronavirus latency during infection was put out by Riffat Mehboob. If we consider that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus is operating through the trigeminal ganglion (TG), which is the principal location for other latent viruses, she has highlighted the possibility of latency in SARS-CoV-2 virus infection. According to her, SP/NK-1R pathway is the key player in inflammation during COVID-19 infection as it may directly affect the ventilatory responses (48). The immune cells, along with other cells in the airways and the lung's epithelial lining, are impacted by the excessive secretion of SP by TG neurons (28). Corona virus may have a less unlikely chance of going latent and controlling the release of different TG peptides, including SP, by entering the TG *via* the trigeminal nerve in the eyes, nose, and mouth but the possibilities cannot be ruled out. The corona virus might be latent or quiescent in TG and could reactivate at any time. The patient could develop an infection as a result and experience no symptoms. After the initial infection, a virus' latency may be broken within the cell (56). Despite blood antibodies to the virus being present, the viral genome may stay in the host cell after primary infection and may be reactivated by any stressor (57).

The mesencephalic trigeminal nucleus and the TG in the brainstem both include some of the primary afferent neurons of the trigeminal nerve. The ocular (V1), maxillary (V2), and mandibular (V3) nerves are the three branches that make up the TrN. Each gives each of their distinct head regions innervation (58). The nociceptors, which are the free nerve ends of the trigeminal sensory afferents, are activated by pain or any other unpleasant stimulus, such as SARS-CoV2. These C-fiber sensory nerve fibers can be myelinated or not, and their cell bodies are found in the TG (42, 58). The trigeminal spinal caudalis (Vc) nucleus of the brainstem receives these impulses *via* afferent fibers. Here, they connect with the second order neurons that send signals to the thalamus and the limbic and somatosensory cortices. Trigeminal afferent neurons' activity can be altered by inflammation of orofacial tissues that the TrN innervates, leading to ectopic firing and increased sensitivity to painful stimuli. Numerous mediators, including neurotrophic factors or neuropeptides at nerve ends, such SP, CGRP, and serotonin, induce sensitization. TG and TrN's SP and CGRP levels rise in response to painful stimuli, including nerve damage (59). Nicotinic stimulation of polyphosphoinositide turnover in rat adrenal medulla slices was studied by Minenko et al. (60) and the influence of adrenal demedullation on stress-related behavior in wistar rats was investigated by Roskte et al. (61), in the same year. One of the stress related parameters measured was SP in addition to blood pressure, pain sensitivity, endogenous opiod system etc.

A study by Henri et al., used computational methods to identify potential drug candidates that can bind to the nucleoprotein N of SARS-CoV-2, the virus that causes COVID-19. The researchers used a new model of N, which was built using an existing model and refined by molecular dynamics simulations. The predicted drug candidates were neuropeptides, such as substance P (1–7) and enkephalin, which bind to a large site on the C-terminal or N-terminal β-sheet of N. The study also found that some variants of N, such as BA4 and BA5, also have large binding sites. The binding sites of the predicted drug candidates were then tested using surface plasmon resonance experiments. The study found that the drugs likely impede RNA binding to N, which could inhibit viral replication. The study suggested that these neuropeptides may play a role in the symptoms of long COVID-19 and that drugs targeting N may help reduce the risk of brain fog and stroke. This link of neuropeptides involved in COVID-19 supports our hypothesis (62).

7. Neurologic manifestations post COVID-19 and role of SP

Clinical manifestations of COVID-19 are variable and hence the neurological symptoms such as anosmia, ageusia, central hemorrhage, infarction also vary depending on age and comorbidities (63). Patients with long COVID-19 infection may experience post-intensive care syndrome, post-viral fatigue syndrome, permanent organ damage and long COVID syndrome (64, 65). Central nervous system disorders in COVID-19 are more than anticipated so far. Peripheral nerves and skeletal muscles are affected to a lesser extent. In majority of the cases, there is no direct attack of the virus toward vulnerable structures, explaining the possibility that why nervous system manifestations manifest favorably to immune suppression (66).

