COVID-19 IN CNS AND PNS: BASIC AND CLINICAL FOCUS ON THE MECHANISMS OF INFECTION AND NEW TOOLS FOR THE THERAPEUTIC APPROACH

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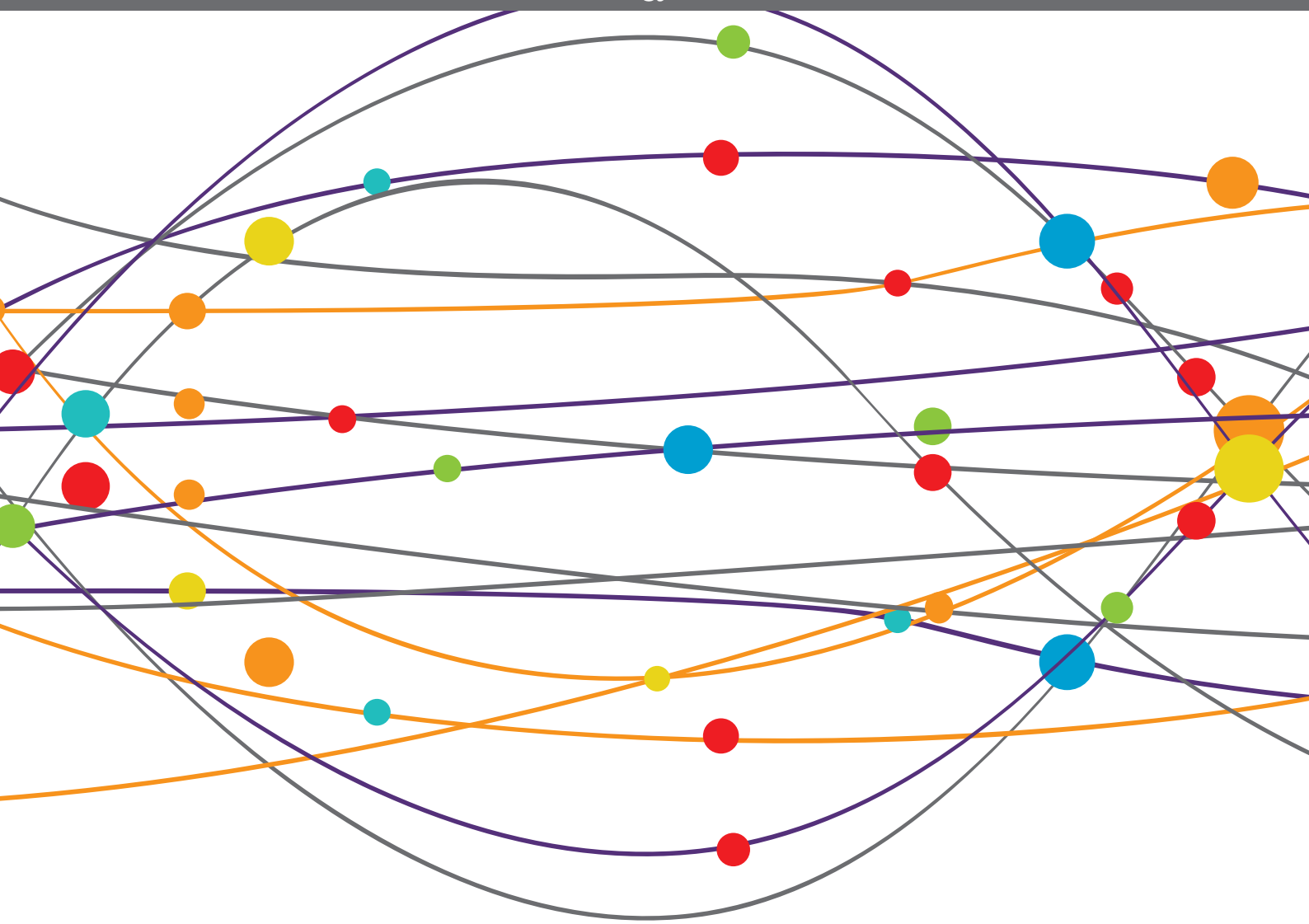

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Table of Contents

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EDITORIAL
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Editorial: COVID-19 in CNS and PNS: Basic and Clinical Focus on the Mechanisms of Infection and New Tools for the Therapeutic Approach

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Keywords: COVID 19, central nervous system, SARS-CoV2, neurological diseases, persistent COVID

Editorial on the Research Topic

COVID-19 in CNS and PNS: Basic and Clinical Focus on the Mechanisms of Infection and New Tools for the Therapeutic Approach

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The COVID-19 pandemic has had significant implications not only for health but also for lifestyles and for organizations both in the healthcare sector and in other spheres. Before December 2019, other coronaviruses had been described that had presented endemically and in some cases caused epidemics, but none spread to such an extent globally; the COVID-19 pandemic is reminiscent of other historic epidemics such as the influenza epidemic of the last century, whose sequelae, including neurological symptoms, were felt for decades (1). Of the other coronaviruses (CoV) affecting humans, 2 are classified as αCoV (HCoV-229E and HKU-NL63) and 4 as βCoV (HCoV-OC43, HCoV-HKU1, SARS-CoV, and MERS-CoV). SARS-CoV and MERS-CoV caused severe infections both in healthy individuals and in people with immune deficiencies, and were associated with high mortality rates, whereas the others are associated with seasonal outbreaks of flu-like symptoms with relatively limited capacity for transmission. However, all of these viruses have either been associated with neurological symptoms or been detected in biological samples taken from the central nervous system (CNS) (2, 3). On 31 December 2019, the World Health Organization reported a novel CoV (SARS-CoV-2) in patients with pneumonia in the city of Wuhan, in the Chinese province of Hubei, which spread rapidly through the rest of the world. The novel virus is a βCoV and bears considerable similarity to SARS-CoV. The main structural differences between SARS-CoV and SARS-CoV-2 are observed in the fusion protein and in accessory proteins, particularly ORF3b and ORF8. As is the case with SARS-CoV, subunit 1 of the novel coronavirus fusion protein also binds to the ACE2 receptor, hence the name SARS-CoV-2 (4). It has been suggested since early in the pandemic that the virus may have direct and indirect effects on the CNS (5), with many researchers and authors beginning to analyze a pandemic with worldwide effects; an enormous amount of information has been generated, largely due to the scientific community's desire for progress. However, much of this information is from case reports, anecdotal series, and many speculative articles based more on opinion than experimentation (6). Despite the great efforts

to generate evidence to enable advances in our understanding of the virus and associated disease, much speculation remains today with regard to the evidence. Such aspects as its epidemiology, transmission factors, and the impact of mass vaccination are conditioned by data from studies that present considerable bias (7) and are not evidence-based.

Since the beginning of the pandemic, there has been debate as to whether SARS-CoV-2 is able to enter the CNS and cause neurological symptoms, trigger or promote pre-existing neurological disease, or remain latent in the brain, making the organ a viral reservoir (5). The study of the potential routes by which the virus enters the CNS (i.e., the hematogenous route or the neuronal route), facilitating this penetration, also constitute an interesting area of research (8, 9) analyzing whether the virus accesses the CNS by infecting endothelial or epithelial cells of the blood-brain barrier; by dissemination from nearby areas; or by axonal transport after infecting neurons in the peripheral nervous system. Another subject of debate is the frequency of neurological symptoms associated with the acute infection (10, 11) and their association with the CNS (12), and associated neurological disorders such as stroke (13, 14) and neuromuscular involvement (15). Furthermore, there is an interesting debate as to whether the virus can trigger neurological disease, for example neurodegenerative disease, in the long term (it has been suggested that the infection may increase the risk of Parkinson's disease or Alzheimer's disease), and by which mechanisms (16, 17), and whether it may facilitate age-related transcriptome or molecular changes (18). Finally, there is controversy regarding the so-called persistent COVID-19 or post–COVID-19 syndrome, referring to symptoms persisting for 3 months after the acute infection, with particular emphasis on cognitive symptoms (19). A recent Delphi consensus study conducted by the World Health Organization establishes 3 major criteria for diagnosing post–COVID-19 condition, 2 of which are based on the presence of neurological symptoms: fatigue and cognitive alterations (20).

The special issue "COVID-19 in CNS and PNS: Basic and Clinical Focus on the Mechanism of Infection and New Tools for the Therapeutic Approach" includes valuable articles addressing these debates, contributing evidence to expand our understanding of the disease and its impact on the CNS.

CONTRIBUTIONS ON NEUROLOGICAL SYMPTOMS DURING THE ACUTE PHASE

Several articles have contributed new information on neurological manifestations of COVID-19. For instance, Yan et al. analyze neurological symptoms in a retrospective series of 1,682 patients from Wuhan, with 30.3% presenting neurological symptoms (12.8% with headache). Tsai et al. reviewed 79 studies, selecting 63 for inclusion in a meta-analysis. These researchers report olfactory alterations in 35% of patients, headache in 10.7%, and stroke in 8.1%. Kushwaha et al. conducted a cross-sectional study analyzing data from 358 patients with neurological symptoms, 69 of whom had suspected SARS-CoV-2 infection, and evaluate the impact of the infection at a center primarily attending neurological patients. Pinzon et al. reviewed 280 studies, selecting 33 for meta-analysis, and report neurological alterations such as myalgia in 19.2% of patients, headache in 10.9%, and stroke in 4.4%; they also review the frequency and characteristics of neurological symptoms during the pandemic. Fiani et al. present an extensive review addressing the impact of neurological manifestations and the underlying mechanisms. The article by Gori et al. specifically studies the frequency and pathogenesis of anosmia, taking a very broad approach, analyzing the possible pathogenic mechanisms and addressing the current controversies; Mathew also contributes an interesting article on anosmia. Hwang et al. analyzed the presence of seizures during SARS-Cov-2 infection in 4 of their own patients and review the literature on the subject, whereas Waters et al. describe the incidence of electroencephalographic seizures. Zito et al. analyzed Guillain-Barré syndrome in patients with COVID-19 in a case report and meta-analysis of 29 articles. Jungbauer et al. analyze vocal cord palsy associated with SARS-CoV-2 infection. The study by Varela Rodríguez et al. addresses neuropsychiatric symptoms in patients with COVID-19 and history of alcohol abuse. Román et al. present a comprehensive clinical review of transverse myelitis associated with COVID-19 and vaccination against the disease, a highly informative contribution. Severa et al. discuss treatment with interferon beta in the context of COVID-19. Together, these articles offer a panoramic view of the neurological symptoms associated with COVID-19.

CONTRIBUTIONS ON THE MECHANISM OF CENTRAL NERVOUS SYSTEM INVASION

This special issue includes several key articles. Huang et al. review the association between biological and molecular factors associated with the viral infection and their potential effects on the CNS. They propose the murine hepatitis virus as a model for studying the role of coronaviruses in the CNS; Sanclemente-Alaman et al. also highlight this model in their review of experimental models of SARS-CoV-2. Wang et al. review the action mechanisms of viruses affecting the CNS in an article on neurological symptoms of COVID-19 and offering an overview of the clinical manifestation and infection mechanisms. Reza-Zaldívar et al. analyze specific mechanisms associated with SARS-CoV-2. Gomes de Assis et al. address the underlying mechanisms of neurological symptoms associated with COVID-19 after reviewing 484 articles according to a pre-established methodology addressing different fields, such as host factors, immune mechanisms, and virology. This comprehensive study presents both experimental and clinical data. Guadarrama-Ortiz et al. conducted an exhaustive review of neurological manifestations, viral entry routes, and potential immune and virological mechanisms, discussing a broad range of potential neurological alterations affecting both the central and the peripheral nervous systems. This interesting review presents an overview of the relationship between SARS-CoV-2 and neurological symptoms. The virus is very difficult to detect or undetectable in the cerebrospinal fluid of patients

with SARS-CoV-2 infection, even in those with encephalitis, according to the transcriptome analysis of cerebrospinal fluid samples by Placantonakis et al.; Pacheco-Herrero et al. studied neuropathological aspects of the infection.

CONTRIBUTIONS ON HOST FACTORS AND THE RISK OF NEUROLOGICAL DISEASE

Bhaskar et al. comprehensively review the immune response associated with SARS-CoV-2 infection, placing special emphasis on the cytokine storm, as well as immunosenescence, and the role of these mechanisms in complications. Severa et al. analyzed the possible relationship between the use of interferon beta and the risk of infection, as well as the drug's role in the clinical course of the disease, considering its potential therapeutic role in COVID-19, as occurred in previous coronavirus epidemics.

CONTRIBUTIONS ON NEUROLOGICAL SEQUELAE AFTER ACUTE SARS-COV-2 INFECTION AND PERSISTENT COVID SYNDROME

D'Arcy et al. discuss the long-term consequences of the infection. Fiani et al. analyzed persistent neurological symptoms after the infection, calling attention to the need for guidelines addressing sequelae of stroke, intracranial infections, and muscle damage, as well as nutrition-related issues.

MOVING TOWARD A BETTER UNDERSTANDING OF THE EFFECTS OF SARS-COV-2 ON THE CNS AND THEIR LONG-TERM IMPLICATIONS

It remains to be determined whether the virus or its RNA can remain within structures of the CNS or whether it may remain latent or cause disease in the long term; therefore, there is a clear need for necropsy studies (21, 22). It also seems important to identify any areas of the CNS that may present greater vulnerability to infection (though the hippocampus and basal ganglia have been suggested), whether some CNS cells present greater susceptibility to viral structures, and whether the virus or its RNA may be transported by such cell substructures as vesicles or exosomes. It is also unclear whether existing lesions, such as demyelinating plaques in multiple sclerosis, may facilitate the entry of the virus and serve as viral reservoirs. The impact of different types of vaccine on viral invasion of the CNS is another area where further study is needed. The effects of SARS-CoV-2 on the brain constitute a new challenge for neuroscientific research (23), in which we may only advance with the greatest possible quantity of information, like that included in this special issue.

AUTHOR CONTRIBUTIONS

JM-G, UG-P, JAM-G, CG, GP, PR, and AB concept, evaluation, writing, and revision. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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The Neurologic Manifestations of Coronavirus Disease 2019 Pandemic: A Systemic Review

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Objective: Review and integrate the neurologic manifestations of the Coronavirus Disease 2019 (COVID-19) pandemic, to aid medical practitioners who are combating the newly derived infectious disease.

Methods: We reviewed the clinical research, consisting of mainly case series, on reported neurologic manifestations of COVID-19. We also reviewed basic studies to understand the mechanism of these neurologic symptoms and signs.

Results: We included 79 studies for qualitative synthesis and 63 studies for meta-analysis. The reported neurologic manifestations were olfactory/taste disorders (35.6%), myalgia (18.5%), headache (10.7%), acute cerebral vascular disease (8.1%), dizziness (7.9%), altered mental status (7.8%), seizure (1.5%), encephalitis, neuralgia, ataxia, Guillain-Barre syndrome, Miller Fisher syndrome, intracerebral hemorrhage, polyneuritis cranialis, and dystonic posture.

Conclusions: Neurologic manifestations in COVID-19 may alert physicians and medical practitioners to rule in high-risk patients. The increasing incidence of olfactory/taste disorders, myalgia, headache, and acute cerebral vascular disease renders a possibility that COVID-19 could attack the nervous system. The cytokine secretion and bloodstream circulation (viremia) are among the most possible routes into the nervous system.

Keywords: COVID-19, pandemic, neurologic, headache, taste, olfactory, ACE2, cytokine

INTRODUCTION

COVID-19 first occurred in late 2019 in Wuhan, China (1). As of May 01, 2020, the COVID-19 pandemic had infected 3,291,008 worldwide and caused 232,478 deaths (data from the World Health Organization). The most common clinical symptoms are cough, sputum production, fatigue, shortness of breath, and mainly respiratory tract symptoms. However, an increasing number of cases have presented with neurologic manifestations, such as olfactory and taste disorders (2), and the phenomenon requires further attention.

COVID-19 is a new RNA virus strain from the family Coronaviridae (including the Middle East respiratory syndrome CoV [MERS-CoV] and severe acute respiratory syndrome CoV [SARS-CoV]). Phylogenetic analysis of the complete viral genome revealed that the virus was most closely related (89.1% nucleotide similarity) to a group of SARS-like coronaviruses (3). As such, it

was previously termed SARS-CoV-2. In the review article published in 2018 (4), researchers found that the human coronavirus can enter the central nervous system through the olfactory bulb, causing demyelination and inflammation (cultured glial cells have been described to secrete cytokines including IL-6, IL-12p40, IL-15, TNF-a, CXCL9, and CXCL10 upon viral infection). The authors of a recent article (5) investigated the mechanism of COVID-19 nervous system involvement, and they stated that similar to SARS-CoV, the COVID-19 virus exploits the angiotensin-converting enzyme 2 (ACE2) receptor to gain entry inside the cells. The brain has been reported to express ACE2 receptors that have been detected over glial cells and neurons, which makes them a potential target of COVID-19. Recently, the research team in Harvard Medical School identified three main cells co-expressing ACE2 and TMPRSS2 (Type II transmembrane serine protease): lung type II pneumocytes, ileal absorptive enterocytes, and nasal goblet secretory cells (6). And the other research team used single-cell RNA-Seq datasets to suggest possible mechanisms through which CoV-2 infection could lead to anosmia or other forms of olfactory dysfunction (7).

METHODS

We searched the MEDLINE, CENTRAL, and EMBASE databases for eligible publications from December 2019 to April 30, 2020 written in English, using the following keywords: COVID-19, SARS-CoV-2, neuro, clinical, characteristics, manifestations. We also checked the reference lists of relevant studies to identify any missing publications. We reviewed the clinical researches, including case series and case reports, for neurologic manifestations of COVID-19 and organized them into tables. A confirmed case of COVID-19 (SARS-CoV-2) was defined and mostly diagnosed using the triple algorithm (epidemiological history, clinical symptoms, and laboratory or radiological findings) as a standard procedure proposed by the World Health Organization. We also reported data from the Taiwan Centers for Disease Control until May 01, 2020. Then we did the meta-analysis of all the case series to pool the data together and make it easier to understand. We used the software of Comprehensive Meta-Analysis Software (CMA), version 3, and chose the model of one group event rate, random effect, to draw the Forest Plot (**Supplementary Figures 2–8**).

RESULTS

We used Preferred Reporting Items for Systematic reviews and Meta-Analyzes (PRISMA) guidelines for searching and listed our flowchart (**Supplementary Figure 1**). Then we made a list of the neurologic manifestations in the current COVID-19 pandemic (**Table 1**). We included 9 case series and 4 case reports of olfactory or taste disorders. We pooled the case series together and found around 35.6% of patients got these symptoms (**Supplementary Figure 2**). We included 43 studies of myalgia, about 18.5% of patients had this symptom (**Supplementary Table 1** and **Supplementary Figure 3**). And 45 studies of headache, the percentage was 10.7% (**Supplementary Table 2** and **Supplementary Figure 4**); 2 studies of acute cerebral vascular disease, the percentage was 8.1% (**Supplementary Figure 5**); 7 studies of dizziness, the percentage was 7.9% (**Supplementary Figure 6**); 4 case series and 2 case reports of altered mental status, the percentage was 7.8% (**Supplementary Figure 7**); and 2 studies of seizure, the percentage was 1.5% (**Supplementary Figure 8**). And still other case reports of encephalitis, neuralgia, ataxia, Guillain-Barre syndrome, Miller Fisher syndrome, intracerebral hemorrhage, polyneuritis cranialis, and dystonic posture.

In addition, we reviewed some basic studies (4, 5, 44, 45) to determine the mechanism of these neurologic symptoms and signs. The cartoon figure summarized the possible mechanism (**Figure 1**).

DISCUSSION

The COVID-19 pandemic is currently progressing, and neurologists and medical practitioners worldwide will face additional challenges from the neurologic complications of the disease (46). An updated review focusing on the neurologic features may help clinicians early identify potential patients.

Interestingly, previous coronavirus infections, including MERS and SARS, did not have a large proportion of patients with olfactory and taste disorders (47). However, patients with COVID-19 frequently complain of abnormalities in smell and taste. In our analysis of data from Taiwan (9), we found that between January 21 and March 24, 2020, a total of 216 patients were confirmed to have COVID-19 infection, and 5 of them (2.3%) had olfactory or taste disorders. Between March 25 and May 01, 48 cases in 213 patients (22.5%) had olfactory or taste disorders. In the beginning, most COVID-19 patients had a contact history related to Wuhan. But after the government of China locked down many big cities, Taiwan's COVID-19 cases mostly originated from travelers from Europe, the Middle East, or the United States. Besides, according to 88 cases series (see **Supplementary Tables 1**, **2**) in China (from December 2019 to April 25, 2020), only one study (2) conducted by neurologists in Wuhan reported olfactory or taste disorder.

On the other hand, an Italian researcher reported that 33.9% of COVID-19 patients in Italy experienced this problem (8). In the Middle East, researchers in Iran found a surge in the outbreak of olfactory dysfunction during the COVID-19 epidemic (based on an online checklist of 10,069 voluntary cases between March 12 and 17, 2020) (48). The different incidence of the olfactory and/or taste dysfunction by the timing and geographic distribution might reveal important information that the virus may carry the potential to alter its affinity to the central nervous system (49–51). However, the possibilities of a higher detection rate of olfactory dysfunction in patients diagnosed by certain sub-specialists, such as neurologists (2) or otolaryngologists, cannot be completely excluded. For example, the study conducted by otolaryngologists (17) found olfactory/taste disorders in more than 80% of the patients.

TABLE 1 | List of the neurologic manifestations in the current COVID-19 pandemic.

Neurologic manifestation	Patient numbers (% in total participants)	Total participants	Age: mean [SD] or median [IQR]	Published journal [reference]
With olfactory or/and taste disorders	20 (33.9%)	59	60 [50–74]	Clin Infect Dis (8)
	53 (12.4%)	429	32 [4–88]	Taiwan CDC (9)
	128 (75.7)	169	43 [34–54]	Int Forum Allergy Rhinol (10)
	25 (20%)	126	43.5 [3–87]	Trav Med Infect Dis (11)
	62 (19.4%)	320	No data	Laryngoscope (12)
	31 (39.2%)	79	61.6 [17.4]	Eur J Neurol (13)
	130 (64.4%)	202	56 [45–67]	JAMA (14)
	1	Case report	80	Eur J Case Rep Intern Med (15)
	1	Case report	50	Neurology (16)
Olfactory disorder only	3 (5.1%)	59	60 [50–74]	Clin Infect Dis (8)
	11 (5.1%)	214	52.7 [15.5]	JAMA Neurol (2)
	357 (85.6%)	417	36.9 [11.4]	EUR ARCH OTO-RHINO-L (17)
	1	Case report	85	Eur J Case Rep Intern Med (15)
Taste disorder only	5 (8.5%)	59	60 [50–74]	Clin Infect Dis (8)
	12 (5.6%)	214	52.7 [15.5]	JAMA Neurol (2)
	342 (82%)	417	36.9 [11.4]	EUR ARCH OTO-RHINO-L (17)
	1	Case report	39	Neurology (16)
Dizziness	1 (12.5%)	8	48.1 [13–76]	Clin Infect Dis (18)
	13 (9.4%)	138	56 [42–68]	JAMA (19)
	37 (8.1%)	452	58 [47–67]	Clin Infect Dis (20)
	21 (8%)	274	62 [44–70]	BMJ (21)
	5 (7%)	69	42 [35–62]	Clin Infect Dis (22)
	1 (4.17%)	24	32.5 [5–95]	Sci China Life Sci (23)
	2 (2%)	81	49.5 [11]	Lancet (24)
Altered mental status	9 (52.9%)	17	86.5 [68.6–97.]	J Infect (25)
	9 (9%)	99	55.5 [21–88]	Lancet (26)
	1 (5.9%)	17	75 [48–89]	J Med Virol (27)
	3 (0.7%)	452	58 [47–67]	Clin Infect Dis (20)
	1	Case report	No data	Radiology (28)
	1	Case report	74	J Med Virol (29)
Seizure	1 (4.8%)	21	70 [43–92]	JAMA (30)
	1 (0.5%)	214	52.7 [15.5]	JAMA Neurol (2)
Acute cerebrovascular disease	3 (23%)	13	63	N Engl J Med (31)
	6 (2.8)	214	52.7 [15.5]	JAMA Neurol (2)
	5	No data	40.4 [5.6]	N Engl J Med (32)
Neuralgia	5 (2.3%)	214	52.7 [15.5]	JAMA Neurol (2)
Ataxia	1 (0.5%)	214	52.7 [15.5]	JAMA Neurol (2)
Guillain-Barre syndrome	5 (0.4%)	1,000–1,200	No data	N Engl J Med (33)
	1	Case report	61	Lancet Neurol (34)
	1	Case report	65	J Clin Neurosci (35)
	1	Case report	71	Neurol Neuroimmunol Neuroinflamm (36)
Encephalitis	1	Case report	24	Int J Infect Dis (37)
	1	Case report	56	Travel Med Infect Dis (38)
	1	Case report	74	Cureus (39)
	1	Case report	No data	Brain Behav Immun (40)
	1	Case report	41	Brain Behav Immun (41)
Intracerebral hemorrhage	1	Case report	79	New Microbes New Infect (42)
Miller Fisher Syndrome	1	Case report	50	Neurology (16)
Polyneuritis cranialis	1	Case report	39	Neurology (16)
Sustained upward gaze, dystonic bilateral leg extension and altered responsiveness	1	Case report	6 week	Neurology (43)

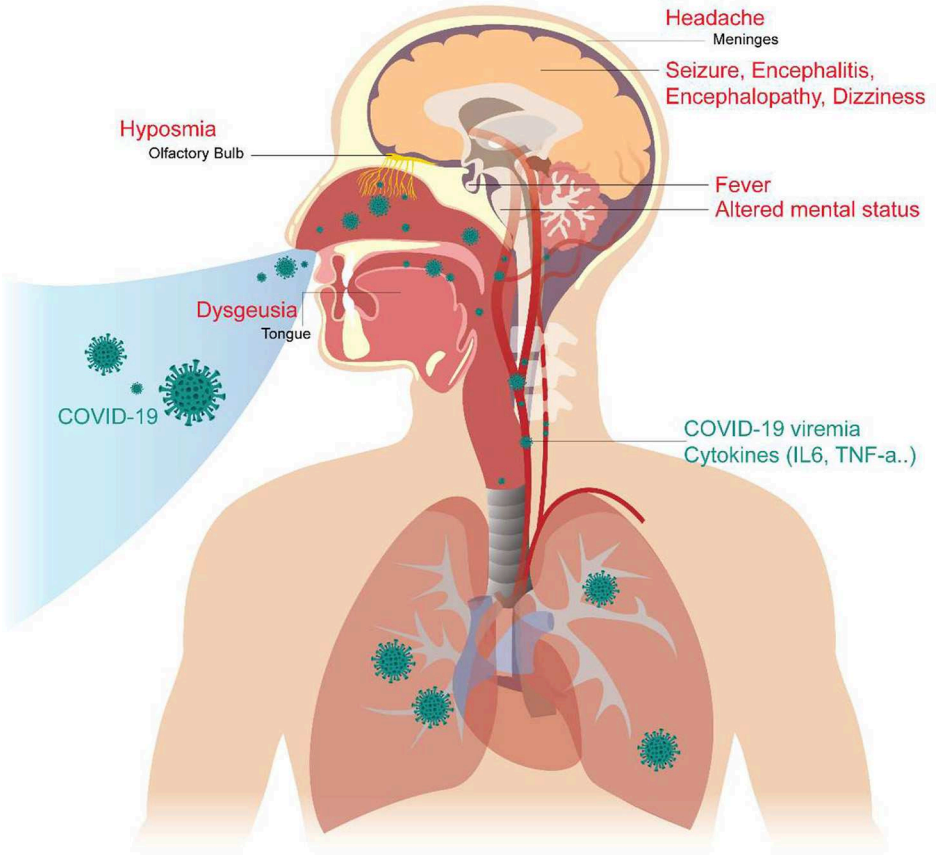

FIGURE 1 | Neurological Manifestations of COVID-19 and the proposed mechanism. The COVID-19 virus may cause neurologic manifestations by cytokines secretion, general circulation (viremia), or direct invasion via the numerous ACE2 receptors in the olfactory epithelium. The olfactory disorder may cause by the olfactory epithelium damage. Fever was believed to be caused by the effect of cytokines or hypothalamus functional pertubation. The seizure may cause by cytokines storm, severely illed condition, or the brain parenchyma involvement, especially the mesial temporal lobe. Altered mental status may be a consequence of multiple organ failure, severe infection, or brainstem involvement. Headache is caused by meningeal irritation.

Fever is generally known as an elevation in body temperature caused by a cytokine-induced upward displacement of the set point of the hypothalamic thermoregulatory center. Small elevations in body temperature appear to enhance immune function and inhibit pathogen growth (52). In 2005, pathologists in Beijing performed autopsies of SARS patients and found signals of the SARS viral genome detected in numerous neurons in the hypothalamus (53). As a result, it is conceivable that fever may be caused mainly by the effect of cytokines or possible direct viral invasion to the hypothalamus.

Concerning seizure in viral infection, generally the paroxysmal spell may be a consequence of multiple complications of systemic disease, such as metabolic disturbances, hypoxia, etc. Considering the viral encephalitis, it frequently manifests with seizures in its acute phase (54). The most widely reported virus was HSV-1 (herpes simplex virus), which involves the highly epileptogenic mesial temporal lobe structures, including the hippocampus (54). In the two case reports (37, 39), both had mesial temporal lobe involvement (one by acute inflammation, one by previous ischemic stroke). Since the case number is limited, we can only speculate that seizures may be caused by the generalized poor condition, cytokine storm (55), or mesial temporal lobe involvement in severe COVID-19 patients.

Several countries are currently encountering a crisis of ventilator shortage. The respiratory failure of COVID-19 infected patients may be partly related to brainstem failure. The COVID-19 virus passes into the cell via the ACE2 receptor (5). ACE2 is expressed in the brain and is mainly found in the brainstem, specifically in the nuclei associated with cardio-respiratory control (56, 57). In the previous research on SARS-CoV-1 and MERS-COV, the brainstem was severely infected, which possibly contributes to the degradation and failure of respiratory centers (45). Besides, the ascending reticular activating system (ARAS), which is responsible for human consciousness, also originates from the brainstem (and then advances into the thalamus and cortex) (58). This may partly explain the altered mental status of COVID-19 patients. However, the maintenance of consciousness is complex. Considering many COVID-19 patients were severely ill with multi-organ failure, both the cytokine effect and systemic impact of organ dysfunction can also lead to the consciousness disturbance.

Both dizziness and headache are considered to be general non-specific symptoms. Etiologies attributed to infectious causes are important secondary causes of headache (59). It is known that cytokines induced by viral infection increase the permeability of vessels. This causes cerebral swelling and meningeal irritation. The meningeal irritation stimulates the trigeminal nerve terminals and triggers pain sensation (60).

Ischemic stroke also occurs in COVID-19 patients because the infection may cause D-dimer elevation, thrombocytopenia, and hypercoagulable state (61–66). Besides, the exaggerated systemic inflammation or a "cytokine storm" (55), cardioembolism from virus-related cardiac injury (67) could further increase the risk of stroke (68).

Most cases of Guillain-Barre syndrome appeared with a lag time from the primary infection of COVID-19 (33, 34); the pathogenesis is therefore likely to be postinfectious immune-mediated.

This review is obviously constrained by the current information and limited reports. And there was considerable heterogeneity in the data. In addition, the researches of the novel pandemic emerge fastly. We could only review the results up to April 30, 2020 in this regard. The cause of neurologic manifestation may be a cytokine storm, multiple organ failure, or direct viral infection. However, the detailed pathophysiology of causing COVID-19 nervous system involvement remains to be elucidated. We sincerely hope the review can help the first line clinicians identify the emerging neurologic manifestations when combating the viral pandemic.

AUTHOR CONTRIBUTIONS

S-TT and M-KL did the literature search and drafted this manuscript. C-HT initiated this review and integrated the clinical and basic research. SS did the meta-analysis and made all the Forest Plot figures.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00498/full#supplementary-material

Supplementary Figure 1 | Preferred Reporting Items for Systematic reviews and Meta-Analyzes (PRISMA), our searching strategy.

Supplementary Figure 1 | Forest plot for olfactory/taste disorder.

Supplementary Figure 3 | Forest plot for myalgia.

Supplementary Figure 4 | Forest plot for headache.

Supplementary Figure 5 | Forest plot for acute cerebral vascular disease.

Supplementary Figure 6 | Forest plot for dizziness.

Supplementary Figure 7 | Forest plot for altered mental status.

Supplementary Figure 8 | Forest plot for seizure.

Supplementary Table 1 | Patients presented with myalgia.

Supplementary Table 2 | Patients presented with headache (ordered by cases recruitment date).

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frontiers in Neurology

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Neurologic Characteristics in Coronavirus Disease 2019 (COVID-19): A Systematic Review and Meta-Analysis

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Importance: Coronavirus disease 2019 (COVID-19) is a newly emerging infectious disease that has caused a global pandemic. The presenting symptoms are mainly respiratory symptom, yet studies have reported nervous system involvement in the disease. A systematic review and meta-analysis of these studies are required to understanding the neurologic characteristic of the disease and help physicians with early diagnosis and management.

Objective: To conduct a systematic review and meta-analysis on the neurologic characteristics in patients with COVID-19.

Evidence Review: Authors conducted a literature search through PubMed from January 1st, 2020 to April 8th, 2020. Furthermore, the authors added additional sources by reviewing related references. Studies presenting the neurologic features of COVID-19 patients in their data were included. Case reports and case series were also included in this review. The quality of the studies was assessed based on the Oxford Center for Evidence-Based Medicine guidelines. Selected studies were included in the meta-analysis of proportion and the heterogeneity test.

Finding: From 280 identified studies, 33 were eligible, with 7,559 participants included. Most of the included studies were from China (29 [88%]). Muscle injury or myalgia was the most common (19.2%, 95%CI 15.4–23.2%) neurologic symptom of COVID-19, followed by headache (10.9%, 95%CI 8.62–13.51%); dizziness (8.7%, 95%CI 5.02–13.43%); nausea with or without vomiting (4.6%, 95%CI 3.17–6.27%); concurrent cerebrovascular disease (4.4%, 95%CI 1.92–7.91%); and impaired consciousness (3.8%, 95%CI 0.16–12.04%). Underlying cerebrovascular disease was found in 8.5% (95%CI 4.5–13.5%) of the studies.

Conclusion: Neurologic findings vary from non-specific to specific symptoms in COVID-19 patients. Some severe symptoms or diseases can present in the later stage of the disease. Physicians should be aware of the presence of neurologic signs and symptoms as a chief complaint of COVID-19, in order to improve management and prevent a worsening outcome of the patients.

Keywords: COVID-19, symptoms, characteristics, neurologic, review, meta-analysis

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INTRODUCTION

In December 2019, three patients with pneumonia were observed and linked to the outbreak of respiratory infection cases detected from Wuhan, China. Later on, the cause of pneumonia was found to be a viral infection known as novel coronavirus disease (COVID-19). In March 2020, the World Health Organization (WHO) declared COVID-19 as an emerging infectious disease caused by the virus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and declared a global pandemic. As of April 7, 2020, globally reported cases are 1.279.722 confirmed cases with more than 70.000 deaths (1, 2).

The disease's main presentations are usually similar to symptoms of upper respiratory tract infection such as fever, dry cough, and myalgia or malaise. In severe cases, manifestations of pneumonia such as shortness of breath (dyspnea), abnormal lung imaging findings, and acute respiratory distress syndrome (ARDS) can be found (3). Multiple studies have also reported the nervous system involvement in the disease. A retrospective study in China found that over 36.4% of hospitalized patients had neurologic symptoms and that these commonly present in severe patients (4, 5).

Studies also suggested that physicians should be aware of the other system involvement, including neurological events, to reduce mortality and morbidity rate in affected individuals. This review aims to provide a systematic report of the neurologic characteristics in patients with COVID-19 based on the latest reported studies.

METHODS

Literature Searching

We performed a systematic literature review, followed by meta-analysis, of the available studies from one scientific database (PubMed), published from January 1st, 2020, to April 8th, 2020, using Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) reporting guideline. The following inclusion criteria were: (1) original studies (e.g., randomized controlled trial studies, cohort studies, case-control studies, cross-sectional studies, case reports, and case-series) on patients with COVID-19; (2) Studies with a focus on clinical manifestations or symptoms in patients with COVID-19; and (3) The literature was restricted to English language articles only. We excluded the following studies: non-original articles, such as review articles including meta-analyses, letters, comments, or consensus documents (6).

We included clinical characteristics studies as long as they contained neurologic data of COVID-19. As the disease is an urgent topic and a newly emerging disease, we also included case reports or case series in the review.

Two co-authors (V.O.W and A.A) independently screened the titles in each study from the search results for eligibility. Each of the abstracts were examined when eligibility was not clear from the title. The search was performed by using terms "COVID-19" AND "characteristics," as well as their derivations from the selected articles. We minimized our search keywords to expand our findings and to obtain more studies. We prioritized our aim to collect the neurological characteristics data of the disease. Finally, additional articles were added based on the bibliography of the articles retrieved through the outlined search strategy and were manually screened to refine this review.

Data Extraction

Two independent reviewers (P.N.N and R.B.B) then assessed the text articles that passed the first screening process to ensure their eligibility and compliance with inclusion and exclusion criteria. Then, the reviewers identified every article bibliography, within each document that discussed the clinical characteristics in COVID-19, and specifically searched for the neurologic characteristics in the text, to be added into the additional records. When the reviewers could not reach consensus, the main author would assess the review relevance for a final decision.

The following data were recorded and tabulated from all reviewed articles: author names, study design, country location, study group, age, neurologic symptoms, key findings, and study limitations.

Study Quality Assessment

We assessed the quality of each study using The Oxford Center for Evidence-Based Medicine Quality ratings. The ratings ranged from 1 to 5, with 1 representing properly powered and adequate randomized controlled trial (RCT) and 5 representing opinions and case reports (7).

Analysis

We conducted the meta-analysis with prevalence estimates, that had been transformed using the Freeman-Tukey transformation (arcsine method), to calculate the weight proportion under the random-effects model. A pooled prevalence figure was calculated with 95% CI. The pooled prevalence of neurologic manifestations was estimated from the reported prevalence of eligible studies. Forest plots were generated, displaying prevalence for each study. The overall random-effects pooled estimate with its CI was reported. We limited the articles included in the meta-analysis to those manifestations that were present in more than one study and excluded the case reports (8).

The meta-analysis was performed using a random-effects model to account for heterogeneity. Heterogeneity between estimates was assessed using the I^2 statistic, which describes the percentage of variation not because of sampling error across studies. An I^2 value above 75% indicates high heterogeneity. Statistical significance was declared at $I^2 > 50\%$ and $p < 0.05$. The analysis was done using MedCalc V.19.2.0 software (8).

RESULTS

Methodological Quality of Included Studies

Most of the studies included were observational studies. Of the 33 included studies, 19 (58%) were cohort studies, 10 (30%) were retrospective case series or cross sectional studies, and four (12%) were case reports. Individual study quality ratings are presented in **Supplementary Table 1**.

Study Results and Patient Characteristics

Initially, we identified 280 studies acquired from the database and additional records, of which 169 were excluded because of duplication and a review of the titles and abstract. Additional articles were identified in the reference lists of included studies. We screened the full text of 111 studies for relevance and excluded 78. Finally, 33 papers were selected for final review. **Figure 1** shows the PRISMA flow diagram for studies included in the review. A total of 7,559 patients were included. The total number of patients in each study ranged from 5 to 1,590, except for the case reports. The mean age of eight studies ranged from 34.9 to 55.5 years, median age of 20 studies ranged from 32.5 to 73.5 years, and 4 case reports studies ranged from 24 to 74 years. Most of the included studies were from China, with 29 studies (7,528 cases), followed by the United States (US) with 2 (2 cases), Japan with 1 (1 case), and South Korea with 1 (28 cases).

Thirty-one studies (3, 4, 9–37) used reverse transcription-polymerase chain reaction (RT-PCR) as a laboratory-confirmed diagnosis for COVID-19, and one study (38) used RT-PCR and clinical-confirmed diagnosis. Of the remaining study, one study (5) did not mention the diagnosis method. Twenty-five studies (3–5, 9, 11–25, 27–31, 38) reported the specimens for laboratory testing were obtained from a throat swab. Others were from sputum, with four (15–17, 37) from a nasopharyngeal swab and two (4, 37) from a cerebrospinal fluid (CSF) sample (26). Six studies (10, 32–36) did not report the specimen used. Five studies (4, 14–16, 37) used more than one type of specimen collected. **Supplementary Table 1** summarizes the characteristics of included studies.