SP is also involved in post COVID olfactory dysfunction. Schirinzi et al., research study investigated the activity of two inflammatory pathways, SP and Prokineticin-2 (PK2), within the olfactory neurons (ONs) of patients to understand the mechanisms of persistent olfactory dysfunction (OD) post-COVID-19. The study collected ONs from 10 patients with persistent post-COVID-19 OD and 10 healthy controls using non-invasive brushing. Gene expression levels of SP, Neurokinin receptor 1, Interleukin-1β (IL-1β), PK2, PK2 receptors type 1 and 2, and Prokineticin-2-long peptide were measured in ONs by Real Time-PCR and correlated with residual olfaction. Immunofluorescence staining was also performed to quantify SP and PK2 proteins. The results showed that patients with OD had increased levels of both SP and PK2 in ONs compared to healthy controls, with the latter being proportional to residual olfaction. This study provides preliminary evidence that both SP and PK2 pathways may have a role in persistent post-COVID-19 OD. The sustained activation of SP, lasting months after infection's resolution, might foster chronic inflammation and contribute to hyposmia, while the PK2 expression could instead support the smell recovery (67).

As the research on COVID-19 infections continued to evolve, various possible meachnisms of virus attack on CNS was suggested. One such mechanism was angiotensin-converting-enzyme-2 receptor as a potential modulator of coronavirus related CNS damage and suggested that it damages the cerebrovascular endothelium and brain parenchyma, the latter predominantly in the medial temporal lobe, resulting in apoptosis and necrosis (68). Neurons and glial cells express ACE2 receptors in the CNS, and recent studies suggest that activated glial cells contribute to neuroinflammation and the devastating effects of

SARS-CoV-2 infection on the CNS. The SARS-CoV-2-induced immune-mediated demyelinating disease, cerebrovascular damage, neurodegeneration, and depression are some of the neurological complications (69). We have also proposed a novel theory that coronavirus may stimulate nociceptive pathways after entering the trigeminal ganglion of the brainstem where it trigers the release of SP. SP binds to Neurokinin-1 Receptor and initiate cytokine storming in lungs leading to complications related to COVID-19 infection. Virus may also become latent in trigeminal ganglion (70).

Evidence of possible routes of SARS-CoV-2 neuroinvasion through systemic circulation and crossing the blood-brain barrier making its way to the central nervous system is still lacking (71). Pathophysiology and neurological manifestations of COVID-19 post infection was discussed by Bobker et al. with focus on headache. Many variations in the neurological symptoms of patients were observed and more researches were suggested for better understanding (71). Persistent post-COVID-19 OD is an unknown syndrome that could lead to neurological complications. Because of the potential long-term neurological consequences, persistent olfactory dysfunction (OD) is one of the most common and concerning problems of long-term COVID-19. OD patients had higher amounts of SP than controls. There is preliminary evidence that SP pathways may play a role in chronic post COVID-19 OD, making both of them potential therapeutic targets (72).

8. Future aspects

The conclusion in one of our previous paper (28), "actually it is not the virus that is fatal and causing mortalities, but the cytokine storming activated and initiated by SP is bringing the disaster," we believe this situation with COVID-19 could be explained by the historical views of Rudolf Virchow (1821–1902), Robert Koch (1843–1910), Max von Pettenkofer (1818–1901), and Oscar Liebreich (1839–1908) on the proper control of epidemics (26, 73). At the end of these discussions, in conjunction with the cholera epidemics of the time, was the statement that the germ is not the disease, but that disease germ, vector, and human mutually influence each other and must, therefore, be considered equally (74).

While contaminated water was the main vector for the cholera epidemics in former times, air is the main vector for the corona pandemics today. The air vector is certainly a multi-layered problem. In addition to viruses as pathogens, the air today contains a large number of pollutants that must be taken seriously. It is significant for the further scientific work that air and respiratory tract are closely related. The findings presented in this paper show that the neuropeptide SP has a defense function in the respiratory tract (75).

At this point, here are some perspective thoughts connected with the goal of linking Substance P research more closely with research on corona diseases. A first thought is that the viruses (or pollutants) entering *via* the respiratory tract are to be understood as stressors. The respiratory tract has the task to recognize these stressors as such to organize the local defense, to impede or block further penetration and, if possible, to destroy the stressors. In this context, the respective state of the immune system is certainly decisive for the subsequent outcome. From the findings of Mehboob et al. (28, 43, 47, 72), and the Oehme group (45, 53), it is clear that the different infection rates for COVID-19 infections or the frequencies of sudden death infant syndromes correlate with the SP plasma level. In addition, from the experience of the current corona epidemic, children are equally likely to become infected with corona but are much less likely to develop corona than adults. In addition, the extensive studies by Oehme and Hecht (see Section 2.2) on experimental animals and humans show that there is a clear relationship between stress sensitivity and SP levels: Low SP levels = high stress sensitivity (12, 28). It would be useful to follow up on these findings and investigate in adult Corona-infected individuals whether the frequency of transition from infection to disease correlates with SP levels in plasma or bronchial lavage.

A second thought: it has been demonstrated by Mehboob et al. (28, 76) for Aprepitant that this SP antagonist, in combination with dexamethasone, improves symptomatology in severe corona course. This finding is explained by a reduction in cytokine storming triggered by SP in the deeper pulmonary alveoli. However, the primary response of coronavirus occurs in the upper nasopharynx. Here, SP (antidrome) is also released to trigger defense processes. Since these local processes determine to a large extent the further course of the infection, they should be investigated in depth. Of particular interest would be whether N-terminal dipeptides, especially Lys-Pro, are cleaved from SP1–11 by enzymatic cleavage during this local SP release. For this dipeptide, both stress-protective effects (see Sections 2.2 and 2.3) and positive effects in the respiratory tract (see Section 3) have been demonstrated. In addition, Lys-Pro was found to stimulate nerve fiber growth in tissue culture (74). Therefore, a Lys-Pro derivative was also applied for a patent with the indication "wound healing" (77).

Drug repurposing, the process of identifying existing drugs that can be used to treat new conditions, has several potential benefits for COVID-19 treatment development. These include shorter development time, reduced costs, and faster regulatory approval. A study by Egieyeh et al., used computational methods to predict drugs from the Drug Bank that may bind to the SARS-CoV-2 spike protein on the human ACE2 receptor and inhibit the protein-protein interaction required for infection. The predicted drugs, which include peptide-based drugs like Sar9 Met (O2)11-Substance P and BV2, may have potential for treating COVID-19 and have already been investigated for other indications such as ARDS and viral infections. The study also explored the current and proposed pharmacological uses of the predicted drugs, finding that some have been investigated for treatment of acute respiratory distress syndrome (ARDS), viral infection, inflammation, and angioedema, as well as stimulation of the immune system and enhancement of antiviral agents against influenza virus. Similar computational study can also be performed on SP/NK1R to test its potential in treating COVID-19 (78).

To prevent and contain this epidemic, it is imperative that new medicinal approaches be developed. A novel class of medications called NK-1R antagonists has antidepressant,

antiemetic, and anxiolytic effects. Aprepitant, Rolapitant, Casopitant, Netupitant, Maropitant, and Fosaprepitant are a few examples of NK-1R inhibitors (79). In 2003, the FDA approved aprepitant as the first NK-1R antagonist (80). It could be a part of a viral respiratory disease therapy plan. In a phase 2 trial (VOLCANO-1) for the treatment of persistent refractory cough, orvepitants had dramatically reduced the symptoms (81). It is well established that NK-1R antagonists have an anti-inflammatory effect on rats, and that SP and NK-1Rs are both increased during the inflammatory processes (82). It may be advantageous to pharmacologically suppress SP-signaling in COVID-19 infection. The use of NK-1R antagonists may be advised to alleviate SP-related symptoms. In patients with viral myocarditis, SP-receptor antagonism may also be suggested as a treatment approach (83). Riffat Mehboob and her team just completed a randomized clinical trial in which they saw very encouraging patient outcomes for COVID-19 treatment. There were two arms; one received standard treatment and the other received the NK-1R antagonist, aprepitant, in addition. Both groups also received dexamethasone treatment. 52 patients were placed in control group A and 67 patients were placed in intervention group B out of a total 119 patients who were randomly assigned to both of these arms. Before and after the intervention, blood parameters examined in both groups. Patients who received a combination of aprepitant and dexamethasone medication demonstrated improved clinical results, laboratory findings, and decreased levels of the inflammatory marker C-reactive protein (76). Here, we propose that the pathogenesis of COVID-19 infection brought on by SARS-CoV-2 is mediated by SP/NK-1R. As in other airway infections, it might be brought on by cytokine storming exacerbating the inflammatory pathways. Corticosteroids, antibiotics, purified intravenous immunoglobulins, and anti-cytokine therapy should all be used together as part of the suggested treatment plan (84). Overall, further contact between SP and corona research would be enlightening and could promote greater collaboration between environmental and medical research.