Neurologic Manifestations in COVID-19

Studies have identified the presence of neurological symptoms in COVID-19 patients. These manifestations were then grouped into several categories based on their symptoms, including nonspecific symptoms, specific symptoms, consciousness disturbance, and skeletal muscle problems. One study (5) particularly examined the neurologic manifestations in COVID-19, with the prevalence of nervous system disease at 36.4% from 214 patients. As for onset, most neurologic symptoms occurred in the early stages of disease (median time, 1–2 days), apart from stroke and impaired consciousness (median time, 8–9 days). The prevalence of neurologic manifestations as reported in these studies are shown in **Supplementary Table 1**. The overall results

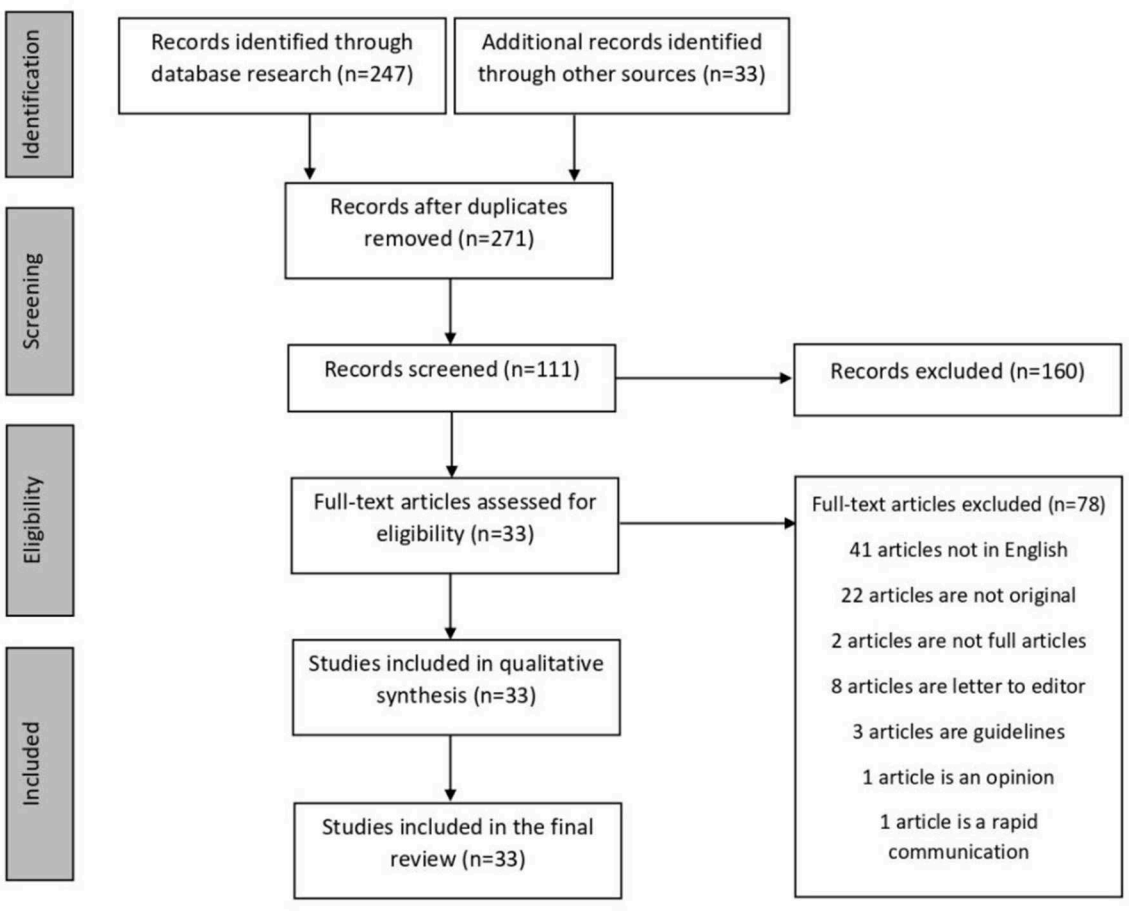

FIGURE 1 | Search algorithm for reviewed articles.

TABLE 1 | Results of meta-analysis of prevalence based on each neurological manifestation.

Variables	Number of studies	Prevalence (%)	95% CI (a)	Pooled sample size	I^2 (b)	*p*-value
Headache	21	10.9	8.62–13.51	6,486	87.8%	<0.0001
Dizziness	6	8.77	5.02–13.43	1,088	81.7%	<0.0001
Nausea with/without Vomiting	13	4.6	3.17–6.27	5,410	82.8%	<0.0001
Cerebrovascular disease	2	4.4	1.92–7.91	435	58.8%	0.1195
Consciousness Disturbance	2	3.8	0.16–12.04	3,848	94.8%	<0.0001
Muscle Problem	25	19.2	15.4–23.2	6,498	92.6%	<0.0001
Cerebrovascular disease comorbidity	13	8.5	4.5–13.5	4148	95.5%	<0.0001

95% CI: 95% Confidence Interval.
I^2 Index for the degree of heterogeneity.

of the meta-analysis of neurologic characteristics proportions are shown in **Table 1**.

Non-specific Neurologic Manifestations

The primary manifestations of COVID-19 are typically respiratory symptoms. However, physicians have found neurological symptoms at the time of diagnosis as an initial symptom(s). Non-specific symptoms may lead to difficulty of diagnosis when it is the only symptom presented, therefore a differential diagnosis should always be considered to avoid delayed or misdiagnosis.

Headache was one of the most common neurologic symptoms in COVID-19 after myalgia, which will be discussed in a later section. Twenty-one studies (3–5, 9, 10, 12–16, 19–21, 23, 27, 28, 30, 33, 35, 37, 38) reported the prevalence of headache ranging from 3.5 to 34% among COVID-19 patients in their baseline characteristics. The overall pooled prevalence of headache was 10.9% (95% CIs: 8.62–13.51) with a high level of heterogeneity (I^2 = 87.8%) from 21 studies with a total number of 6,486 total cases (**Table 1**). A forest plot of prevalence (%) of headache is included in **Figure 2**. These findings may be indicative that headache can be found in the early stages of the disease. A retrospective study (37) described that headache was more common among patients with aggravation of illness during follow up (19 vs. 14.6%).

Dizziness was reported in 6 studies (5, 11, 13, 16, 19, 27). The overall pooled prevalence of dizziness was 8.77% (95% CIs: 5.02–13.43) with a high level of heterogeneity (I_2 = 81.7%) from six studies, with a total number of 1088 total cases (**Table 1**). Forest plot of prevalence (%) of dizziness is included in **Figure 3**. In one study, dizziness (16.8%) was the most common central nervous system manifestation of COVID-19 followed by headache (13.1%). Dizziness and headache were often observed in earlier disease as typical symptoms of COVID-19 (5).

Nausea with or without vomiting was reported in 13 studies (4, 12, 13, 16, 17, 19–21, 27, 30, 34, 37, 38) with the prevalence ranging from 1.25 to 8.7%, although vomiting without nausea was reported in one study among non-critically ill patients. The overall pooled prevalence of nausea with or without vomiting was 4.6% (95% CIs: 3.17–6.27), with a high level of heterogeneity (I^2 = 82.8%) from 13 studies with a total number of 5410 total cases (**Table 1**). The prevalence forest plot (%) of nausea is included in **Figure 4**.

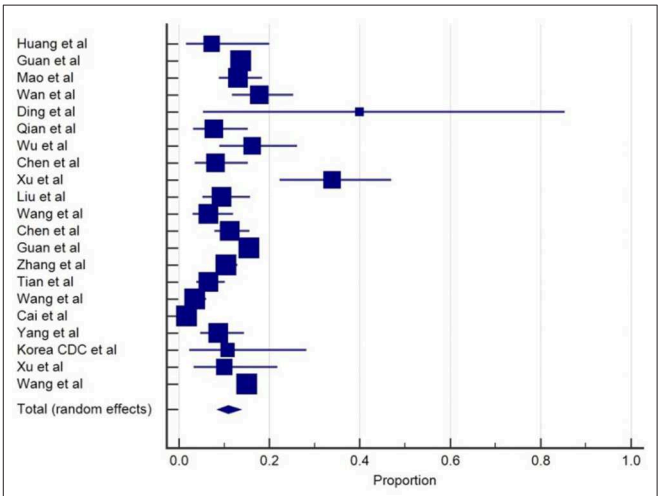

FIGURE 2 | Proportion estimates of headache in COVID-19 patients.

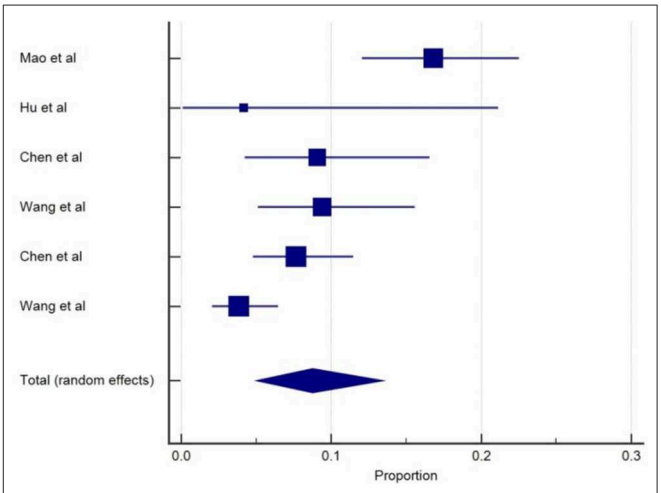

FIGURE 3 | Proportion estimates of dizziness in COVID-19 patients.

Most of the studies were conducted during the outbreak period of COVID-19. Therefore, advanced imaging and diagnostic procedures such as magnetic resonance imaging

(MRI) and electroencephalography (EEG) were avoided or limited unless the symptoms were specific for a disease (e.g., hemiparesis or seizure). Hence, it is difficult to distinguish the origin of these neurologic symptoms, whether it is caused directly by the virus or indirectly from other organ injury, such as gastrointestinal manifestation (5).

Specific Neurologic Manifestations

More specific manifestations related to COVID-19 were also observed, such as impairment of smell or taste (hypogeusia) or vision, limb weakness, acute cerebrovascular disease, and seizure. Some specific symptoms were only reported in one study (5) that included impairment of taste (5.6%), smell (5.1%), and vision (1.4%), ataxia (0.5%), and neuralgia (2.3%).

Seizure was less common in COVID-19 and only reported in two case reports (5, 26). However, the diagnosis for seizure was based on clinical founding, without further diagnostic tests. Only one study reported seizure characteristics with a sudden onset of limb twitching, foaming at mouth, and altered consciousness, which lasted for 3 minutes (5). Convulsion in COVID-19 is also associated with an incidence of encephalopathy (26).

In a case report (31) of a 61-year-old female, the patient presented with acute weakness in both legs and severe fatigue progressing within 1 day. Neurological examination showed symmetrical weakness grade 4/5 and areflexia in both legs and feet. The nerve conduction studies showed delayed distal latencies and absent F waves in early course, supporting demyelinating neuropathy, and the patient was diagnosed with Guillain-Barre syndrome (GBS). Interestingly, the onset of weakness precedes the typical COVID-19 symptoms (fever and respiratory symptoms). This report might be an indication that neurologic symptoms could occur in an early stage of the disease.

The incidence of acute cerebrovascular disease (CVD) was reported in two studies (5, 18). The overall pooled prevalence of acute cerebrovascular disease was 4.4% (95% CIs: 1.92–7.91) with a moderate level of heterogeneity ($I^2 = 58.8\%$) from two studies with a total number of 435 total cases (**Table 1**). A forest plot of prevalence (%) of cerebrovascular disease is included in **Figure 5**. In a retrospective study (18) among 221 patients with COVID-19, 5.9% of patients had a new onset of CVD during hospitalization stay. Median duration from the first symptoms of infection to a sudden onset of hemiplegia was 9 to 10 days (5, 18). The most common type was ischemic stroke (84.6%), followed by cerebral venous thrombosis (7.7%) and hemorrhage stroke (7.7%). The onset of CVD was more likely to present with those of an older age, severe disease, and history of underlying diseases such as hypertension and diabetes mellitus. This study also showed various inflammatory biomarkers including elevated levels of white blood cells, C-reactive protein, and D-dimers in COVID-19 patients with stroke. In a study by Mao et al. (5), six patients (2.8%) were reported to have CVD and severe patients were more likely to present with CVD than non-severe cases. However, association between COVID-19 and the incidence of cerebrovascular events is lacking and unclear.

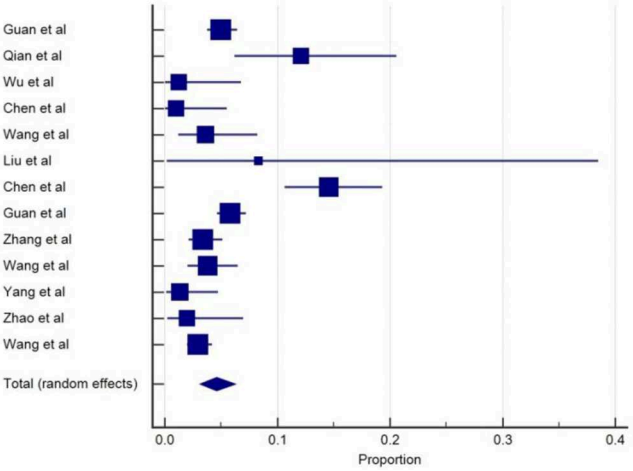

FIGURE 4 | Proportion estimates of nausea with/without vomiting in COVID-19 patients.

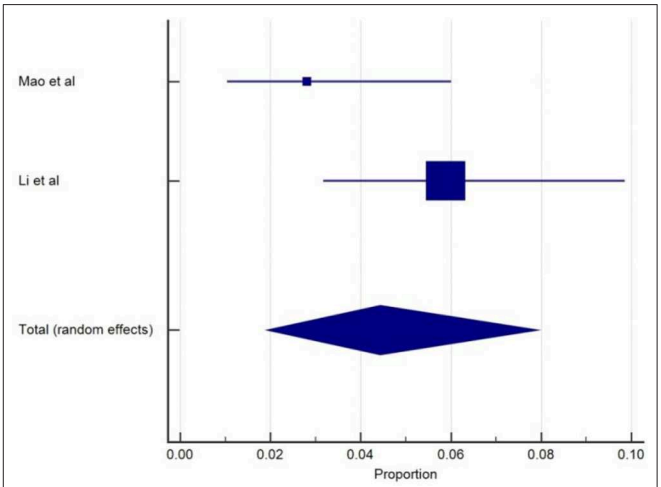

FIGURE 5 | Proportion estimates of cerebrovascular disease in COVID-19 patients.

Consciousness Disturbances

Impaired consciousness was detected in five studies (5, 20, 25, 26, 32). The overall pooled prevalence of consciousness disturbance was 3.8% (95% CIs: 0.16–12.04) with a high level of heterogeneity ($I^2 = 94.8\%$) from two studies, with a total number of 2848 total cases (**Table 1**). A forest plot of prevalence (%) of consciousness disturbance is included in **Figure 6**. Three studies were excluded from analysis because those studies were case reports. One study (20) stated that patients with comorbidity on admission were more likely to present with unconsciousness (2.5 vs. 1%). Findings from this limited study have been confirmed in other reports, showing that underlying diseases were associated with the incidence of consciousness disturbance.

A study reported the onset of impaired consciousness (median time, 8 days) to hospital admission was longer

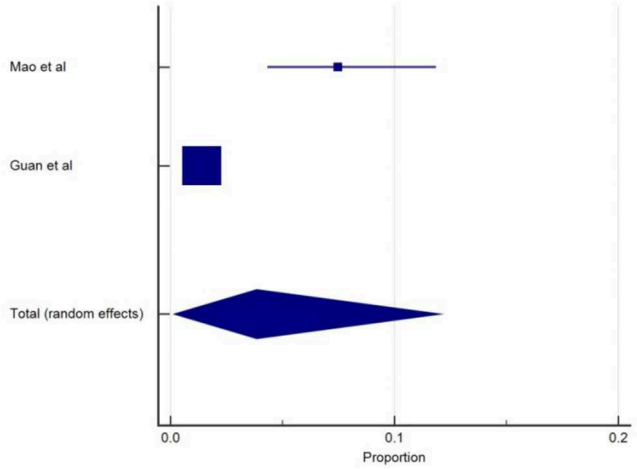

FIGURE 6 | Proportion estimates of consciousness disturbance in COVID-19 patients.

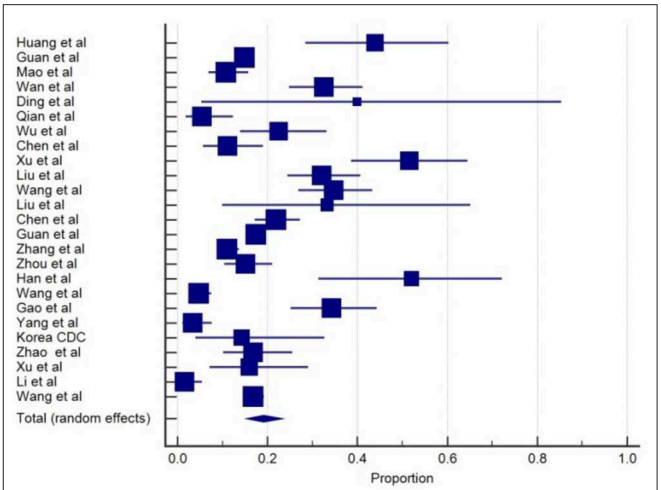

FIGURE 7 | Proportion estimates of muscle problem in COVID-19 patients.

compared to other neurologic symptoms (5). In contrast, three case reports (25, 26, 32) described that consciousness disturbance occurs as a presenting symptom of COVID-19. These case reports described altered consciousness on admission was linked to the presence of SARS-CoV-2 infection in the central nervous system, associated with encephalopathy and meningitis/encephalitis. These findings could be helpful to distinguish whether consciousness disturbance has a pure neurologic origin or is caused by an indirect process from organ failure based on the symptom onset, yet the association between consciousness disturbance with SARS-CoV-2 infection remains uncertain.

A case report (32) from the United States reported a 74-year-old man presented to the emergency department with an altered mental status, with prior symptoms of fever, headache, and cough. Electroencephalography shows diffuse slowing and focal slowing sharply contoured waves in the left temporal region, which indicates encephalopathy, and the patient was tested positive for COVID-19. The patient was transferred to ICU with poor outcomes.

An interesting case report (26) from Japan found impaired consciousness followed by convulsion was associated with meningitis/encephalitis, with duration from first symptoms (e.g., headache, fever) to unconsciousness at 9 days. Interestingly, the presence of SARS-CoV-2 infection in this case was detected only through CSF specimen and negative from throat swab. Loss of consciousness associated with seizure was also reported in another retrospective study (5, 26).

Altered mental status was also related to a rare complication of viral infection. This report by Poyiadji et al. (25) described a 50-year-old female who was brought in with 3 days history of fever, cough, and altered mental status. The laboratory-confirmed positive for COVID-19. CSF findings were normal. Non-contrast head CT found hypoattenuation within the bilateral medial thalamic, whereas an MRI showed rim enhancing lesions with hemorrhage within the bilateral thalami, medial temporal lobes, and sub-insular regions. This is the first reported case of COVID-19–associated acute necrotizing encephalopathy (ANE).

Skeletal Muscle Problems and Indicator of Muscle Injury in COVID-19

SARS-CoV-2 infection appears to affect the muscles and cause skeletal muscle problems. Muscle injury and myalgia as a manifestation of COVID-19 were reported in 25 from the total of 33 studies and commonly appears alongside several other symptoms with the prevalent, ranging from 2 to 52% (3–5, 9, 10, 12–17, 19–22, 24, 27, 29, 30, 33–38). The overall pooled prevalence of skeletal muscle problems was 19.2% (95% CIs: 15.4–23.2) with a high level of heterogeneity (I^2 =92.6%) from 25 studies with a total number of 6,498 total cases (**Table 1**). A forest plot of prevalence (%) of skeletal muscle problems of studies is included in **Figure 7**.

Some COVID-19 patients showed malaise, muscle soreness, and elevated muscle enzyme levels, which may be related to the inflammation and muscle injury caused by the virus. The higher levels of creatine kinase (CK) levels in blood have been generally considered to be an indicator of muscle damage and inflammatory response (39). From the total, 25 studies that reported muscle injury as a manifestation of COVID-19. Of these, 16 studies had patients with COVID-19 who also had higher levels of CK serum. However, some of these studies used different standards in determining elevated levels of CK serum. Several studies were using range >185 units per liter (U/L) for elevated CK levels (3, 13, 14, 17, 22), four studies set the value at >200 U/L (4, 9, 24, 30), and one study set the value at >310U/L (12). Of these, six studies (5, 16, 19, 21, 27, 29) mentioned an elevation in CK levels but did not report the normal value to assess elevated CK levels. Seven studies (3–5, 9, 16, 19, 27) comparing CK levels between severe and non-severe cases found that CK levels tend to be higher in severe cases, including ICU patients, or among deceased patients.

Of the included studies, the authors did not perform electromyography (EMG) or other diagnostic tests to indicate myopathic changes. Therefore, it remains difficult to differentiate between inflammation-related muscle injury and other neuromuscular disorders or myopathy.

Neurological Features in Severe COVID-19

It has been investigated whether, for those in the severe stage of the disease, clinical deterioration may be associated with neurologic events. A total of six6 studies (4, 5, 9, 23, 28) reported neurologic symptoms were more common in severe cases. Mao et al. (5) reported that patients with more severe disease were more likely to present with nervous system symptoms (45.5 vs. 30.2%, $p < 0.05$); including impaired consciousness (14.8 vs. 2.4%), acute cerebrovascular events (5.7 vs. 0.8%), and muscle injury (19.3 vs. 4.8%). One population-based survey (4) of laboratory-confirmed COVID-19 patients reported nationwide clinical characteristics of COVID-19 in 1,099 patients. In severe cases, patients were more likely to present with headache (15 vs. 13.4%), nausea or vomiting (6.9 vs. 4.6%), myalgia or arthralgia (17.3 vs. 14.5%), and CVD comorbidity (2.3 vs. 1.2%). Following SARS-CoV-2 infection, patients with neurological involvement were more likely to require intensive care unit (ICU) interventions (5, 16).

A study categorized neurologic findings based on their system, including central and peripheral nervous systems. In the severe group, the central nervous system symptoms (e.g., dizziness, headache, cerebrovascular events) were more common compared to peripheral nervous system manifestations (30.7 vs. 8%).

Patients with severe disease were also more likely to experience myalgia compared to the non-severe group (17.3% vs. 14.5%) (4). Similarly, six studies (3, 5, 9, 27, 29, 37) also reported that muscle problem was more common among severe cases or non-survivors. In a retrospective study (20), COVID-19 patients with comorbidity were more likely to have muscle pain. In terms of comorbidity, cerebrovascular disease (CVD) was commonly reported as a neurologic comorbidity in COVID-19 patients. We found 13 studies (4, 11–14, 16, 19, 20, 27, 29, 30, 34, 38) that reported the presence of CVD as an underlying disease in COVID-19 patients. The rate of CVD comorbidity ranged from 1.4 to 40 %. The overall pooled prevalence of CVD comorbidity was 8.5% (95% CIs: 4.5–13.5) with a high level of heterogeneity ($I^2 = 95.5\%$) from 13 studies with a total number of 4,148 cases (**Table 1**). A forest plot of prevalence (%) of CVD comorbidity of studies is included in **Figure 8**.

CVD comorbidity was also a predictive factor of poor outcomes. In a retrospective study (27) on 339 hospitalized COVID-19 patients, the patients' prognostic factors were evaluated based on a 4-week follow-up; 21 (6.2%) patients had CVD comorbidity and this was more prevalent in patients who died (10/65 or 15.6%). Similarly, two studies (19, 29) reported that patients with CVD comorbidity on admission had a higher mortality rate (4 vs. 0%) and was more prevalent among non-survivors (17.6vs. 3.5%), respectively. A complication of hypoxic encephalopathy occurrence following COVID-19 was also observed in 20% of patients and was likely to occur in patients who died (20% vs. 1%) than patients who survived (19).

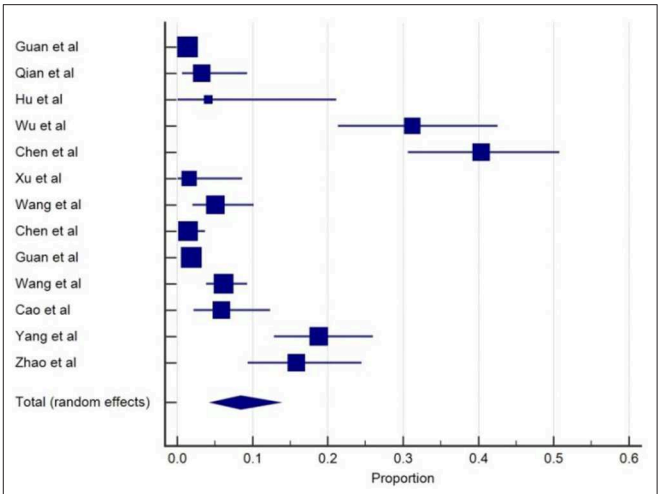

FIGURE 8 | Proportion estimates of cerebrovascular disease comorbidity in COVID-19 patients.

DISCUSSION

As a newly emerging disease, COVID-19 has become a pandemic disease since its outbreak in December 2019. The infection caused by SARS-CoV-2 mainly targets the respiratory tract. However, studies also reported the involvement of the nervous system as presenting symptoms, especially in different stages of disease (40). During the early phase of the disease, symptoms were commonly mild or asymptomatic. Therefore, the diagnosis of the disease was often difficult at the time of presentation (4). Patients may be referred to the neurologic clinic without respiratory symptoms, with infection that may be hard to detect or that could be misdiagnosed.

Pathophysiology of Neurologic Manifestations in COVID-19

SARS-CoV-2 used the angiotensin-converting enzyme 2 (ACE2) receptor to enter the host cells, the same as SARS-CoV infection in 2003. The pathologic mechanism COVID-19 has on the nervous system can happen through several pathways, including the hematogenous pathway, retrograde neural pathway, hypoxia, immune injury, and ACE-2 enzyme (41). The virus entering blood circulation can cause the immune system to produce cytokine as a physiological response; the increasing cytokine production may cause an increase in blood-brain barrier permeability, thereby facilitating the virus to enter the CNS (41). This may explain why patients with more severe COVID-19 might have cytokine storm syndrome.

A case report of a rare complication of viral infections has been related to intracranial cytokine storms, which result in blood-brain barrier disruption without direct viral invasion. This theory has been linked to the first reported case of COVID-19-associated acute necrotizing encephalopathy (ANE) (42).

A recent study on COVID-19 patients with a neurologic manifestation found that some patients had smell impairment

(5). This finding presents the fact that COVID-19 can invade the central nervous system through the olfactory nerve and gain access to the central nervous system (41). Hypoxia injury happens when a virus begins to proliferate in the lung cells, causing alveolar gas exchange disorder and thus leading to hypoxia of the CNS and increasing the rate of anaerobic metabolism, which leads to the accumulation of acid and can cause cerebral vasodilation, interstitial edema, swelling of brain cells, or obstruction of cerebral blood flow that leads to headache as a result of congestion and ischemia (5).

The past study suggested the neurotropic potential of COVID-19 (43). The neurotropic virus had the ability to incite the activation of glial cells and invoke a proinflammatory state. Furthermore, an elevated level of proinflammatory cytokine in serum may cause chronic inflammation and lead to damage in skeletal muscle and brain (5, 40). ACE-2 is a protecting factor that plays a major role in anti-atherosclerosis and blood pressure regulation. COVID-19's capability to bind ACE-2 receptors may result in elevated blood pressure and increase the feasibility of cerebral hemorrhage (40).

Laboratory Abnormality and Neurologic Manifestations in COVID-19

Severe SARS-CoV-2 infection has been associated with increased immune-inflammatory response, including higher white blood cell counts, neutrophil counts, lower lymphocyte counts, and increased C-reactive protein levels compared with non-severe infection. Some laboratory findings were also associated with the neurologic manifestations of the disease. Patients with central nervous symptoms involvement had lower lymphocyte levels, platelet counts, and higher blood urea nitrogen levels compared with those without CNS symptoms. However, there were no significant differences in laboratory findings of patients with PNS manifestations and those without PNS (5). This finding may be a link to the immunosuppression among patients with CNS symptoms, especially in severe patients.

Skeletal muscle injury was described as skeletal muscle pain or myalgia with an elevated level of serum creatine kinase (CK) more than 200 U/L as a manifestation of an increased inflammatory response (5). The cellular disturbances caused by the infection or direct muscle injury by the virus can induce creatinine kinase to leak from intra cells into the blood. Assessment of serum CK levels are a valuable indicator of the occurrence of muscle and tissue damage due to disease or trauma. This finding may be associated with the ACE-2 receptor in skeletal muscle (5, 40).

Additionally, the coagulation system was also affected by the SARS-CoV-2 infection, causing the elevated level of D-dimer more than $\geq$0.5 mg/L and platelet abnormalities, which increases the risk of cerebrovascular events among patients. Elderly populations are at high risk of account for the majority of strokes, especially in more severe patients. Moreover, some studies reported the increased level of D-dimer was more prevalent in more severe COVID-19, which could be the source of embolic cerebrovascular diseases (4, 18, 40).

Neurologic Symptoms Characteristics in COVID-19

One population-based survey of laboratory-confirmed COVID-19 patients reported nationwide clinical characteristics of the disease in 1,099 patients. The study found some of the common symptoms in COVID-19 patients, including fever, cough, nausea, fatigue, myalgia, and headache (4). Patients with fever or headache may present to the neurology clinic after initially being ruled out of COVID-19 by routine examination. However, several days later, patients presented typical COVID-19 symptoms such as cough, throat pain, lower lymphocyte count, and pneumonia appearance on lung imaging. These findings showed that COVID-19 often presents with non-specific symptoms and leads to delayed and inappropriate management (40).

Mao et al. (5) reported that neurologic symptoms in COVID-19 can range from specific symptoms (e.g., hypogeusia, hyposmia, or stroke) to more nonspecific symptoms (e.g., headache, impaired consciousness, dizziness, or myalgia). Nonspecific symptoms were more commonly present in mild or early stages of the disease. However, future studies are required to identify which manifestations are truly neurologic in origin or just a response of systemic inflammation of the disease in patients (44).

As known from previous studies, COVID-19 puts the elderly population and patients with pre-existing comorbidity and prior neurological conditions (e.g., history of cerebrovascular disease) at a higher risk of developing more severe symptoms, such as encephalopathy, altered mental status, and new onset of stroke on admission. A recent study reported four COVID-19 patients with acute stroke as a presenting symptom. The patients admitted to the hospital with a positive PCR test and imaging confirmed acute stroke. The patients presented neurological symptoms such as altered mental status, facial drop, slurred speech, hemiparesis, hemiplegic, and aphasia. The pathophysiology behind this may be related to the infection or hypoxia that leads to brain ischemia (45). Severe COVID-19 patients were also more likely to develop more specific neurologic manifestations. Moreover, underlying cerebrovascular disease is related to poor prognosis (5, 18).

COVID-19 may also invade and disrupt the intracranial component. Researchers have detected the incidence of encephalopathy and meningitis in COVID-19 with symptoms of impaired consciousness and seizures (22, 25, 26). This may happen because of COVID-19 binding to ACE-2 receptors in the brain and causing damage in the brain tissue, thus leading to impaired consciousness and seizures (26). COVID-19 also induces the intracranial cytokine storms, which result in the breakdown of the blood-brain barrier and leads to damage in brain tissue (25). Importantly, the virus can be found in the cerebrospinal fluid through nucleic acid examination using PCR. Some studies even reported the central nervous system manifestations preceded respiratory symptoms (25). Therefore, physicians should stay aware and look at the potential signs of intracranial or other organ involvement.

Neurologic Symptoms Characteristics in SARS-CoV and MERS-CoV

Similar to SARS-CoV-2 that caused COVID-19, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) patients also present several neurological symptoms. According to previous studies, myalgia (45–61%), headache (20–56%), dizziness (4.2–43%), nausea, and vomiting (20–35%) are among the neurological symptoms found in SARS-CoV patients, which are similar to neurological symptoms frequently found in COVID-19 patients (46). Several studies also reported neurological manifestations such as stroke, myopathy, polyneuropathy, and rhabdomyolysis in SARS-CoV patients (47–49). Middle East Respiratory Syndrome Corona Virus (MERS-CoV) patients present several neurological symptoms similar to SARS-CoV and COVID-19, such as myalgia, headache, nausea, and vomiting (50). The neurological complication of MERS-CoV reported in previous studies consists of stroke, Bickerstaff's encephalitis (BBE), Guillain-Barré syndrome (GBS), and polyneuropathy (51–53).

Limitations

Our study has several limitations. First, the search keyword used in this systematic review was limited to the terms of "characteristics," so there is a possibility that relevant studies were missed by the search. Second, our study was also limited to the English language. Third, there were variations among laboratory value findings in the studies included. Some studies used a different standard of laboratory normal value range, which may lead to misinterpretation. Fourth, several studies included in this research are lacking in severity degree of neurological symptoms. Another important limitation was the variation in the methodologic quality of the included studies. Most of the included studies were observational studies and the data used was obtained from medical records. Therefore, comparability among literature was also limited. Furthermore, the majority of the studies retrieved were from China, which led to a lack of data from other countries. Therefore, further studies are needed to provide a different perspective from neurologic findings of patients with COVID-19 from other countries.

CONCLUSION

As a pandemic, COVID-19 has become common globally. Physicians are expected to be prepared for and confronted with these patients in the upcoming period. The disease can range from mild disease with asymptomatic or non-specific symptoms to severe disease with respiratory distress. The neurologic symptoms are more commonly found in the later or severe stage of the disease and may not be found at the early stage or mild disease, yet physicians should stay vigilant and aware of those symptoms as signs of nervous systems complicity (40).

It is hoped that this brief review may provide a spectrum of neurologic manifestations in COVID-19. Therefore, early diagnosis and management can prevent further deterioration from neurologic complications in the later stage of disease. Our review could be a reference for physicians in management and detection of neurological symptoms in COVID-19 patients.

AUTHOR CONTRIBUTIONS

RP: study concept and design, supervision, writing of the initial draft, and final revision. VW: study concept and design, writing of the initial draft, data extraction, analysis, and interpretation. RB and PN: full text review, analysis and interpretation, and manuscript preparation. AA: abstract screening, data extraction, analysis, and interpretation.

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OPINION
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Three Decades of Interferon-β in Multiple Sclerosis: Can We Repurpose This Information for the Management of SARS-CoV2 Infection?

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INTRODUCTION

While waiting for a vaccine, therapeutic alternatives to slow or stop the ongoing COVID-19 pandemic caused by the newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) are urgently needed. Given their wide and unspecific antiviral and immunoregulatory properties, Interferon (IFN)-α and β are among the many drugs under evaluation all over the world for their repurposing potential in this context (1). They are presently tested in clinical trials at different dosages and routes of administration in combination with other compounds such as Remdesivir, Lopinavir, and Ritonavir; chloroquine and hydroxychloroquine (2, 3) were also tested before trials halted due to safety concerns (4, 5). Many *in vitro* and *in vivo* studies in the CoV field indicate that IFN-β1a and IFN-β1b are more potent than an IFN-α subtype in the inhibition of SARS-CoV and MERS-CoV replication (2). Very recently, type I IFN's ability to suppress SARS-COV2 infection in Vero cells was also reported (6).

IFNs have been classified into three types based on their receptor usage: in humans, type I IFN contains 13 IFN-α, -β, -ω, -ε, and -κ; type II IFN includes a single IFN-γ; and type III IFN consists of IFN-λ1, -λ2, and -λ3. The effects of IFN are mediated through the induction of around 2,000 IFN-stimulated gene (ISG) products, the expression of which is mainly regulated by the JAK/STAT pathway(s). At the cellular and systems levels, in addition to their definitive antiviral and antibacterial effects, IFNs regulate, through the induction of several ISG, cell proliferation, cell cycle, survival/apoptosis, cell differentiation, and migration. While type II IFN, i.e., IFN-γ, whose expression is dramatically increased in MS, is linked to activation and maintaining of inflammation, type I IFNs (mainly IFN-αs and IFN-β) are abundantly secreted in response to viral infection, acting early during the immune response to potentiate antiviral responses and to prime and maintain adaptive immunity (7). Multiple recombinant IFN-α and IFN-β formulations have been clinically approved all over the world.

Here, we briefly review the knowledge acquired in the last 27 years of IFN-β usage for the treatment of relapsing-remitting forms of multiple sclerosis (RRMS) to hypothesize the impact of this treatment on COVID-19.

ALTERATION OF TYPE I IFN SYSTEM IN MS

Normally, low amounts of constitutive type I IFN accumulate in the tissue in absence of infection to support cell priming and responses to other cytokines, antiviral, and antitumor immunity, and immune homeostasis. The perturbation of this so-called "tonic," the constitutive production of IFN (8), has been linked to the development of a number of different autoimmune diseases, including systemic lupus erythematosus, Sjögren's syndrome, or type I diabetes mellitus, often correlating with increased disease severity. Additionally, in MS, evidence on low serum levels of IFN in most patients (9) poses the basis for IFN use in MS treatment and paves the way for the investigation of endogenous defect of IFN signaling in MS. In line with this observation, the presence of SNPs and rare variants in genes involved in type I IFN signaling and antiviral pathways, namely IRF-8, STAT3, SOCS1, TYK2, ZC3HAV1, and OAS1, which may display transcriptional dysregulation in blood cells at distinct MS stages (10), were found to be altered by several published GWA studies in MS, confirming the hypothesis that an alteration of IFN-regulated antiviral responses could be linked to MS pathogenesis (11–13).

The efficacy of IFN-β, the first approved therapy for RRMS whose mechanisms of action, only partially understood, appears to be mainly related to its multifaceted pleiotropic effects resulting in sustained broad anti-inflammatory action (7), may also rely on the capacity to rescue these fine endogenous molecular defects.

In spite of the identification of MS-associated genetic alteration linked to the IFN system, only a few genomics studies have addressed in detail whether these pieces of evidence could be also highlighted at the transcriptional level in therapy-free MS patients compared to healthy donors (14). Most information on IFN gene signatures in MS has, indeed, identified them mainly as IFN-β treatment-related biomarkers in human peripheral blood mononuclear cells (PBMC) of MS patients analyzed before and after IFN-β therapy, e.g., in Malhotra et al. (15) and other references. Only recently it was shown that some ISG found expressed in PBMC or CSF of MS subjects specifically cluster with other indicators of inflammation with clinical and sex parameters of analyzed patients (16). Thus, to better understand whether dysregulation of IFN-regulated genes and pathways may be related to MS development and maintenance, we performed a systematic analysis of datasets from transcriptomes obtained from more than 400 human PBMC at distinct MS stages as well as CNS tissues and encephalitogenic CD4 T cells derived from the murine model of MS. These data indicate impaired ISG transcription profiles especially in the RR form of disease and myelin-reactive T cells and identifies a core of 21 transcripts concordantly dysregulated in all MS stages (17). In addition, recent evidence highlights dysregulations in endogenous IFN system in specific immune cell subsets, critically impacting on immune functions (18). In particular, paired analysis of B cells and monocytes from sex and age-matched control and treatment-naïve MS subjects underlined several altered previously uncharacterized ISG and pathways in MS. Notably, this study describes for the first time that expression of several ISG strictly involved in antiviral responses is strongly reduced in MS B cells and involves a profound multi-level defect in type I IFN pathway due to the low level of IFN receptors, weak STAT1 and 2 expression, and activation of and selective impairment in responses to type I but not type II IFN (18). B cells, particularly the memory subset, are human reservoirs of Epstein-Barr virus (EBV) infection. The contribution of EBV to MS pathogenesis is still being fervently debated; however, its epidemiological association with MS is clear. In line with this view and with our results pointing to an anti-viral failure, *in vitro* infection of MS B cells with EBV highlights that these cells display an altered EBV expression program and propagation, resulting in reduced containment of its infection in MS. Importantly, *in vitro* and *in vivo* exposure to IFN-β potentiates type I IFN signaling machinery in MS in turn, activating the antiviral responses and reducing the frequency of EBV-infected and proliferating B cells in MS but not healthy cultures (18).

In MS, different therapeutic strategies targeting memory B cells, including B-cell-depleting therapy, significantly reduce disease activity. The basis for this effect appears to be related to decreased production of pro-inflammatory cytokines or reduced antigen presentation by these cells. Importantly, we have reported that IFN-β therapy mediates a marked and specific reduction of memory B cells in peripheral blood of treated MS patients via a mechanism requiring a FAS-R-mediated caspase-3-dependent apoptosis, and this memory B-cell decrease is associated with reduced expression of the latent EBV gene *LMP2A* in PBMC of MS patients under IFN-β treatment (19). Altogether, this evidence points to a double-face scenario for IFN-β efficacy in MS treatment, combining anti-inflammatory and immunomodulatory actions with marked antiviral properties (20). Another important aspect in these pandemic times is that the decrease in the $CD27^{+}$ memory B-cell compartment correlates with the concomitant increase in the $CD27^{-}$ naïve cells, likely as a result of a renewal of circulating B cells in the peripheral blood of MS patients, offering opportunity for expansion of new virus-specific clones of antibody secreting plasma cells (19). Accordingly, *in vitro* stimulation with a TLR7 ligand (simulating viral RNA) promotes IgM and IgG production in PBMC cultures derived from IFN-β-treated MS patients as compared to the same individuals before therapy (21).

WHAT IFN-β USAGE IN MS WOULD TEACH US IN THE COVID-19 ERA

International recommendations on immunization of MS patients do not indicate a specific risk for vaccination of these individuals or an increased risk for future MS development for those who vaccinate [reviewed in (22)]. Furthermore, while the recommendation does outline that patients treated with some MS therapeutics, such as fingolimod, glatiramer acetate, mitoxantrone, and rituximab, have lower responsiveness to influenza vaccination, many trials indicate that IFN-β-treated MS patients achieve significant responses and comparable protection to non-treated patients and healthy controls (22). Hence, despite

the lower vaccination response rate under some treatments, MS patients contribute to the overall herd immunity toward common vaccine-preventable diseases, including (hopefully soon) a COVID-19 vaccine.

In this context, the combined properties of IFN-β as antiviral and immunoregulatory molecule could be exploited in the pandemic Phase 2 when a protective humoral immunity is desired to limit SARS-CoV2 re-infections and also from the perspective of later phases of COVID-19 management when a vaccine will be available. This key consideration implies better preservation of a functionally active and protective B-cell-mediated humoral immunity. Thus, by replenishing MS immune system with IFN-regulated functions, IFN-β therapy, alone or in continuous or cyclic combination regimen with other drugs (2, 23), may represent a treatment that combines safety and efficacy in the COVID-19 era.