9. Conclusions

SP release from trigeminal nerve as a consequence of a nociceptive stimulus is directly related to the respiratory complications in COVID-19 and other respiratory illnesses. It causes an increased inflammation and must be blocked by using Aprepitant, a neurokinin 1 receptor antagonist along with glucocorticoid, dexamethasone. Dexamethasone will activate the enzyme neutral endopeptidase which is responsible for degradation of SP and Aprepitant may block the NK-1R. Hence, the cytokine storm will be inhibited by blocking this pathway and the disease progression too. This therapeutic strategy may be effective as a successful clinical trial was conducted on COVID-19 patients in Pakistan by Riffat Mehboob and her team (76). These findings urge for more investigation on this drug, further clinical trials in other countries and a drug should be formulated based on this strategy. It will be a new and effective treatment for COVID-19.

Overall, this summarized review urges further research into this magical regulatory peptide (regulide) which can be used as treatment strategy for various diseases by maintaining an optimal balance and regulation of this peptide within plasma. We can foresee future of treatments into peptides and peptide researches and should be investigated on priority basis.

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 authors.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

RM is the founder and research director of Lahore Medical Research Center and LMRC Laboratories, LLP.

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

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. von Euler US, Gaddum JH. An unidentified depressor substance in certain tissue extracts. *J Physiol.* (1931) 72:74–87.

2. von Euler US. Historical notes. In: Euler US, Pernow B, editors. *Nobel Sympoium 37, Substance P.* New York, NY: Raven Press (1977), p. 1–3.

3. Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J Biol Chem.* (1970) 245:4784–90.

4. Tregear G, Niall HD, Potts JT, Leeman SE, Chang MM. Synthesis of substance P. *Nature.* (1974) 232:87–9.

5. Lembeck F, Gamse R, Juan H. *Substance P and Sensory Nerve Endings*. New York, NY: Raven Press (1977), p. 169–81.

6. Nilson G, Dahlberg K, Brodin E, Sundler F, Sandberg K. *Distribution and Constrictor Effect of Substance P in Guinea Pig Tracheobronchial Tissue*. New York, NY: Raven Press (1977). p. 7581.

7. Sundler F, Aluments J, Brodin E, Dahlberg K, Nilson G. *Perivascular Substance P-Immunoreactive Nerves in Tracheobronchial Tissue*. New York, NY: Raven Press (1977). p. 271–3.

8. Oehme P, Bergmann J, Bienert M, Hilse H, Piesche L, Minh Thu P, Scheer E. *Biological Action of Substance P—Its Differentiation by Affinity and Intrinsic Efficacy*. New York, NY: Raven Press (1977). p. 327–35.

9. Oehme P, Hilse H, Morgenstern E, Göres E. Substance P: Does it produce analgesia or hyperalgesia? *Science*. (1980) 208:3305–307.

10. Görne RC, Morgenstern E, Oehme P, Bienert M, Neubert K. Wirkung von Substanz P und Substanz P—fragmenten auf die Schmerzschwelle von Mäusen. *Pharmazie*. (1982) 37:299–300.

11. Hecht K, Oehme P, Kolometseva IA, Lyovshina I, Poppei M, Airapetjanz MG. Effect of Substance P analogue on chronic deprivation of sleep of Wistar rats under stress. In: Marsan CA, Traczyk S, editors. *Neuropeptides and Neural Transmission, International Brain Research Organization (IBRO) Mongraph Series*. New York, NY: Raven Press (1980). p. 159–64.

12. Oehme P, Hecht K, Piesche L, Hilse H, Morgenstern E, Poppei M. *Substance P as a Modulator of Physiological and Pathological Processes*. New York, NY: Raven Press (1980). p. 73–84.

13. Nieber K, Oehme P. Effect of substance P (SP) and the N-terminal SP-analogue SP (1–4) on the pre- and postsynaptic transmitter release in rat adrenal gland slices. *Biomed Biochim Acta*. (1987) 46:103–9.

14. Cheung NS, Karlsson P, Wang J-X, Bienert M, Oehme P, Livett BC. Functional studies with substance P analogues: effects of N-terminal, C-terminal, and C-terminus-extended analogues of substance P on nicotine-induced secretion and desensitization in cultured bovine adrenal chromaffin cells. *J Neurochem*. (1994) 62:2246–53.

15. Oehme P, Krivoy WA. Substance P: a peptide with unusual features. *TIPS*. (1983) 4:521–3.

16. Minenko A, Oehme P. Substance P action on inositol phospholipids in rat adrenal medulla slices. *Biomed Biochim Acta*. (1987) 46:461–7.

17. Foreman JC, Jordan CC, Oehme P, Renner H. Structure-activity relationships for some substance P-related peptides that cause wheal and flare reactions in human skin. *J Physiol*. (1983) 335:449–65.

18. Renner H, Oehme P. *Histaminfreisetzung aus Mastzellen Durch Substanz P*. New York, NY: Springer (1983).

19. Theoharides TC. Potential association of mast cells with coronavirus disease 2019. *Ann Allergy Asthma Immunol*. (2021) 126:217–8. doi: 10.1016/j.anai.2020.11.003

20. Lundberg JM, Saria A, Brodin E, Martling C-R, Hökfelt T, Rosell S. Mucosal oedema and bronchoconctrictioniduced by irritation of capsaicin-sensitive Substance P afferents. In: Scrabanek P, Powell D, editors. *Substance P*. Dublin: Boole Press (1983). p. 86–9.

21. Pernow B. Substance P: present status and future prospects. In: Jordan CP, editors. *Substance P: Metabolism and Biological Actions*. London: Taylor&Francis (1985). p. 187–96.

22. Schreiber J, Slapke J, Nieber K, Oehme P. Rolle von Substanz P in der Regulation der Bronchomotorik und der Pathogenese der bronchialen Hyperreagibilität. *Z Erkrank Atm*. (1989) 172:90–8.

23. Paegelow I, Werner H, Bienert M, Oehme P. Influence of substance P and substance P: sequences on immunocompetent cells. *Pharmazie*. (1989) 44:145–6.

24. Schreiber J, Slapke J, Nieber K, Oehme P. Influence of capsaicin on the reagibility of the isolated guinea pig trachea. *Biomed Biochim Acta*. (1990) 49:97–101.

25. Nieber K, Baumgarten CR, Rathsack R, Furkert J, Oehme P, Kunkel G. Substance P and beta-endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. *J Allergy Clin Immunol*. (1992) 90:646–52.

26. Oehme P. Aktuelle Probleme der Peptidforschung. In: *Sitzungberichte der Akademie der Wissenschaften der DDR, 10 N*. Berlin: Nachdruck De Gruyter (2022)

27. Oehme P, Hecht K. *Reflektionen zur Substanz P-Forschung*. Cambridge: Eigenverlag (2022). Available online at: https://leibnizsozietaet.de/aktuelle-ueberarbeitung-der-reflektionen-zur-substanz-p-forschung/ (accessed March 17, 2022).

28. Mehboob R. Neurokinin-1 Receptor as a potential drug target for COVID-19 treatment. *Biomed Pharmacother*. (2021) 143:112159. doi: 10.1016/j.biopha.2021.112159

29. Sarzi-Puttini P, Giorgi V, Sirotti S, Marotto D, Ardizzone S, Rizzardini G, et al. COVID-19, cytokines and immunosuppression: what can we learn from severe acute respiratory syndrome? *Clin Exp Rheumatol*. (2020) 38:337–42. doi: 10.55563/clinexprheumatol/xcdary

30. Karamyan VT. Between two storms, vasoactive peptides or bradykinin underlie severity of COVID-19? *Physiol Rep*. (2021) 9:e14796. doi: 10.14814/phy2.14796

31. Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2(-) mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging Dis*. (2020) 11:216–28. doi: 10.14336/AD.2020.0228

32. Bozic CR, Lu B, Höpken UE, Gerard C, Gerard NP. Neurogenic amplification of immune complex inflammation. *Science*. (1996) 273:1722–5.

33. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol*. (2004) 201:167–80. doi: 10.1002/jcp.20061

34. Graefe SB, Mohiuddin SS. Biochemistry, Substance P. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing (2022).

35. Mazzone SB, Geraghty DP. Respiratory actions of tachykinins in the nucleus of the solitary tract: effect of neonatal capsaicin pretreatment. *Br J Pharmacol*. (2000) 129:1132–9. doi: 10.1038/sj.bjp.0703173

36. Ramkissoon SH, Patel HJ, Taborga M, Rameshwar P, G. protein-coupled receptors in haematopoietic disruption. *Expert Opin Biol Ther*. (2006) 6:109–20. doi: 10.1517/14712598.6.2.109

37. Bost KL. Tachykinin-mediated modulation of the immune response. *Front Biosci*. (2004) 9:3331–2. doi: 10.2741/1484

38. Kaczynska K, Zajac D, Wojciechowski P, Kogut E, Szereda-Przestaszewska M. Neuropeptides and breathing in health and disease. *Pulm Pharmacol Ther*. (2018) 48:217–24. doi: 10.1016/j.pupt.2017.12.001