DISCUSSION

Efforts are ongoing to understand the effects of disease modifying therapies, including IFN-β, on the risk and severity of COVID-19 in persons with MS (24, 25). Although these preliminary data are still insufficient to draw firm conclusions, they have not, so far, exhibited signals of overt danger (24, 26). This case series will soon reach the sample size that is needed to provide reliable answers to persons with MS. At the same time, it could be also verified whether IFN-β or other treatments may exert some protection against SARS-CoV2. Interestingly, an impaired IFN-α2 production in about 20% of critically-ill COVID patients has been recently described, indicating that a defective innate immune response may be associated with a poor outcome and, thus, suggesting that the timing of IFN exposure may be critical to control the virus replication and limit immune-pathogenesis (27). Further studies are required to overcome the limitation of this study, given the small number of included patients and the technical difficulties for IFN-β and IFN-λ detection, as well as to define individual genetic susceptibility that could be predictive of a molecular target for novel therapeutic strategies and treatments (27). However, in line with this view, a recent paper by Blanco-Melo et al. highlighted an unbalanced inflammatory response characterized by a reduced IFN-I and -III response to SARS-CoV-2 coupled to elevated chemokines and high expression of IL-6 in cell and animal models of SARS-CoV-2 infection and in transcriptional and serum profiling of COVID-19 patients (28). Collectively, these data provide a new and dynamic view of COVID-19-related immunopathogenic features that should be taken into consideration to pinpoint and adjust new immunomodulating therapeutic strategies.

At present, we can conclude that IFN-β remains an option in the treatment of MS, particularly during this difficult pandemic period. Ongoing clinical trials in COVID-19 and the growth of clinical data collections in MS will tell us, hopefully soon, whether this evergreen molecule may have a new role in COVID-19 treatment.

AUTHOR CONTRIBUTIONS

MSe wrote the article and contributed to the discussion. CF and MSa revised the article and contributed to the discussion. EC wrote the article, proposed the subject, and organized the article.

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REVIEW
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Potential of SARS-CoV-2 to Cause CNS Infection: Biologic Fundamental and Clinical Experience

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SARS-CoV-2 is a novel coronavirus leading to serious respiratory disease and is spreading around the world at a raging speed. Recently there is emerging speculations that the central nervous system (CNS) may be involved during SARS-CoV-2 infection, contributing to the respiratory failure. However, the existence of viral replication in CNS has not been confirmed due to the lack of evidence from autopsy specimens. Considering the tropism of SARS-CoV-2, ACE2, is prevailing in CNS, and the neuro-invasive property of human coronavirus was widely reported, there is a need to identified the possible complications during COVID-19 for CNS. In this review, we conduct a detailed summary for the potential of SARS-CoV-2 to infect central nervous system from latest biological fundamental of SARS-CoV-2 to the clinical experience of other human coronaviruses. To confirm the neuro-invasive property of SARS-CoV-2 and the subsequent influence on patients will require further exploration by both virologist and neurologist.

Keywords: COVID-19, human coronavirus, SARS-CoV-2, central nervous system, viral infection

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INTRODUCTION

Since the appearance of the first pneumonia patient in early December 2019 in China, the COVID-19 has resulted in a global pandemic. By the end of April 30, over 3,000,000 cases were reported all around the world and leading to over 200,000 deaths (1). Compared with previous coronavirus outbreak, SARS and MERS, COVID-19 has certainly induced a much larger global pandemic. After the application of oxygen therapy, mechanical ventilation, and antiviral therapies, over 90% of the cases have been cured and discharged from hospitals after twice verified absence of virus in respiratory tract by nucleic acid amplification tests (2). Although human coronaviruses mainly lead to respiratory tract infection, previous researches have indeed demonstrated the potential of the coronavirus to spread to extra-pulmonary organs, involving nervous system, gastrointestinal tract, and kidney, as widely observed in SARS and MERS cases. According to the latest reports, some patients with COVID-19 presented neuro-infection symptoms such as olfactory and taste disorders (3), which may be attributed to the olfactory nerve damage. However, the mechanism and route for SARS-CoV-2 to lead to neuro-infection, especially central nervous system (CNS), still remain to be explored. In this review, we summarize the proves for possible SARS-CoV-2 related CNS damage basing on the previous experience of coronavirus infection and the latest outcomes of SARS-CoV-2 biology.

THE MOLECULAR BASIS FOR COVID-19 NEURO-TROPISM

Coronavirinae is a group of enveloped, spherical virus in the Coronaviridae family, Nidovirales order, which is further divided into the genus of alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus, and were called by a joint name, coronavirus (4). Coronavirus is the largest RNA virus extending to a diameter of 120 nm, with a huge monopartite, linear, single chain, positive RNA genome ranging from 27 to 32 kb inside. The first two strains of coronavirus, namely HCoV-229E and HCoV-OC43, was acquired in 1960's from the organ culture of patients diagnosed with upper respiratory tract disease, and was regarded as a novel kind of pathogen for common cold (5–7). So far, over 3,000 strains of coronavirus are discovered, but most of them prevail in vertebrates such as bats and porcine, and, before the outbreak of COVID-19, only six species of them cause infective disease to human, including HCoV-229E and HCoV-NL63 in alphacoronavirus genus, and HCoV-HKU1, HCoV-OC43, SARS-CoV, and MERS-CoV in betacoronavirus genus (8–12). In most of time, coronavirus infects the epithelium cells of upper respiratory tract, and were regarded as opportunistic pathogens leading to mild infections in immunocompetent individuals, and accounting for about 15% of the common colds all around the world (13). However, the coronavirus can also result in devastating clinical outcomes. Animal coronavirus may sometimes manage to efficient cross-species infection after host-adaptation by novel virus strains and cause zoonotic infections, usually accompanied by lethal cytokine storm. A case in point is the outbreak of pandemic respiratory syndrome caused by SARS-CoV in 2003 and MERS-CoV in 2012. These two strains of coronavirus are no longer restricted in the upper respiratory tract, but also spread to the trachea, bronchi, lung in immunocompetent individuals, and leading to lethal acute respiratory failure. For infants, the aged and patients with disordered immunity, coronavirus sometimes may spread to extra-pulmonary organs, including central nervous system, in which the patients may present high fever and headache with a mortality over 60% (14). In 2019 December, the breakout of zoonotic viral pneumonia revealed a novel kind of human- targeting coronavirus, which was named COVID-19 (15). COVID-19 shares a lot of similarity to the SARS in 2002, both in viral tropism and clinical symptoms, indicating that the COVID-19 may have similar complication with SARS, such as CNS infection. Computer modeling showed the COVID-19 has the same viral receptor as SARS-CoV, which is the angiotensin-converting enzyme 2 (ACE2) (16). The strong binding affinity of spike of SARS-CoV-2 to ACE2 was further proved by biochemical interaction studies and crystal structure analysis (17). As a result, the distribution of ACE2 in tissues plays an important role in SARS-CoV-2 tissue tropism.

Spike Protein and Viral Tropism

The shape of coronavirus under electron microscopy is quite distinctive, in which the virus was found to be surrounded by a number of projections connected to viral envelope, and make the virus look like a royal crown (18). These projections are called "Spike(S)," which are proteins determining the tropism of coronavirus. The *S* protein is a glycosylated transmembrane protein, consisting of two subunits, S1 and S2, which are separately responsible for the viral attachment and membrane fusion during the virus enter its host cell (19). During the process of viral entry, the virus makes use of the receptor binding domain locating in the distal S1 subunit to attach to the viral receptor on the membrane of host cell. Then the S2 subunit is cleaved by the transmembrane protease, leading to an irreversible conformational change and the subsequent membrane fusion of virus and the host cell (20). As a result, the entry of coronavirus into host cell turns out to be a multistep process, and each of the mutation in the functional domain of S1 or S2 subunit may result in different viral tropism or even infectivity. In the latest research conducted by Vincent Munster et al., the author created various COVID-19 pseudotypes with spikes from different lineage B coronaviruses, some of which infect animals, and revealed that the absence of 431–437 and 456–473 residues in the RNA coding sequence of receptor binding domain, which is widely observed in betacoronaviruses that infect non-human animals, deprived the infectivity of modified COVID-19 to infect cells overexpressed human angiotensin-converting enzyme 2 (hACE2), the receptor for COVID-19 (21). The infectivity recovered after trypsin digestion, indicating these regions are related to the intact function of human transmembrane protease, and act as a major barrier for mutual infections between different species, at least in some of the animal betacoronaviruses. Considering the huge coronavirus reservoir in wild animals may acquire the necessary RNA fragment from human coronavirus by recombination, it is no doubt that zoonotic coronavirus infections will appear at a much higher frequency in the near future without manual intervention (22). Although the viral replication cycle is driven by a series of proteins or organelles in host cell and the absence of each of them can lead to disrupted viral propagation, the entry of the virus to the host cell is the primary step for all of the subsequent process. As a result, using neutralizing antibody or recombinant protein to keep the virus from entry into the host cell is the most common strategy to treat and prevent viral infection. The indispensable function of the *S* protein during viral entry makes it an ideal target for antibody or vaccine development (23). As shown in the case that Weiner et al. produced a plasmid-based MERS-CoV S protein vaccine using 293T cells. Vaccine-immunized mice showed increased T cell number, elevated T cell response, protective antibody, and resistance to MERS-CoV infection (24).

The Function and Distribution of ACE2

Discovered in 2000 as the first homolog of human angiotensin-converting enzyme, angiotensin-converting enzyme (ACE2) is a transmembrane carboxypeptidase, which removes carboxyterminal amino acid from peptide substrates by peptide hydrolyzation reaction (25). ACE2 shares about 42% similar coding sequence with ACE, and was proposed to be the product of a fusion gene consist of partial ACE and collectrin. As a result, ACE2 turn out to be a multi-functional protein with two separate functional domains. On one hand, physically, as a member of the renin angiotensin system, ACE2 converts angiotensin I to

Ang 1-9, a peptide whose function remains to be explored, and angiotensin II to angiotensin 1-7, which is a vasodilator as the ligand for the G-protein coupled receptor MAS1 (26, 27). On the other hand, its collectrin domain collaborates with amino acid transporter B0AT1 to transfer neutral amino acids on the brush border of intestinal epithelial cells, and was found to be essential for the absorbance of several amino acids, especially tryptophan (28, 29). According to the immunohistochemistry, the ACE2 mainly distributes in the vascular endothelial cells of the heart, kidney, testes, alveoli, gastrointestinal, and, at a lower expression level, brain (30–34). The ACE2 in brain was also found in the neurons of subfornical organ, an area lacking the blood-brain barrier and sensitive to blood-borne circulating peptides (35).

The recognition of the existence of ACE2 in brain is much earlier than the identification of ACE2 molecule. In 1988, Santos et al. observed a continued transformation of ANG-I to Ang-(1–7) even after using ACE inhibitor in the brainstem of dog, which indicated the Ang-(1–7) was synthesized via a different route bypass ACE, which was later confirmed to be ACE2 pathway (36). Soon the same group verified that Ang-(1–7) produced by this ACE homolog in hypothalamo-neurohypophysial system (HNS) induced the release of arginine vasopressin (AVP) when Ang-(1–7) or angiotensin II (Ang II) was added to the explants from rats (37). One year later, Campagnole-Santos et al. provided the first *in vivo* proof for the biological functions of central Ang-(1–7) (38). After the injection of Ang-(1–7) into the nucleus tractus solitarii (NTS), the rats present a significant reduction in blood pressure. The function of Ang-(1–7), the products of ACE2, in central nervous system was further confirmed to be of great importance in baroreflex modulation and the central control of the blood pressure by the Ang-(1–7) antagonist, its analog D-Ala7-ANG-(1–7) (39–41). In spontaneously hypertensive rat models, selectively overexpressing ACE2 in the rostral ventrolateral medulla or paraventricular nucleus induced a significant relief on blood pressure (42, 43). Besides, the ACE2 and the renin-angiotensin system (RAS) also play an important role in neuro-inflammation. Zheng et al. used triple transgenic mice selectively overexpressing ACE2 in neurons, SARA, to study the role of ACE2 in ischemic stroke (44). After *in vitro* deprivation of oxygen and glucose for the brain slices from transgenic mice and control mice, they found less swelling, cell death, and ROS production in cerebral cortex and hippocampal CA1 region areas. The latest research also revealed that the ACE2 in brain not only responsible for the body blood pressure regulation, but also present a protective profile for the brain itself in a series of neurologic pathologies, including aging-related neuroinflammation (45), focal cerebral ischemia (46), demyelinating disease (47), Alzheimer's disease (48), and neuropsychiatric disorders (49). In general, the receptor of SARS-CoV-2, ACE2, is widely distributed in the central nervous system, and has been proved to take part in multiple normal physiological processes. As a result, once SARS-CoV-2 successfully invades central nervous system, it can infect neurons by recognition of ACE2, and then leads to central nervous system damage via direct viral replication or disordered immune response.

THE POSSIBLE ROUTES FOR COVID-19 INFECTION FROM RESPIRATORY TRACT TO BRAIN

The cases of respiratory virus induced CNS infection have been widely reported, such as adenovirus, influenza virus and measle virus. As another respiratory virus, SARS-CoV-2 also has the potential to enter the CNS via retrograde transport or circulatory system. In this section, we introduce the common pathway, retrograde transport and circulatory route, for respiratory virus-induced CNS infection, in which there may be clues for clinical researchers to find viral replication (**Figure 1**).

Retrograde Transport Through Olfactory Nerve

Neurologic viral infection is very common, but most of the neural infections remain in peripheral nervous system and do not lead to serious damage to healthy individuals. It is estimated that about 70–80% of healthy adults are infected by human simplex virus (HSV) including HSV-1 and HSV-2, both of which lead latent but lifelong infection in the cell bodies of neurons, such as trigeminal ganglion. However, when virus enter the central nervous system through neuronal dissemination, which is named retrograde transport, it may induce life-threatening diseases, such as herpes simplex encephalitis, which has a rate of fatality as high as 70% in untreated patients (50). Other than HSV, many viruses can take use of the retrograde transport to enter the central nervous system, such as influenza virus (51), measle virus (52), vesicular stomatitis virus (53), and rabies (54). These viruses may first infect the local tissue, and then spread to the peripheral nerves by binding to the specific viral receptor on the axons or dendrites of the neurons. Once the viruses enter the neurons, they reside in the endosomal vesicles, which is formed by the cytomembrane during viral entry, and engage a motor protein, dynein, to transport the endosomal vesicles along the microtubule to the centrosome locating beside the nucleus (55). The capsid of virus gradually disassembles according to the change of the gradually decreasing PH value in the endosomal vesicle, and then the nucleic acids of the virus are released from endosomal vesicle to the cytoplasm (56). After that, most of the DNA virus will dock into the nucleus, where there are transcriptase and substrates for mRNA and DNA synthesis, while most of the RNA virus stay in the cytoplasm and start to form the inclusion body, where RNA virus replicates. Finally, viral nucleic acids and viral protein are transported to synaptic membrane for further assembly and trans-synapse transmission to the next neuron and ultimately to the central nervous system.

Given the nerve endings distribute in all types of tissues, and the constitutions of different nerve pathways are not the same, the retrograde transport in specific disease can undergo different transport process and lead to varied clinical features according to the location of the primary lesion. Since SARS-CoV-2 distributes in the respiratory tracts, the olfactory nerve may servesas a major retrograde route for the spread of virus to central nervous system, which has been widely reported in vesicular stomatitis virus and influenza virus (57–59). During the transduction of odor

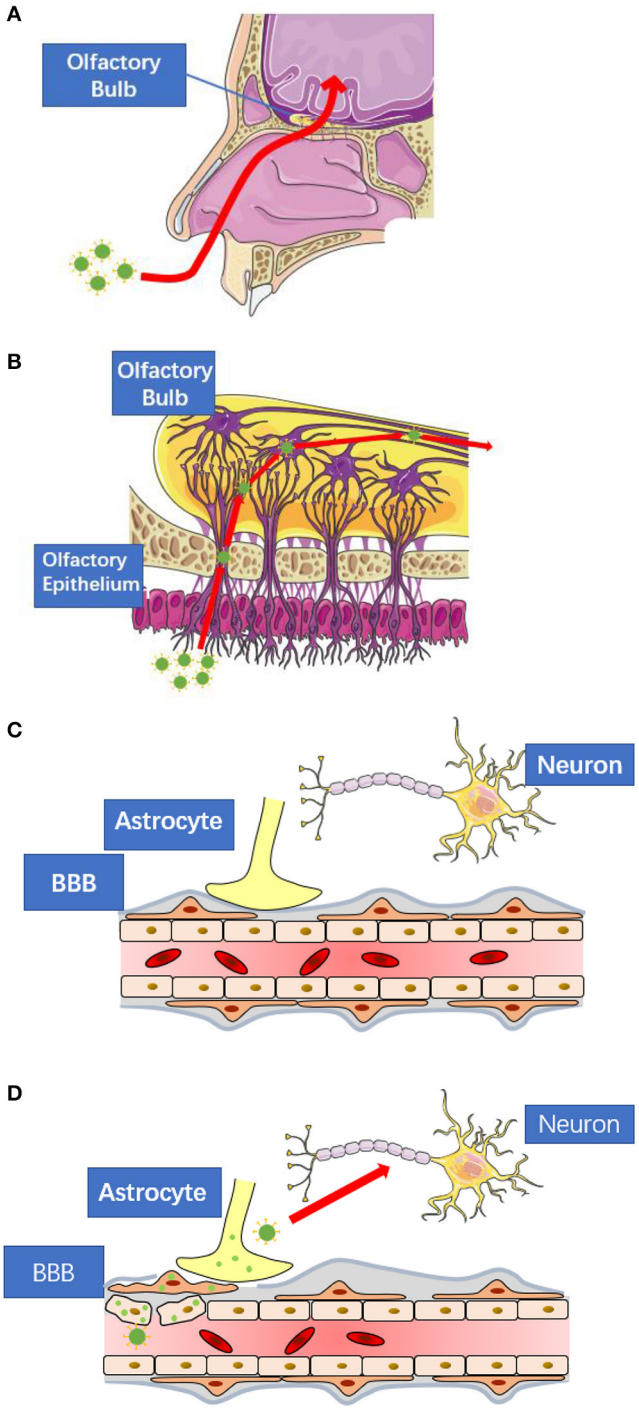

FIGURE 1 | The possible routes for SARS-CoV-2 to enter central nervous system. **(A)** SARS-CoV-2 may spread to the central nervous system via retrograde transport through the olfactory nerve as seen in the case of other respiratory viruses. **(B)** The nerve endings of olfactory receptor neurons distribute in the nasal mucosa. These neurons are exposed to SARS-CoV-2 and are connected to the olfactory bulb. **(C)** The blood-brain-barrier consists of tightly connected endothelial cells, basement membrane, and the end feet of astrocytes. All of them prevent large molecules to enter the central nervous system directly. **(D)** When viral infection occurs in the endothelial cells, pericytes, or astrocytes, the blood-brain-barrier will lose its protective function. These figures are modified and reproduced under the permission of Creative Commons Attribution 3.0 License.

signal, the stimulation starts from the dendrites of the OSN, which scatter under the olfactory mucosa on the roof of the nasal cavity (60), then transmits along the axon of the neuron, which combined together into bundles, to the central olfactory apparatus, including olfactory bulb and rhinencephalon (61). To confirm the SARS-CoV-2 related CNS infection, the viral replication in historical specimens of these location should be paid most attention.

Viral Spread Through Circulatory System

Another possible pathway for COVID-19 is to enter the CNS through the circulatory system, in which the involvement of CNS acts as part of the systematic infection derived from the primary lesion. This pathway starts from the release of the virus from the basolateral side of the infected epithelial cells. Different from the systematic spread of viral infection commonly seen in severe infection cases to marrow or kidney, due to the protection of blood-brain barrier, neurons are not in direct contact with circulatory system. Instead, virus take use of two types of intermediate host cell before spreading into CNS from circulatory system, namely the endothelium and leukocytes (62).

The blood-brain barrier (BBB) is a highly selective structure separating CNS from the circulating blood to avoid most of the large molecules entering CNS, including virus. This barrier consists of three layers (63). The first layer is formed by endothelial cells lining around the brain capillaries, which has a distinct tight junction connection different from endothelium in other tissue. The second layer is a thick, intact basement membrane, and finally the astrocyte end-feet projections surrounding the capillaries forms the third layer (64, 65). During viremia, some viruses can directly infect the epithelial cells of blood-brain barrier, which is of the most exposure to the circulating virions among the three layers. The viral replication and the subsequent inflammation cytokines eventually deprive BBB of the normal function by increasing its permeability, and lead to the CNS infection. This pathway is confirmed in the cases of Encephalitic Alphaviruses (66), Hepatitis E Virus (67), and HSV (68). Considering the viral receptor, ACE2, is highly expressed in the epithelial cells, it is possible for COVID-19 to cause direct damage to the BBB, and thus enter the CNS.

Instead of penetrating the BBB, another group of viruses, including human immunodeficiency virus (69) and Zika virus (70), take use of the circulating leukocytes to carry them across the BBB, which is called "Trojan horse" mechanism (71). This pathway is best explored in the case of AIDS-related dementia (72). During HIV infection, the virus accumulates in the monocytes, which usually do not undergo a rapid replication-induced cytolysis and are able to traverse the BBB physically (73). After entering the CNS, HIV infects and activates the microglia to secret chemokines, which in turn induce a larger size of monocytes infiltration, and increase the permeability of BBB as widely seen in the normal process of inflammation (74, 75). The ability to infect leukocytes is also reported in coronavirus infection. For example, HCoV-229E can lead to restricted infection in monocytes/macrophages (76, 77), peritoneal macrophages and dendritic cells (78–80), and induce chemokine secretion (81), which means coronavirus has the same potential as HIV to constitute a reservoir in leukocytes and use

them as vectors to spread into the other tissues outside the respiratory tracts.

THE NEURO-INVASIVE PROPERTY OF CORONAVIRUS

Although traditionally coronaviruses were regarded as respiratory viruses for a long time, there are indeed accumulating proves about the neuro-invasive property of coronavirus these years. Among the six strains of coronaviruses before the breakout of COVID-19, three of them, HCoV-229E, HCoV-OC43, and SARS-CoV, have ever been reported to cause CNS infection so far. Given the prevalent existence of the coronavirus in healthy individuals and the lethal outcomes of the central nervous system infection, increasing efforts are devoted into the research of coronavirus neurological pathologies. In this section, we summarize the previous researches on coronavirus CNS infection, including clinical cases, histopathology, and animal models, and mainly focus on the SARS-CoV. Since SARS-CoV shares the same viral receptor as SARS-CoV-2 and also induced a serious pandemic lethal pneumonia, we suppose SARS-CoV-2 can also lead to CNS infection with similar mechanism observed in SARS cases.

The Neuro-Invasive Property of Murine Hepatitis Virus (MHV)

Actually, some animal-oriented coronaviruses have long been regarded as neuro-invasive viruses and were used in the research of virus-related neurological disease. A case in point is murine hepatitis virus (MHV) is a highly infectious coronavirus, which was discovered and isolated in 1949 and prevailing in many mouse colonies throughout the world (82). These zoonotic pathogens are mainly transmitted via respiratory route, and lead to hepatitis and encephalitis. After intracerebral injection of the MHV, the rats presented acute panencephalitis and demyelinating foci, and the virus RNA was detected in both neurons and oligodendroglial cells (83). This discovery in animal models reminded people that this respiratory virus may also have the potential to induce central nervous system, but the routes were still remained unclear until 1988 when the olfactory neural pathway was confirmed to be the routes for CNS infection caused by respiratory virus instead of trigeminal nerve pathway (84, 85). Later it was found that MHV has a strong correlation with the autoimmune neurogenic inflammation and the subsequent multiple sclerosis, indicated that the coronavirus may result neurologic disorder via distorted immune attack other than direct viral replication (86). Nowadays, MHV has been widely used in the construction of animal models of multiple sclerosis or neurological infections to study the functional change and mechanism of neurological diseases (87–89).

Neuro-Invasive Property of HCoV-229E and HCoV-OC43

HCoV-229E and HCoV-OC43 are the first two strains of coronaviruses been isolated. In most of the time these two coronaviruses induce mild infection confined in upper respiratory tracts. Since the discovery of the neuro-invasive property of the MHV in mice, some researchers supposed human coronaviruses may also lead to human neurological disease, especially multiple sclerosis. In 1981, two strains of coronaviruses which serologically related to HCoV-OC43 and murine coronavirus A59 were isolated from the fresh autopsy brain tissue of patients diagnosed with multiple sclerosis (90). These two strains of coronaviruses shared cross-reactivity to the OC43 antiserum, but due to the lack of polymerase chain reaction (PCR) technology, these two viruses could not be clearly identified. Until 1992, Stewart JN et al. confirmed the existence of HCoV-OC43 and HCoV-229E in the brain tissue of multiple sclerosis patients by total RNA extracting and reverse transcription-polymerase chain reaction, in which the HCoV-OC43 but not HCoV-229E was found in specimens of all the patients (91). Actually, HCoV-229E and HCoV-OC43 present a different infectivity to the cells of the central nervous system. Both of the two coronaviruses can lead to acute infections to the cell lines of neuroblastoma, glioblastoma, glioblastoma, astrocytoma, and oligodendrocytes (92), but only HCoV-OC43 can result in a persistent infection (92). Nowadays, HCoV-OC43 is considered to be related to a series of chronic neurological disorders such as medullary atrophy, Parkinson's disease, polyneuropathy, senile dementia, and headache (93). According to the results from susceptible mice model, this coronavirus mainly uses the olfactory route and neuron-to-neuron transmission to enter the CNS, and concentrates in the piriform cortex, the brain stem, and spinal cord (94). In immunocompetent adults, the pathogenicity of HCoV-OC43 are mainly attributed to the subsequent autoimmunity instead of viral replication, which undergoes a chronic and latent procedure for years, but in infants or immunocompromise individuals, this virus may lead to lethal acute encephalitis (14, 95, 96).

Neuro-Invasive Property of SARS-CoV

In 2003, a previously uncharacterized coronavirus, SARS-CoV, was isolated from a cook in Guangdong, China. Different from the previously discovered HCoV-229E and HCoV-OC43, this type of coronavirus was not confined in the upper respiratory tracts, but lead to progressed pneumonia, which resulted in reduced alveolar diffuse function and the subsequent acute respiratory distress syndrome (ARDS). This coronavirus soon induced a global pandemic, and involved in 8,096 patients and 774 deaths (97, 98). Given the distinct syndromes compared with common viral pneumonia, this pandemic was finally named Severe Acute Respiratory Syndrome (SARS), and thus the pathogen was named SARS-CoV. SARS-CoV is a zoonotic virus originating from bats, and infected the palm civet as the second carrier, then finally managed to get across the species barrier to human via nosocomial transition and resulted in lethal pneumonia (99). The outbreak of SARS-CoV for the first time demonstrated the lethal pathogenicity of coronavirus, and the prevalence of coronavirus reservoirs in bats.

During SARS infection, an important complication is the multiple organ failure, usually observed in severe infection cases (100). This systemic damage of SARS used to be simply

regarded as a result of immune dysregulation such as systemic inflammatory response syndrome (SIRS), until viral replication was detected in gastrointestinal, kidney, immune cells, and even brain, demonstrating that the SARS-CoV also produced direct toxicity to the organs by local viral replication (101). According to the reported autopsy results, SARS in brain tissue mainly confined to hypothalamus and cortex, leading to edema and degeneration of neurons. Indeed, the SARS central nervous infection presented a strong correlation with mortality risk, as the SARS was detected in all of the brain tissue autopsies. Another research found necrosis of neurons and hyperplasia of gliocytes in brain tissue specimen collected from SARS patient with the symptoms of significant central nervous infection, and anti-viral immune response was verified by increased immune cells and elevated cytokines level (102). Experimental data from hACE2 transgenic mice revealed that intranasal inoculation of SARS induced a delayed central nervous system infection, conforming to the process of secondary infection. Different from the regional distribution of SARS-CoV in brain specimens from clinical cases, once getting across the brain-blood barrier, the virus spread throughout the brain tissue of transgenic mice via the connection of neurons at a high effiency, especially in the regions of cortex, basal ganglia, and midbrain. Olfactory nerve retrograde and hematogenous routes were both suspected to account for the spread from respiratory tract to central nervous system, as virions were detected in blood and olfactory nerve (103, 104). Considering the brain infection presented a high correlation to death, it may exert neurogenic contribution to the respiratory failure, clinically the most common cause of death for SARS victims. Besides, the neuro-invasion and neuro-toxicity of SARS-CoV can also lead to chronic diseases and disorders even years after recovery from pneumonia. A series of somatic and psychologic symptoms relating to central nervous system prevailed among the survivor of SARS, including fatigue, sleep physiological changes, sleep disordered breathing and musculoskeletal pain (105). Sometimes the lesion can be latent and uncommon, such as a case of SARS-CoV induced olfactory dysfunction, which is rarely reported in typical peripheral neuropathy and leads to permanent anosmia (106). It needs to be pointed out that SARS-CoV shared the same receptor, ACE2, with SARS-CoV-2, and are genetically identical to the SARS-CoV-2, which means that these two viruses may undergo similar pathogenicity progress.

Neuro-Invasive Property of SARS-CoV-2

SARS-CoV-2 shares a number of similarities to SARS-CoV, according to the clinical symptoms, viral sequence, infectivity, viral receptor, and possibly the neuro-invasive property. So far, SARS-CoV-2 has been found to cause extra-pulmonary infection, such as kidney, gastrointestinal and possibly heart (107–109). For neuro-invasive property of SARS-CoV-2, there are some cases about the neurological damage in COVID-19 patients, such as olfactory disorder and ocular abnormalities, both of which are related to peripheral nervous system disfunction, with no direct proves manifesting the viral replication in these tissue by RT-PCR (110). As for central nervous system, in the reports conducted by Chen et al., encephalopathy was seen in 23 of the 113 deceased patients, in which the patients presented anorexia, myalgia, and disorders of consciousness (111). However, there is still a lack of evidence from autopsy confirming the viral replication in brain tissue or cerebrospinal fluid, so it remains unclear whether these neurologic symptoms are led by viral infection or simply as a result of pulmonary encephalopathy. To confirm the existence of SARS-CoV-2 in central nervous system, more convincing proves from autopsy histologic examination is needed, especially the lesions in the olfactory bulb, rhinencephalon, paraventricular nucleus, and brain stem.

CONCLUSION

In this review, we have summarized the possible mechanism for SARS-CoV-2 induced CNS infection, and conduct analysis on the potential of SARS-CoV-2 to cause CNS infection. At present the neuro-invasive property of SARS-CoV-2 are still unclear, but it seems that its infectivity in CNS is not as common as the previous SARS-CoV. It is no doubt that the central nervous system serves as an ideal target for SARS-CoV-2, with necessary receptor for viral tropism and feasible pathway for viral spread. At present, whether the SARS-CoV-2 can induce acute or chronic damage to CNS system, whether the SARS-CoV-2 can remain long-term latent infection in CNS system, and whether the latent SARS-CoV-2 will revive under given situation are all remaining to be explored in the follow-up visit. Considering the large number of patients involved, which is far more than the victims of SARS and MERS, clinicians should pay attention to the neurologic symptoms in COVID-19 patient, and act on an early stage. It should be pointed out for clinicians that the clinical manifestation of SARS-CoV-2 CNS infection could be staged, with possible existence of encephalitis in the acute phase (112), postinfectious symptoms in the subacute phase (113), and even involvement in patients vulnerable to neurodegenerative diseases in the chronic phase (114).

AUTHOR CONTRIBUTIONS

LZ conjointly conceptualized the idea for the review. JH and MZ performed the literature search, analyzed cited studies, and wrote the article. XT, YC, and AT critically revised the work and made changes and additions to its intellectual content. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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REVIEW
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A Contemporary Review of Neurological Sequelae of COVID-19

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Coronavirus 2019 (COVID-19) is currently the center of what has become a public health crisis. While the virus is well-known for its trademark effects on respiratory function, neurological damage has been reported to affect a considerable proportion of severe cases. To characterize the neuro-invasive potential of this disease, a contemporary review of COVID-19 and its neurological sequelae was conducted using the limited, but growing, literature that is available. These neurological squeal are based on the manifestations that the virus has on normal central and peripheral nervous system function. The authors present the virology of the SARS-CoV-2 agent by analyzing its classification as an enveloped, positive-stranded RNA virus. A comprehensive timeline is then presented, indicating the progression of the disease as a public health threat. Furthermore, underlying chronic neurological conditions potentially lead to more adverse cases of COVID-19. SARS-CoV-2 may reach ACE2 receptors on neuronal tissue through mode of the general circulation. The CNS may also be susceptible to an immune response where a "cytokine storm" can manifest into neural injury. Histological evidence is provided, while symptoms such as headache and vertigo are highlighted as CNS manifestations of COVID-19. Treatment of these symptoms is addressed with paracetamol being recommended as a possible, but not conclusive, treatment to some CNS symptoms. The authors then discuss the peripheral nervous system sequelae and COVID's impact on causing chemosensory dysfunction starting with viral attack on olfactory sensory neurons and cells types within the lining of the nose. Histological evidence is also provided while symptoms such as anosmia and ageusia are characterized as PNS manifestations. Possible treatment options for these symptoms are then addressed as a major limitation, as anecdotal, and not conclusive evidence can be made. Finally, preventive measures of the neurological sequelae are addressed using a multidirectional approach. Postmortem examinations of the brains of COVID-19 patients are suggested as being a possible key to formulating new understandings of its neuropathology. Lastly, the authors suggest a more comprehensive neurological follow-up of recovered patients, in order to better characterize the neurological sequelae of this illness.

Keywords: COVID 19, coronavirus, neurological sequelae, neurological symptoms, neuroinfection

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INTRODUCTION

Epidemiology

The emergence of the coronavirus and its viral agent, severe respiratory syndrome coronavirus 2 (SARS-CoV-2), has derived into one of the most unprecedented and considerable pandemics of the modern era. The third zoonotic coronavirus this century, its pathogenic nature and large scale adverse medical effects has cemented its status as one of history's greatest public health threats. This virus was first isolated in December 2019 after many patients in Wuhan, China, were diagnosed with pneumonia of unknown etiology (1–3). Following its global dispersion, the World Health Organization (WHO) declared the coronavirus as a pandemic due to its rapidly transmissible nature, increasing mortality rate, and limited treatment options. Currently, there is an ongoing outbreak as the number of COVID-19 cases exponentially increases with a reported low to moderate mortality rate internationally. As of late May 2020, there are over 5.7 million cases confirmed worldwide dispersed throughout 212 countries and territories, including almost 353,000 deaths (1, 2).

Until recently, COVID-19 was investigated as primarily a respiratory illness. However, as the number of cases continue to grow, more reports are starting to show that the virus is capable of not only lung damage, but also neurological damage. In a case series from Wuhan, neurological symptoms were noticed in 36.4% of patients with severe infections (4). This notable proportion of cases presents a question on what neurological symptoms are manifested along with how the virus manages to present them. As of May 1st, 2020, remdesivir has been approved for some emergency treatment usage. However, no other clinically approved vaccine or specific antiviral treatments has provided conclusive relief to COVID-19 and its physiological manifestations. Therefore, understanding the clinical impact of SARS-CoV-2, along with its underlying mechanisms is of great importance to promote the development of effective, preventative, and therapeutic counteragents. Herein, we will characterize the virus, along with its neurological impact, through a thorough a review of the literature to date.

Virology

Coronaviruses (CoVs; family *Coronaviridae*, subfamily *Coronavirinae*) circulate in a diverse array of mammalian and avian reservoirs. CoVs are classified into four genera and are enveloped, positive-stranded, RNA viruses known to have large genomes susceptible to recombination and mutations that lead to the emergence of novel viruses. They can cause respiratory, enteric, hepatic, and neurological diseases (5–7). There are still some inconsistencies related to the origin of SARS-CoV-2, with research evidence suggesting that it could be from similar origins of its viral relatives. Both betacoronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), had originated in bats, and it is likely that SARS-CoV-2 did as well from an unknown intermediate host (3, 5, 6).

The estimated reproduction number (R_0) for SARS-CoV-2 ranges from 2.2 to 5.5 (3, 8, 9), meaning one person can potentially transmit the disease to 5–6 people, making it highly transmissible. Additionally, SARS-CoV-2 research has revealed specific physiochemical and thermal sensitivities. Properties of SARS-CoV-2 can be inactivated by UV light or at a temperature of 56°C for 30 min. Disinfectants such as diethyl ether, 75% ethanol, chlorine, peracetic acid, and chloroform destabilize its functional integrity (8). The virus has the longest viability on stainless steel and plastic surfaces and can be detected up to 72 h after initial contact to these surfaces (8). Genomic studies of the virus have revealed that it mutated to produce two variants, L and S. The L-type was more prevalent during early stages of the outbreak and can replicate faster while being more transmissible. The S-type is considered an older and milder variant (8).

Timeline: From History to Today

The first human coronavirus was detected by Tyrrell and Bynoe in the 1960s in human embryonic tracheal organ cultures obtained from the respiratory tract of an adult with a common cold. After performing electron microscopy on the samples of this new group of viruses, the morphology denoted a crown-like appearance of the surface projections and thus was named coronavirus (3, 10). Since then, SARS-CoV-2 is the seventh coronavirus that is known to infect humans. The first pandemic of the 21st century was caused by SARS-CoV followed by MERS-CoV. In 2002, the SARS-CoV emerged in Guangdong Province, China, spreading to 37 countries, and its subsequent global epidemic was associated with 8,096 cases and 774 deaths. A decade later, the MERS-CoV spread to 27 countries, causing 2,494 infected cases and 858 deaths worldwide (**Figure 1**) (2, 6).

SUSCEPTIBILITY

According to the Centers for Disease Control and Prevention, risk of susceptibility to COVID-19 can be stratified into risk of exposure and risk of severe illness (11). Increased risk of exposure to SARS-CoV-2 has been shown to occur in places with ongoing community spread, such as between healthcare workers, close contacts of persons with COVID-19, and travelers from affected international locations. Of those infected, increased severity of illness can be seen more commonly in people aged 65 years and older, those living in a nursing home or long-term care facility, and in people with underlying medical conditions such as hypertension, chronic lung disease, moderate to severe asthma, cardiac disease, immunocompromised states (i.e., cancer, smoking, HIV or AIDS, steroids), BMI > 40, diabetes, chronic kidney disease, and liver disease (11–16). Moreover, The Chinese Center for Disease Control and Prevention similarly reported from one of their largest studies of 72,314 cases that high case-fatality rates came from those with similar comorbidities: cardiovascular disease (10.5%), diabetes (7.3%), chronic respiratory disease (6.3%), hypertension (6%), and cancer (5.6%) (17). In addition, those with cerebrovascular disease alongside coronary artery disease had a higher relative

Abbreviations: WHO, World Health Organization; COVID-19, Coronavirus Disease-2019; ICTV, International Committee on Taxonomy of Viruses; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus.

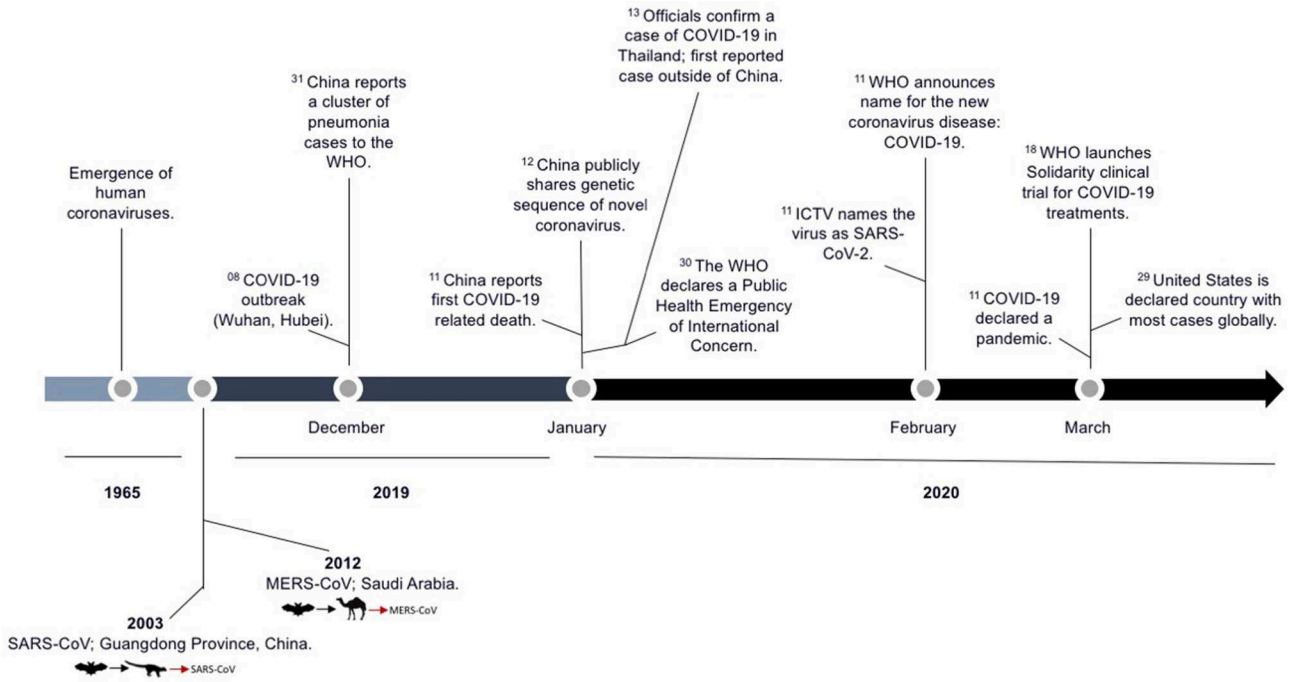

FIGURE 1 | Timeline of the SARS-CoV-2 outbreak. Significant epidemiological events and scientific advances during this time period are highlighted and adapted from the WHO and recently published data (1–3, 10). The superscript number adjacent to the text indicate the calendar date of each event described.

risk of acquiring the pathogen more severely than those without this comorbidity (18). Finally, and most applicable to the area of interest, those with chronic neurological pathologies present an added risk of developing a more severe case of COVID-19 (19). Neurological or neurodevelopment conditions (such as cerebral palsy, epilepsy, stroke, intellectual disabilities, spinal cord injury, or muscular dystrophy), present additional risk factors that increase susceptibility to a severe course of COVID-19 through mechanisms that add physiological stress (19). The current epidemiology demonstrates the impact SARS-CoV-2 can have on the health and well-being of people with underlying health conditions, highlighting its potential effects on multiple organ systems in the body. However, due to this virus being relatively new, not all risk factors have been identified adding limitation to this understanding.