39. Bellis A, Mauro C, Barbato E, Trimarco B, Morisco C. The rationale for angiotensin receptor neprilysin inhibitors in a multi-targeted therapeutic approach to COVID-19. *Int J Mol Sci*. (2021) 21:8612. doi: 10.3390/ijms21228612

40. Aguirre-Siancas EE, Colona-Vallejos E, Ruiz-Ramirez E, Becerra-Bravo M, Alzamora-Gonzales L. Substance P, proinflammatory cytokines, transient receptor potential vanilloid subtype 1 and COVID-19: a working hypothesis. *Neurología*. (2021) 36:184–5. doi: 10.1016/j.nrleng.2020.10.002

41. Lavezzi AM, Mehboob R, Matturri L. Developmental alterations of the spinal trigeminal nucleus disclosed by substance P immunohistochemistry in fetal and infant sudden unexplained deaths. *Neuropathology*. (2011) 31:405–13. doi: 10.1111/j.1440-1789.2010.01190.x

42. Mehboob R. Substance P/neurokinin 1 and trigeminal system: a possible link to the pathogenesis in sudden perinatal deaths. *Front Neurol*. (2017) 8:82. doi: 10.3389/fneur.2017.00082

43. Mehboob R, Lavezzi AM. Neuropathological explanation of minimal COVID-19 infection rate in newborns, infants and children: a mystery so far. New insight into the role of Substance P. *J Neurol Sci*. (2021) 420:1–3. doi: 10.1016/j.jns.2020.117276

44. Mehboob R, Hashmi AM, Ahmad FJ. Vertebrate specific oncogenic TAC1 has unconventional networking properties. *Healthmed*. (2014) 8:843–9.

45. Scholle S, Zwacka G, Glaser S, Knöfel B, Scheidt B, Oehme P, et al. Substance P, mean apnoea duration and the sudden infant death syndrome. *Biomed Biochim Acta*. (1990) 49:249–56.

46. Hayashi M, Sakuma H. Immunohistochemical analysis of brainstem lesions in the autopsy cases with severe motor and intellectual disabilities showing sudden unexplained death. *Front Neurol*. (2016) 7:93. doi: 10.3389/fneur.2016.00093

47. Mehboob R, Kabir M, Ahmed N, Ahmad FJ. Towards better understanding of the pathogenesis of neuronal respiratory network in sudden perinatal death. *Front Neurol*. (2017) 8:320. doi: 10.3389/fneur.2017.00320

48. Szereda-Przestaszewska M, Kaczynska K. Serotonin and substance P: synergy or competition in the control of breathing. *Auton Neurosci*. (2020) 225:102658. doi: 10.1016/j.autneu.2020.102658

49. Tomaki M, Ichinose M, Miura M, Hirayama Y, Yamauchi H, Nakajima N, et al. Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis. *Am J Respir Crit Care Med*. (1995) 151:613–7.

50. Adcock IM, Peters M, Gelder C, Shirasaki H, Brown CR, Barnes PJ. Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids. *J Mol Endocrinol*. (1993) 11:1–7.

51. Bertrand C, Geppetti P. Tachykinin and kinin receptor antagonists: therapeutic perspectives in allergic airway disease. *Trends Pharmacol Sci*. (1996) 17:255–9.

52. Bright FM, Vink R, Byard RW, Duncan JR, Krous HF, Paterson DS. Abnormalities in substance P neurokinin-1 receptor binding in key brainstem nuclei in sudden infant death syndrome related to prematurity and sex. *PLoS ONE*. (2017) 12:e0184958. doi: 10.1371/journal.pone.0184958

53. Scholle S, Zwacka G, Scheidt B, Glaser S, Oehme P, Rathsack R. Screeningprogram zur Erfassung von Kindern mit einem erhöhten SIDS-Risko (Plötzlicher und unerwarteter Kindstod). *Klein Pädiatr*. (1989) 201:377–81.

54. Bright FM, Byard RW, Vink R, Paterson DS. Normative distribution of substance P and its tachykinin neurokinin-1 receptor in the medullary serotonergic network of the human infant during postnatal development. *Brain Res Bull*. (2018) 137:319–28. doi: 10.1016/j.brainresbull.2018.01.009

55. Bright FM, Vink R, Byard RW. The potential role of substance P in brainstem homeostatic control in the pathogenesis of sudden infant death syndrome (SIDS). *Neuropeptides.* (2018) 70:1–8. doi: 10.1016/j.npep.2018.02.006

56. Villarreal L. *Viruses and the Evolution of Life*. Washington, DC: ASM Press (2005). doi: 10.1128/9781555817626

57. Dimmock NJ, Leppard KN. *Introduction to Modern Virology*. 6th edn. Hoboken, NJ: Blackwell Publishing (2007).

58. Bista P, Imlach WL. Pathological mechanisms and therapeutic targets for trigeminal neuropathic pain. *Medicines.* (2019) 6:91. doi: 10.3390/medicines6030091

59. Goto T, Iwai H, Kuramoto E, Yamanaka A. Neuropeptides and ATP signaling in the trigeminal ganglion. *Jpn Dent Sci Rev.* (2017) 53:117–24. doi: 10.1016/j.jdsr.2017.01.003

60. Minenko A, Kiselev G, Tulkova E, Oehme P. Nicotinic stimulation of polyphophionositide turnover in rat adrenal medulla slices. *Pharmazie.* (1987) 42:341–3.

61. Roske I. Influence of adrenal demandullation on stress-related behaviour in wister rats. *Pharmazie.* (1987) 42:253–5.

62. Henri J, Minder L, Mohanasundaram K, Dilly S, Goupil-Lamy A, Di Primo C, et al. Neuropeptides, new ligands of SARS-CoV-2 nucleoprotein, a potential link between replication, inflammation and neurotransmission. *Molecules.* (2022) 27:8094. doi: 10.3390/molecules27228094

63. Lin JE, Asfour A, Sewell TB, Hooe B, Pryce P, Earley C, et al. Neurological issues in children with COVID-19. *Neurosci Lett.* (2021) 743:135567. doi: 10.1016/j.neulet.2020.135567

64. Mahase E. Long COVID could be four different syndromes, review suggests. *Br Med J.* (2020) 371:3981. doi: 10.1136/bmj.m3981

65. Fernández-de-Las-Peñas C, Palacios-Ceña D, Gómez-Mayordomo V, Cuadrado ML, Florencio LL. Defining post-COVID symptoms (post-acute COVID, long COVID, persistent post-COVID): an integrative classification. *Int J Environ Res Public Health.* (2021) 18:2621. doi: 10.3390/ijerph18052621

66. Finsterer J, Scorza FA. Clinical and pathophysiologic spectrum of neuro-COVID. *Mol Neurobiol.* (2021) 58:3787–91. doi: 10.1007/s12035-021-02383-0

67. Schirinzi T, Lattanzi R, Maftei D, Grillo P, Zenuni H, Boffa L, et al. Substance P and Prokineticin-2 are overexpressed in olfactory neurons and play differential roles in persons with persistent post-COVID-19 olfactory dysfunction. *Brain Behav Immun.* (2023) 108:302–8. doi: 10.1016/j.bbi.2022.12.017

68. Aghagoli G, Gallo Marin B, Katchur NJ, Chaves-Sell F, Asaad WF, Murphy SA. Neurological involvement in COVID-19 and potential mechanisms: a review. *Neurocrit Care.* (2021) 34:1062–71. doi: 10.1007/s12028-020-01049-4

69. Mahalakshmi AM, Ray B, Tuladhar S, Bhat A, Paneyala S, Patteswari D, et al. Does COVID-19 contribute to development of neurological disease? *Immun Inflamm Dis.* (2021) 9:48–58. doi: 10.1002/iid3.387

70. Bratosiewicz-Wasik J. Neuro-COVID-19: an insidious virus in action. *Neurol Neurochir Pol.* (2022) 56:48–60. doi: 10.5603/PJNNS.a2021.0072

71. Bobker SM, Robbins MS. COVID-19 and headache: a primer for trainees. *Headache J Head Face Pain.* (2020) 60:1806–11. doi: 10.1111/head.13884

72. Mehboob R, Kurdi M, Bamaga A, Aldardeir N, Nasief H, Moshref LH, et al. Substance P/neurokinin-1 receptor, trigeminal ganglion, latency, and coronavirus infection-is there any link? *Front Med.* (2021) 8:727593. doi: 10.3389/fmed.2021.727593

73. Labisch A. *Die bakteriologische und die molekulare Transition der Medizin—Historizität und Kontinenz als Erkennungsmittel. In: Labisch A, editor. Historizität Erfahrung und Handeln—Geschichte und Medizin, Hsg*. Stuttgart: Franz Steiner Verlag (2004). p. 214–8.