NEUROLOGICAL IMPACT

Central Nervous System

With much being said about SARS CoV-2's hallmark impact on respiratory function, gradual advances in research have found properties of neuroinvasive potential, particularly in the invasion of the central nervous system (CNS). It is widely understood that the mode of transmission of COVID-19 is through direct contact or respiratory droplets from an infected individual. However, indirect contact through fomites can also act as a vehicle of transmission. Fomites are inanimate surfaces that contain the virus (through direct contact), and based on the viability of the virus on to an inanimate surface, it can lead to the rampant spread onto another host. As soon as the virus enters the body, its effects are highly dependent on its viral structure. The viral structure of COVID-19 is contained within the nucleocapsid which is surrounded by a nuclear envelope. The nuclear envelope is derived from the host cell's membrane and embedded within that membrane are glycoprotein spikes, known as S-proteins (20). It is these S-protein spikes that allow the cell to attach to a new cell and infect it (20). To carry through its function, S-proteins contain an S1/S2 activation cleavage site that is activated by the serine endoprotease, furin (21). Furin is a key protein that can cause normal and abnormal physiological conditions. It is important neurologically because it is responsible for activating neural growth factors that are key to homeostasis (22). However, in the case of SARS-CoV-2, furin plays the role of a viral activator. This activation occurs through furin's enzymatic activity, which allows for the irreversible cleavage of precursor proteins to enter their biologically active state (21). With regards to SARS-CoV-2, furin autocleavage helps the S-protein's subunits to separate, and allow for the virus to break open and enter host cells (21). This is done after the S-proteins have interacted with the cell surface receptor, angiotensin-converting enzyme 2, or ACE2 (21). The ACE2 receptor is present along multiple cell organs such as the heart, kidney, lunges, while also being found in both the central and peripheral nervous system. This makes it a valuable target in understanding the neural-potential of the SARS-CoV-2 antigen.

The pathophysiology of neural infections in the CNS from COVID-19 is a phenomenon that is still being characterized. Previous studies have shown that SARS-CoV-2 may reach ACE2 receptors on neuronal tissue through mode of the general

circulation. Viremia causes the virus to pass into the cerebral circulation and reach the brain to induce neurotropic effects. The virus may also reach the brain through routes of the nasal cavity, specifically as the virus passes through the cribriform plate. Upon entry into the neural cavity, the virus will encounter ACE2 receptors located on the endothelial lining between the blood capillaries and the brain (23). The virus may also encounter the expression of ACE2 receptors over glial cells and neurons, allowing for multiple sites of invasion. The interaction of the glycoproteins of SARS-CoV-2 to the ACE2 receptor can cause subsequent cycles of viral budding, allowing for neuronal damage to manifest in neural tissues and the blood-brain-barrier (BBB) (23). The breaking of the BBB may allow for cerebral edema, subsequently compressing the brainstem and compromising involuntary respiratory activity (24). This cerebral edema may also be caused by apoptosis of brain cells following elevated intracranial pressure and therefore remains a theoretical concern of the virus. Evidently, patients exhibiting acute SARS-CoV illnesses have presented the virus in cerebrospinal fluid analyses (23). The cerebrospinal fluid (CSF) is a bodily fluid that surrounds the brain and serves an array of functions ranging from acting as a shock absorber from neurological trauma to circulating nutrients around the CNS. Its role in homeostasis and neurological metabolism has made it a great reporter of the neurological environment; therefore, it can be clinically used to detect infectious agents and diseases (25). Furthermore, viral implications on the immune system could induce indirect effects on the CNS. The breakage in the blood-brain barrier could leave an immune response of cytokines (particularly IL-6) becoming overly abundant along infected tissue of the brain (26). This leads to a hypercoagulable state in what is known as a "cytokine storm." Depending on the severity of the case, this could leave COVID-19 patients at risk of developing acute necrotizing encephalopathy (ANE) and hemorrhages (27). Most recently, a third theory discusses how neural injury is manifested from COVID-19 as a result of a cascade effect following respiratory stress. A loss of oxygen due to lung damage can subsequently result in multisystem organ failure leading to a cascade effect that results in neural injury (28). Ultimately, what these three theories provide are multiple mechanisms through which neural manifestations of COVID-19 can result. These theories also highlight the limitations in addressing neurological manifestations in COVID-19, as research remains ongoing.

A recent case of a female patient in Detroit, Michigan showed the possible long-term impact of neurological manifestations of COVID-19 (27) while also presenting some histological significance. This patient was jointly diagnosed with COVID-19 and ANE due to possible subsequent neural injury. CT scans of her brain revealed symmetrical tissue damage of the thalamus while her MRI also showed damage to the thalamus along with the cerebral cortex and the brain tissue beneath its gyri (folds within the cerebral cortex) (27). Histological significance is evident in her CT image as hypodense areas are present, indicating tissue damage had occurred. This decrease in density is a result of cerebral edema, when excess fluid floods brain tissue after neural injury, a possible consequence of severe CNS manifestations of COVID-19 (27). As previously stated, the cause of this cerebral edema is possibly through a "cytokine storm," apoptosis of brain cells, or even from breakage in the BBB. In addition, other severe cases aside from ANE and hemorrhage are prevalent, as the first case of meningitis has been associated with COVID-19 (29). A male patient was found to have this condition after his MRI showed hypersensitivity along the right lateral ventricle, as well as hyperintesnse signal changes in the right mesial temporal lobe and hippocampus (29). He was confirmed to have COVID-19 after SARS-CoV-2 was found in his CSF, however, there was no evidence of cerebral edema like the previous case mentioned (29). Thus, the variability in histological evidence could link theories of pathophysiology regarding COVID-19's impact on the CNS.

TABLE 1 | Overview of COVID-19's impact on the central and peripheral nervous system, as known to date.

	CNS Overview of COVID-19	**PNS Overview of COVID-19**
How is it transferred?	Directly: respiratory droplets & direct contact with an infected individual Indirectly: Fomites/Surfaces	Directly: respiratory droplets & direct contact with an infected individual Indirectly: Fomites/Surfaces
Pathophysiology theories	1. Viral Entry into the Brain 2. Adverse-Immune Responses 3. Respiratory Stress	1.Chemosensory Dysfunction
Histological significance found:	Hypodense areas in CT scans, following positive testing for COVID-19	Falling out of hair-like receptors on olfactory tissue
Main symptoms:	Headache and Vertigo	Anosmia and Ageusia
Major symptoms:	Stroke, Meningitis, ANE, Hemorrhages	Guillain-Barre Syndrome (GBS) and Miller Fisher Syndrome
Treatment/ Management/ Recovery:	No conclusive treatment: Paracetamol has been seen to provide headache and vertigo relief without worsening COVID-19 symptoms	No conclusive treatment: Nasal Spray being developed for medication delivery

Limitations are prevalent as a neurological understanding is yet to be fully confirmed.

While the more severe symptomatic manifestations of the CNS may include ANE, meningitis, and hemorrhage, the more commonly reported CNS symptoms are vertigo, cephalgia, impaired consciousness, seizures, ataxia, and acute cerebrovascular disease (30) (**Table 1**). In a study published to JAMA Neurology, patients with CNS manifestations most commonly exhibited headache and dizziness (30) as the major symptoms. These manifestations have been reviewed and confirmed by trained neurologists, with most neurological symptoms occurring during the early stages of the illness (30). Laboratory findings of these patients reported lower lymphocyte and platelet counts and higher blood urea nitrogen levels. However, in more mild cases, no significance was found between patients with or without CNS manifestations of COVID-19 (4). Therefore, it was concluded that patients with severe cases were more susceptible to neurological symptoms. Moreover, the more severe cases had higher fibrin degradation product

levels (D-dimer), linking these cases to a higher likelihood of cerebrovascular disease (4). Furthermore, recent findings have reported that a portion of patients are developing symptoms of stroke. Specifically, reports coming out of New York health systems have reported that large vessel strokes can be manifested, even in individuals under the age of 50 (31). A large vessel stroke is an interruption of blood flow in one of the larger arteries in the brain. The suspected cause of these strokes are investigated as an immunohematological issue in allowing for blood clotting throughout a patient's body (31).

Treatment of COVID-19 and its CNS manifestations are still ongoing. However, if the coronavirus is breaching the blood-brain barrier, this could complicate the recovery process (32). The role of the BBB is to serve as the gatekeeper in cerebral passage, therefore medication delivery to the BBB would be difficult because medications are considered as antigens to it, thereby preventing their therapeutic effects (32). Since differences in genetic makeup can increase susceptibility to viral invasion of the BBB, technologies such as CRISPER/CAS9 presents a possible way to target these adverse genetic differences. By targeting specific sequences to engineer candidate risk genes in the BBB, one can assess if BBB permeability is strengthened (33). With regards to the symptomatic prevalence of the disease, hydroxychloroquine has been notably investigated as a possible therapeutic avenue. However, in certain patients, this antimalarial drug may induce adverse neuropsychiatric effects, such as worsening of CNS symptoms like headaches, vertigo, and increased anxiety (30). This makes hydroxychloroquine a less viable treatment for symptoms pertaining to the CNS, and alternative therapies should be considered for patients suffering from these symptoms or from chronic mental instability (22). Furthermore, the NHS recommends paracetamol for CNS-like symptoms such as fevers and headaches due to its reported symptom relief and low likelihood of complications. However, ibuprofen and other NSAIDs are still being evaluated after being ruled out as a therapeutic option (34). They were initially ruled out due to reports that anti-inflammatory drugs may exacerbate COVID-19 symptoms, due to possibly causing a dampened immune response to viral attack (35). These reports have since been rejected by the World Health Organization due to a lack of evidence suggesting that anti-inflammatory treatments are a direct threat to a patient's immunity (34). However, this does not present NSAIDs as a potential therapy, especially when symptoms become more complicated. With regards to blocking viral activity and ultimately CNS manifestations, furin inhibitors have also been investigated as a possible treatment by preventing the activation of the S-proteins on the viral surface (30). However, with furin's important role in normal physiology, researchers are evaluating whether a molecule could be secreted to separate its activity from the virus. Unfortunately, there are still considerable limitations in finding a viable treatment for COVID-19's CNS manifestations, as clinical trials will continue until a vaccine becomes readily available.

Peripheral Nervous System Involvement

COVID-19, like its pathogenic relatives, could also specifically compromise the activity of the peripheral nervous system. Just like the CNS, PNS manifestations arise following direct contact through respiratory droplets or indirect contact through fomites. However, as an individual starts to touch mucosal membranes, such as their eyes, nose, or mouth, it presents a portal of entry for the virus to disrupt neurological sensations that pertain to these sites. Moreover, just like the CNS manifestations, furin's endoproteolytic activity is maintained in order to activate the virus following its interactions with ACE2 receptors. These consistencies are not just present within the nervous system but along all physiological systems that get affected through COVID-19. However, COVID-19, has a peripheral neural involvement that occurs when ACE2 receptors are interacting with the viral agent. These ACE2 receptors have been found along the tongue, mouth, and nasal passage showing the possibility of the virus targeting multiple neurons and influencing those senses tremendously (36).

It was recently discovered that two cell types in the nose are the most likely initial infection points for viral entry into the PNS. These two cell types are called goblet cells and ciliated cells (37). Goblet cells are epithelial cells that can produce mucus on the surface of the mucosal membrane, thereby lubricating the epithelium and protecting it against foreign invaders (38). They are found in several physiological systems, including the respiratory system, consequently leading to their involvement in the pathophysiologies of several respiratory diseases such as COVID-19 (38). On the other hand, ciliated cells are another cell type that is known for having tiny hair-like projections anchored along the lining of the epithelial respiratory tract. Ciliated cells aid in mucus transport and stopping foreign bacteria from reaching the lunges (39). These two cell types are related in the sense that they both contain high concentrations of the ACE2 receptor that allows SARS-CoV-2 to enter host cells (38). In addition, they contain high concentrations of another protease called TMPRSS2 (38). TMPRSS2 is a cell surface protease that can have cumulative effects with furin to promote SARS-CoV-2 activation and entry (40). To understand what types of cells are infected following initial viral entry, it is important to understand that the sense of smell and taste are closely associated with one another. Both rely on the same type of sensory cells, called chemoreceptors, which are activated when they meet certain types of chemical stimuli. SARS-CoV-2 could disrupt these chemoreceptors by reaching the olfactory mucosa (which contains epithelium cells, blood vessels, and axons from olfactory neurons) and initiate an inflammatory response (24). This area is joined with the olfactory bulb by the cribriform plate, which allows for the transmission of olfaction senses at the base of the frontal lobe (41). Here, neurons can be infected while endangering deeper cerebral tissue (24). This highlights the linkage between the CNS and PNS during the viral invasion through the nasal cavity (**Figure 2**). Moreover, should defects in respiration be considerable, then PNS chemoreceptic dysfunction may be presented due to the proximity of sensory neurons in the olfactory bulb to deeper brain tissue (42).

Histological significance pertaining to the PNS involvement was found following analysis of infected olfactory tissue. Professor Carl Philpott, director of medical affairs and research at Fifth Sense, states that while looking at the olfactory tissue

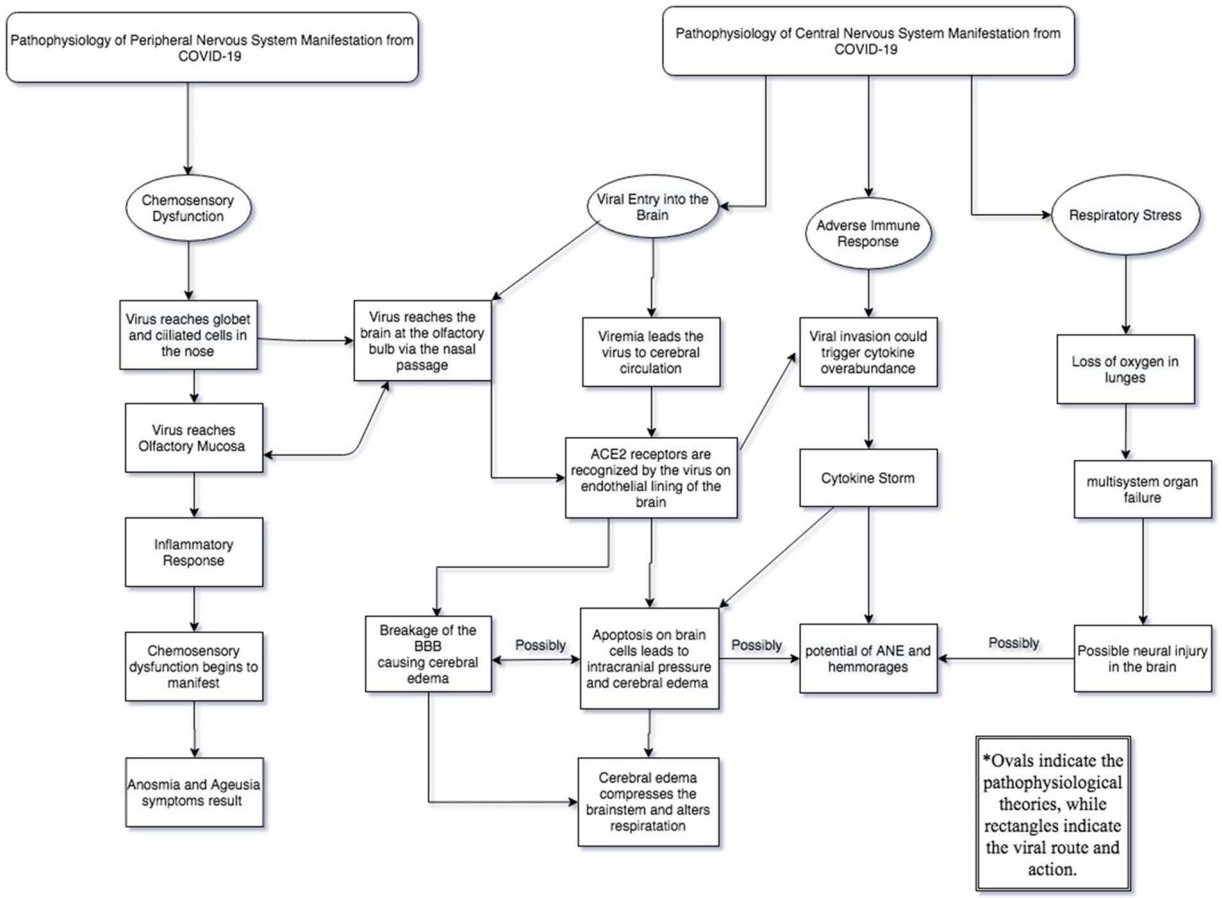

FIGURE 2 | The pathway of the virus in the central and peripheral nervous system which are connected when the virus reaches the olfactory bulb.

microscopically, one will notice the fine hair-like endings of the receptor cells had fallen, thus disabling the cells from receiving olfactory senses from the nose (43). This is a byproduct of the virus interacting with the chemoreceptors in the mucosal membrane and compromising neural sensations. Further histological evidence on peripheral tissue and other mucosal membranes have not been actively reported, thus highlighting limitations to understanding peripheral involvement following viral activity.

According to the American Academy of Otolaryngology—Head and Neck Surgery, otolaryngologists in the U.K have reported that more than two-thirds of confirmed COVID-19 cases in Germany had anosmia (loss of smell) (44). While in South Korea, 30% of patients testing positive have had anosmia as their primary symptom in otherwise mild cases (44). Similar reports have been found globally, as anosmia and ageusia are highly reported as primary symptoms of COVID-19. In another study, patients with confirmed cases of COVID-19 were recruited from 12 European hospitals to investigate olfactory and gaustory dysfunctions (45). Out of the 417 patients studied, between 85.6 and 88% of patients reported these dysfunctions, with significantly more cases in females (45). 44% of the participants also reported their symptoms early in their pathology (45). Physicians state that it's not surprising that the olfactory system might be heavily influenced, given that loss of smell and changes in taste are a rather common complaint of people who get influenza as well (46). Other symptoms may include hyposmia, neuralgia, and skeletal muscular symptoms, however, these symptoms are not considered primary peripheral manifestations due to a lack of supporting evidence. Additionally, a single case suggests a possible association between Guillain-Barré Syndrome (GBS) and SARS-CoV-2 infection (47, 48). A female patient who returned from Wuhan, China, underwent neurologic examination and was diagnosed with GBS while also testing positive for SARS-CoV-2 (48). This was followed with five more cases also displaying COVID-19 with GBS complications (49). GBS is a rare autoimmune disorder that attacks nerves in the PNS and can lead to paresthesia, areflexia, ataxia, and muscle and facial weakness, or paralysis (49). Despite being a unique condition, viral infections can be triggers of GBS, especially when these infections reach a severe state. Primary symptoms of GBS, such as tingling, leg weakness, and facial weakness, were noticed in COVID-19 patients around 5–10 days after the initial common COVID symptoms (49). After a

few days, more adverse symptoms such as quadraparesis and paralysis were recognized (49). Moreover, a variant of GBS, known as Miller Fisher Syndrome, has also been reported in two cases of COVID-19 from Madrid, Spain (50). Miller-Fisher Syndrome is characterized by areflexia and paralysis of the eyes and may also be proceeded by a viral agent's neurological activity (51). While still being a rare condition, the association between COVID-19, GBS, and Miller-Fisher Syndrome warrants further epidemiological data to support a causal relationship between them (47).

As far as treatment, research is ongoing with clinical trials still being administered to determine the most viable form of care. Once the virus has started impacting neuronal activity, it becomes very difficult to treat, meaning the best mode of treatment is by preventing or reducing the viral entry. However, The Center of Disease Control recently stated: "There are no drugs or other therapeutics approved by the US Food and Drug Administration to prevent or treat COVID-19" (52). This adds further limitations to addressing PNS neural manifestations of COVID-19. The British Rhinological Society has advised against the use of oral steroids in the treatment of anosmia in COVID-19 patients (53). This precaution comes with the concern that corticosteroid use may increase severity of the pathogen. However, due to the concentrated presence of the coronavirus in the nasal tissues, a nasal spray has been suspected as the best mode in medication delivery (54). Recent reports from Utah have started addressing the efficacy of chlorpheniramine maleate (CPM) combined into a nasal spray to study cultures with SARS-CoV-2 (54). CPM is an antihistamine with previous studies suggesting that it acts well in the antiviral treatment against strains of influenza (54). The initial trials have showed relative success with reduced amounts of the virus being reported *in vivo* following CPM administration (54). However, this requires further testing in order to accurately and effectively treat COVID patients. With more severe patients who may have acquired GBS with COVID-19, intravenous immunoglobulin therapy (IVIG) have been known as the most common treatment for these patients (49). IVIG treatment allows for the administration of healthy antibodies and has been suggested to be taken with antivirals to further combat the COVID like symptoms (49). Plasmapheresis with antiviral treatment has also been suggested as another treatment option to enhance the autoimmune system while reducing viral activity (49). With regards to the two cases with Miller-Fisher Syndrome, the first was treated with IVIG while the second with acetaminophen. After two weeks, it was seen that both patients made a complete neurological recovery, except for primary symptoms of anosmia and ageusia from their COVID-19 diagnosis (50). However, these reports have yet to be confirmed as a definite viable treatment and may not directly lead to every patient's recovery.

To better combat PNS manifestations today, ENT UK released an info-sheet encouraging those who experience sudden loss of smell or taste during the COVID-19 pandemic to assume they have the virus until tests prove negative. The management plan included: self-isolation, avoiding visits to medical professionals or hospitals unless symptoms require proper treatment, and try smell training to improve senses. Furthermore, The AAO-HNS created a web-based reporting tool for any health-care professionals to file reports of patients experiencing anosmia and ageusia related to COVID-19. Until a viable treatment is released to the public, these guidelines will help those with chemosensory dysfunction to be informed and aware of what conditions to follow.

AVOIDANCE OF DETRIMENTAL NEUROLOGICAL SEQUELAE AND MULTIDISCIPLINARY APPROACH

Following the emergence of more literature findings about the neurological manifestations of COVID-19, an increasing number of studies are now urging clinicians to screen for neurological presentations of this disease (1, 4, 23, 55, 56). As previously mentioned, the symptoms present a neurological pathology, while those with more severe cases have been susceptible to neurological complications. Thus, these studies are urging clinicians to comprehensively assess the neurological symptoms in patients coming into acute healthcare settings, to effectively triage patients, delay diagnosis and misdiagnosis, and prevent transmission of the virus. Although preliminary, studies suggest that surveillance of the neurological manifestations of COVID-19 may have guiding significance for the prevention and treatment of COVID-19 induced respiratory failure and death (4, 55).

Knowledge and clinical guidelines surrounding treatment of COVID-19 is rapidly evolving as more information becomes available. At this time, knowledge surrounding the prevention and treatment of neurological complications of COVID-19 is limited. One study published several clinical guidelines for neurologists treating patients with suspected or confirmed cases of COVID-19(57). These guidelines were pertaining to the management of acute cerebrovascular disease, intracranial infection, and muscle damage as a result of SARS-CoV-2.

In the context of SARS-CoV-2, neurologists may encounter two phenotypes of patients with acute cerebrovascular diseases—acute ischemic stroke patients, and hypertensive patients with increased risk of intracranial hemorrhage. The authors recommend that emergency treatment be jointly offered by neurologists and infectious disease specialists when admitting an acute ischemic stroke patient with suspected or confirmed SARS-CoV-2 diagnosis, and preventive anticoagulation is recommended for ischemic stroke patients with high D-dimer levels (57). Hypertensive patients with SARS-CoV-2 may be particularly challenging to treat, as they may encounter blood pressure fluctuations (since SARS-CoV-2 specifically binds to ACE2 receptors), which may increase their risk of intracranial hemorrhage. Additionally, patients with severe infection may have severe thrombocytopenia, which is another risk factor for cerebral hemorrhage. Per the guidelines, clinicians treating hypertensive patients with SARS-CoV-2 should consider calcium channel blockers, diuretics and other classes of antihypertensive drugs instead of ACE inhibitors or angiotensin II receptor blockers (ARBs) (57).

Further surveillance of intracranial infection for COVID-19 patients would provide further insight in a neurological

prognosis. MRI scans (with and without contrast) of the cranium along with lumbar puncture procedures to collect cerebrospinal fluid would highlight such a neuroinvasive association to the pathogen (57). For patients with definitive intracranial infection, suggested treatment strategies are controlling for cerebral edema, treating and preventing seizures, and treating psychotic symptoms (57). Lastly, for patients experiencing symptoms associated with muscle damage, strengthening nutritional support is recommended on top of active treatment for the virus (57).

CONCLUSION

In summary, the SARS-CoV-2 pandemic remains one of the greatest threats to public health to date. Although the virus is known to directly invade the lungs, emerging research shows central and peripheral nervous system involvement in the pathology of the disease and clinical worsening in patients. Patients with nervous system involvement as the presenting symptoms in the early stages of infection may be misdiagnosed, and may inadvertently spread the virus. Furthermore, studies suggest that the time it takes for SARS-CoV-2 to advance from initial symptoms, such as coughing, tiredness, and fever, to difficulty breathing, is roughly five days, which is long enough for the virus to enter and damage medullary neurons involved in respiratory control (4, 55). Thus, healthcare providers and neurologists should work closely to monitor the neurological manifestations of the disease and carry a high index of suspicion when evaluating patients in an endemic area. In some cases, tele-neurology as a substitute for face to face interactions with patients to monitor their clinical status may be useful.

Going forward, to elucidate the involvement of the nervous system in the progression of this disease, postmortem examinations of the brain may be a valuable facet in understanding the neurological mechanisms the virus manifests. Currently, few, if any, autopsies are being performed due to the fear of contracting the disease. Furthermore, a more comprehensive neurological follow-up of patients who recovered from the disease may be warranted to screen for long-lasting neurological impacts of the illness and save lives.

AUTHOR CONTRIBUTIONS

BF was the primary author contributing to the outline/direction of the paper, literature review, and writing, editing, and overseeing of the paper as a whole including submission process. RJ played a major role in writing and editing the paper. MS, AD, and CC focused their efforts on writing. All authors contributed to the article and approved the submitted version.

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Cytokine Storm in COVID-19—Immunopathological Mechanisms, Clinical Considerations, and Therapeutic Approaches: The REPROGRAM Consortium Position Paper

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Cytokine storm is an acute hyperinflammatory response that may be responsible for critical illness in many conditions including viral infections, cancer, sepsis, and multi-organ failure. The phenomenon has been implicated in critically ill patients infected with SARS-CoV-2, the novel coronavirus implicated in COVID-19. Critically ill COVID-19 patients experiencing cytokine storm are believed to have a worse prognosis and increased fatality rate. In SARS-CoV-2 infected patients, cytokine storm appears important to the pathogenesis of several severe manifestations of COVID-19: acute respiratory distress syndrome, thromboembolic diseases such as acute ischemic strokes caused by large vessel occlusion and myocardial infarction, encephalitis, acute kidney injury, and vasculitis (Kawasaki-like syndrome in children and renal vasculitis in adult). Understanding the pathogenesis of cytokine storm will help unravel not only risk factors for the condition but also therapeutic strategies to modulate the immune response and deliver improved outcomes in COVID-19 patients at high risk for severe disease. In this article, we present an overview of the cytokine storm and its implications in COVID-19 settings and identify potential pathways or biomarkers that could be targeted for therapy. Leveraging expert opinion, emerging evidence, and a case-based approach, this position paper provides critical insights on cytokine storm from both a prognostic and therapeutic standpoint.

Keywords: COVID-19, cytokine storm, immunological mechanisms, autoimmunity, neuroimmunology, immunotherapies, guidelines, critical care

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*† The COVID19 pandemic is causing an unprecedented public health crisis impacting healthcare systems, healthcare workers, and communities. The COVID-19 Pandemic Health System **RE**silience **PROGRAM** (**REPROGRAM**) consortium is an international not-for-profit think-tank for global pandemic preparedness and action*

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has caused a public health crisis with profound long-term socioeconomic fallout. COVID-19 results from infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus (1). Although the vast majority of patients experience mild to moderate symptoms, the disease remains fatal in a significant proportion of those infected (2–4). Much of the critical illness associated with SARS-CoV-2 infection is believed to be the result of a hyperinflammatory process referred to as hypercytokinemia or a "cytokine storm" (5). A full understanding of the immunopathogenesis, of cytokine storm in COVID-19 patients has the potential to guide future strategies to improve early diagnosis and implement therapeutic strategies to mitigate cytokine storm-associated morbidity and mortality risks (5, 6). This article discusses the implications of hypercytokinemia for COVID-19 patients, including the risk factors for cytokine storm, potential therapeutic strategies (6), and clinical considerations, with special emphasis on patients with cancer, autoimmune diseases, and those undergoing immunosuppressive therapies.

COVID-19 AND CYTOKINE STORM

Pathophysiology

Observations from the first cohort of 41 COVID-19 patients in Wuhan, which led to the discovery of the novel SARS-CoV-2 virus, revealed a cytokine profile similar to that of secondary hemophagocytic lymphohistiocytosis (sHLH), a hyperinflammatory condition triggered by viral infection (2). Patients who were admitted to intensive care unit (ICU) had higher levels of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP1A), and tumor necrosis factor alpha (TNFα) compared to those who were not admitted to ICU (2). Observations from another 150 patients in Wuhan revealed that those who died of COVID-19 complications had higher serum levels of C-reactive protein (CRP), interleukin (IL)-6 and ferritin, suggesting an underlying hyperinflammatory process (3). A combination of these markers may therefore be used as prognostic markers to determine COVID-19 severity. Another study showed that patients experiencing COVID-19-related cardiac injury with the elevated levels of troponin T (TnT) also demonstrated significantly higher CRP and procalcitonin levels (up to 3–4 times more) and experienced increased morbidity and mortality (4).

Patients who die from severe COVID-19 disease experience endothelial cell infection and an endotheliitis affecting many organs (7, 8). The SARS-CoV-2 S protein binds to angiotensin converting enzyme 2 (ACE2) to enter host cells. Most COVID-19 patients present with respiratory symptoms because ACE2 receptors are expressed in vascular endothelial cells of the lower respiratory tract (9). In severe COVID-19 cases, hypercytokinemia in the lungs leads to diffuse alveolar damage, hyaline membrane formation, thrombus formation [confirmed in small vessels at autopsy (10)], fibrin exudates, and fibrotic healing. These pathologic changes result in acute lung injury and manifest clinically as acute respiratory distress syndrome (ARDS) (11). Forty percent of COVID-19 patients experience proteinuria and haematuria, suggesting kidneys infection and injury (12). COVID-19-related kidney injury occurs because ACE2 receptors are found in the kidney in the brush border of proximal tubular cells (12). Although the kidneys of COVID-19 patients examined post-mortem reveal SARS-CoV 2 antigens in the proximal tubules, the role of cytokine storm in causing kidney injury is not yet clear (13).

ACE2 receptors are also present in cardiac tissue and in the gastrointestinal tract, arguably explaining the cardiac and gastrointestinal clinical manifestations in some COVID-19 patients. Available data suggests that those with underlying cardiovascular disease, hypertension, severe dyslipidaemia, obesity, and diabetes are at high risk for severe COVID-19 disease (14), whilst other data indicates that SARS-CoV-2 infects the heart, resulting in myocarditis and myocardial infarctions (6, 7, 15–17). Patients with underlying cardiovascular disease are at increased risk of cytokine storm (4, 18) and poor outcomes. COVID-19 patients with underlying cardiovascular disease are also at higher risk of myocardial injury [with cardiac troponin (TnT) increase], as well as both atherosclerosis-related and thromboembolic events such as stroke, plaque instability, vasculitis, and myocardial infarction (7, 15, 19). COVID-19 has also been presumably linked to central nervous system (CNS) symptoms and conditions including acute necrotizing encephalitis, myalgia, and headache among others although the pathogenesis is uncertain (20–25). Owing to the lower ACE2 expression levels in the CNS tissues, it has been hypothesized that the SARS-CoV-2 *per se* can generate little inflammation (26). Recent autopsy studies found scarce evidence of inflammation (26–30). Whether the transfer of SARS-CoV-2 to CNS tissues potentiate or exacerbate cytokine storm is a subject of ongoing debate (28, 29).

Immunosenescence and Cytokine Storm

Elderly patients, especially older males, with comorbidities, demonstrate increased susceptibility to poor prognosis or increased risk of severe condition or even fatality from COVID-19 (31). Aging is associated with a decline in immune function or "immunosenescence" (32–36). With age, the immune system can present with a series of changes, characterized by immunosenescence markers (34–36), a decrease in the generation of CD3+ T cells, an inversion of the CD4 to CD8 (CD4/CD8) T cells ratio due to the loss of CD8+ T cells (35) (increased CD4/CD8 ratio), an increase in regulatory T cells (Treg) and a decrease in B lymphocytes (34). It is postulated that COVID-19 induced cytokine storm may be contributing to the poor outcomes in elderly patients due to immunosenescence. T lymphocytes can be potentially infected by the virus (37), reducing their number due to their apoptosis. It is currently not known whether the infection of the lymphocytes themselves potentiate cytokine storm or otherwise. In a recent study employing immunomodulatory therapeutic strategy, intravenous transplantation of mesenchymal stem cells (MSCs) was effective, especially in critically severe cases, in a series of 7

patients with COVID-19 pneumonia (38). Immunomodulatory therapies targeting cytokine storm show potential for such approaches in improving outcomes and reducing mortality due to COVID-19 in elderly patients (5, 39). Future studies are required to further evaluate the efficacy of immunomodulatory therapies in preventing cytokine storm induced severe illness in COVID-19 patients in general, and elderly patients in particular (38).

Significance of Cytokine Storm

Hypercytokinemia is an unregulated hyperinflammatory response that results from the systemic spread of a localized inflammatory response to viral or bacterial infection. Elevated cytokine levels result in endothelial dysfunction, vascular damage, and paracrine/metabolic dysregulation, thereby damaging multiple organ systems. Levels of acute-response cytokines (TNF and IL-1β) and chemotactic cytokines (IL-8 and MCP-1) rise early in hypercytokinemia, facilitating a sustained increase in IL-6. IL-6 binds to either membrane bound IL-6 receptor (mIL-6R) or soluble IL-6 receptor (sIL-6R), forming a complex that acts on gp130, regulates levels of IL-6, MCP-1 and GM-CSF via the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, and thereby perpetuates the inflammatory processes (39). IL-6, along with other pleiotropic cytokines, drives an acute phase response that elevates serum ferritin, complement, CRP, and pro-coagulant factors, many of them measurable through commercially available blood tests. The acute phase response of cytokine storm is relatively over-exaggerated. Since high serum levels of cytokines are inversely related to the total lymphocyte count, low levels of cytotoxic T cells may contribute to reduced viral clearance (40). Blocking upstream events related to or at the level of cytokine response, such as JAK-STAT signaling of macrophages to reduce IL-1 and IL-6 production, offers a potential therapeutic target for the cytokine storm. Cell-based target strategies may also be considered, but the time to therapeutic effect of anti-B lymphocytes directed therapies such as rituximab may be too long to be clinically relevant. Therefore, targeting the upstream events may be relatively more effective.

In reaction to SARS-CoV-2 infection, macrophages (41) and dendritic cells trigger an initial immune response, including lymphocytosis and cytokine release. However, the inflammatory response results in the destruction of lymphocytes attempting to stop SARS-CoV-2 infection. Lymphopenia ensues, especially in patients severely affected enough to require ICU admission (42). Cytokine production becomes rapidly dysregulated, damaging healthy cells typically first in the lungs but potentially spreading to other organs including the kidneys, heart, blood vessels, and brain. The cascade of cytokine storm-associated damage begins with disruption of the epithelial barrier in the lungs. Activation of NOD, LRR-, and pyrin domain-containing protein 3 (NLRP-3) inflammasome and the relative blunted response of histone deacetylase 2 on nuclear factor kappa betta (NFκB) complex has been suggested to be associated with cytokine storms. The epithelial barrier disruption exposes the lungs or other tissues to bacterial infection. Pathophysiological mechanisms associated with COVID-19 induced cytokine storms are shown in **Figure 1** (11, 43–50).

We propose that the immune system cytokine network may also communicate with the central nervous system (CNS) cytokine network, especially when the blood-brain-barrier (BBB) is compromised. Microglia and IL-1 activation can cause increased reactive oxygen species (ROS) production, phagocytosis, apoptosis, and increased cytokine expression (see **Figure 2**) within the CNS (43), leading to neural tissue damage through neuroinflammation, increased oxidative stress and excitotoxicity, and dysfunction in synaptic pruning. The systemic immune system cytokine network and the CNS cytokine network influence each other through the neuropeptidergic pathway involving neurokinin C and B, neuroendocrine peptides (NPY)/gastrin-releasing peptide (GRP), SPA-GRP {SPA: [(D-Arg, D-Trp, Leu)Substance P], a derivative of substance P}, and vasoactive intestinal polypeptide (VIP). Activation of macrophages and phagocytosis, chemotaxis with neutrophils and degranulation of mast cells, and activation and proliferation of T-cells activate this pathway. Inflammatory cytokines are also be transported through the blood, which could further amplify the cytokine storm (11). We postulate that the overlapping immune and CNS cytokine networks may drive "immune hijack." In light of these mechanisms and potentially devastating impact of COVID-19 on "high-risk" patients, specific clinical considerations for medical conditions have been discussed in the following section.

CYTOKINE STORM AND HIGH-RISK PATIENTS—CLINICAL CONSIDERATIONS FOR SPECIFIC MEDICAL CONDITIONS

Some of the severe complications associated with COVID-19 are acute respiratory distress syndrome (ARDS) (prevalence of 17–29%), acute cardiac injury including myocarditis, myocardial infarction and cardiac arrest, sepsis, multi-organ failure, ischemic stroke, Kawasaki-like syndrome in children, acute pulmonary embolism, sHLH, and secondary infections such as bacterial pneumonia (**Supplementary Table 1**) (3). Therefore, special considerations must guide management of patients at high risk of severe COVID-19 disease and cytokine storm, including patients with underlying coronary artery disease, obesity, cancer, primary immunoglobulin deficiencies, autoimmune conditions, as well as those receiving immunosuppressive therapies. A risk-based strategy to identify high risk patients is presented in **Table 1**. Measuring the viral load at different time points as well as the immune response may help optimize treatment strategies. Next, we will discuss the radiological findings consistent with hyperinflammation or cytokine storm in COVID-19 cases.

Cytokine Storm and Radiological Findings

Although the association between cytokine storm and the radiological manifestations of COVID-19 pneumonia infection require further investigation, computer assisted tomography

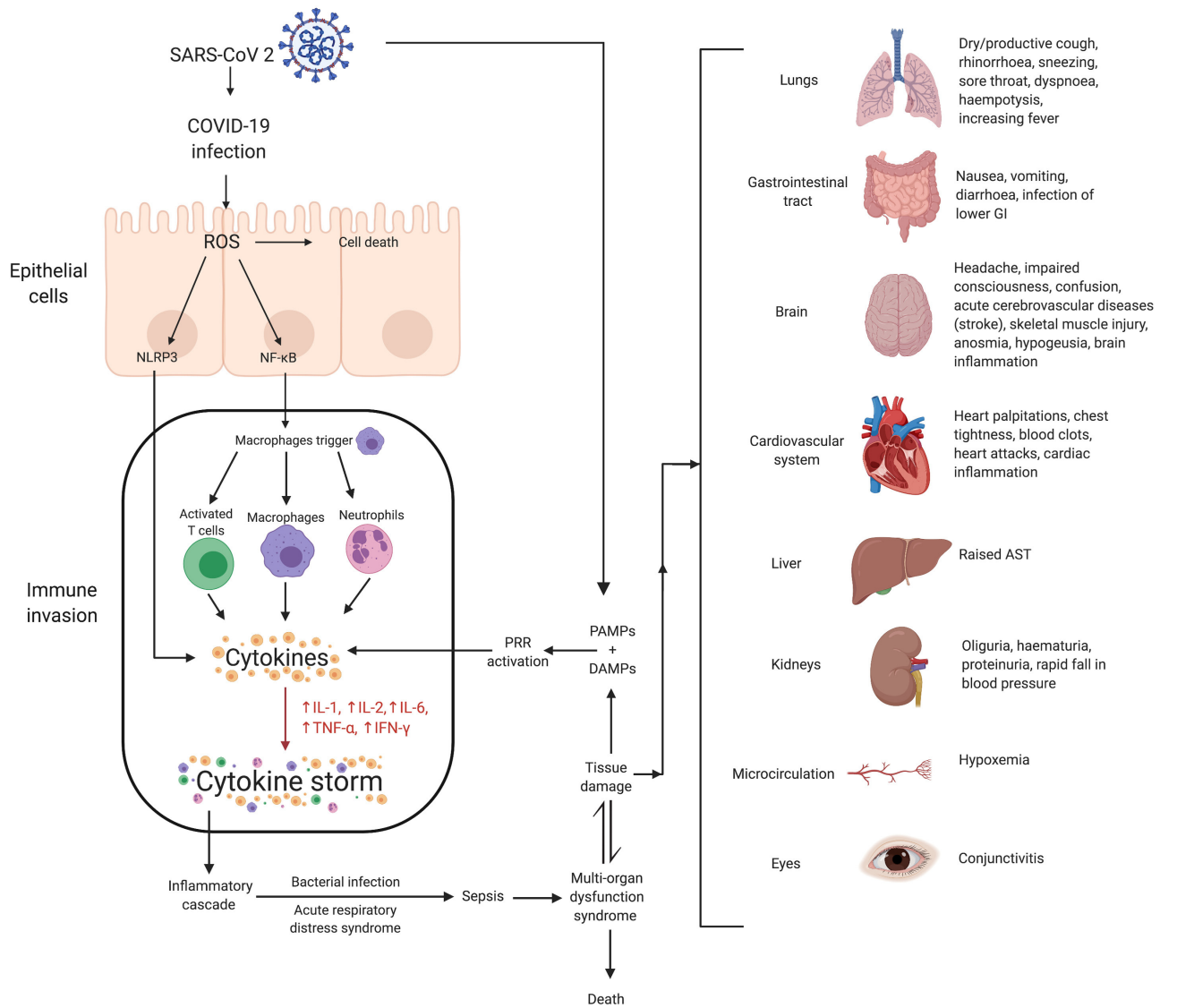

FIGURE 1 | Mechanisms of SARS-CoV-2 associated cytokine storm and associated damages. Infection with SARS-CoV 2 can stimulate a hyperinflammatory immune response wherein epithelial-cell-mediated production of reactive oxygen species (ROS) can cause cell death. ROS can also stimulate the synthesis of NLRP3 and NF-κB which contribute to increased cytokine levels, and thus, the cytokine storm. This essentially causes immune invasion which can lead to clinically relevant conditions such as ARDS, sepsis, MODS and potentially even death. The organs affected as a result of MODS, and their associated symptoms, have been shown. Lower gastrointestinal (GI) is rich in ACE2 receptors and hence at higher risk of infection due to COVID-19. Twenty percent of COVID-19 patients have diarrhea as symptoms. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ROS, reactive oxygen species; NLRP3, (NOD)-like receptor protein 3 inflammasome; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; PRR, pattern recognition receptors; AST, aspartate aminotransferase; MODS, multiple organ dysfunction syndrome.

(CT), the lungs of patient with COVID-19 pneumonia typically demonstrate findings typical of underlying hyperinflammatory pathway (53, 54). On CT chest, the lungs typically demonstrate ground-glass opacities (subpleural, peripheral and bilateral) (53), bronchovascular thickening within lesions, smooth or irregular interlobular or septal thickening, air space consolidation, traction bronchiectasis, ill-defined margins, air bronchograms, and thickening of the adjacent pleura (54). IL-1β induces the production of bronchoalveolar lavage fluid, creating the ground-glass appearance (40). CT findings evolve over time (54–60). Normal CT scans may be seen in the first 3–4 days. During the intermediate stage, septal thickening and increased ground glass opacities appear (57). During the advanced stage, which is usually at 9–13 days of the disease, the features seen in the intermediate stage consolidate. After 14 days of the disease, during the resolution stage, fibrous stripes appear and typically resolve after 1 month (58–60).

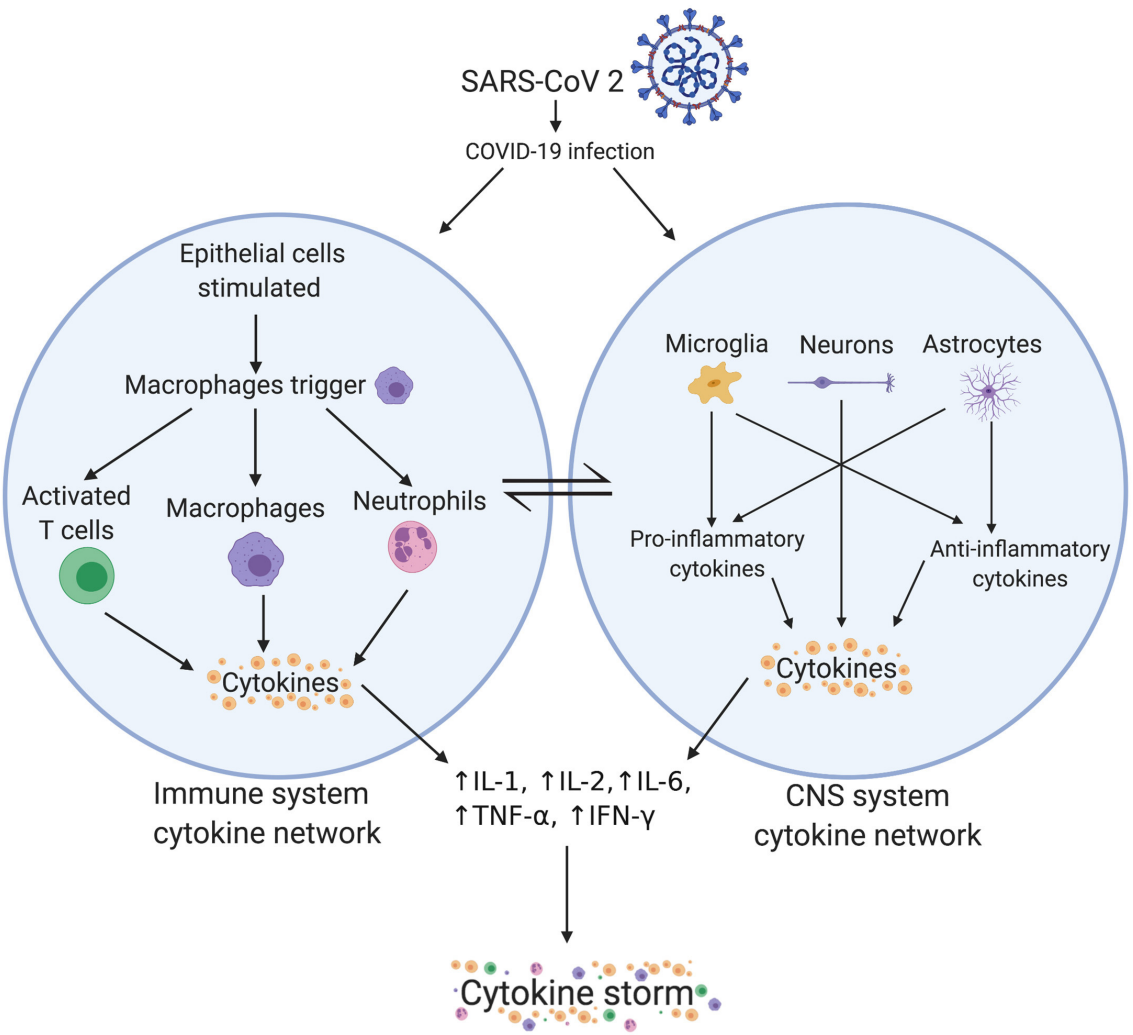

FIGURE 2 | Crosstalk between immune system and CNS system cytokine networks**.** There is a supposed link between the immune system cytokine network and the CNS system cytokine network. Peripheral cytokines can cross the blood brain barrier to enter the CNS. Alternatively, microglia and astrocytes can also produce cytokines. Potential involvement of neurons in regulation of cytokines for example brain-derived neurotrophic factor (BDNF) and interleukin-6 levels is also plausible (51). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; CNS, central nervous system; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon.

COVID-19 Associated Coagulopathy and Its Complications

Patients with COVID-19, especially younger patients, are at a higher risk of hypercoagulability and thereby experience higher rates of arterial and venous thromboses (61–64). A case series reported large vessel ischemic stroke in young asymptomatic or mildly symptomatic COVID-19 patients (62). Critically ill patients appear to experience high rates of acute venous thromboembolism (VTE) (63, 64). In 54 consecutively admitted ICU patients treated with prophylactic low molecular weight heparin since admission, 22.2% experienced VTE [predominantly deep vein thrombosis (DVT)] (63). In a retrospective study of severe COVID-19 patients admitted to ICU ($n = 81$), 25% of the patients not receiving pharmacologic VTE prophylaxis developed lower extremity DVT, and 40% of those patients died. A D-dimer level of >1.5 μg/mL predicted VTE with high sensitivity and specificity (64). Another study of 191 patients reported significantly higher mortality in patients with D-dimer >1.0 μg/mL compared to those whose level was <1.0 μg/mL (65). A 31% cumulative incidence of thrombosis (from ischemic stroke, DVT, acute pulmonary embolism, myocardial infarction, systemic arterial embolism) has been reported, with pulmonary embolism being the most common thrombotic complication (81%) (66). A prothrombin time >3.0 s and a prolonged aPTT >5 s have also been reported to independently predict thrombotic complications (66).

An autopsy series microscopically confirmed the presence of platelets and thrombi in small vessels, thrombi in small vessels in the peripheral aspect of lungs, and scattered areas of diffuse alveolar damage (67). Gross pathological examination

TABLE 1 | Patient at "risk" of severe outcomes after COVID-19 associated cytokine storm.

High-risk patients	Risk category in COVID-19 settings	Therapeutic strategy
Hemodynamic instability or cardiogenic shock	Very high risk	Invasive STEMI* pathway
Cardiac arrest/life-threatening arrhythmia	Very high risk	Invasive STEMI pathway
Acute heart failure	Very high risk	Invasive STEMI pathway
Recurrent intermittent ST elevation	Very high risk	Invasive STEMI pathway
Mechanical complications of myocardial infarction	Very high risk	Invasive STEMI pathway
Established diagnosis of NSTEMI based on cardiac troponins AND at least one of the following (52): 1. recurrent symptoms 2. dynamic ST/T changes (silent or symptomatic)	Very high risk	Testing followed by invasive STEMI strategy
Acute stroke	Very high risk	Acute stroke pathway
Acute meningitis/encephalitis	Very high risk	Respiratory care and ongoing monitoring; increased intracranial pressure pathway; epileptic seizure monitoring
Age $\geq$ 75	High risk	Respiratory care and ongoing monitoring
Solid organ or stem cell transplant patients	High risk	Respiratory care and ongoing monitoring
HIV patients	High risk	Respiratory care and ongoing monitoring
Inherited immune conditions	High risk	Respiratory care and ongoing monitoring
On immunomodulatory therapy	High risk	Respiratory care and ongoing monitoring
Undergoing cancer treatment	High risk	Respiratory care and ongoing monitoring
Obesity	High risk	Respiratory care and ongoing monitoring
Diabetes	High risk	Respiratory care and ongoing monitoring
Established diagnosis of NSTEMI based on cardiac troponins AND at least one of the following (52); 1. Diabetes mellitus or renal insufficiency (estimated glomerular filtration rate $<$ 60 mL/min/1.73 m^2); 2. Left ventricular ejection fraction (LVEF) $<$ 40% or congestive heart failure; 3. Early post infarction angina or prior PCI/CABG	High risk	Non-invasive testing using CCTA, respiratory care and ongoing monitoring
Epileptic seizures, status epilepticus	High risk	Respiratory care and ongoing monitoring; seizure/status epilepticus treatment
Coronary artery disease (CAD)	Intermediate risk	Respiratory care and ongoing monitoring
Cerebrovascular disease	Intermediate risk	Respiratory care and ongoing monitoring
Cardiovascular disease (CVD)**	Intermediate risk	Respiratory care and ongoing monitoring
Pre-existing Hypertension	Intermediate risk	Respiratory care and ongoing monitoring
Smoking	Intermediate risk	Respiratory care and ongoing monitoring
Pneumonia	Intermediate risk	Respiratory care and ongoing monitoring

**NSTEMI, non-ST segment elevated MI; MI, myocardial infarction; CVD, cardiovascular disease; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; CCTA, cardiac computed tomography angiography.*

***CVD is linked to inflammation and oxidative stress, and patients who are not adherent to the anti-inflammatory therapy (Angiotensin-converting-enzyme inhibitors (ACEIs) or angiotensin-II-receptor-antagonists (ARBs) and statins), viruses such as SARS-CoV2 might immediately cause degranulation of macrophages and monocytes in the damaged endothelium, causing atheroma plaque instability, and increased coagulopathy.*

revealed small firm clots in sections of peripheral parenchyma of the lungs. Other autopsy series have revealed microthrombi in small pulmonary arterioles and diffuse alveolar damage in the majority of cases. In light of the findings of high-frequency pulmonary micro thrombosis on histology, the hypothesis of COVID-19 induced coagulopathy or hypercoagulation merits further discussion. In a large retrospective analysis of consecutive severe cases ($n = 449$), elevated D-dimer and prothrombin time were correlated with a higher mortality rate (68). However, neither aPTT nor platelet count was significantly different between mildly and severely affected patients. Elevated levels of D-dimer level may indicate secondary fibrinolysis, contributing to clinically severe manifestations of COVID-19 infections. It is noteworthy that anticoagulation significantly reduced mortality in patients with the International Society for Thrombosis (ISTH) sepsis-induced coagulopathy score of $\geq$4 (40.0 vs. 64.2%) (68). However, there are variations in the incidence of VTE in ICU patients across several centers. A meta-analysis of 9 studies demonstrated that D-dimer level were elevated and coagulopathy more prevalent in patient with severe disease as compared to those with mild disease (69).

The American Society of Haematology recommends VTE prophylaxis with LMWH or fondaparinux (alternative to unfractionated heparin to reduce exposure) in all hospitalized

COVID-19 patients unless the risk of bleeding outweighs thrombosis risk (70). Fondaparinux can also be used in patients with a history of heparin-induced thrombocytopenia (HIT), as those patients are at 5-fold increased risk of severe COVID-19. Mechanical thromboprophylaxis should be used when anticoagulation is either contraindicated or unavailable. According to the recent guidelines of the ISTH, all patients with an elevated D-dimer (typically a 3 to 4-fold increase) should be admitted to the hospital. Fibrinogen levels should be monitored at the later stages of the disease (day 10–14) with >2.0 g/L in both bleeding and non-bleeding patients indicating disseminated intravascular coagulation. The guidelines recommend consideration of LMWH in all patients requiring hospital admission for COVID-19 except those in whom anticoagulation is contraindicated (71). Contraindications for anticoagulation with LMWH are platelet count <25 × 109/L, active bleeding, or severe renal impairment (71). Notably, either an abnormal PT nor aPTT was not listed as a contraindication for anticoagulation with LMWH. Further studies of the association of elevated D-dimer and other coagulopathy markers with cytokine storm and severity of COVID-19 clinical manifestations are warranted. Optimal anticoagulation strategies aimed at correcting or preventing coagulopathy should also be expeditiously studied (14, 18, 19).

Immunosuppressed/Cancer Patients

Certain cancer patients, especially those with hematopoietic or lymphoid malignancies, are at higher risk of severe COVID-19 disease because they are immunocompromised (72, 73). Global Radiation Oncology has made specific recommendations about treating several types of cancers during the COVID-19 pandemic (72). Although patients undergoing chemotherapy or radiotherapy are temporarily immunocompromised, colony stimulating factors can be administered to strengthen their immune system (73). Generally, oncologists are accustomed to managing infections. However, for cancer patients infected with COVID-19, benefit to risk ratio-based chemotherapy is followed in the absence of guidelines and prospective Phase 2 evidence (74). It is recommended that cancer-related treatment be delayed if treatment provides only modest benefit and the biology of the cancer allows for delay. If radiation is being administered for palliative purposes, all alternatives including maximizing analgesics and bisphosphonates should be explored. In situations like painful bone metastases, radiation cannot be avoided (73). In such scenarios, a single 8 Gy fraction should be used because it is as effective as multiple fraction courses (74).

Hematopoietic stem cell transplantation recipients should practice self-isolation prior to transplantation. If such a patient becomes infected with SARS-CoV-2, the procedure should be delayed (75). Complete immunological recovery following stem cell transplantation may take 3 to 6 months, so self-isolation after the procedure is necessary as well. Everyone who comes in direct contact with either a stem cell or an organ transplant recipient should be vaccinated for common respiratory viruses (76). A cancer patient presenting with symptoms suggestive of COVID-19 should also be evaluated for mimics. Pneumonitis from radiation therapy for example, can be treated with corticosteroids, but this same treatment may cause pulmonary injury in COVID-19 patients (75).

HIV-infected patients should be provided sufficient supply of medications to avoid treatment gaps and to allow them to maintain a viral load below the level of detectability. HIV-infected patients who display symptoms of COVID-19 should be prioritized for diagnostic testing because they are at risk for severe complications (77).

Autoimmune Conditions

Patients with systemic autoimmune conditions, including systemic lupus erythematosus (SLE), vasculitis, multiple sclerosis, progressive systemic sclerosis, or rheumatic disease affecting the lungs are at a greater risk of developing complications secondary to respiratory viruses (78). This increased susceptibility to lung disease may be the result of either the underlying disease or immunosuppressive treatments (79). Patients with active autoimmune conditions should continue immunosuppressive treatment because the risk of relapse is more detrimental than the risk of SARS-CoV-2 infection. Stable patients should be maintained on their current therapeutic regimen. Therapy should be changed in stable patients only if they are at a higher risk for exposure to COVID-19 or if they become infected with the disease. In this situation treatment should gradually be reduced and halted consistent with the guidelines from the American College of Rheumatology (80) and the German Society of Rheumatology (81). Corticosteroid injections into joints or soft tissues should only be reserved for severe cases (78, 82). Because psychological stress can induce flare-ups in patients with rheumatic diseases, patients experiencing anxiety, depression, or suicidal thoughts should be referred for mental health support. This can be done through telemedicine to reduce risk of COVID-19 transmission. Patients should be encouraged to maintain their daily routine, such as sleeping a consistent amount of time and maintaining a healthy diet, within the isolation of their homes (78).

THERAPEUTIC STRATEGIES TO TARGET CYTOKINE STORM

Various stages of the cytokine storm pathway can be targeted for therapeutic effects (**Table 2** and **Figure 3**) (110). Cytokine storm has an inciting trigger (viral infection), as well as factors potentiating pathogenic effects and perpetuating the cycle of hyperinflammation. Immunomodulation may improve outcomes even without antiviral drugs (11). A list of ongoing clinical trials targeting cytokine storm and hyperinflammation is presented (**Supplementary Table 2**). The patient's immune and comorbidity profile may modulate response to therapy. Drug interactions with medications used for SARS-CoV2 therapy as well as strategies targeting cytokine storm with antivirals, antiretrovirals, antimalarials (e.g., chloroquine and hydroxychloroquine), or immunomodulators (e.g., tocilizumab) may be considered on a case-by- case basis.

TABLE 2 | Various immunomodulatory strategies targeting cytokine storm in COVID-19 patients.

Strategies or agents	Studies and indications	Safety/drug to drug interactions*	References
Cyclooxygenase (COX) inhibitors	The use of COX inhibitors in COVID-19 has not been evaluated. It should be used on a case-by-case basis.	Should not be used in patients with previous history of stroke, or prior heart bypass surgery (coronary artery bypass graft, or CABG). Cox-2 inhibitors (Celecoxib) have less gastrointestinal side effects than non-steroidal anti-inflammatory drugs (NSAIDS). Similar cardiovascular event risk profiles of Cox-2 and non-selective NSAIDS (ibrufen, diclofenac and naproxen). Cox inhibitors increases risk of cardio-thrombotic events, congestive heart failure.	(83)
Corticosteroids	The use of high-dose corticosteroids is not recommended in cases of COVID-19.	Mild to intermediate dose may be considered to reduce inflammation in initial treatment of cytokine storm and in specific cases of COVID-19-induced pneumonia.	(83)
Anti-tumor necrosis factor (TNFa) therapy	Anti-TNFa is widely used for several autoimmune diseases. Its use in COVID-19 should be explored.	May be protective against SARS-CoV-2 pneumonia.	(84)
Intravenous immunoglobulin (IVIg) therapy	Due to its lack of side effects, IVIg may be beneficial in COVID-19 patients especially in settings of cytokine storm or hyperinflammatory state and septic shock.	Could be explored as an alternative to corticosteroids. Low dose IVIg may require complement activation; whereas, high doses of IVIg may act directly on immune cells (85).	(86)
Angiotensin-converting-enzyme inhibitors (ACEIs) or angiotensin-II-receptor-antagonists (ARBs)	The use of ACEI/ARB is associated with lower mortality in COVID-19 in-patients.	ARBs preferable in preventing kidney failure in patients with established (diabetic) nephropathy (87). ACEIs advantageous in prevention of new onset albuminuria ACEIs have relatively higher mortality benefit than ARBs in the setting of prior MI, coronary artery disease or heart failure. ACEIs (as a monotherapy or with a diuretic) have greater benefit in recurrent stroke prevention. ARBs can be considered in patients who do not tolerate ACEIs owing to cough and angioedema.	(88)
Peroxisome proliferator-activated receptor (PPAR) agonists	PPAR agonists increase the production of anti-inflammatory cytokines and thus, may be beneficial in COVID-19 patients. PPAR-γ agonists are often used in treatment of type 2 diabetes.	PPAR-γ agonist thiazolidinediones (TZDs), like pioglitazone and rosiglitazone, have anti-inflammatory properties with potential for corrective effects on severe viral pneumonia. Nutritional ligands of PPAR-γ, such as lemongrass, pomegranate, and curcuma may be used in conjunction with PPAR pharmacological agents (89).	(90)
5′ adenosine monophosphate-activated protein kinase (AMPK) activators	AMPK activators such as metformin have direct anti-inflammatory effects. Could have benefit in reducing cytokine storm in COVID-19 infected patients.	AMPK activators has shown to increase survival rates in influenza infected animal models. Combination with pioglitazone could have added survival benefits (91).	(92)
Macrolide	The antiviral effects of macrolide may benefit COVID-19 patients.	Macrolide may reduce inflammation in infected patients.	(93)
Arbidol	Arbidol is an antiviral that has been shown to prevent COVID-19 infection.	Studies on animal model of influenza has shown benefits in reducing mortality, inflammation and lung lesion formation (94). Arbidol could be explored in targeting cytokine storm.	(95)
OX40 (CD134)	There are several limitations in the therapeutic use of OX40.	OX40–immunoglobulin fusion protein treatment has previously demonstrated clinical benefit in influenza animal models by eliminating weight loss and cachexia without preventing virus clearance (96).	(97)
Antioxidants	Antioxidants, such as vitamin C, have anti-inflammatory effects when administered intravenously.	Could be used in combination with other anti-inflammatory agents to target cytokine storm.	(98)
Suppressor of cytokine signaling (SOCS)	SOCS is involved in regulating antiviral immunity.	Could be protective against severe cytokine storm during severe COVID-19 infection.	(99)
Extracorporeal therapy	Extracorporeal therapy is a proposed mechanism to remove cytokines in septic patients	Extracorporeal cytokine removal may have protective effects on vascular integrity and could reverse cytokine storm (100).	(101)
Pyrrolidinedithiocarbamate (PDTC) ammonium	Inhibits IκB phosphorylation and thus blocks NF-κB translocation to the nucleus and reduces the expression of downstream cytokines.	PDTC ammonium may have a role in limiting cytokine storm by inhibiting reactive oxygen species (ROS) production (102).	(103)
Diacerein	An inhibitor of IL-1B—an acute response cytokine which appears in hypercytokinemia.	Diacerein attenuates inflammation in severe sepsis, and hence improves survival (104). Could be beneficial in reducing sepsis induced insulin resistance as an alternative to insulin therapy in severe sepsis cases where intensive insulin therapy is associated with adverse outcomes. Diacerein could be beneficial in managing diabetes patients infected with SARS-CoV-2.	(105)

(Continued)

TABLE 2 | Continued

Strategies or agents	Studies and indications	Safety/drug to drug interactions*	References
Tranilast	An anti-allergic drug which inhibits NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) which plays an important role in the pathogenesis of COVID-19.	Tranilast has been shown to attenuate ischemia reperfusion injury by inhibiting inflammatory cytokine production and PPAR expression (106).	(107)
Statin	*In-silico* evidence on efficacy of statins as SARS-CoV-2 Mpro inhibitors	As drugs of choice—Rosuvastatin (with preference for starting a low dose and titrating up) and Fluvastatin should be administered.	(16, 108)
Chloroquine/Hydroxychloroquine	Strong anti-inflammatory activity may be of use in targeting cytokine storm.	Not recommended currently by Food and Drug Administration (FDA) and Europeans Medicine Agency (EMA) outside of the hospital setting or a clinical trial due to risk of heart rhythm problems and fatal conditions including congestive heart failure.	(109)

**With standard therapy used for SARS-CoV-2 infection: antiviral drugs (remdesivir), antiretroviral drugs (lopinavir/ritonavir), macrolides (mainly azithromycin), anti-malaria (chloroquine and hydroxychloroquine) and anti-rheumatoid (tocilizumab).*

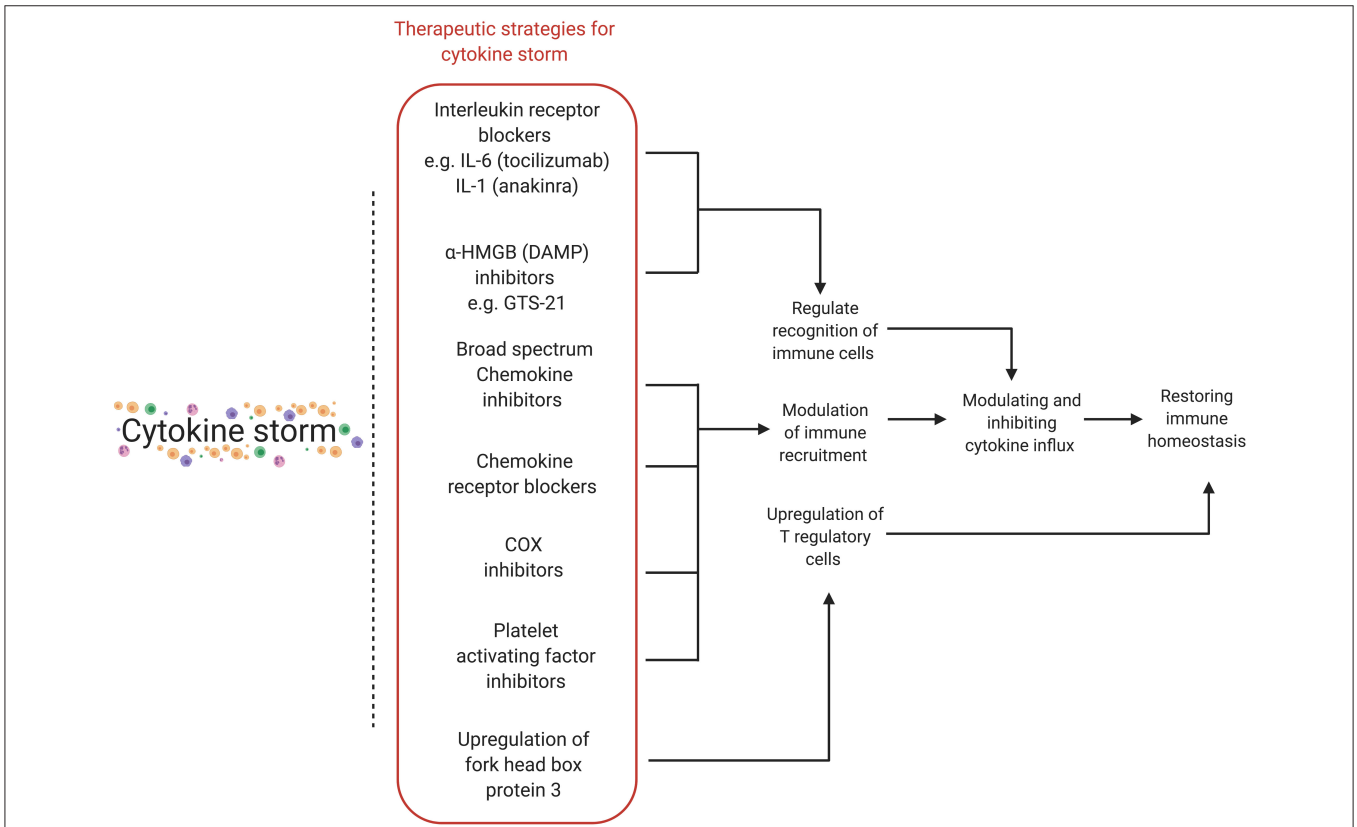

FIGURE 3 | Various therapeutic strategies for targeting cytokine storm. Different stages of the hyperinflammatory immune response can be targeted for therapeutic purposes, with the final aim of modulating and inhibiting cytokine influx in order to restore immune homeostasis. HMGB, high-mobility group protein 1; DAMP, damage-associated molecular pattern; COX, cyclooxygenase.

Corticosteroids and NSAIDs

Corticosteroids and NSAIDs can effectively suppress hyperinflammatory responses; however, delayed viral clearance could lead to further complications and also increase the risk of transmission. Although corticosteroids could be used acutely to target cytokine storm, their use in respiratory viral infection is associated with increased mortality, increased risk of secondary bacterial or fungal infections, and prolonged ICU admission. Furthermore, corticosteroids may mask COVID-19 related fever. As such, corticosteroids and NSAIDs are not recommended for routine management of COVID-19 patients (40), despite a theoretical benefit in reducing cytokine storm risk.

Targeting Interleukins

Interleukin-6 (IL-6) plays a key role in cytokine storm (102). Blocking IL-6 is another potential therapeutic strategy (**Figure 4**) (44). Tocilizumab is a monoclonal antibody against IL-6 receptor

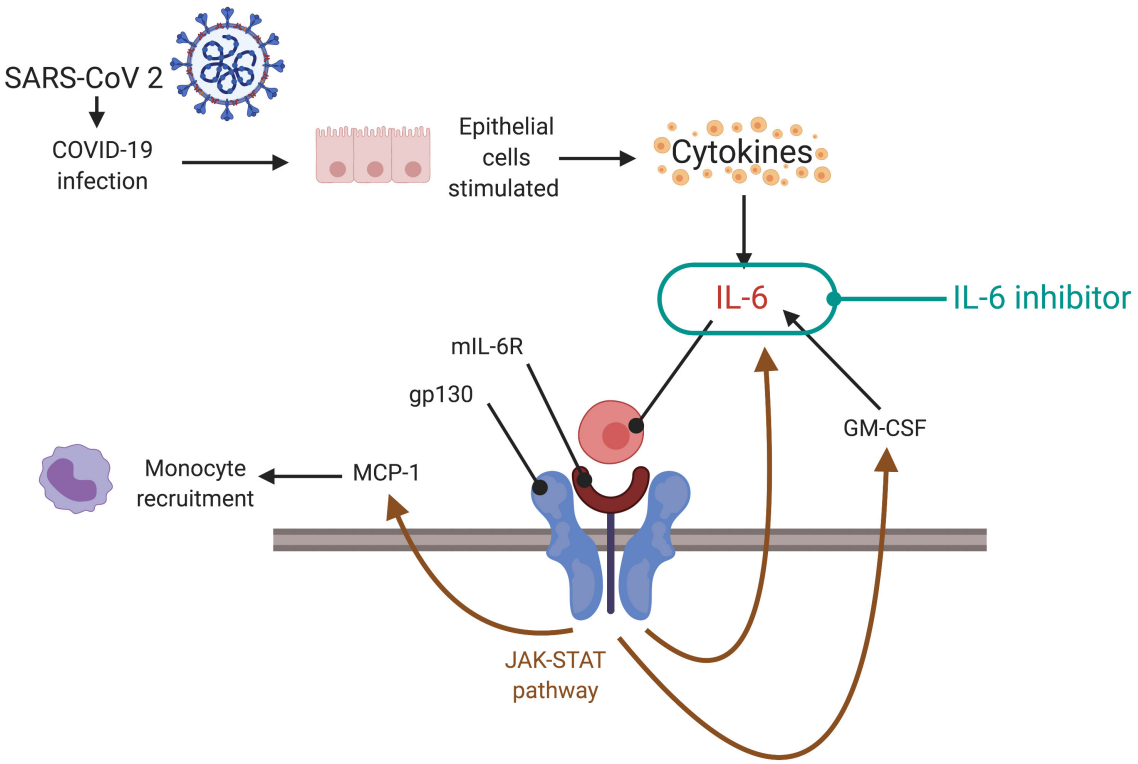

FIGURE 4 | Targeting cytokine storm via the JAK-STAT pathway. During a cytokine storm, there are increased levels of IL-6 which can form a complex with mIL-6R to act on gp130. Gp130 regulates levels of IL-6, MCP-1, and GM-CSF via the JAK-STAT pathway. This could facilitate the cytokine storm. Inhibition of the JAK-STAT pathway, potentially using IL-6 inhibitors or direct inhibition of signaling, can be a therapeutic strategy (depending on the timing—indicated preferably at later stages of illness, not in early phase, or at clinical signs of cytokine storm). SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; IL, interleukin; mIL-6R, membrane bound interleukin-6 receptor; gp 130, glycoprotein 130; MCP-1, monocytes chemoattractant protein-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; JAK-STAT, janus kinase/signal transducer and activator of transcription.

(IL-6-R) that binds to membrane-bound and soluble IL-6-Rs (mIL-6R and sIL-6R), thus preventing the downstream signal transduction of IL-6 on binding to membrane protein gp130 (102). A study of 21 tocilizumab-treated COVID-19 patients revealed that clinical manifestations improved following administration (111). Tocilizumab is undergoing phase IV clinical trials (ChiCTR2000029765) and has been approved for use in treating COVID-19 pneumonia and raised IL-6 levels in China. The Italian Regulatory Drug Agency is undertaking phase II trials of tocilizumab in COVID-19 patients (TOCIVID-19) (44). The pro-inflammatory effects of IL-6 occur via the trans-signaling pathway using sIL-6R. On the other hand, the anti-inflammatory and regenerative effects of IL-6 involve the cis-signaling pathway via the mIL-6R, present on macrophages, neutrophils, some T lymphocytes and hepatocytes. Tocilizumab is not selective for the sIL-6R and may inhibit mIL-6R, thereby causing negative side effects such as upper respiratory tract infections. Recombinant soluble gp130 protein (sgp130) may be an alternative to tocilizumab because it binds to sIL-6R, thereby reducing its pro-inflammatory effects when it binds to IL-6 (112). IL-1 inhibitors may be an alternative for treating COVID-19 hypercytokinemia. A phase III clinical trial of anakinra showed survival benefit without increased adverse effects (40). IL-37 and IL-38 could be evaluated as therapeutic options for COVID-19 because they inhibit the pro-inflammatory effects of IL-1 (113).

Janus Kinase Inhibitors

SARS-CoV-2 enters host cells via receptor-mediated endocytosis, which is regulated by numb-associated kinases (NKA) such as adaptor complex protein 2 (AP2)-associated protein kinase (AAK1) and G-associated kinase (GAK). The high affinity AAK1 blocker ruxolitinib is under investigation for treating COVID-19 (ChiCTR2000029580). To achieve NAK inhibition, toxic doses of AAK1 blockers are required. Baricitinib can inhibit both AAK1 and GAK (approved dosage of 2–4 mg daily) and can selectively inhibit JAK 1 and 2, thus reducing the inflammatory effects of Il-6 via the JAK-STAT signaling pathway. Furthermore, baricitinib can be considered in combination antiviral and anti-inflammatory therapies due to its minimal interaction with cytochrome P450 (CYP) enzymes and low plasma protein binding (40, 114). Early reports show promise of baricitinib combined with antiviral therapy in COVID-19 patients (115).

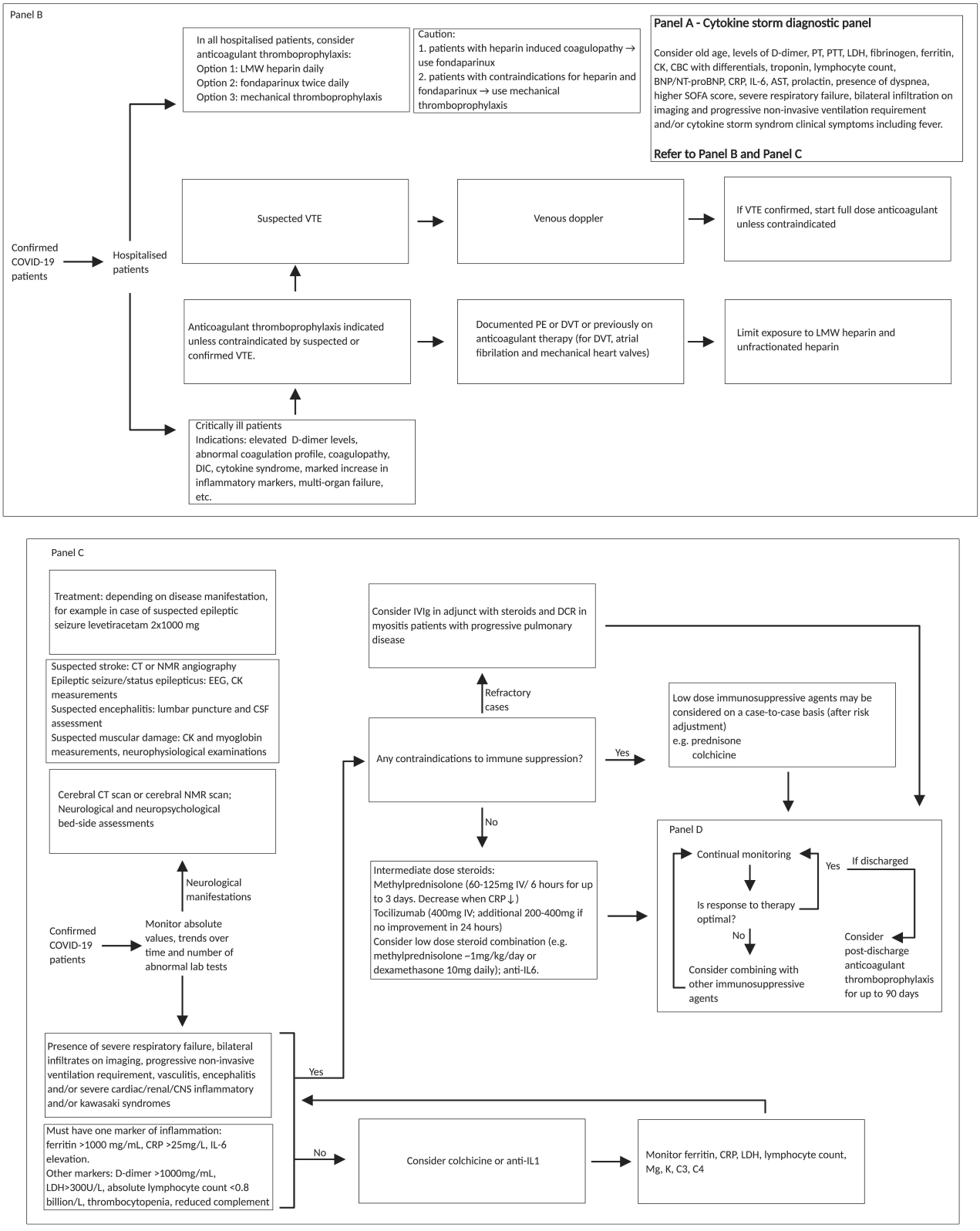

FIGURE 5 | REPROGRAM consortium pathway for targeting cytokine storm in severe or critically ill COVID-19 patients. Diagnostic panel for risk factor assessment of cytokine storm associated prognosis of COVID-19 patients could include (Panel A: on top right) (99): older age, dyspnoea, higher SOFA score, IL-6, lymphocyte

(Continued)

FIGURE 5 | count; cardiac troponin; BNP/NT-proBNP (if clinical suspicion of heart failure); one marker of inflammation (Ferritin > 1,000 mg/mL, CRP > 25 mg/L, and Il-6 elevation); presence of severe respiratory failure, bilateral infiltration on imaging and progressive non-invasive ventilation requirement, D-dimer > 1,000 mg/mL; LDH > 300 U/L; absolute lymphocyte count < 0.8 billion/L; PCT level (>0.5 ng/mL), and AST > 40 U/liter (61, 116–119). In low-resourced settings, cytokine release syndrome clinical symptoms could be used in the absence or limited availability of diagnostic panels. Fondaparinux is a synthetic pentasaccharide factor Xa inhibitor. Fondaparinux binds antithrombin and accelerates its inhibition of factor Xa. It is chemically related to low molecular weight heparins. Patients with CNS involvement should have cerebral CT or MRI scan and in the if a stroke is suspected also a CT angiography or MRI angiography, in case of epileptic seizures or status epilepticus an EEG and in case of suspected encephalitis a lumbar puncture for cerebro-spinal fluid assessment. Also, bedside neuropsychological assessments are of value. In addition, assessment of CK and myoglobin are of value (neurophysiology as well, but this is not so important acutely). Treatments should include: antiepileptics (for example, levetiracetam 2x1000 mg) and depending on disease condition. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; IV, intravenous; PT, prothrombin time; PTT, partial thromboplastic time; LDH, lactate dehydrogenase; CK, creatine kinase; CBC, complete blood count; BNP, brain natriuretic peptide; NT-proBNP, N-terminal pro hormone brain natriuretic peptide; CRP, c-reactive protein; IL, interleukin; AST, aspartate aminotransferase; SOFA, sequential organ failure assessment score; LMW, low molecular weight; VTE, venous thromboembolism; PE, pulmonary embolism; DVT, deep vein thrombosis; DIC, disseminated intravascular coagulation; CT, computed tomography; NMR, nuclear magnetic resonance; EEG, electroencephalogram; CSF, cerebrospinal fluid; IVIg, intravenous immunoglobulin; DCR, direct current cardioversion; CNS, central nervous system; Mg, magnesium; K, potassium; C, complement component; PCT, procalcitonin.

DECISION MAKING BASED ON CYTOKINE STORM

Because of the association between cytokine storm and severe COVID-19 complications, we propose a cytokine storm-based diagnosis and management workflow for patients with or suspected of COVID-19 (**Figure 5** and **Table 1**) (120). Our proposal expands on the multidisciplinary evidence-based guidelines currently used in the diagnosis and treatment of cytokine storm linked macrophage activation syndrome and sHLH (121). The Surviving Sepsis Campaign COVID-19 panel recommends the use of moderate-dose steroids for intubated patients with ARDS (10 mg dexamethasone daily, or 60 mg/day methylprednisolone) (122).