74. Larsson O, Tengroth L, Xu Y, Uddman R, Georén SK, Cardell LO. Substance P represents a novel first-line defense mechanism in the nose. *J Allergy Clin Immunol.* (2018) 141:128–36.e3. doi: 10.1016/j.jaci.2017.01.021

75. Lindner G, Jentzsch KD, Oehme P, Wenzel M. Wirkung von Derivaten des Substanz P-Dipeptids3-4 auf das Nervenfaserwachstum in der Gewebekultur. *J Hirnforsch.* (1986) 27:639–49.

76. Mehboob R, Ahmad FJ, Qayyum A, Rana MA, Gilani SA, Tariq MA, et al. *Aprepitant as a Combinant with Dexamethasone Reduces the Inflammation Via Neurokinin 1 Receptor Antagonism in Severe to Critical COVID-19 Patients and Potentiates Respiratory Recovery: A Novel Therapeutic Approach*. Lahore: Bahria International Hospital (2020). doi: 10.1101/2020.08.01.20166678

77. Shivakumar P, Gupta MS, Jayakumar R, Gowda DV. *Preparation for Promoting Wound Healing and Preparation Method Thereof* (1984).

78. Egieyeh S, Egieyeh E, Malan S, Christofells A, Fielding B. Computational drug repurposing strategy predicted peptide-based drugs that can potentially inhibit the interaction of SARS-CoV-2 spike protein with its target (humanACE2). *PLoS ONE.* (2021) 16:e0245258. doi: 10.1371/journal.pone.0245258

79. Ibrahim MA, Preuss CV. *Antiemetic Neurokinin-1 Receptor Blockers, in StatPearls*. Florida: Treasure Island (2022).

80. Quartara L, Altamura M. Tachykinin receptors antagonists: from research to clinic. *Curr Drug Targets.* (2006) 7:975–92. doi: 10.2174/138945006778019381

81. Smith J, Allman D, Badri H, Miller R, Morris J, Satia I, et al. The neurokinin-1 receptor antagonist orvepitant is a novel antitussive therapy for chronic refractory cough: results from a phase 2 pilot study (VOLCANO-1). *Chest.* (2020) 157:111–8. doi: 10.1016/j.chest.2019.08.001

82. Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in cancer progression. *Peptides.* (2013) 48:1–9. doi: 10.1016/j.peptides.2013.07.024

83. Robinson P, Taffet GE, Engineer N, Khumbatta M, Firozgary B, Reynolds C, et al. Substance P receptor antagonism: a potential novel treatment option for viral-myocarditis. *Biomed Res Int.* (2015) 2015:645153. doi: 10.1155/2015/645153

84. Shoenfeld Y. Corona (COVID-19) time musings: our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning. *Autoimmun Rev.* (2020) 19:102538. doi: 10.1016/j.autrev.2020.102538

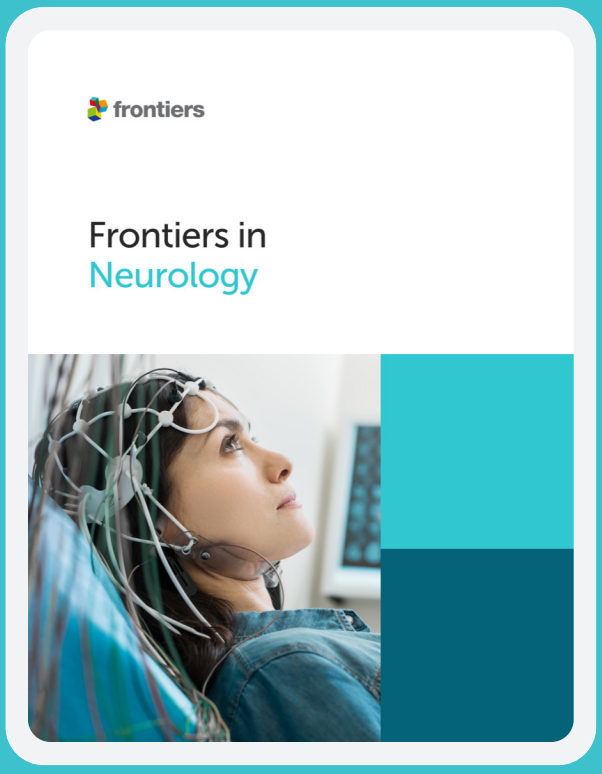
Frontiers in Neurology

Explores neurological illness to improve patient care

The third most-cited clinical neurology journal explores the diagnosis, causes, treatment, and public health aspects of neurological illnesses. Its ultimate aim is to inform improvements in patient care.

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