CONCLUSION AND DISCUSSIONS

The diagnosis and management of cytokine storm are clinically challenging and controversial due to lack of proven treatment. Our proposed algorithm may be used as a possible approach (**Figure 5**) (120–122); however clinical decision should be based on individual patient profile and disease severity. Immunosuppressive agents such as steroids or immunomodulating drugs such as anti-IL6 monoclonal antibodies like tocilizumab, are relatively high priced, unavailable in low resource setting, and may be in short supply during the COVID-19 pandemic even in developed countries. The neuroinvasive potential of COVID-19 (24, 25) and the association between neuroinvasion and cytokine storm need further consideration (123). We recommend longitudinal follow-up of COVID-19 patients with and without the cytokine storm to understand the specific immunopathological mechanisms and biomarkers for severe disease. After cytokine storm resolves, an immunologic memory of the SARS-CoV-2 infection will likely persist (124), raising the possibility of either relapse or reinfection in previously COVID-19 positive patients who subsequently cleared the infection. Limiting damage from a hyperimmune response during both the acute phase and the cytokine storm is a target for further research. There could be a decoupling mechanism of cytokines that may attenuate the cytokine storm and preserve memory (110). Understanding the various pathophysiological mechanisms linked to cytokine storm could be used to develop targeted diagnostic and therapeutic strategies for critically ill 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

SB devised the project, the main conceptual ideas, including the workflow targeting cytokine storm, proof outline, and coordinated the writing and editing of the manuscript. SB and AS wrote the first draft of the manuscript. SB encouraged AS to investigate and supervised the findings of this work. All authors discussed the results and recommendations and contributed to the final manuscript. The opinions expressed in this article are those of the authors and do not necessarily represent the decisions, official policy, or opinions of the affiliated institutions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.01648/full#supplementary-material

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CASE REPORT
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COVID-19 and Guillain–Barré Syndrome: A Case Report and Review of Literature

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During the recent coronavirus disease 2019 (COVID-19) outbreak in Northern Italy, we observed a 57-year-old man developing acute motor-sensory axonal neuropathy, a variant of Guillain–Barré syndrome (GBS), 12 days after severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection. Similarly to other bacterial and viral infections, dysregulation of the immune system due to post-infectious mechanisms, such as the molecular mimicry, could lead to an indirect damage of the peripheral nervous system related to SARS-CoV-2. GBS causes motor dysfunctions that are not easily recognizable in non-neurological settings or in patients requiring ventilatory assistance. Several reports also suggested that GBS and Miller Fisher syndrome (MFS) could be neurological complications of COVID-19. Therefore, we performed a review of the 29 articles so far published, describing 33 GBS cases and five MFS cases associated with SARS-CoV-2 infection. We recommend awareness of this rare, but treatable, neurological syndrome, which may also determine a sudden and otherwise unexplained respiratory deterioration in COVID-19 patients.

Keywords: guillain–barré syndrome, miller fisher syndrome, COVID-19, SARS-CoV-2, AMSAN, post-infectious

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) outbreak started at the end of 2019 in Wuhan, the capital of Hubei province, in China. The novel coronavirus was designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To date, millions of cases have been confirmed worldwide, and Italy has been one of the most affected countries.

Neurological manifestations have been described in one third of patients with COVID-19. Some of these neurological symptoms have proved to be quite specific, e.g., loss of smell or taste, but other ones are non-specific, e.g., headache, dizziness, or reduced level of consciousness (1). However, whether the neurological symptoms associated with SARS-CoV-2 are attributable to secondary mechanisms (i.e., multiorgan dysfunction or systemic inflammation), an abnormal immune response or the direct injury of the virus is still unknown.

Recently, several case reports have suggested a relationship between the occurrence of Guillain–Barré syndrome (GBS) and a previous SARS-CoV-2 infection, which preceded the GBS onset by up to 4 weeks. Therefore, a post-infectious dysregulation of the immune system, triggered by SARS-CoV2, appears to be the most probable cause.

Conversely, a recent pathological study on postmortem human brain tissues found that anosmia and dysgeusia, described in up to 20% of patients, are more likely due to the direct viral invasion of the olfactory nerve and bulb (2). In addition and in line with this observation, a COVID-19 patient with anosmia showed magnetic resonance imaging (MRI) abnormalities in the olfactory bulb and in the inferior frontal lobe, as a conceivable result of the direct invasion of SARS-CoV-2 through the olfactory pathway *via* trans-synaptic retrograde spreading (3).

Here we describe a patient with an axonal variant of GBS following COVID-19, and we review the available reports in the literature on other GBS cases related to SARS-CoV-2 infection.

CASE REPORT

A 57-year-old man developed dysgeusia, cough, and fever of up to 39°C lasting for 5 days. At 12 days after the resolution of the symptoms, he complained of numbness and tingling in the feet and, a few days later, also in the hands. Over 10 days, the patient developed distal limb weakness and severe gait impairment, so he was referred to the emergency department. A neurological examination showed weakness in the dorsiflexion of the foot and the extension of the toes [Medical Research Council (MRC) score: 3/5 on the right side and 4/5 on the left side], weakness in the extension of hand and fingers (MRC score: 4/5 bilaterally), gait ataxia, loss of touch and vibration sensation in the feet and ankles, weak tendon reflexes in the upper and the lower limbs, but absent ankle jerk reflex. The cranial nerves were spared. The chest radiography was negative for pneumonia, and a nasopharyngeal swab testing for SARS-CoV-2 with real-time polymerase chain reaction assay (RT-PCR) was negative, too.

At this stage, the patient was admitted to our unit for further diagnostic workup. The nerve conduction studies, performed 4 weeks after the neurologic onset, showed reduced or absent compound muscle action potentials and sensory nerve action potentials in the lower limbs, absent F wave response in the lower limbs, and prolonged F wave response in the upper limbs. The electromyography showed very rich spontaneous activity (fibrillation potentials and positive sharp waves) in the lower limb muscles (**Table 1**). The cerebrospinal fluid (CSF) examination disclosed normal cell count and normal proteins, normal CSF/serum albumin ratio, and absence of oligoclonal banding. Serum SARS-CoV-2 IgG was detected (Maglumi, Snibe). Anti-GM1, anti-GD1b, and anti-GQ1b IgG and IgM were negative (ELISA, Bühlmann). The laboratory investigations demonstrated high C-reactive protein (18.9 mg/dl). The serological tests for HIV, syphilis, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and *Mycoplasma pneumoniae* (MP) were negative, except for anti-EBV, anti-CMV, and anti-MP IgG. An intravenous immunoglobulin (IVIG) cycle at 0.4 g/kg/day over 5 days was started, leading to a significant improvement of the weakness in the upper limbs and the left foot but a poor benefit on the right foot and gait ataxic. The patient was then transferred to the rehabilitation unit. He slowly improved through physiotherapy and, after 1 month, he was able to walk without aid and was discharged. **Figure 1** shows a timeline of the clinical milestones of the patient.

DISCUSSION

We reported a patient showing a stepwise progression of numbness, tingling, and weakness 12 days after the resolution of fever, cough, and dysgeusia. The clinical features and the electrophysiological findings along with the epidemiological context and the presence of IgG to SARS-CoV-2 supported the diagnosis of post-COVID-19 GBS. In particular, the neurophysiological examination was consistent with an acute motor-sensory axonal GBS (AMSAN) variant, with level 2 diagnostic certainty for GBS according to the Brighton Criteria (consistent clinical features and supporting nerve conduction study, but not CSF) (4, 5). Active SARS-CoV-2 infection was excluded by a complete recovery of the typical antecedent symptoms, absence of the viral genome in the nasopharyngeal swab, and negative chest radiography. The detection of serum IgG to SARS-CoV-2 is in line with the chronological profile of the antibody appearance. Indeed IgG seroconversion in COVID-19 patients is reached within a median of 13 days from the clinical onset (6).

TABLE 1 | Neurophysiological findings.

Antidromic sensory NCS	Latency (ms)	Amplitude (μV)	Velocity (m/s)	Motor NCS	Latency proximal/distal (ms)	Amplitude proximal/distal (mV)	Velocity (m/s)
Sural: posterior ankle	R = NE; L = NE	R = NE; L = NE	R = NE; L = N	Tibial: medial malleolus-abductor hallucis brevis; popliteal fossa-medial malleolus	R = NE; L = NE	R = NE; L = NE	R = NE; L = NE
Radial: thumb	R = 4.2; L = NE	R = 0.13; L = NE	R = 31; L = NE				
Ulnar: digit 5	R = 2.3; L = 2.6	R = 6.2; L = 1.5	R = 52.2; L = 46.2	Common peroneal: ankle-extensor digitorum brevis; below fibula-ankle	R = 11.9/4; L = NE	R = 0.1/0.1; L = NE	R = 36.7; L = NE

Electromyography showed fibrillation potentials and positive sharp waves in the tibialis anterior and gastrocnemius medialis muscles, bilaterally.
L, left; NCS, nerve conduction study; NE, not evocable; R, right.

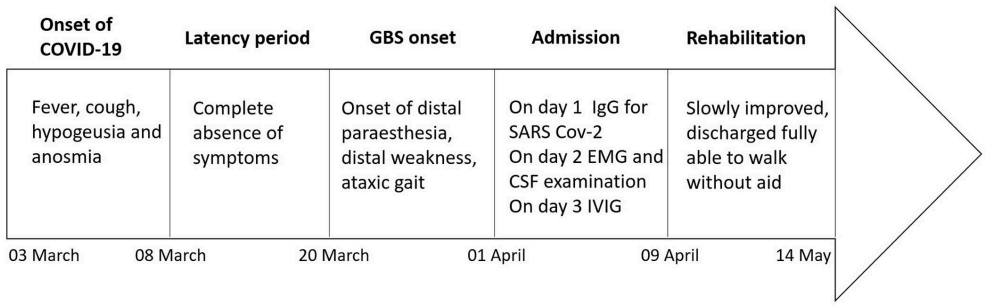

FIGURE 1 | Timeline of clinical events, diagnostic-therapeutic approach, and clinical outcome.

More than half of GBS cases appear 1 to 2 weeks after an underlying infection. *Campylobacter jejuni* is the most frequent precipitant of GBS, but viral infections, including EBV,CMV, and Zika virus, are also frequently reported (7). The association of GBS with other coronaviruses was described only in two cases (8, 9). Recently, SARS-CoV-2 has also been related to GBS and Miller Fisher syndrome (MFS), wherein an autoimmune post-infectious mechanism, such as molecular mimicry or bystander activation, targeting self-ganglioside epitopes in spinal roots and peripheral nerves, might be involved, in analogy with all the other post-infectious cases.

We carried out a literature search in MEDLINE *via* PubMed for all articles published using the keywords or MeSH terms "COVID-19" or "SARS-CoV-2," together with "Guillain–Barre syndrome," "GBS," "AIDP," "AMAN," "AMSAN," "Miller Fisher syndrome," or "MFS." At the time of writing this manuscript, we found in literature 29 articles reporting 33 patients with GBS and five cases of MFS associated with SARS-CoV-2 infection, which are summarized in **Table 2** (10–38).

The age of the patients ranged between 23 and 77 years (mean ± standard deviation: 59 ± 12), with a male prevalence (63.2%). The severity of COVID-19 manifestations, defined as mild, severe, and critical, according to a previously described classification (39) was as follows: mild in 30/38 (78.9%), severe in 5/38 (13.2%), and critical in 3/38 (7.9%). The time elapsed from onset of the COVID-19 symptoms to the clinical GBS manifestations ranged between 3 and 28 days (mean 12 ± 6). Notably, the timing of the majority of cases was consistent with the parainfectious profile rather than a post-infectious paradigm. In two patients, the onset of GBS actually preceded by a few days the first manifestations of COVID-19, but an earlier presentation of COVID-19 characterized by very mild or even absent symptoms could be taken into account in both cases.

The main clinical, electrophysiological, and CSF features of the patients so far reported are summarized in **Table 3**. With regard to GBS subtypes, the main clinical variant was the classical sensory-motor GBS (30/38), the second phenotype was MFS (5/38), the third was featured by facial diplegia with sensory deficits (2/38), and in only one case the pharyngeal–cervical–brachial variant was observed.

Following the first reported cases of GBS related to SARS-CoV-2 (10), a more common axonal rather than demyelinating variant has been suggested. However, unlike the initial reports, the electrophysiological features in other cases did not show a higher prevalence of axonal variants in these patients. By contrast, the demyelinating and the mixed forms were more often observed.

The examination of CSF samples obtained from 32 patients showed an albumin-cytological dissociation in 68.4% of cases. All RT-PCRs for SARS-CoV-2 on CSF were negative, suggesting the lack of a direct causative role of the virus.

Anti-ganglioside antibodies were detected only in two cases of MFS, showing a borderline positivity for IgM anti-GM1 and a positivity for IgG anti-GD1b, respectively.

Brain and/or spinal cord MRI was performed in 20 patients and showed contrast enhancement of nerve roots at the level of the cauda equina, of brachial and lumbosacral plexus, and also of single or multiple cranial nerves. In addition, a brainstem and cervical leptomeningeal enhancement, which is an atypical feature in GBS, was seen in one case.

Almost all patients were treated with IVIG and/or plasma exchange, whereas one mild case received only symptomatic treatment. The recovery timing and the outcomes varied widely, but in 14/38 cases, a rapid improvement was reported. In 12/38 cases, the improvement was instead slower and required admission to rehabilitation facilities, whereas 6/38 patients had a poor outcome (prolonged stay in the intensive care unit and long-lasting severe disabilities). Two cases were fatal, and in 4/38 cases, the outcome was not available. Of relevance is the fact that it does not seem that COVID-19 severity at onset is correlated with GBS outcomes.

Moreover, the clinical features of post-COVID-19 GBS did not differ from those of cases related to other viruses, with the notable exception of a remarkable respiratory involvement (**Table 3**). It is indeed crucial to highlight that respiratory failure was present in 15 /38 cases (39.5%) of GBS related to SARS-CoV-2, a percentage higher than that observed in previous GBS cohorts (ranging from 20 to 30%) (5). This suggests that COVID-19 pneumonia may overlap with GBS-associated respiratory muscle weakness and increase the number of cases needing a respiratory support. The reported observations indicate that physicians should always consider GBS in the differential diagnosis of a respiratory insufficiency in COVID-19 patients, especially in cases of normocapnic

TABLE 2 | Guillain–Barré syndrome (GBS) cases related to SARS-CoV-2.

Patient	Time between events	Clinical features	EMG	CSF	MRI	Treatment/outcome	References
GBS cases							
77 years old, w	7 days	Tetraplegia, areflexia, paresthesia in upper limbs, facial diplegia, dysphagia, tongue weakness, and respiratory failure	AMSAN	ACD	Spine: enhancement of caudal nerve roots	Two cycles of IVIG; poor outcomes	(10)
23 years old, m	10 days	Facial diplegia, areflexia, lower limbs paresthesia, and ataxia	AMSAN	ACD	Brain: enhancement of facial nerve bilaterally	IVIG, slow improvement	(10)
55 years old, m	10 days	Tetraparesis, areflexia, paresthesia in all limbs, facial diplegia, and respiratory failure	AMAN	ACD	Spine: enhancement of caudal nerve roots	Two cycles of IVIG, poor outcomes	(10)
76 years old, m	5 days	Tetraparesis, areflexia, and ataxia	AIDP	N	Brain and spine: N	IVIG, slow improvement	(10)
61 years old, m	7 days	Tetraplegia, areflexia, lower limb paresthesia, facial diplegia, dysphagia, and respiratory failure	AIDP	ACD	Spine: N	IVG, PE, poor outcomes	(10)
61 years old, w	8 days[a]	Tetraparesis, areflexia, and sensory loss in all limbs	AIDP	ACD	n.a.	IVIG, rapid improvement	(11)
60 years old, m	20 days	Tetraparesis, areflexia, and ipopallesthesia in lower limbs, paresthesia in all limbs, facial diplegia, hypophonia, and dysarthria	AIDP	N	Cervical spine: N	IVIG, slow improvement	(12)
43 years old, m	14 days	Paraparesis, areflexia, apallesthesia, and sensory deficit in all limbs; ataxia and right peripheral facial	AIDP	ACD	Brain: multiple cranial neuritis Spine: radiculitis, and brachial and lumbar plexitis	IVIG, rapid improvement	(13)
70 years old, w	7 days	Tetraparesis, areflexia, paresthesia in all limbs, and perioral, left peripheral facial palsy, respiratory failure.	AIDP	ACD	n.a.	IVIG, rapid improvement	(13)
72 years old, m	7 days	Tetraparesis, neck flexor weakness, paresthesia, sensory loss in all limbs, respiratory failure, dysautonomia, and SIADH	AIDP	ACD	n.a.	IVIG, poor outcomes	(14)
55 years old, m	20 days	Facial diplegia, hyporeflexia, dysphagia, bilateral masseter weakness, and dysphonia	AIDP	N[b]	Brain: N	IVIG, rapid improvement	(15)
60 years old, m	20 days	Tetraparesis, areflexia and massive dysautomia (gastroplegia, paralytic ileus, and hypotension)	AMSAN	N[b]	n.a.	IVIG, rapid improvement	(15)
65 years old, m	14 days	Tetraparesis, facial diplegia, areflexia, hypopallestesia and sensory deficit in lower limbs	AMSAN	n.a.	Brain and Cervical spine: N	IVIG, n.a	(16)
70 years old, w	24 days	Tetraparesis, areflexia, distal paresthesia in all limbs and respiratory failure	AIDP	ACD	n.a.	IVIG; poor outcomes	(17)
53 years old, w	n.a[a]	Paraparesis, areflexia, paresthesia in lower limbs, dysarthria and jaw weakness	AIDP	ACD	Spine: radiculitis of cervical and lumbar spine	PE, slow improvement	(18)
76 years old, w	8 days	Tetraparesis, areflexia, distal paresthesia sensory loss in lower limbs, dysphonia, dysphagia and respiratory failure	n.a	n.a	n.a	IVIG, death	(19)
52 years old, w	15 days	Tetraparesis, areflexia, distal paresthesia and sensory loss, ataxia, dysautonomia and respiratory failure	AIDP	ACD	Spine: N	IVIG, poor outcomes	(20)
63 y years old, w	7 days	Tetraparesis, areflexia, and distal paresthesia	AIDP	N	n.a.	IVIG, slow improvement	(20)
61 y years old, w	22 days	Tetraparesis, areflexia, hypopallestesia and allodynia, facial diplegia, dysphagia, and dysautonomia	AIDP	ACD	Spine: lumbosacral nerve root enhancement	IVIG, slow improvement	(20)
50 years old, m	28 days	Paraparesis, areflexia, distal paresthesia, hypopallestesia in lower limbs, ataxic gait and facial diplegia	AIDP	N	Brain: N	IVIG, rapid improvement	(21)

(Continued)

TABLE 2 | Continued

Patient	Time between events	Clinical features	EMG	CSF	MRI	Treatment/outcome	References
54 years old, m	10 days	Tetraparesis, areflexia, paresthesia and respiratory failure	n.a.	n.a.	Spine: N	IVIG, slow improvement	(22)
58 years old, m	20 days	Facial diplegia, areflexia and paresthesia in lower limbs, dysarthria with labial sounds	AIDP/Blink reflex absent	ACD	Brain: bilateral facial enhancement	IVIG, slow improvement	(23)
68 years old, m	10 days	Tetraparesis, areflexia, sensory loss in lower limbs and respiratory failure	AIDP	ACD	Spine: N	IVIG, PE, slow improvement	(24)
57 years old, m	7 days	Tetraparesis, areflexia, paresthesia, hypopallestesia and sensory loss in lower limbs, dysphagia and respiratory failure	AIDP	ACD	n.a.	IVIG, slow improvement	(25)
64 years old, m	11 days	Tetraparesis, areflexia, paresthesia in all limbs, hypo/apallesthesia in all limbs, dysphagia and respiratory failure.	AIDP	ACD	n.a	IVIG, n.a	(26)
70 years old, w	3 days	Tetraplegia, areflexia, paresthesia in all limbs, bilateral positive Lasègue sign.	AMSAN	ACD	n.a	IVIG, n.a	(27)
43 years old, man	10days	Tetraparesis, areflexia, sensory loss in all limbs, facial diplegia and dysphagia	AIDP	n.a.	n.a.	IVIG, rapid improvement	(28)
70 years old, m	10 days	Paraparesis, areflexia, urinary retention and constipation	AIDP	ACD	Spine: N	IVIG, rapid improvement	(29)
71 years old, m	7 days	Tetraparesis, areflexia, paresthesia and hypesthesia in all limbs, respiratory failure	AIDP	ACD	n.a.	IVIG, death	(30)
64 years old, m	23 days	Paraparesis, areflexia, ipopallesthesia and sensory loss in all limbs	AIDP	ACD	n.a.	IVIG, rapid improvement	(31)
54 years old, w	21 days	Paraparesis, areflexia, paresthesia in all limbs and dysphagia	AIDP	ACD	n.a.	IVIG, rapid improvement	(32)
66 years old, w	10 days	Tetraparesis and areflexia	AIDP	ACD	n.a.	IVIG, rapid improvement	(33)
55 years old, w	15 days	Tetraparesis, areflexia, paresthesia in all limbs and perioral, facial diplegia, dysphonia, dysphagia and respiratory failure	AIDP	ACD	Brain: lepto-meningeal enhancement in medulla	IVIG, slow improvement	(34)
Miller Fisher syndrome cases							
50 years old, m	5 days	Ophthalmoparesis, areflexia, perioral paresthesia and ataxia	n.a	ACD	n.a.	IVIG, rapid improvement	(35)
39 years old, m	3 days	Ophthalmoparesis, areflexia	n.a	ACD	n.a.	Acetaminophen, n.a.	(35)
36 years old, m	4 days	Ophthalmoparesis (CN III and CN VI), ataxia, and hyporeflexia and sensory loss in lower limbs	n.a.	n.a.	Brain: enlargement, prominent enhancement CN III	IVIG, rapid improvement	(36)
54 years old, m	14 days	Ophthalmoparesis, tetraparesis, areflexia, distal paresthesia, facial diplegia, dysautonomia and respiratory failure	AIDP	n.a.	Spine: N	IVIG, slow improvement	(37)
74 years old, w	15 days	Ataxia and areflexia.	Increase F-wave latencies	ACD	n.a.	IVIG, rapid improvement	(38)

ACD, albumin-cytological dissociation; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; CSF, cerebrospinal fluid; EMG, electromyography; GBS, Guillain–Barré syndrome; IVIG, intravenous immunoglobulin; m, man; MFS, Miller Fisher syndrome; MRI, magnetic resonance imaging; N, normal investigation; n.a., not available; PE, plasma exchange; w, woman.

[a] *GBS manifestation precedes COVID-19.*

[b] *Those cases have low serum albumin, 2.9 and 2.6 mg/dl, respectively. Furthermore, a mirror-pattern oligoclonal banding was observed in both cases.*

or hypercapnic respiratory failure (pointing to a restrictive respiratory pattern in contrast with the interstitial pattern of COVID-19 pneumonia) or when a discrepancy between chest imaging and respiratory parameters occurs. An additional explanation for this latter scenario is that the respiratory failure in GBS associated with COVID-19 may also be driven by a dysfunction of the cardiorespiratory centers in medulla oblongata directly induced by the virus (40) since the SARS-CoV-2 genome has also been detected in the human brainstem (2).

TABLE 3 | Clinical, neurophysiological, and CSF features of Guillain–Barré syndrome/Miller Fisher syndrome cases.

Clinical features	*N*	%
Tetraparesis	24/38	63.2
Paraparesis	7/38	18.4
Ophthalmoparesis	4/38	10.5
Hypo/areflexia	38/38	100
Facial weakness	15/38	39.5
Paresthesia and/or sensory loss	30/38	78.9
Ataxia	9/38	21.1
Bulbar	11/38	28.9
Dysautonomia	6/38	15.8
Respiratory failure	15/38	39.5
SIADH	1/38	2.6
NCS		
AIDP	26/38	68.4
AMSAN	5/38	13.2
AMAN	1/38	2.6
n.a.	5/38	13.2
Only F wave delay	1/38	2.6
CSF		
ACD	25/38	65.8
Mirror OB	2/38	5.3
Normal	5/38	13.2
n.a.	6/38	15.8

ACD, albumin-cytological dissociation; AIDP, acute inflammatory demyelinating polyneuropathy; AMAN, acute motor axonal neuropathy; AMSAN, acute motor-sensory axonal neuropathy; NCS, nerve conduction studies, CSF, cerebrospinal fluid; OB, oligoclonal band; n.a., not available; SIADH, syndrome of inappropriate antidiuretic hormone secretion.

The early recognition of GBS symptoms is critical, given the associated high mortality as well as severe motor disabilities that may seriously limit the quality of life of these patients (41). Deficits induced by this neurological condition could be reasonably included among the post-COVID-19 sequelae, which requires an accurate evaluation by the neurologists, similarly to what has been suggested in the past outbreaks, for instance, in cases of post-polio syndrome (42).

CONCLUSION

Our case report and review of literature contribute to raise awareness of the possible association between GBS and SARS-CoV-2 infection. The underlying mechanism of injury could be an autoimmune reaction against peripheral nerve antigens, in light of the lack of a viral genome in the CSF.

The main clinical, electrophysiological, and CFS features of the patients so far reported proved to be similar to GBS cases related to other infectious diseases. Nevertheless, respiratory involvement is more frequent in GBS related to SARS-CoV2, and a reasonable explanation for this finding could be the coexistence of COVID-19 interstitial pneumonia and GBS respiratory muscle weakness since the majority of cases had a parainfectious profile.

However, the relationship between SARS-CoV-2 and GBS, actually described only in single case reports and small case series, should be confirmed in larger observational studies in order to evaluate the temporal correlation between GBS clusters and the COVID-19 epidemic curve in each affected country.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Written informed consent was obtained from the patient for the publication of this case report, including any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AZ, MC, and AC were involved in the work-up of the patient, planning and conducting investigations, and providing clinical care. AZ planned the case report and drafted the initial manuscript. EA and MT performed the electrophysiological investigation. DF and MG carried out the laboratory testing. AZ, MT, and MCo reviewed the literature. EA, DF, MG, MT, MCo, MC, and AC revised the manuscript. All the authors approved the final manuscript as submitted.

DEDICATION

This paper is dedicated to the loving memory of our colleague, Prof. Arrigo Moglia, neurologist and neurophysiologist at our Institute, who recently died from COVID-19.

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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 in Neurology

SYSTEMATIC REVIEW
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Respiratory Syndrome Coronavirus Infections: Possible Mechanisms of Neurological Implications—A Systematic Review

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Within the context of the worst pandemic of the century—Covid-19—which emerged in China and has spread across the entire globe over the last 6 months, increased knowledge about viral behavior that be prognostic is crucial. Following the patterns of other coronaviruses (CoVs), particularly those infecting the respiratory tract, neurological manifestations have been reported in patients with Covid-19. Such manifestations highlight the neurovirulence of this severe acute respiratory syndrome (SARS)-CoV2. In order to collect all available information on the implications and mechanisms of infections by respiratory CoVs, a systematic review was designed following the PRISMA protocol. The following PICO strategy (patient, problem, or population; intervention; comparison, control, or comparator; outcomes) was adopted: P included healthy individuals, patients, and animal models susceptible to human-specific viruses; I included molecular, cell culture, and comparative experimental studies; C included healthy, diseased, and immunized conditions; and O represented the virulence and pathogenicity of respiratory CoVs and their effects on the central nervous system (CNS). Searches were conducted in PubMed databases from March 30 to April 1, 2020. Results indicate the involvement of the CNS in infections with various CoVs. Infection typically begins in the airway epithelia with subsequent alveolar involvement, and the virus then spreads to the CNS via neuronal contacts with the recruitment of axonal transport. Neuronal infection and regulated cell death are the main factors causing a generalized encephalitis.

Keywords: Covid-19, SARS-CoV2, coronavirus, neurovirulence, pathogenicity

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INTRODUCTION

Viral neurotropism with the potential for acute and/or chronic consequences to the central nervous system (CNS) has been identified since the late 1950s with findings of the involvement of a murine hepatitis virus (MHV) in encephalomyelitides in humans (1, 2). The name coronavirus (CoV) emerged in a small note published in 1968 by a group of virologists who published their papers in the *Nature* journal. They showed that the viral particles are more or less rounded, although they noted a certain degree of polymorphism, with a fringe of projections, which are rounded or petal-shaped, rather than sharp or pointed. This appearance, resembling the solar

corona, inspired the name that was adopted for MHV and several viruses recovered from humans at the time (3).

Coronaviruses (in this study referred only to those human-specific infectious) enclose a group of eukaryotic spherical RNA viruses, which infect animals and humans by fecal-oral and respiratory routes, as well as mechanical transmission. Several species of CoVs have been transmitted among humans, causing epidemics of various proportions. In just 6 months, the current Covid-19 (Coronavirus disease-2019) pandemic, caused by infection with the respiratory CoV named "SARS-CoV2" (severe acute respiratory syndrome coronavirus), has become the greatest global public health and economic crisis seen in generations. Most recently, reports of medical doctors operating on the front line of the ongoing pandemic suggest the incidence of neuropathological manifestations associated with SARS-CoV2 infections (4, 5).

Similarities between SARS-CoV2 and other respiratory CoVs have also been reported. For instance, SARS-CoV2, like other respiratory CoVs that infect humans, binds to the angiotensin-converting enzyme 2 (ACE2) receptor, which is widely distributed throughout the respiratory tract epithelium, lung parenchyma, and gastrointestinal tract (6). Respiratory distress in patients with Covid-19 may be the result of both pulmonary inflammatory structural damage, as well as damage caused in the respiratory centers of the brain (7). However, reports of such distress require further investigation on the possible mechanisms of neurovirulence associated with CoV infections of the respiratory system.

Furthermore, beyond the pulmonary, renal, cardiac, and circulatory damage that can prove to be fatal in patients with Covid-19, a dominant cerebral involvement with the potential to cause cerebral edema can be a leading cause of death, long before systemic homeostatic dysregulation (8). Thus, a critical view of the scientific evidence of human infections with a wider spectrum of respiratory CoVs is necessary to elucidate the possible mechanisms of SARS-CoV2 interactions with the nervous system.

In order to substantiate possible implications of the Covid-19 pandemic for neurology and gain a better understanding of the virulence and pathogenicity of SARS-CoV2, we performed a systematic review. We collected all available data at present in the PubMed databases on viruses of the CoV family, which cause respiratory infections in humans and have implications for the nervous system.

METHODS

In order to retrieve all available data on respiratory infections with CoVs that have an effect on the nervous system, a search strategy was conducted in the Medical Subject Headings PubMed platform from March 30 to April 1, 2020, using the following combinations of terms: neurons vs. coronavirus; neural stem cells vs. coronavirus; nervous system vs. coronavirus; SARS virus vs. neurons; SARS virus vs. neural stem cells; and SARS virus vs. nervous system. A total of 484 papers were retrieved and downloaded in the Mendeley software and duplicates were removed. Studies were subjected to double-blinded screening by their titles and abstracts for inclusion criteria regarding the following PICO strategy:

P (patient, problem, or population): healthy individuals and patients with neurological disorders;
I (intervention): immunology and molecular tests, cell cultures, and comparative experimental studies of respiratory CoV in humans;
C (comparison, control, or comparator): healthy vs. diseased conditions, health vs. immunized conditions;
O (outcomes): virulence and pathogenicity of the virus in the CNS.

Reviews, letters, commentaries, non-interventional studies, articles not written in English, and animal studies without a focus on human retroviruses were all excluded (**Figure 1**).

All reports of human CoV infections found at this stage were displayed on a timeline (**Figure 2**). A total of 30 articles were included for synthesis without meta-analysis (10). This review followed the PRISMA (preferred reporting items for systematic reviews and meta-analyses) protocol. The studies' findings are summarized in **Table 1**.

RESULTS

Six strains of infectious bronchitis virus (IBV) were detected in embryonic tracheal organ cultures from patients with colds. McIntosh et al. (11) demonstrated that two of these "IBV-like" strains were able to grow in newborn inoculated mice, and caused an encephalitic syndrome. Complement-fixation tests of human convalescent sera and the specific mouse immune sera were homologous to the brain suspensions from affected mice. The strains were shown to be identical with each other and distinct from IBV and strain CoV-229E (another respiratory CoV, morphologically similar to IBV). Kaye and Dowdle (12) discovered two strains of IBV-like CoVs and named them "OC38" and "OC43."

Pearson and Mims (13) investigated selective cell vulnerability to CoV-OC43 infection. Using cell-type-specific markers and neural cultures derived from various areas of the CNS, they showed that neurons from the dorsal root ganglia produced both viral antigen and infectious virus, while astrocytes and fibroblasts produced only viral antigen, and oligodendrocytes produced neither the infectious virus nor viral antigen. Human embryo brain cells, including astrocytes, are susceptible to OC43 infection but production of infectious virus has not been reported.

Collins (14) showed that cortical neuronal cells support the replication of both CoV-229E and CoV-OC43 serotypes. Using a human cerebral neuron cell line, the results of that study showed that neurons, which express the aminopeptidase-N receptor (CD13) for CoV-229E at nerve synapses, are much more susceptible to direct infection by CoV-229E than by CoV-OC43. Both viruses induced the synthesis of viral antigens.

Using antibodies to CoV-229E and CoV-OC43 viruses, and cell markers in human neural primary cultures cells,

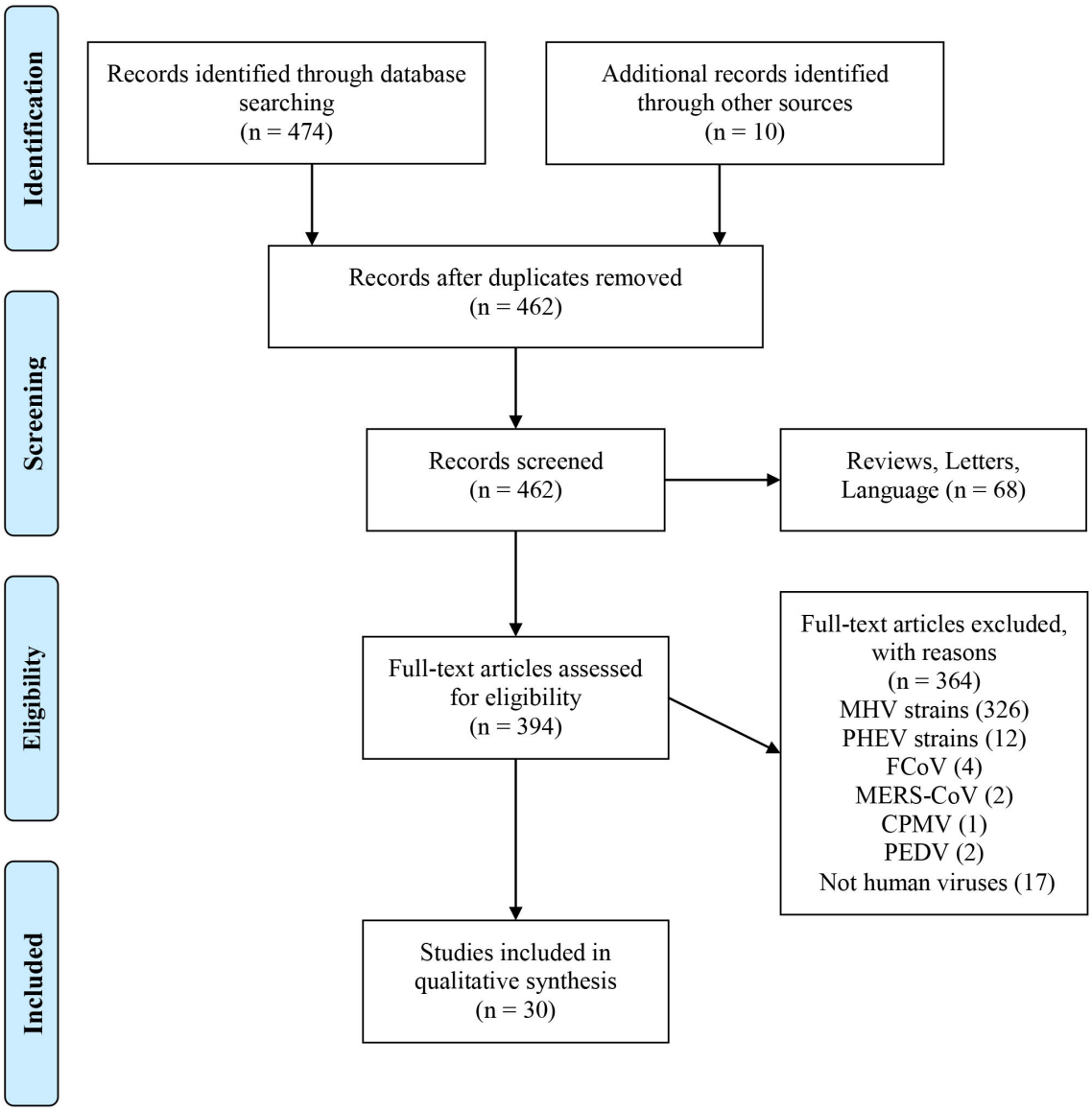

FIGURE 1 | PubMed data extraction flow chart. From Moher et al. (9). For more information, visit www.prisma-statement.org.

Bonavia et al. (15) was able to demonstrate viral neuroinvasion in fetal astrocytes, and in adult microglia and astrocytes, by the strain OC43. Furthermore, RNA amplification also confirmed infection of fetal astrocytes, adult microglia, and a mixed culture of adult oligodendrocytes and astrocytes with the strain 229E. In this study, infectious virus was released only from fetal astrocytes, with higher titers for CoV-OC43.

Lachance et al. (16) tested whether CD13 is utilized as a receptor for CoV-229E infection inhuman neural cell lines. They proved that CD13 expression on the surfaces of various neuronal and glial cell lines, which are susceptible to CoV-229E infection, correlate with the level of viral attachments. They also showed that CD13 is expressed and serves as a receptor for CoV-229E infection in neuronal and glial cells.

Arbour et al. (17) provided evidence of CoV-229E neurotropism, and possible viral persistence in the CNS by showing that astrocytoma, neuroblastoma, neuroglioma, and oligodendrocytic cell lines are all susceptible to infection by CoV-229E. The oligodendrocytic and neuroglioma lines sustained a persistent viral infection, which was monitored by detection of the viral antigen and infectious viral progeny.

Arbour et al. (40) characterized CoV RNA in a large panel of human brain autopsy samples. Amplified CoV-229E and CoV-OC43 strains from the samples of donors with various neurological diseases 39 with multiple sclerosis (MS), 26 with other neurological diseases, and 25 controls) reported that 44% (40 of 90) of donors were positive for CoV-229E and 23% (21 of 90) were positive for CoV-OC43. A higher prevalence of

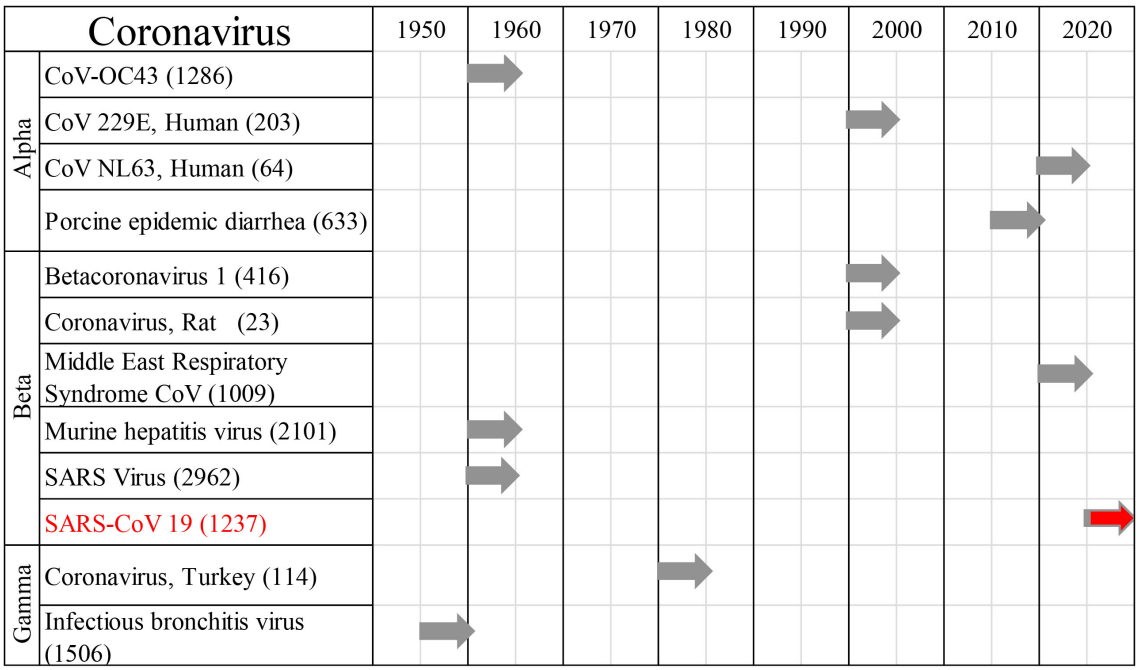

FIGURE 2 | Human coronaviral infections. First reports on the different types of Coronaviruses infecting humans throughout history, and the number of published studies.

CoV-OC43 was noted in patients with MS (35.9%; 14 of 39) than in the controls (13.7%; 7 of 51). Viral RNA was found in brain parenchyma, but outside of the blood vessels.

Evidence of a CoV-induced MS-like in rodents, which plays a role in the inflammatory system, led Edwards et al. (18) to analyze the expression of cytokines and chemokines in CoV-OC43-infected human astrocytes and immortalized microglial cell lines. An up-regulation of IL-6, TNF-a, and MCP-1 mRNA expression was observed in the astrocytes infected with CoV-OC43. The virus also modulated the activity of matrix metalloproteinases-2 and -9 and augmented nitric oxide production in microglial cells.

Brain tissue samples from 25 patients with MS and 36 controls were tested for the prevalence of CoV (19). Four PCR assays with primers specific for the N-protein gene of CoV-229E and three PCR assays specific for the nucleocapsid protein gene of CoV-OC43 were performed. Some sporadic positive PCR assays were observed in both patients and controls. Results were not reproducible and no significant difference was reported in the proportion of positive signals from the patients with MS compared with the controls.

Jacomy and Talbot (20) developed an experimental model of CoV-OC43-inoculated mice and characterized the neurotropic properties of the virus. The virus led to a generalized infection of the entire CNS. The acute infection targeted neuronal cells, which underwent vacuolation and degeneration, during strong microglial reactivity and inflammatory reactions. Damage to the CNS was not immunologically mediated and the microglial reactivity was instead, a consequence of direct virus-mediated neuronal injury.

Lau et al. (21) reported a case of a 32-years-old healthy woman in week 26 of pregnancy, who was admitted to the hospital with myalgia, fever, chills, and rigor for 2 days. She had tested positive for the "new" severe acute respiratory syndrome CoV (SARS-CoV) in cerebrospinal fluid collected from day 22, after generalized tonic-clonic convulsions with a loss of consciousness.

St-Jean et al. (22) uncovered six mutations scattered throughout the CoV-OC43 genome giving rise to two amino acid substitutions. The two CoV-CO43 variants were able to reach the CNS after intranasal inoculation in mice. The stability of the virus in the environment was highlighted, when the two variants were isolated from cells, 40 years apart. Genomes of the two CoV-OC43 variants displayed 71, 53.1, and 51.2% identity with MHV A59, the SARS-CoV Tor2 strain, and CoV-229E, respectively. Furthermore, CoV-OC43 has well-conserved motifs, like the genome sequence of the Tor2 strain of SARS-CoV, suggesting that CoV-OC43 and SARS-CoV may share several important functional properties.

Glass et al. (23) produced a model of SARS-CoV infection in mice. Intranasally introduced virus replicated transiently to high levels in the lungs, with a peak on day 3 post-infection and clearance by day 9. Viral RNA was localized to the bronchial and bronchiolar epithelium and mRNA expression for the ACE2 receptor was detected in the lung, following infection. The expression of the proinflammatory chemokine genes, *CCL2, CCL3, CCL5, CXCL9,* and *CXCL10*, and receptor genes (especially *CXCR3*) was up-regulated in the lungs with differential kinetics. However, T-cell cytokine mRNAs (Th1 and Th2) were not detectable.

TABLE 1 | Studies and main findings.

Authors and Title	Virus	Model	Main findings
(11) "From Patients with Upper Respiratory Tract Disease"	IBV-like virus strains	Suckling mouse and tracheal cell culture	Two of the six "IBV-like" strains caused an encephalitic syndrome in inoculated mice
(12) "Some Characteristics of Hemagglutination of Certain Strains of 'IBV-like' Virus"	IBV-like	Mouse brain harvests, human, chicken, mouse, rat rhesus and guinea pig cells, erythrocytes	Human cells were agglutinated without spontaneous elution at an optimal hemagglutination temperature
(13) "Differential Susceptibility of Cultured Neural Cells to the Human Coronavirus OC43"	CoV-OC43	Neural cell cultures	Human embryo brain cells, including astrocytes, were susceptible to OC43 infection but did not produce infectious virus
(14) "Interferon γ Potentiates Human Coronavirus OC43 Infection of Neuronal Cells by Modulation of HLA Class I Expression"	CoV-229E CoV-OC43	Human cortical neuron, neuroblastoma, and diploid lung cell lines	CoVs were able to replicate in neurons
(15) "Infection of Primary Cultures of Human Neural Cells by Human Coronaviruses 229E and OC43"	CoV-229E CoV-OC43	Human neural cell lines	Microglial cells did not produce infectious progeny viruses after CoV-OC43 infection
(16) "Involvement of Aminopeptidase N (CD13) in Infection of Human Neural Cells by Human Coronavirus 229"	CoV-229E	Human embryonic lung and neural cell lines	Expression of aminopeptidase-N receptor in neurons, astrocytes, and oligodendrocytes might explain their susceptibility to CoV-229E infection
(17) "Persistent Infection of Human Oligodendrocytic and Neuroglial Cell Lines by Human Coronavirus 229E"	CoV-229E	Human neural cell lines	Oligodendrocytic and neuroglioma cell lines also sustained a persistent viral infection
(17) "Neuroinvasion by Human Respiratory Coronaviruses"	CoV-229E CoV-OC43	Human brain autopsy samples–various neurological diseases	Higher prevalence of CoV-OC43 in patients with MS compared with the controls
(18) "Activation of Glial Cells by Human Coronavirus OC43 Infection"	CoV-OC43	Immortalized human microglial cells and human astrocyte cell line	CoV infection of glial cells may be indirectly associated with CNS pathologies
(19) "Coronaviruses in Brain Tissue from Patients with Multiple Sclerosis"	CoV-229E CoV-OC43	Brain tissue from patients with MS	Evidence for chronic infection with CoV-229E or OC43 in the brain tissue of patients with MS or controls has not been found
(20) "Vacuolating Encephalitis in Mice Infected by Human Coronavirus OC43"	CoV-OC43	Mice	Damage to the CNS was not immunologically mediated and the microglial reactivity was a consequence of neural infection
(21) "Possible Central Nervous System Infection by SARS Coronavirus"	SARS-CoV	32-years-old woman, week 26 of pregnancy, previously in good health	Generalized tonic-clonic convulsions and positive SARS-CoV in cerebral spinal fluid suggested infection of the CNS with SARS-CoV
(22) "Human Respiratory Coronavirus OC43: Genetic Stability and Neuroinvasion"	CoV-OC43	Cell culture	Described the complete genome sequence of CoV-OC43 strains
(23) "Mechanisms of Host Defense Following Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) Pulmonary Infection of Mice"	SARS-CoV	Mice	SARS-CoV generated a transient non-fatal systemic infection in the lungs which was disseminated to the brain
(24) "Multiple organ infection and the pathogenesis of SARS"	SARS-CoV	Brain tissue, full autopsy of 39-years-old patient with SARS	Neuroinvasion by SARS-CoV, evidenced by viral morphology, genetic identification, and the viral antigen (N protein) found in the brain
(25) "Susceptibility of Human and Rat Neural Cell Lines to Infection by SARS Coronavirus"	SARS-CoV	Human oligodendroglioma, Rat glioma, Human intestine, Canine kidney, and Rabbit kidney cell lines	Human and rat neural cells were susceptible to SARS-CoV infection, with no apparent cytopathic effects
(26) "Murine Encephalitis Caused by HCoV-OC43, a Human Coronavirus with Broad Species Specificity, Is Partly Immune-Mediated"	CoV-OC43	Mice	Rapidly increase in virulence after passage in the brain is likely to occur via selection of mutations in the S-glycoprotein

(Continued)

TABLE 1 | Continued

Authors and Title	Virus	Model	Main findings
(27) "Human Coronavirus OC43 Infection Induces Chronic Encephalitis Leading to Disabilities in BALB/C Mice"	CoV-OC43	Mice neural cell lines	Results support the theory that CoV-OC43 has a preferential tropism for neurons
(28) "Lethal Infection of K18-HACE2 Mice Infected with Severe Acute Respiratory Syndrome Coronavirus"	SARS-CoV	Mice transgenic for ACE2 receptor	Transgenic mice developed a rapidly lethal infection that spread to the brain, after intranasal inoculation with SARS-CoV
(29) "Severe Acute Respiratory Syndrome Coronavirus Infection of Mice Transgenic for the Human Angiotensin-Converting Enzyme 2 Virus Receptor"	SARS-CoV	Mice transgenic for ACE2 receptor	Lungs and brain were the major sites of viral replication, particularly in transgenic mice
(30) "Long-Term Human Coronavirus-Myelin Cross-Reactive T-Cell Clones Derived from Multiple Sclerosis Patients"	CoV-229E CoV-OC43	T-cell clones (TCC) of patients with MS	TCC from the blood of patents with MS could be activated by either viral or myelin antigen and sometimes by both
(31) "Pathological Changes in Masked Palm Civets Experimentally Infected by Severe Acute Respiratory Syndrome (SARS) Coronavirus"	SARS-CoV	Masked palm civets	SARS-CoV caused a multi-organ pathology in civets similar to that observed in human patients with SARS
(32) "Severe Acute Respiratory Syndrome Coronavirus Infection Causes Neuronal Death in the Absence of Encephalitis in Mice Transgenic for Human ACE2"	SARS-CoV	Mice transgenic for ACE2 receptor	Neurons are highly susceptible targets for SARS-CoV infection and the absence of cell receptors prevents severe murine brain disease
(33) "Neuroprotective Effect of Apolipoprotein D against Human Coronavirus OC43-Induced Encephalitis in Mice"	CoV-OC43	Human apolipoprotein D(apoD) transgenic mice	Overexpression of apoD in neurons resulted in an increased number of survivors to CoV-OC43 infection
(34) "Glutamate Excitotoxicity is Involved in the Induction of Paralysis in Mice after Infection by a Human Coronavirus with a Single Point Mutation in Its Spike Protein"	CoV-OC43	Mice	The AMPA receptor antagonist led to reduced microglial activation, which was believed to improve the regulation of CNS glutamate homeostasis
(35) "Human Coronavirus-Induced Neuronal Programmed Cell Death Is Cyclophilin D Dependent and Potentially Caspase Dispensable"	CoV-OC43	Human neuronal cell lines	Mitochondrial apoptosis-inducing factor and cyclophilin D appears to be pivotal in CoV-OC43-induced programmed cell death, while caspases do not appear to be essential
(36) "Novel Treatment with Neuroprotective and Antiviral Properties against a Neuroinvasive Human Respiratory Virus"	CoV-OC43	Mice	Memantine improved clinical scores related to the motor disabilities and attenuated mortality rates in virus-infected mice
(37) "Pivotal Role of Receptor-Interacting Protein Kinase 1 and Mixed Lineage Kinase Domain-Like in Neuronal Cell Death Induced by the Human Neuroinvasive Coronavirus OC43"	CoV-OC43	Human neuroblastoma cell lines and mice	A CoV-OC43 variant, harboring two-point mutations in the S-glycoprotein (S2) was more neurovirulent than the CoV-OC43 in mice and induced more cell death in murine and human neuronal cells
(38) "The OC43 Human Coronavirus Envelope Protein Is Critical for Infectious Virus Production and Propagation in Neuronal Cells and Is a Determinant of Neurovirulence and CNS Pathology"	CoV-OC43	Human neuronal cell lines and mouse neuronal cell lines	CoV-OC43 envelope protein is critical for the production of infectious virions
(39) "Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43"	CoV-OC43	Human neuronal cell lines and mice	Both passive diffusion of released viral particles and axonal transport are valid propagation strategies used by the virus

CoV, coronavirus; CNS, central nervous system; MS, multiple sclerosis; ACE2, angiotensin-converting enzyme 2; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid.

Minimal local accumulation of leukocytes was observed without obvious clinical signs of pulmonary dysfunction. Infection also spread from the lungs to the brain without leukocyte accumulation. Mice showed normal clearance of the virus.

Gu et al. (24) isolated a SARS-CoV strain from brain autopsies. Fifteen cytokines and chemokines were detected in the blood of a patient. Amplifications of SARS-CoV specific fragments from the brain tissue of eight patients confirmed the presence of the virus. Pathologic examination showed necrosis of neuronal cells and

extensive hyperplasia of gliocytes. The *CXCL9* gene was expressed in gliocytes and the infiltration of monocytes/macrophages and T lymphocytes was observed in the brain mesenchyme.
Both *CXCL10* and *CXCL9* expression levels were highly elevated in the blood, although the levels of other cytokines and chemokines were close to normal. Chest radiographs indicated that the pathologic change in the brain was independent of pulmonary superinfection. Neuroinvasion by SARS-CoV was confirmed by viral morphology observed under the microscope, as well as genetic identification, and the presence of viral antigen (N protein) in the brain.

Yamashita et al. (25) showed that SARS-CoV infection in human neural cells can yield viral infectivity of 10^{2-5} per mL, with no apparent cytopathic effects. Infection of the intestinal cell line "CaCo-2" also induced no apparent cytopathic effects, with the production of lower levels of the virus. The SARS-CoV receptor, ACE2, was expressed at higher levels on CaCo-2 cells, but at undetectable levels in neural cells.

Butler et al. (26) showed that CoV-OC43 is highly virulent in the suckling mouse brain and can cause a uniformly fatal encephalitis in adult mice. A spike glycoprotein (S-glycoprotein) on the surface of the CoV-OC43 membrane was revealed as a major virulence factor in CoV infections. The ability to rapidly gain virulence after passage in the murine brain is likely to occur via the selection of mutations in the S-glycoprotein, which optimize both binding and entry to target cells. Three changes in the CoV-OC43 S-glycoprotein, in the domain of the protein responsible for binding to host cells, correlated with enhanced neurovirulence in the model. Such adaptability has facilitated the isolation of CoV-OC43 variants with markedly differing abilities to infect animals and tissue culture cells. This adaptability also appears to be a mechanism by which CoV-OC43 and SARS-CoV can readily adapt to growth in cells from heterologous species.

Jacomy et al. (27) used primary cell cultures to determine the susceptibility of each type of neural cell toCoV-OC43 infection. They showed that neurons are the target cells undergoing degeneration during infection, in part due to apoptosis. Intracerebral inoculation with CoV-OC43 in susceptible mice led to an acute encephalitis, with neuronal cell death by necrosis and apoptosis. Infectious viral particles were apparently cleared from surviving animals, whereas viral RNA persisted for several months. After the acute encephalitis, some of the surviving animals presented an abnormal limb-clasping reflex and reduced motor activity starting several months post-infection.

McCray et al. (28) produced transgenic mice that expressed the human ACE2 receptor in the airway and other epithelia. Mice developed a rapidly lethal infection after intranasal inoculation with SARS-CoV. Infection began in the airway epithelia, with subsequent alveolar involvement, and extrapulmonary virus spread to the brain. Furthermore, infection resulted in the infiltration of macrophages and lymphocytes into the lungs and an upregulation of proinflammatory cytokines and chemokines in both the lungs and brain.

In the study Tseng et al. (29), the best model of transgenic mice expressing the human ACE2 receptor showed clinical manifestations within 8 days post-intranasal SARS-CoV infection. High viral titers were detected in the lungs and brains on days 1 and 3. Inflammatory mediators in these tissues coincided with the high levels of viral replication. Lower viral titers were detected in the blood. Infected non-transgenic mice survived, without showing signs of clinical illness. Extensive CNS involvement likely determines whether the animal dies, rather than the presence of viral pneumonia. Mouse lineages, in which the transgene expression is considerably less abundant than it was in this model, showed that viral replication is largely restricted to the lungs but not the brain.

Boucher et al. (30), after reporting the isolation of CoV-229E/myelin basic protein (MBP) cross-reactive T cell lines (TCL) in patients with MS, checked for antigenic cross-reactivity. A total of 155 long-term T-cell clones (TCC) were derived from 32 patients with MS by *in vitro* selection against MBP, proteolipid protein, or CoV (strains 229E and OC43). Overall results showed that 114 TCC were virus-specific, 31 were specific for the myelin antigen, and 10 were CoV/myelin cross-reactive. Additionally, 28 virus-specific TCC and seven myelin-specific TCC were obtained from six healthy donors. The TCC derived from the blood of patients with MS could have been activated by either viral or myelin antigen, and sometimes by both.

Xiao et al. (31) elucidated the histopathological changes induced by SARS-CoV in an infected civet (*Paguma larvata*) model. *In-situ* hybridization detected viral RNA in the animal's lung, small intestine, and brain alone. Apoptosis detection within the brain showed evidence of neuronal degeneration and mild neuronophagia from days 3 to 13 after infection. In two cases, mild edema was also observed around the small veins and nerve cells. No microscopic changes were observed in these tissues after 13 days. Furthermore, no evidence of apoptosis was observed within the myocardium. Those results indicated that SARS-CoV can cause a multi-organ pathology in civets, similar to that observed in human patients with SARS.

Netland et al. (32) experimented with transgenic mice and the ACE2 receptor. They showed that the virus enters the animal's brain primarily via the olfactory bulb and the infection results in a rapid trans-neuronal spread to other connected areas of the brain. Extensive neuronal infection was the main cause of death, considering that intracranial inoculation with low doses of the virus results in a uniformly lethal disease, even when little infection is detected in the lungs. Death likely results from dysfunction and/or death of infected neurons, especially those located in cardiorespiratory centers in the medulla. The SARS-CoV induced minimal cellular infiltration in the brain. The results of that study confirmed that neurons are highly susceptible targets for SARS-CoV infection, in which only the absence of the cell receptor prevented severe murine brain disease.

Do Carmo et al. (33) showed that apolipoprotein D (apoD) expression is regulated during acute encephalitis induced by CoV-OC43 infection. The upregulation of human apoD expression in transgenic mice coincided with glial activation. Both apoD expression and glial activity returned to normal levels when the virus was cleared. Overexpression of apoD in the neurons of mice resulted in a 3-fold increase in

the number of mice surviving the CoV-OC43 infection. The overexpression of apoD also correlated with up-regulated glial activation, a limited innate immune response (associated with cytokines and chemokines), and T-cell infiltration into infected brains.

Brison et al. (34) showed that a single point mutation in the S-glycoprotein of CoV-OC43, acquired during viral persistence in human neural cells, led to a hindlimb paralytic disease in mice. In the S-glycoprotein mutant mice, the inhibition of glutamate excitotoxicity, using a 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptor antagonist, improved clinical scores associated with paralysis and motor disabilities. Glutamatergic inhibition also protected the CNS from neuronal dysfunction, as illustrated by the restoration of phosphorylation of neurofilaments. Expression of the glial glutamate transporter (GLT-1), responsible for glutamate homeostasis, was down-regulated following infection, while the AMPA receptor antagonist restored GLT-1 steady-state expression levels. Treatment with the AMPA receptor antagonist led to reduced microglial activation, which was believed to improve the regulation of CNS glutamate homeostasis.

After observing that CoV-OC43 infection of neurons activates the unfolded-protein response and caspase-3, and induces cell death with involvement of the S-glycoprotein, Favreau et al. (35) elucidated possible mechanisms of cell death following CoV-OC43 infection. They reported a more neurovirulent and cytotoxic CoV-OC43 variant, harboring two-point mutations in the S-glycoprotein (S2) in human neuronal cells. Caspase-3 and -9 were both activated after infection, but caspase inhibitors neither reduced nor delayed virus-induced cell death. The proapoptotic proteins, BAX and cytochrome c (CytC), and the apoptosis-inducing factor were re-localized toward the mitochondria, cytosol, and nucleus, respectively, after infection with the variants of both viruses. Neuronal cells treated with cyclosporine (an inhibitor of the mitochondrial permeabilization transition pore), or knocked down for cyclophilin D (a protein known for regulating mitochondrial function) were completely protected from CoV-OC43-induced neuronal death. However, knockdown of S2 in infected cells had a moderate effect on programmed cell death The results supported the theory that mitochondrial apoptosis-inducing factor and cyclophilin D are central to CoV-OC43-induced cell death, while caspases do not appear to be essential.

Brison et al. (36) demonstrated that glutamate recycling, via GLT-1 and glutamine synthetase, is central to the dysregulation of glutamate homeostasis and development of motor dysfunctions and paralytic disease in CoV-OC43-infected mice. Furthermore, memantine (an N-methyl-D-aspartate receptor antagonist, widely used in the treatment of neurological diseases) improved clinical scores related to motor disabilities, by partially restoring physiological neurofilament phosphorylation in virus-infected mice and attenuating mortality rates. Reduced CoV-OC43 replication was observed in the CNS in a dose-dependent manner.

Meessen-Pinard et al. (37) verified whether knockdown of BAX (BCL2-associated X protein) or RIP1 (a key regulator of necroptosis) altered the percentage of neuronal cell death following CoV-OC43 infection. They reported that BAX-dependent apoptosis did not play a role in regulated cell death following infection, once the inhibition of BAX expression, mediated by RNA interference, did not confer cellular protection against the cell death process. Both RIP1 and mixed lineage kinase domain-like (MLKL) were involved in neuronal cell death, as RIP1 knockdown and chemical inhibition of MLKL increased cell survival after infection. The results indicated that RIP1 and MLKL contribute to necroptotic cell death, following CoV-OC43 infection, to limit viral replication. This regulated cell death can lead to neuronal loss and accentuate the neuroinflammatory process, reflecting the severity of neuropathogenesis.

Stodola et al. (38) used recombinant viruses, either devoid of the structural envelope (E) protein or harboring mutations in the putative transmembrane domain or PDZ-binding motif. They demonstrated that with CoV-OC43 infection of cell lines from the human CNS and mouse CNS, the E protein is critical for efficient and optimal viral replication and propagation, and therefore, neurovirulence.

Dubé et al. (39) showed a route of neuropropagation from the nasal cavity to the olfactory bulb and piriform cortex, and then to the brain stem in mice. A neuron-to-neuron propagation was identified as one underlying mode of viral spread in cell culture. Both passive diffusion of released viral particles and axonal transport were propagation strategies used by the virus. The presence of viral platforms with static dynamism was consistent with the viral assembly sites revealed along the axons. The CoV-OC43 modes of propagation might be modulated by selected CoV-OC43 proteins and axonal transport.

DISCUSSION

Cumulative data indicate that respiratory infection with different species of CoV can evolve to CNS disturbances, sequelae, and possibly chronic disease (**Figure 3**). Respiratory CoVs have been identified for more than eight decades, with the most devastating scenario created by the current Covid-19 pandemic following the SARS-CoV2 outbreak. Search results showed that the last 6 months of the pandemic has already produced more speculation than the entire body of scientific literature on any other CoV. Historically, IBV-like, CoV-OC43, CoV-229E, and SARS-CoV have been shown to interact with the CNS (**Figure 2**).

Reports of respiratory infections with CoVs among several studies show similar levels of neuropathogenicity. The binding of these viruses to the ACE2 receptor is putative for viral neuroinvasion, even though neuronal viral infection may be mediated by the CD13 receptor. IN addition, CoV-229E, CoV-OC43, and SARS-CoV have been shown to have the capacity to infect neurons, astrocytes, glial cells, and fibroblasts with a common response of encephalitis, and highest viral production in neurons.

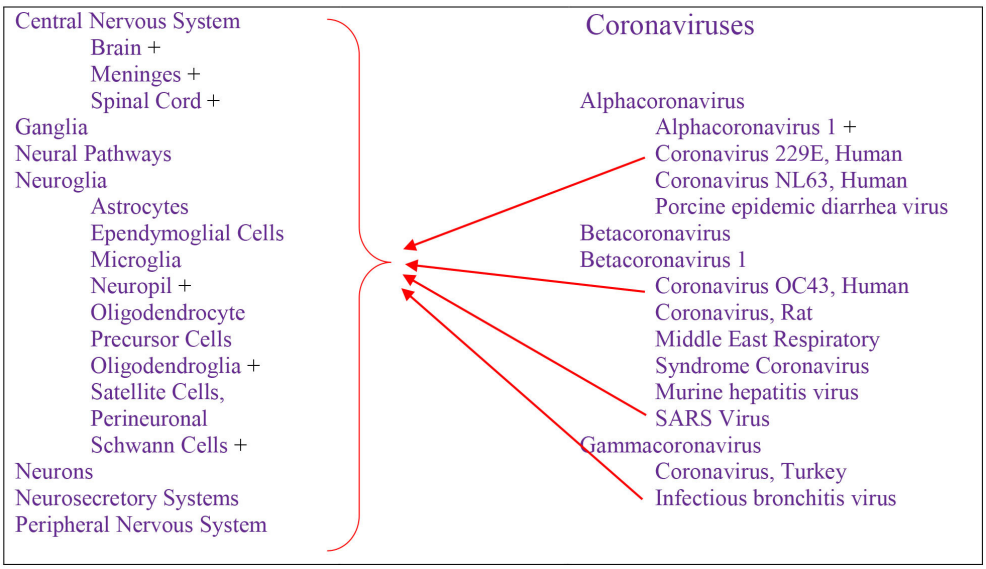

FIGURE 3 | Respiratory-Neurovirulent Coronaviruses detected in human—PubMed source.

The consensus that human infection with respiratory CoVs can cause encephalitis is supported by animal models, which have facilitated descriptions of this route of transmission. The infection begins in the airway epithelia, with binding of the virus to ACE2 receptors and subsequent alveolar involvement. Extrapulmonary viral particles are then spread to the CNS, possibly through nuclei that regulate the respiratory rhythm in the brain stem, or through the olfactory bulbs. The mechanism of neuroinvasion in respiratory CoVs may occur via neuronal contacts and the recruitment of axonal transport.

Although other neural cells expressing ACE2 and CD13 receptors are also susceptible, as neurons are the main targets of CoV neuroinvasion, the neuronal losses induced by regulated cell death are probably the main neurovirulence factor causing generalized encephalitis (14, 16). Such losses likely occur via mitochondria-associated caspase-independent apoptosis. However, glutamate homeostasis dysregulation and toxicity could be additional factors associated with brain damage, cardiorespiratory center inhibition, and microglial inflammatory reactions (32, 34–36).

The inflammatory processes involved in the CNS response to CoV infection remains unclear. While immune cell infiltration is weakly supported (24), the participation of astrocytes and other glial cells in these processes have been extensively reported. The expression of inflammatory cytokines and chemokines, together with an upregulation of their receptors, have revealed the role of microglial activation in the inflammatory reactions of the CNS (18, 23, 24, 28, 29).

The neurovirulence of respiratory CoVs appears to be associated with the duration of viral exposure, which is crucial for the severity of pathogenicity. The persistence of CoV may lead to a spectrum of conditions from acute encephalitis without noticeable sequelae, to paralysis or chronic disease, as illustrated by Lau et al. (21), Arbour et al. (17), Brison et al. (34), and Tseng et al. (29). The findings of those reports show that viral reactivation does not lead to increased inflammation, increased response of virus-specific T cells, or re-expression of cytolytic effector function. Virus-specific Tcells within the brain retain the ability to secrete viral antigen but are unable to prevent CNS viral reactivation.

While interferon-γ is crucial for viral clearance during acute infection, it is insufficient to control viral reactivation. Such inability to enhance T-cell effector function is attributed to the decreased ability of peripheral Tcells to access the CNS, and an inability of antigens to leave the CNS and reactivate peripheral Tcells (41, 42). These effects all lead to an absence of T cell-mediated cytokines in the brain (23) and an insufficiency of the Tcell-mediated immune response to CoVs in the CNS.

Several viruses can infect neural tissue cells and possibly participate in the induction of neurological diseases, even though their primary site of infection in humans may not be the CNS (43). For instance, neurodegenerative diseases, such as dementia-related disorders and/or sequelae might also be linked to viral infection. The association of CoVs with MS has been reported in some studies (18, 40) and immune cell virus cross-reactivity could be one of the pathological mechanisms by which it arises (30).

One notable point is the mutagenicity of CoVs. Even a couple mutations designed in the laboratory were able to increase the specificity of the virus to human CNS cells (22, 26, 34, 35, 38). This finding strongly supports an analogous process in SARS-CoV2 during the pandemic of Covid-19, through which we may be confronted with new neural-specific viral infections. Nevertheless, retrospective analysis of the data on CoV infections of the neural system could facilitate the

prediction of viral behavior, indicate the weak points, and inform the prognosis of cases, within the context of future medical challenges.

Limitations

Although previous findings on respiratory CoVs are useful for this Pandemic moment, more novel, in-depth studies on SARS-CoV2 are required to gain a better understanding of the events following the recent epidemics of Covid-19. All available studies this submission date referred to strains of CoV viruses that cause respiratory infections other than SARS-CoV2.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are from articles publically available on the PubMed database (https://pubmed.ncbi.nlm.nih.gov/).

AUTHOR CONTRIBUTIONS

GA: conceptualization and data collection and curation; GA and EG: writing—original draft preparation; EM-C and PC: writing—review & editing; PC: supervision; PC and EM-C: project administration and funding acquisition. All authors contributed to the article and approved the submitted version.

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Neurological Implications of Non-critically Ill Patients With Coronavirus Disease 2019 in a Fangcang Shelter Hospital in Wuhan, China

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Background: Coronavirus disease 2019 (COVID-19) is a new viral respiratory disease and has become a pandemic. Fever, weakness, and dry cough are the main clinical manifestations. However, little is known about neurological symptoms of non-critically ill COVID-19 patients.

Objective: To investigate the neurological symptoms and implications of patients with non-critically ill COVID-19 patients.

Materials and Methods: This retrospective cohort study investigated all COVID-19 patients admitted to Wuhan East-West Lake Fangcang shelter hospital. Demographic data, clinical manifestations, comorbidities, radiological data, the result of nucleic acid test, and treatments were collected and analyzed.

Results: Among 1,682 patients with confirmed non-critically ill COVID-19, 509 patients (30.3%) had neurological symptoms, including myalgia (311, 18.5%), headache (216, 12.8%), fatigue (83, 4.9%), and dizziness (15, 0.9%). One hundred and fourteen patients (6.8%) were the expansion of pulmonary infection according to their chest CT images and medical history. Compared with patients without neurological symptoms, patients with neurological symptoms had a significantly longer length of hospital stay, time of nucleic acid turning negative, and the mean time from onset of symptom to hospital admission ($p < 0.05$). Patients with neurological symptoms were more likely to occur the expansion of pulmonary infection compared with the patients without neurological symptoms (46/509 [9.0%] vs. 68/1,173 [5.8%]).

Conclusions: Non-critically ill COVID-19 patients commonly have neurological symptoms. Neurological symptoms are significantly associated with the processes of COVID-19. Early identification and aggressive treatment are particularly important for COVID-19 patients with neurological symptoms.

Keywords: neurologic symptoms, COVID-19, SARS-CoV-2, CNS, neurological implications

INTRODUCTION

COVID-19 is a potentially severe acute respiratory infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has spread rapidly around the world (1–4). On March 11, the World Health Organization (WHO) declared that the COVID-19 outbreak can be characterized as a pandemic (5). As of 27 June 2020, the data from WHO indicate that there have been 9,653,048 confirmed cases, including 491,128 deaths in just 4 months, which has affected more than 200 countries and regions.

The human respiratory system is recognized as the SARS-CoV-2 primary target. Fever, cough, and dyspnea are the most common clinical symptoms at the onset of illness (6, 7). Moreover, several studies have confirmed that SARS-CoV-2 is highly similar to SARS-CoV; the latter can invade human multiple systems including lung, trachea/bronchus, stomach, small intestine, brain, and liver, not just the respiratory system (8–11). Recent researches have shown that digestive symptoms are common in patients with COVID-19, including nausea, vomiting, and diarrhea, and the fecal–oral route may be a potential route for SARS-CoV-2 infection (12–16). Some experts hold the view that SARS-CoV-2 may invade the brain and central nervous system by the ACE2 receptor (17, 18), which is present in the nervous system and skeletal muscles.

A recent study showed that 81% of SARS-CoV-2 nucleic acid-positive patients were classified as mild (non-critically ill patients), which displays non-pneumonia or only mild pneumonia (19). In order to address the drawbacks of home isolation and ease pressures on the designated traditional hospitals, 15 Fangcang shelter hospitals special for non-critically ill COVID-19 patients were rapidly established by the urgent renovation of stadiums and exhibition centers. The diagnostic and classification criteria of COVID-19 are based on the diagnosis and treatment program for novel coronavirus pneumonia issued by the National Health Commission (NHC) of the People's Republic of China (Trial Version 6). All patients who are nucleic acid positive, have mild to moderate symptoms (respiratory rate $<$30 breaths/min and blood oxygen saturation $>$93% at resting state), and are quarantined at home are unified transferred to nearby Fangcang shelter hospitals by the government (20–23).

At present, most of the studies focused on the clinical characteristics of severe or critically ill patients. However, specific information characterizing neurological symptoms of non-critically ill COVID-19 patients remains obscure. The present study aims to investigate the neurological implications of non-critically ill COVID-19 patients and provide references for the prevention and treatment of the disease.

MATERIALS AND METHODS

This single-center retrospective cohort study was performed at Wuhan East-West Lake Fangcang shelter hospital, which is the first largest shelter hospital designated by the government to admitting non-critically ill COVID-19 patients in the city, constructed and led by Zhongnan Hospital of Wuhan University from February 4, 2020, to March 10, 2020. We retrospectively analyzed all COVID-19 patients admitted to Wuhan East-West Lake Fangcang shelter hospital. Moreover, we only excluded a few patients with incomplete data. All patients were confirmed COVID-19 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in designated hospitals before admission. Throat-swab specimens were obtained for the nucleic acid test.

The diagnostic and classification criteria issued by the National Health Commission (NHC) of the People's Republic of China (Trial Version 6) classified the disease severity of patients to 4 levels: mild, moderate, severe, and critical. The mild grade represents patients with mild clinical symptoms and no sign of pneumonia on imaging. The moderate grade represents patients who have a fever and respiratory symptoms with radiologic findings of pneumonia. The severe grade represents patients meet any of the following criteria: (1) respiratory distress ($\geq$30 breaths/min); (2) oxygen saturation $\leq$93% at rest; and (3) PaO_2FiO_2 $\leq$300 mm Hg or chest imaging showing obvious lesion progression $>$50% within 24–48 h. The critical grade represents patients meeting any of the following criteria: (1) respiratory failure and requiring mechanical ventilation; (2) shock; (3) having other organ failures that require ICU care (24).

Data Collection

A trained team of physicians reviewed electronic medical records, nursing records, laboratory findings, and radiologic examinations for all patients during the epidemic period. Patient data including demographic, medical history, chest CT imaging, treatments, the result of nucleic acid test, and comorbidities were collected and analyzed. Clinical symptoms from onset to hospital admission were provided by COVID-19 patients who were conscious, cognitively, and mentally normal.

Statistical Analysis

Continuous variables were described as mean ± standard deviation (SD) or median (interquartile range, IQR) and compared with independent group *t*-test or the Mann–Whitney U test if appropriate. Categorical variables were described as a percentage and compared by χ^2 test or Fisher's exact test if appropriate. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc. Chicago, IL). A value of $P < 0.05$ at two-sided was considered statistically significant.

RESULTS

Clinical Characteristics of Patients With Non-critically Ill COVID-19

A total of 1,682 patients with COVID-19 were included in the analysis, and 17 patients were excluded due to incomplete data. The demographic and clinical characteristics are shown in **Table 1**. The median [IQR] age was 50 [39–58] years (range, 5–89), and 859 patients (51.1%) were female. Of these patients, the median [IQR] day of hospital stay was 19 (14–23) days (range 1–29). The median [IQR] day of nucleic acid turning negative for all patients was 9 (5–13) days (range 0–25). The majority of these non-critically ill COVID-19 patients have no comorbidities. The most common comorbidities were

TABLE 1 | Clinical characteristics of patients with non-critically ill COVID-19.

Characteristics	No. (%)
No. of patients	1,682
Age, median [IQR], (range), years	50 [39–58], (5–89)
<30	125 (7.4)
30–45	508 (30.2)
46–65	892 (53.0)
≥65	157 (9.3)
Gender	
Female	859 (51.1)
Male	823 (48.9)
From onset of symptom to hospital admission, median [IQR] (range), days	10 (7–15), (1–60)
Hospital stay, median [IQR] (range), days	19 (14–23), (1–29)
Comorbidities	
Hypertension	118 (7.0)
Diabetes	53 (3.2)
Lung disease	
Chronic bronchitis	30 (1.8)
Bronchiectasis	3 (0.2)
Tuberculosis	3 (0.2)
Coronary heart disease	15 (0.9)
Cerebral infarction	5 (0.3)
Turning to nucleic acid negative, median [IQR] (range), days	9 (5–13), (0–25)
Treatments	
Traditional Chinese Medicine (Qingfei Paidutang, QPD)	1,617 (96.1)
Lianhua Qingwen capsule	1,372 (81.4)
Antibiotics	899 (53.4)
Moxifloxacin hydrochloride	863 (51.3)
Cephalosporin	103 (6.1)
Azithromycin	12 (7.1)
Antivirals	1,355 (80.6)
Umifenovir	1,316 (78.2)
Lopinave/Litonawe (62, 3.7%)	164 (9.8)
Oseltamivir	62 (3.7)
Tenofovir	2 (0.1)
Glucocorticoid (budesonide)	2 (0.1)
Expansion of pulmonary infection	114 (6.8)

IQR, interquartile range; COVID-19, Coronavirus disease 2019. The results were presented as median [IQR] (range) for continuous variables and number (%) for categorical variables.

hypertension (118, 7.0%), followed by diabetes (53, 3.2%); lung disease (36, 2.2%), such as chronic bronchitis (30, 1.8%), bronchiectasis (3, 0.2%), and tuberculosis (3, 0.2%); coronary heart disease (15, 0.9%); and cerebral infarction (5, 0.3%). After admission, antiviral and antibiotic basic medical care is provided. There were 1,617 patients (96.1%) receiving traditional Chinese medicine (Qingfei Paidutang, QPD) treatment, which is recommended by the National Health Commission (NHC) of the People's Republic of China; 1,372 (81.4%) on Lianhua Qingwen capsule; 1,355 (80.6%) on antivirals including umifenovir (1316, 78.2%), Lopinave/Litonawe (164, 9.8%), oseltamivir (62, 3.7%), and tenofovir (2,0.1%), and 899 (53.4%) on antibiotics including moxifloxacin hydrochloride (863,51.3%), cephalosporin (103, 6.1%), and azithromycin (12,7.1%), 2 (0.1%) on glucocorticoids (budesonide). Based on the results of chest CT images and medical history, 114 patients (6.8%) made up the expansion of pulmonary infection during hospitalization.

TABLE 2 | Clinical symptoms of patients with non-critically ill COVID-19 on admission.

Symptoms	No. (%)
No. of patients	1,682
Respiratory system symptoms	1,559 (92.7)
Fever	1,177 (70.1)
Cough	951 (56.6)
Chest tightness or dyspnea	425 (25.3)
Expectoration	363 (21.4)
Chills	285 (16.9)
Sore throat	254 (15.1)
Nasal obstruction	113 (6.7)
Runny nose	107 (6.4)
Nervous system symptoms	509 (30.3)
Myalgia	311 (18.5)
Headache	216 (12.8)
Fatigue	83 (4.9)
Dizziness	15 (0.9)
Gastrointestinal symptoms	305 (18.1)
Diarrhea	233 (13.6)
Abdominal pain	73 (4.3)
Nausea or vomiting	73 (4.3)
Poor appetite	23 (1.4)

IQR, interquartile range; COVID-19, Coronavirus disease 2019. The results were presented as number (%) for categorical variables.

Clinical Symptoms of Patients With Non-critically Ill COVID-19 on Admission

The median [IQR] day of the onset of symptoms to hospital admission for all patients was 10 (7–15) days (range 1–60). Clinical symptoms of all patients on admission are summarized in **Table 2**. Respiratory system symptoms are the most common clinical symptoms (1,559, 92.7%), including fever (1,177, 70.1%), cough (952, 56.6%), chest tightness or dyspnea (425, 25.3%), expectoration (363, 21.4%), chills (285, 16.9%), sore throat (254, 15.1%), nasal obstruction (113, 6.7%), and runny nose (107, 6.4%). The second prevalence of clinical symptoms were neurological symptoms (509, 30.3%), including myalgia (311, 18.5%), headache (216, 12.8%), fatigue (83, 4.9%), and dizziness (15, 0.9%). Of these 1,682 patients, 305 patients (18.1%) admitted to the hospital were found to present with one or more digestive symptoms, including diarrhea (233, 13.9%), abdominal pain (73, 4.3%), nausea or vomiting (73, 4.3%), or poor appetite (23, 1.4%).

Characteristics of Patients With Non-critically Ill COVID-19 With/Without Neurological Symptoms

Table 3 shows the characteristics of patients with/without neurological symptoms. Among these 509 COVID-19 patients with neurological symptoms, 258 patients (55.1%) were female. The median [IQR] age of patients with COVID-19 with neurological symptoms was 50 [40–58], ranging from 15 to 79 years of age. There was no significant difference in age and gender between the two groups. Compared with patients without neurological symptoms, patients with neurological symptoms had a significantly longer length of hospital stay (median [IQR], 20 (16–23) vs. 18 (14–22) days; $P = 0.002$), time of nucleic acid turning negative (median [IQR], 10 (5–14) vs. 8 (5–13) days; $P = 0.036$). Although the median [IQR] time from onset of symptom to hospital admission of the two groups is basic consistent (10 (7–15) vs. 10 (7–14) days), the mean time of patients with neurological symptoms is longer (mean ± SD [12.01 ± 6.099] vs. [10.68 ± 6.245] days; $P = 0.001$). Patients with neurological symptoms had significantly higher rates of comorbidities including hypertension (46 [9.1%] vs. 72 [6.1%]) and diabetes (29 [5.6%] vs. 24 [2.0%]). The non-critically ill COVID-19 patients with hypertension and diabetes were more likely to appear neurological symptoms.

Compared with patients without neurological symptoms, those with neurological symptoms presented with significantly higher rates of respiratory system symptoms, including fever (374 [73.5%] vs. 803 [68.2%]), cough (313 [61.5%] vs. 638 [54.4%]), chest tightness or dyspnea (195 [38.3%] vs. 231 [19.7%]), expectoration (161 [31.6%] vs. 202 [17.2%]), chills (178 [35.0%] vs. 107 [9.1%]), sore throat (129 [25.3%] vs. 125 [10.7%]), nasal obstruction (71 [14.0%] vs. 42 [3.6%]), and runny nose (63 [12.4%] vs. 44 [3.8%]). Patients with neurological symptoms also had significantly higher rates of gastrointestinal system symptoms including abdominal pain (57 [11.2%] vs. 16 [1.4%]), vomiting (40 [7.8%] vs. 21 [1.8%]), and poor appetite (17 [3.3%] vs. 6 [0.5%]). Additionally, we found that patients with neurological symptoms were more likely to occur expansion of pulmonary infection compared with the patients without neurological symptoms (46 [9.0%] vs. 68 [5.8%]).

DISCUSSION

Recent studies revealed that fever, cough, and dyspnea are the most common and typical clinical symptoms in COVID-19 patients (25, 26). However, atypical clinical manifestations including the digestive and nervous system were significant differences in different study populations (27). In this study, we found that neurological symptoms are common in patients with non-critically ill COVID-19. Among 1,682 non-critically ill COVID-19 patients, 509 patients (30.3%) had at least one neurological symptom. Compared with patients without neurological symptoms, patients with neurological symptoms had a significantly longer length of hospital stay and time of nucleic acid turning negative. Patients with neurological symptoms were more likely to occur the expansion of pulmonary infection compared with the patients without neurological symptoms (46/509 [9.0%] vs. 68/1,173 [5.8%]). These results suggest that neurological symptoms are significantly associated with the disease processes of COVID-19. Moreover, compared with patients without neurological symptoms, although the median [IQR] time of the two groups is basic consistent, the mean time from onset of symptom to hospital admission is longer in patients with neurological symptoms, it suggests that nervous system-related symptoms have not garnered much attention. Based on this finding, early identification and aggressive treatment are particularly important for COVID-19 patients with neurological symptoms.

The current study demonstrates that patients with hypertension and diabetes comorbidities are more prone to experience neurological symptoms during COVID-19. The exact pathophysiological mechanism underlying nervous system injury caused by COVID-19 is not fully understood. Genomic analysis reveals that SARS-CoV-2 belongs to betacoronavirus (βCoV) family that includes severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Research has confirmed that the infection of other CoVs, such as in SARS-CoV and MERS-CoV can cause neurologic injury by invading the brain. SARS-CoV had been detected in brain tissue and cerebrospinal fluid, and some SARS patients experienced central nervous symptoms and neurological sequelae (28–30). SARS-CoV-2 shares a highly homological sequence with human SARS-CoV (with 82% identity) and infects host cells by the same angiotensin-converting enzyme 2 (ACE2) receptor (31–34). The expression of ACE2 protein was observed in multiple human organs including lung, small intestine, colon, liver, kidney, and brain; thus, these organs have been confirmed to be infected (35, 36). A recent study shows that SARS-CoV-2 leads to neuronal damage by the expression of ACE2 in central nervous system tissue (18). In addition, another study also explained that SARS-CoV-2 invades gastrointestinal tissues, and large quantities of harmful metabolites were absorbed, which causes neurological symptoms through the gut–brain axis (15). These findings indicate the expression and distribution of ACE2 in the nervous system may play a key role in the interaction between SARS-CoV-2 and nervous system injury. Hypertension or diabetes patients are always treated with ACE inhibitors and ARBs, which results in an upregulation of ACE2 expression (37, 38). We speculate that diabetes and hypertension treatment with ACE2-stimulating drugs may increase the risk of developing more neurological symptoms during COVID-19 infection.

The present study detailed investigates the neurological symptoms and implications of non-critically ill COVID-19 patients, which reminds clinicians not to overlook these neurological symptoms during treatment for COVID-19. We found 21 patients (4.1%) presented with only neurological symptoms in the absence of respiratory symptoms and digestive symptoms. In addition, recent reports suggest that anosmia or hypogeusia and dysgeusia are also one of the clinical manifestations. The current study demonstrates that neurological symptoms are significantly associated with the processes of COVID-19.

TABLE 3 | Characteristics of patients with non-critically ill COVID-19 with/without neurological symptoms.

Characteristics	With neurological symptoms No. (%) (n = 509)	Without neurological symptoms No. (%) (n = 1,173)	P-value
Age, median [IQR], (range), years	50 [40–58], (15–79)	50 (38–58), (5–89)	0.413
Gender			0.836
Female	258 (55.1)	601 (60.0)	
Male	251 (44.9)	572 (40.0)	
From onset of symptom to hospital admission			<0.001
Mean ± SD	12.01 ± 6.099	10.68 ± 6.245	
Median [IQR] (range), days	10 (7–15), (1–30)	10 (7–14), (1–60)	
Hospital stay, median [IQR] (range), days	20 (16–23), (1–29)	18 (14–22), (1–29)	0.002
Turning to nucleic acid negative median [IQR] (range), days	10 (5–14), (0–25)	8 (5–13), (0–25)	0.036
Comorbidities			
Hypertension	46 (9)	72 (6.1)	0.033
Diabetes	29 (5.6)	24 (2.0)	<0.001
Lung disease			
Chronic bronchitis	4 (0.7)	26 (2.2)	0.042
Bronchiectasis	0	3 (0.2)	0.254
Tuberculosis	2 (0.4)	1 (0.1)	0.169
Coronary heart disease	10 (2.0)	5 (0.4)	0.794
Cerebral infarction	2 (0.4)	3 (0.2)	0.635
Clinical symptoms			
Respiratory system symptoms			
Fever	374 (73.5)	803 (68.2)	0.039
Cough	313 (61.5)	638 (54.4)	0.007
Chest tightness or dyspnea	195 (38.3)	231 (19.7)	<0.001
Expectoration	161 (31.6)	202 (17.2)	<0.001
Chills	178 (35.0)	107 (9.1)	<0.001
Sore throat	129 (25.3)	125 (10.7)	<0.001
Nasal obstruction	71 (14)	42 (3.6)	<0.001
Runny nose	63 (12.4)	44 (3.8)	<0.001
Gastrointestinal symptoms			
Abdominal pain	57 (11.2)	16 (1.4)	<0.001
Nausea	6 (1.2)	6 (0.5)	0.135
Vomiting	40 (7.8)	21 (1.8)	<0.001
Poor appetite	17 (3.3)	6 (0.5)	<0.001
Clinical outcomes			
Expansion of pulmonary infection	46 (9.0)	68 (5.8)	0.015

IQR, interquartile range; COVID-19, Coronavirus disease 2019. P-values indicate differences between patients with and without neurological symptoms, and $P < 0.05$ was considered statistically significant. The results were presented as median [IQR] (range) for continuous variables and number (%) for categorical variables. The different characteristics between the two groups were tested by the Mann-Whitney U test (continuous variables) or χ^2 test or Fisher's exact test (categorical variables).

Therefore, early identification and aggressive treatment are particularly important for COVID-19 patients with neurological symptoms.

This study has several limitations. First, we only collected and analyzed non-critically ill patients with COVID-19. It would be better to include more patients with severe infection. Second, we only analysis the neurological symptoms. Considering that Fangcang shelter hospitals are not regular hospitals but temporary hospitals in the special circumstances, magnetic resonance imaging, cerebrospinal fluid (CSF) test, electroencephalogram (EEG), and electromyogram (EMG) cannot be implemented. Third, this study only presents a preliminary assessment of neurological symptoms and the implications of non-critically ill COVID-19 patients. Nevertheless, the study findings should supply important information regarding the neurological implications of non-critically ill COVID-2019 patients.

In conclusion, non-critically ill COVID-19 patients commonly have neurological symptoms. Neurological symptoms reflecting nervous system injury are significantly associated with the processes of COVID-19. Early identification and aggressive

treatment are particularly important for COVID-19 patients with neurological symptoms.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by this study complied with the principles of the Declaration of Helsinki and was approved and written informed consent was waived by the ethics committee of the Zhongnan Hospital of Wuhan University (2020090K). The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

JZ had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis, and concept and design. NY, BM, YG, and ZX: acquisition, analysis, or interpretation of data. NY and ZX: drafting of the manuscript and statistical analysis. ZX, DL, and JZ: critical revision of the manuscript for important intellectual content. NY, ZX, and BM: administrative, technical, or material support. ZX and JZ: supervision. All authors contributed to the article and approved the submitted version.

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Experimental Models for the Study of Central Nervous System Infection by SARS-CoV-2

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Introduction: The response to the SARS-CoV-2 coronavirus epidemic requires increased research efforts to expand our knowledge of the disease. Questions related to infection rates and mechanisms, the possibility of reinfection, and potential therapeutic approaches require us not only to use the experimental models previously employed for the SARS-CoV and MERS-CoV coronaviruses but also to generate new models to respond to urgent questions.

Development: We reviewed the different experimental models used in the study of central nervous system (CNS) involvement in COVID-19 both in different cell lines that have enabled identification of the virus' action mechanisms and in animal models (mice, rats, hamsters, ferrets, and primates) inoculated with the virus. Specifically, we reviewed models used to assess the presence and effects of SARS-CoV-2 on the CNS, including neural cell lines, animal models such as mouse hepatitis virus CoV (especially the 59 strain), and the use of brain organoids.

Conclusion: Given the clear need to increase our understanding of SARS-CoV-2, as well as its potential effects on the CNS, we must endeavor to obtain new information with cellular or animal models, with an appropriate resemblance between models and human patients.

Keywords: SARS-CoV-2, experimental models, COVID-19, central nervous system, neurodegenerative disease, multiple sclerosis

INTRODUCTION

On 31 December 2019, the Word Health Organization reported for the first time on an epidemic of lower respiratory tract infection in Wuhan, in the Chinese province of Hubei. The causal agent was soon identified as Severe acute respiratory syndrome coronavirus (SARS-CoV-2), a coronavirus (CoV), and the associated disease was named coronavirus disease 19 (COVID-19) (1). CoVs are positive-sense single-stranded RNA viruses resembling a crown under microscopy due to the presence of spike (S) glycoproteins on the viral envelope. There are four types of CoVs: αCoVs, βCoVs, δCoVs, and γCoVs. SARS-CoV, MERS-CoV, and SARS-CoV-2 are zoonotic (2), first

infecting animals and then spreading to humans. βCoVs, which include SARS-CoV-2, are thought to originate in bats (3, 4), among other species. These viruses can cause respiratory and enteric diseases in different animal species. In humans, HCoV-OC43 and HCoV-HKU1 (αCoVs), and HCoV-229E and HCoV-NL63 (βCoVs) can cause the common cold and self-limiting infection of the lower respiratory tract in immunocompetent individuals in seasonal periods (5). Two previous epidemics have, however, been caused by CoVs; these were similar to the current pandemic, though with higher mortality rates, and were caused by the severe acute respiratory syndrome coronavirus (SARS-CoV) and middle east respiratory syndrome coronavirus (MERS-CoV) and affected many countries (6). Although they led to many research studies, expanding our knowledge of the viruses, the COVID-19 epidemic has had a far greater global impact. One as-yet unknown aspect of SARS-CoV-2 is its potential short- and long-term impact on the central nervous system. In this respect, although the rate of neurological symptoms associated with infection is not high (7), the possibility of subsequent effects of central nerve system (CNS) infection has been suggested by many authors (8–17). To address these questions, we need experimental models based on advances in biomedical research both *in vitro* and *in vivo*. We review these models in the present article.

OBJECTIVES OF THE EXPERIMENTAL MODELS IN THE STUDY OF THE IMPACT OF SARS-COV-2 INFECTION

Previous studies on SARS-CoV already demonstrated that the spike protein facilitates viral invasion of the target cell by interacting with the angiotensin converting enzyme 2 (ACE2) (18). This protein is expressed on the surface of epithelial cells of the lower respiratory tract, endothelial cells of arteries and veins, intestinal mucosa cells, kidney cells, immune cells, glial cells, and neurons (19–21). *In vitro* studies have demonstrated that SARS-CoV-2 also uses this receptor to penetrate cells (22, 23). In fact, sequencing of the SARS-CoV-2 spike protein has shown that it contains residues that may increase its binding affinity to the ACE2 receptor 10- to 20-fold in comparison with SARS-CoV (18). When spike protein interacts with the receptor, cleavage and activation by cell proteases is necessary to enable the viral membrane to fuse with the host cell and the virus to penetrate. *In vitro* studies have shown that SARS-CoV-2 spike proteins are mainly processed by transmembrane protease serine 2 (*TMPRSS2*), by endosomal cathepsins B and L in the absence of *TMPRSS2* (23, 24), and by furin (25, 26). Once the virus is internalized, viral RNA is released into the cytoplasm, and a series of translations and replications create new RNAs and viral proteins that assemble to form new virions (20). These are released from the host cell and can infect new target cells expressing the appropriate receptor on their surfaces (20, 23).

More complex biological systems are, however, needed to study the possible interactions between SARS-CoV-2 and the host. Due to these complex interactions, selecting an appropriate model should be a thoughtful and clearly defined process, in order to provide relevant and translatable scientific data enabling us to address the questions of interest and to ensure the rational use of animals. Although many animals may respond similarly to humans from physiological, pathological, and therapeutic perspectives, we must bear in mind that differences between species may lead to erroneous conclusions (27); it is therefore necessary to understand the relationship between the model and the human disease (28). In the light of the current pandemic, it is essential to have a cellular or animal model mimicking the symptoms and pathological processes identified in patients infected with SARS-CoV-2 (29).

IN VITRO MODELS (TABLE 1)

Vero E6 Cell Line

This cell line was isolated from kidney epithelial cells extracted from an African green monkey (*Chlorocebus aethiops*) in 1979; the Vero E6 cell line has been shown to be very useful for viral propagation and production *in vitro* (30). These cells are permissive to SARS-CoV replication, as they efficiently express the ACE2 receptor (31, 32). Furthermore, they enable persistent infection *in vitro* (33). Vero E6 cells have therefore already been used in studies with SARS-CoV and MERS-CoV (30, 33–36) and also in the development of live-attenuated and inactivated vaccines for human use (37, 38). More recently, they have been used in research into the viral infection mechanism in COVID-19 (23, 39, 40), the effects of the virus on cells, confirmation of viral infection (41–43) and pharmacological research (44–48).

HEK 293T Cell Line

This cell line is a variant of the HEK 293 lineage, which was isolated from kidney epithelial cells extracted from human embryos (49). Both lines are widely used in research due to their high transfectability, gene expression, and production of proteins or recombinant retrovirus (23, 49, 50). The HEK 293T variant expresses the T SV-40 antigen, which enables the amplification of transfected plasmids containing the SV40 origin of replication and thus considerably increases the expression levels of desired gene products (50). These cells have also previously been used in studies of other such viruses as herpes simplex virus, SARS-CoV, and even CoV pseudovirions (34, 51–53). Due to its high efficiency of transfection, research with this cell line is producing significant findings, such as the confirmation of ACE2 as the cell entry receptor for SARS-CoV-2 and the potential role of CD147 as an alternative receptor (23, 24, 39, 40).

BHK-21 Cell Line

This line is a subclone of the fibroblast cell line extracted from 1-day-old Syrian hamster (*Mesocricetus auratus*) kidney cells (54) and is useful for studying virus propagation and plasmid transfection (55). It has been used in the study of HCoV-OC43 and SARS-CoV-2 infection mechanisms (23, 36, 56, 57).

Huh7 Cell Line

This lineage was established in 1982 from a human hepatocellular carcinoma; it is therefore able to produce a great variety of substances secreted by the human liver (58) and has been used in

TABLE 1 | *In vitro* models for the study of SARS-CoV-2.

References	Cell Line	Origin	Virus Dose	Use
(23, 42, 43, 44, 47, 48)	***Vero E6***	African green monkey (*Chlorocebus aethiops*) kidney epithelial cells	• **Multiplicity of infection (MOI) of 0.01 to 0.5**	• *In vitro* amplification of viral particles • Diagnosis of SARS-CoV-2 infection and viral isolation • Study of infection mechanism • Search for new therapeutic targets • Pharmacological screening
(23, 24, 39, 40)	***HEK 293T***	Human embryonic kidney epithelial cells	• **No determined**	• Study of infection mechanism • Search for new therapeutic targets • Pharmacological screening
(23, 39)	***BHK-21***	Baby hamster (*Mesocricetus auratus*) kidney fibroblasts	• **8×10^7 genome equivalents (GE) per 24-well of SARS-CoV-2**	• Study of infection mechanism
(23, 26, 46, 48)	***Huh7***	Human hepatocellular carcinoma	• **MOI of 0.05 to 1**	• Diagnosis of SARS-CoV-2 infection and viral isolation • Study of infection mechanism • Pharmacological screening
(39, 41, 43)	***LLC-MK2***	Rhesus macaque (*Macaca mulatta*) kidney epithelial cells	• **No determined**	• Diagnosis of SARS-CoV-2 infection and viral isolation • Study of infection mechanism
(23)	***Caco-2***	Human colorectal adenocarcinoma	• **No determined**	• Study of infection mechanism
(23, 39, 72)	***Calu-3***	Human lung adenocarcinoma	• **MOI of 0.001 to 0.08**	• Study of infection mechanism • Pharmacological screening
(116, 117, 139, 141)	***Human brain organoids***	Human induced pluripotent stem cells (hIPSCs)	• **MOI of 0.1 to 2.5**	• Nervous tissue infection mechanisms • Neurodegenerative mechanisms • Pharmacological screening

The table includes the main cell lines used in the study of SARS-CoV-2 infection, the origin of the cells, viral dose used in some studies for the infection of cells and their uses. The culture media used for each cell line is specified in ***Supplementary Material****.*

the study of hepatitis C virus, SARS-CoV, and MERS-CoV (27, 34, 58, 59). In the context of SARS-CoV-2, this cell line has been used in the study of infection mechanisms (23, 39), the cytotoxicity of viruses from human hosts (22, 60), and in pharmacological research (26, 46, 48).

LLC-MK2 Cell Line

The LLC-MK2 cell line was established in 1955 from rhesus macaque (*Macaca mulatta*) kidney epithelial cells (61). It has been mainly used in the study of poliovirus, but also to study SARS-CoV, HCoV-NL63 (34, 62, 63), and SARS-CoV-2 to assess the cytopathic effects in patients infected with the virus (41, 43) and to characterize its viral infectious pathway (39).

Caco-2 Cell Line

This line was extracted in 1977 from human epithelial colorectal adenocarcinoma cells; under specific culture conditions, the cells are able to differentiate into small intestine enterocytes (64). This cell line is frequently used to study transfection, invasion, and absorption (65). It has been used in studies of MERS-CoV, SARS-CoV, and HCoV-NL63 (27, 66, 67), and in the analysis of the S protein activation in studies with SARS-CoV-2, due to the fact that these cells express the TMPRSS2 protease (23).

Calu-3 Cell Line

This line was extracted in 1975 from a human lung adenocarcinoma and is used in the study of respiratory viruses (68, 69). It has been used in the study of SARS-CoV and MERS-CoV (27, 70, 71) and infection mechanisms studies and pharmacological research into COVID-19 (23, 39, 72).

Other Cell Lines

Several of the available cell lines susceptible to transfection may also be used in *in vitro* research. For example, the study by Zhou et al. (22) uses the *HeLa* cell line, which does not endogenously express ACE2, and expression plasmids for human ACE2 (hACE2) in the study of SARS-CoV-2.

IN VIVO MODELS (TABLE 2)

Mouse and hamster strains are the main animal models used in research into SARS-CoV and MERS-CoV (73); however, in the case of SARS-CoV-2, research efforts have been focused

TABLE 2 | *In vivo* models for the study of SARS-CoV-2 infection in the CNS.

References	Model	Virus, inoculation route, and dose	Findings
(76)	C57BL/6 *ACE2* knockout mouse	SARS-CoV; intranasal; 7.6×10^6 pfu/ml in DMEM	Mechanisms of primary infection and viral propagation
(73)	C57BL/6 *TMPRSS2–/–* knockout mouse	SARS-CoV; intranasal; 10^5 $TCID_{50}$ in F-musX MERS-CoV; intranasal; 10^6 $TCID_{50}$ in HCoV-EMC 2012	Mechanisms of primary infection and viral propagation in the respiratory tracts
(83, 85)	C57BL/6 *STAT1* knockout mouse	SARS-CoV, SARS-CoV 2; intranasal 7×10^4 pfu	Cytokine-induced biological responses
(79)	hACE2 ICR mouse	SARS-CoV-2; intranasal; 10^5 $TCID_{50}$	Viral antigen in the lower respiratory tract, alveolar infiltrates, and weight loss
(80)	C57BL/6J-hACE2-AAV mouse	SARS-CoV-2; intranasal; 3×10^7 pfu/ml	Pulmonary infiltrate, neutralizing antibodies, interferon-stimulated genes
(81)	C57BL/6 young and aged hACE2 mouse	SARS-CoV-2; intranasal; 4×10^5 pfu. Intragastric; 4×10^6 pfu	Viral antigen in trachea, lungs, and brain. Pulmonary infiltrate. Pathological changes in lungs are more evident in aged mice.
(88)	Young and aged BALB/c mouse	Adapted viruses: SARS-CoV-2 MA; intranasal; 10^5 pfu	Viral replication in lower and upper respiratory tract. Clinical symptoms worsen with age. Prophylactic and therapeutic role of interferon lambda-1a
(87)	Young and aged BALB/cC57 mouse Young C57BL/6 mouse	Adapted viruses: SARS-CoV-2 MASCp6; intranasal; 7.2×10^5 pfu Adapted viruses: SARS-CoV-2 MASCp6; intranasal; 7.2×10^5 pfu	Evaluation of the efficacy of the subunit vaccine consisting of SARS-CoV-2 S protein RBD fused with a human IgG Fc subunit
(92)	Syrian hamster (*Mesocricetus auratus*)	SARS-CoV-2; intranasal; 8×10^4 $TCID_{50}$	Viral antigen in the upper and lower respiratory tracts and intestinal mucosa. Pulmonary infiltrates. Neutralizing antibodies. Transmission by direct contact or aerosols
(146, 148)	C57BL/6 mouse	MHV coronavirus; intranasal, intracerebral, enteral ($1–2.5 \times 10^3$ pfu)	Mechanisms of primary infection and viral propagation, demyelinating lesion model
(93)	Syrian hamster (*Mesocricetus auratus*)	SARS-CoV-2; intranasal; 10^5 pfu in DMEM	Viral antigen in the upper and lower respiratory tracts and intestinal mucosa. Mononuclear infiltrates. Neutralizing antibodies. Transmission by direct contact
(97)	*Mustela putorius furo*	SARS-CoV-2 F13-E and SARS-CoV-2 CTan-H; intranasal; 10^5 pfu	Viral replication in the upper respiratory tract without causing severe disease
(98)	*Mustela putorius furo*	SARS-CoV-2 GISAID ID EPI_ISL 406862; intranasal; 6×10^5 $TCID_{50}$ of SARS-CoV2 virus diluted in 500 μl of PBS	Direct and indirect transmission
(96)	*Mustela putorius furo*	SARS-CoV strain Toronto-2; intranasal; $10^3 TCID_{50}$/mL diluted in medium	Transmission before peak viral load, without symptom onset
(105)	*Macaca fascicularis*	SARS-CoV-2 # 026V-03883, MERS-CoV # 011V-02838; intratracheal, intranasal; $10e^6$ $TCID_{50}$ in PBS	Clinical symptoms similar to COVID-19
(100)	*Macaca mulatta*	SARS-CoV-2 HB-01; intratracheal; 10^6 $TCID_{50}$/mL	More severe clinical symptoms in older monkeys
(106)	*Macaca mulatta*	SARS-CoV-2 MN985325.1; intratracheal, intranasal, conjunctival, oral; 4×10^5 $TCID_{50}$/ml of DMEM	Significant increase in pro-inflammatory interleukins
(109)	*Macaca mulatta*	SARS-CoV-2 WH-09/human/2020/CHN; conjunctival; 1×10^6 $TCID_{50}$/ml	Viral load in the ocular system and respiratory and digestive tracts
(107)	*Macaca mulatta/Macaca fascicularis/Callithrix jacchus*	SARS-CoV-2; intratracheal, intranasal, conjunctival; 10^6 pfu/ml	Rhesus monkey is more susceptible to infection.
(110)	*Macaca mulatta*	MERS-CoV strain HCoV-EMC/2012; intratracheal; 7×10^6 $TCID_{50}$	Viral RNA in the throat soon after infection
(111)	*Macaca mulatta*	SARS-CoV-2/WH-09/human/2020/CHN; intratracheal; 1×10^6 $TCID_{50}$/ml	Rhesus monkeys present no reinfection
(112)	*Macaca mulatta*	SARS-CoV-2 NR-52281; intratracheal, intranasal; 1.1×10^6 pfu or 1.1×10^5 pfu or 1.1×10^4 pfu	No reinfection
(113)	*Macaca mulatta*	SARS-CoV-2 MN985325.1; intratracheal, intranasal, conjunctival, oral; 2.6×10^6 $TCID^{50}$	Treatment study
(115)	*Macaca mulatta*	SARS-CoV-2; intratracheal; 10^6 $TCID_{50}$/ml	Vaccine development

The table includes the characteristics of the animals (species) used, as well as the route of administration, viral dose used in some studies for the infection and the relevance of the model in SARS-CoV-2 research. TCID50, 50% tissue culture infectious dose; pfu, plaque forming units.

on the study of aged mouse strains, the design of humanized mouse models that express the hACE2 receptor, and the creation of knockout mice, with the aim of replicating the mechanisms involved in human infection.

Aged Mouse Strains

Roberts et al. (74) reported that aged BALB/c mouse strains were able to maintain high rates of viral replication, which was associated with clinical illness and pneumonia; the study demonstrated an age-related susceptibility to SARS disease in animals that parallels the human experience. Advanced age has been identified as an independent factor of poor prognosis in COVID-19 and is considered a predictor of mortality in patients with SARS-CoV-2 infection. Other strains like C57BL/6 have also been used (75).

Knockout Mouse Models

K18-hACE2 transgenic mice express hACE2, with regulation by the human cytokeratin 18 (K18) promoter. Specifically, these mice contain 2.5 kb of the K18 genomic sequence, including the promoter, the first intron, and a translation-promoting sequence. Expression of hACE2 is mainly observed in the epithelium of the respiratory tract, which shows a higher incidence of SARS-CoV-2 infection, and in the epithelium of other organs, including the liver, kidneys, and gastrointestinal tract (76). K18-hACE2 mice infected with SARS-CoV-2 show weight loss and viral replication in the lungs, as in humans. Furthermore, they show the typical histopathological findings of interstitial pneumonia with lymphocytic and monocytic infiltration into the alveolar interstitium and an accumulation of macrophages in the alveolar cavities. Alveolar and bronchial epithelial cells show presence of viral antigens; this is not observed in wild-type (WT) mice with SARS-CoV-2 infection. Furthermore, these mice show other pathological changes, such as vasculitis, degeneration, and necrosis of extrapulmonary organs and presence of the viral antigen in the brain (77, 78). Bao et al. (79) recently studied the pathogenicity of SARS-CoV-2 virus in hACE2-expressing ICR transgenic mice and WT mice. This model may be useful in research into drug treatments for COVID-19. Israelow et al. (80) developed an hACE2-adeno-associated virus 9 (AAV) murine model, which was subsequently intranasally infected with SARS-CoV-2. They studied the presence of coronavirus infection, the inflammatory response in the lungs, the presence of neutralizing antibodies, and the type I interferon signaling pathway. The authors reported that this model would be very useful for understanding questions related to infection, replication, and pathogenesis of SARS-CoV-2 and for testing therapeutic strategies.

Sun et al. (81) used *CRISPR/Cas9* technology to create a murine hACE2 model in young and aged animals. After intranasal infection of the animals with SARS-CoV-2, the researchers observed viral replication in the lungs, trachea, and brain of both animals. However, alveolar inflammatory infiltrate and vascular lesions were more evident in aged animals; this is analogous to the pathological changes observed in older patients with COVID-19. This model also showed evidence of respiratory tract infection following intragastric inoculation.

TMPRSS2–/– Knockout Mice

This model was designed using a directional vector to replace exons 10–13, which codify the serine protease domain of the *TMPRSS2* gene. It was constructed by electroporation of embryonic stem cells and their subsequent injection into C57BL/6 blastocysts for at least five generations (82). After experimental infection with SARS-CoV-2, *TMPRSS2*-deficient mouse strains showed reduced body weight loss and viral kinetics in the lungs. Absence of *TMPRSS2* affected the infection sites and virus spread within the respiratory tract; therefore, this is a useful model for COVID-19 research (73).

The *STAT1* Knockout Mouse Model

(129S6/SvEv-STAT1tmRDS) contains a homozygous *STAT1* mutation and completely lacks functional *STAT1* proteins (Pgm1c and Gpi1b alleles of 129S6). The model was created by targeting the *STAT1* gene in GS-1 ES cells and injecting target cells into blastocysts. Heterozygous models of the mutation were produced from the chimeras and were crossed over to generate homozygous models (83, 84). The *JAK-STAT* signaling pathway is involved in the mediation of cytokine-induced biological responses. This is therefore a useful model for determining the role of a variety of cytokines in immune responses, the role of *STAT1* protein in mediating interferon-dependent responses, and its relationship with viral and bacterial pathogens (84–86); the model is also interesting in the analysis of SARS-CoV-2 inflammation mechanisms.

Adapted Mouse Models

Studies have been recently conducted with BALB/c and C57BL/6 mice (87, 88) together with modified SARS-CoV-2 strains. Modification of the virus has led to mouse-adapted SARS-CoV-2 strains, the SARS-CoV-2 MA (88) and SARS-CoV-2 MASCp6 (87) strains, which are able to infect mice with no need for modification of the animals, as these strains efficiently bind to the murine ACE2 receptor in both young and aged mice, causing a disease resembling human COVID-19. In addition to their use in the study of pathogenesis, these models have enabled researchers to trial vaccines and treatments for the disease (87, 88).

Mouse Models for the Induction of Neutralizing Antibodies

BALB/c mice have been used in studies for the development of vaccines, and Wistar rats have been used in studies into immunization with attenuated strains of the virus (89).

Syrian Hamster Models

This animal model (*M. auratus*) has previously been used in the study of SARS-CoV (90, 91), as the hamster presents an ACE2 receptor homologous to the human receptor. Sia et al. (92) intranasally inoculated animals with the β–CoV/Hong Kong/VM20001061/2020 strain and corroborated the presence of viral antigen in the epithelial cells of the nasal and bronchial mucosa with progression to pneumocytes and clearance of infectious particles by day 7 after infection. Presence of mononuclear cell infiltrates was moderate in the

nasal turbinates but was greater in the lungs. The viral antigen was observed in epithelial cells of the duodenum, without signs of inflammation. No infectious particles were detected in the kidney, and no histopathological changes were observed in other organs. Neutralizing antibodies were observed on day 14 after infection. Infected animals presented clear weight loss and researchers corroborated that the main route of viral transmission was through direct contact or aerosols. The authors conclude that SARS-CoV-2 infection in Syrian hamsters presents similar characteristics to those observed in humans with mild infections and that this model represents an opportunity for understanding the transmission dynamics of this novel coronavirus.

Chan et al. (93) confirmed transmission by contact, the progressive decrease in viral load between days 2 and 7 after infection in both lower and upper respiratory tracts, and the expression of viral antigens (protein N) by epithelial cells together with presence of mononuclear infiltrates. On day 7 after infection, regenerative hyperplasia occurring in bronchioles led to the appearance of multiple irregularly arranged epithelial layers. Furthermore, they detected the viral antigen in the intestinal mucosa and observed histopathological changes in the spleen and heart. The pro-inflammatory cytokine cascade in this model normalizes at approximately day 7, and antibodies are observed at 7–14 days. This animal model reproduces the respiratory and enteric symptoms observed in patients with COVID-19.

Ferret Models

Ferrets (*Mustela putorius furo*) are frequently used as animal models to study respiratory diseases caused by such viruses as influenza virus or SARS-CoV (94–96). All studies with SARS-CoV-2 in ferrets have used the intranasal route to inoculate the virus (97–99). Shi et al. (97) studied the susceptibility of ferrets to infection with 2 SARS-CoV-2 strains. In a first stage of the study, the authors detected viral RNA in samples from the nasal turbinates, palate, and tonsils, but no viral load was detected in the other organs analyzed, including the lungs and brain. This suggests that the virus can replicate in the upper respiratory tract of ferrets. In a second stage of the study, analyzing the viral replication dynamics, animals showed viral RNA in nasal washes, whereas rectal swabs showed much lower viral load. Furthermore, ferrets presented anti-SARS-CoV-2 antibodies at days 13 and 20. Histopathological studies showed altered pneumocytes, macrophages, and neutrophils. Finally, when studying replication in the lungs, the authors observed that the virus is able to replicate in the upper respiratory tract up to day 8 after infection without causing severe symptoms. Richard et al. (98) focused on the study of the direct and indirect transmission of the SARS-CoV-2. Ferrets inoculated with SARS-CoV-2 were placed in direct contact with a group of healthy ferrets 6 h after infection. Inoculated animals showed productive infection, and the peak of viral infection was reached on the third day. All animals in direct or indirect contact presented viral RNA at day 1–3 after exposure. Animals exposed to inoculated ferrets were expected to present a lower viral load than the inoculated animals; however, viral load was similar in all cases. Lastly, the authors observed that all animals presented antibodies at day 21 after exposure to the virus, and that their levels were similar, regardless of the form of infection. A similar design was employed by Kim et al. (99), who observed viral load up to 8 days after infection, both in samples from inoculated animals and in samples from animals that were in direct contact with the inoculated ferrets. Animals in indirect contact with infected ferrets showed positive results for infection at day 2 after exposure, which suggests rapid transmission, even before peak viral load was reached and in the absence of clinical symptoms; this correlates with the reported transmission by asymptomatic human patients.

Non-human Primate Models

Severe acute respiratory syndrome coronavirus and middle east respiratory syndrome coronavirus have previously been studied in non-human primates (75, 100–104); the use of these models has been proposed for the study of SARS-CoV-2 (105) especially with a view to the development of vaccines or antiviral treatments (106, 107). Rockx et al. (105) used a combined intratracheal and intranasal route to inoculate the SARS-CoV-2 virus to both young and old adult cynomolgus macaques (*Macaca fascicularis*). The results of this study showed that these animals tolerated viral infection, presenting symptoms similar to those of COVID-19 in humans. The virus efficiently replicates in epithelial cells throughout the upper respiratory tract, which correlates with the ease of transmission of the virus, whereas replication in the lower respiratory tract correlates with the development of the disease (105–108). Yu et al. (100) obtained similar results with intratracheal inoculation of young and old rhesus monkeys (*Macaca mulatta*). The authors report that, although viral replication was more active in older adult monkeys, both groups developed interstitial pneumonia together with edema, which was more severe and diffuse in older adult monkeys. Munster et al. (105) developed a COVID-19 model using rhesus monkeys, inoculating them with the virus through four different but combined routes (intranasal, intratracheal, conjunctival, and oral). The animals presented clinical signs compatible with COVID-19 from day 1 after the inoculation to symptom resolution, between days 9 and 17. All animals presented weight loss, low-grade fever, and pulmonary infiltrates, in addition to a significant increase in IL-6 and IL-10, among other findings; IgG antibodies were present at detectable levels from day 7 after the infection. Nasal, pharyngeal, and rectal samples showed high levels of viral RNA. Furthermore, histopathological analyses showed similar alterations to those caused by SARS-CoV and MERS-CoV (97, 98). Deng et al. (109) inoculated rhesus monkeys with SARS-CoV-2 through the conjunctival route. They did not observe significant changes in the animals' weight or body temperature. Antibody analyses detected presence of IgG at days 14 and 21. Furthermore, radiographs of these animals showed bilateral alterations in the upper lobes and right lower lobe, which correlates with the moderate interstitial pneumonia observed microscopically. Histopathological studies showed viral load in the ocular and nasolacrimal system, as well as in the respiratory and digestive

tracts. Results of a comparative study of three non-human primate species [rhesus monkey, crab-eating macaque, and common marmoset (*Callithrix jacchus*)], which were inoculated with the virus through three different pathways (intratracheal, intranasal, and conjunctival), showed that almost all animals presented clinical signs compatible with COVID-19, although there were differences between species in the histopathological findings (107). The rhesus monkey is the most susceptible species to viral infection, and therefore a good model for the study of COVID-19, as well as for the development of vaccines and pharmacological studies (107, 108). De Wit et al. (110) studied rhesus monkeys intratracheally inoculated with the virus, finding that viral RNA was detectable in the throat during the first days, but with levels subsequently decreasing until becoming undetectable. Bao et al. (111) and Chandrashekar et al. (112) intratracheally and intranasally inoculated rhesus monkeys with the SARS-CoV-2 virus to study the possibility of reinfection. Williamson et al. (113) used rhesus monkeys that had previously been inoculated through the intranasal, oral, ocular, and intratracheal routes for pharmacological research. These animals have also been used for the development of vaccines (106, 107, 114, 115).

MODELS FOR CNS RESEARCH

Coronaviruses have been found in autopsy studies in the CNS of patients with multiple sclerosis, Parkinson's disease, and Alzheimer disease. Experimental studies have shown that human CoVs can infect neurons, astrocytes, and microglia in primary cultures as well as immortalized human microglial cells (6). The suggestion that SARS-CoV-2 may use the brain as a reservoir (10), potentially favoring the development of neurodegenerative diseases (11), underscores the need to specifically analyze the effect of the virus on the CNS.

CNS CELL LINES

The information currently available is from research on SARS-CoV, which has 78% nucleotide homology with SARS-CoV-2 (18). Although CNS tropism has been described (20), no specific models of neural cell lines have been developed for the study of SARS-CoV-2; however, neural progenitor cells (NPCs), neurons and microglia derived from human induced pluripotent stem cells (hIPSCs) have already been used in *in vitro* studies of SARS-CoV-2 viral infection, demonstrating the virus potential to infect CNS cells (116–118). Previous studies with other neurotropic human coronaviruses showed possible neural cell lines susceptible to infection by SARS-CoV-2 that may be useful in studying the possible mechanisms by which the virus infects the CNS. Studies into neural susceptibility to SARS-CoV infection have used cell lines including HOG, a line derived from a human oligodendroglioma that expresses proteins characteristic of oligodendrocytes and is very widely used in the study of neurons (119, 120) and the C6 cell line, derived from a glioma induced in Wistar Furth rats exposed to N-nitroso-N-methylurea, which is morphologically similar to glioblastoma multiforme when injected into the brains of neonate rats (121, 122). Although both cell lines have been shown to be susceptible to SARS-CoV infection, low levels of viral replication have been observed in comparison with such other susceptible cell lines as Vero E6 or Caco-2 (123). Other cell lines used to study the virulence of HCoV-229E and HCoV-OC43 in the CNS include human H4 brain neuroglioma cells, the LA-N-5 human neuroblastoma cell line, the CHME-5 human fetal microglia cell line, and the U-373 MG and U-87 MG astrocytic lines derived from a human glioblastoma and an astrocytoma, respectively, among many others (124–129). Cultures of human primary neurons, astrocytes, oligodendrocytes, and microglia have been used to study these viruses (130, 131). All these neural cell lines may be useful in the near future to study SARS-CoV-2.

BRAIN ORGANOIDS AS A MODEL OF CNS INFECTION BY SARS-COV-2

Organoids are miniaturized, simplified, three-dimensional versions of an organ produced *in vitro*, partially recreating the cellular structure and the functioning of that organ (132, 133). Classic cell culture systems present certain limitations, such as the inability to study complex and dynamic responses or cell-cell interactions. The use of organoids enables us to study complex physiological or pathological processes in structures bearing much greater resemblance to *in vivo* conditions, including SARS-CoV-2 infection, tropisms and potential treatments (133–135). To date, SARS-CoV-2 infection has been studied in human organoids of lung, liver, intestine, blood vessels, and kidney (42, 118, 136–138). Human brain organoids have also been used; these present strong cellular and structural similarities to some mammalian brain regions, such as a neural epithelium containing NPCs, that align to form a ventricular zone-like layer, cortical neurons, that contribute to the formation of a cortical plate-like layer and glial cells, such as astrocytes or oligodendrocytes (139–141). These organoids are useful for the study of early stages of human neurodevelopment and network formation, key cellular processes such as proliferation, differentiation, apoptosis, synaptogenesis or myelination, CNS function such as electrophysiological activity, neurodegenerative diseases, potential treatments, and have been already used for the study of other virus such as ZIKA virus or HIV (139, 141–144).

Ramani et al. (116) observed that in these brain organoids, the virus mainly infects mature cortical neurons and presents a perinuclear distribution within these cells. Furthermore, neurodegenerative effects have been observed in cells infected by SARS-CoV-2, including cell death and hyperphosphorylation, as well as mislocation of Tau protein; these alterations are observed in such conditions as tauopathies or Alzheimer disease (116). However, no productive replication of the virus was observed in these cells, at least in the first 4 days after infection (116), which would support the hypothesis that the CNS may act as a long-term reservoir of the virus (10). In contrast, Bullen et al. (139) observed an incremented accumulation of viral particles in

neural cells of brain organoids between 6 and 72 h after SARS-CoV-2 infection, suggesting an active replication and productive infection of the virus in neural cells during the first days. Viral particles were detected mainly in the neuronal soma and, in some cells, also into the neurites (139). Similar to Ramani et al., Mesci et al. (141) used these brain organoids and observed that the virus was able to infect neurons, including NPCs and mature cortical neurons, and cause cell death accompanied by the impairment of excitatory synapses. Furthermore, this work tested the efficiency of Sofosbuvir, an FDA-approved brain-penetrant antiviral drug for positive-sense single-stranded RNA viruses (145), as a treatment for the SARS-CoV-2 infection and observed that this drug was able to rescue the altered synaptogenesis and decrease neuronal death and viral accumulation in these brain organoids (141). Song et al. (117) also demonstrated that SARS-CoV-2 has neuroinvasive capacity in human brain organoids, particularly of NPCs and mature cortical neurons. Infected cells showed a hypermetabolic state and viral particles were accumulated within endoplasmic reticulum-like structures, indicating the virus ability to use the neural cell machinery to replicate (117). In addition, a hypoxic environment and extensive neuronal cell death were observed in high density SARS-CoV-2 infected areas, suggesting that virus infection could promote death of nearby cells (117). Finally, this study detected IgG antibodies against SARS-CoV-2 in the cerebrospinal fluid of a COVID-19 patient that were able to block SARS-CoV-2 infection in brain organoids (117). All these studies show that SARS-CoV-2 can directly infect neural cells and trigger damaging consequences that could cause neurologic symptoms. Also, these studies expose the great potential of the human brain organoids for the study of the SARS-CoV-2 effects in the CNS.

MHV-COV INFECTION IN MICE

Mouse hepatitis virus (MHV) is a βCoV that poses no risk to humans but presents a great similarity with other viruses from the same family, such as SARS-CoV, MERS-CoV, and SARS-CoV-2. It penetrates the CNS, causing white matter lesions; it has therefore been proposed as a viral model of demyelinating disease (146, 147). The virus has been shown to remain in the white matter and to be able to replicate in the CNS (148); therefore, it is a good model for the study of CNS infection by coronaviruses. Neurotropic strains of MHV-CoV have been used extensively to induce acute and chronic demyelinating disease mediated by neuroinflammation (149). Depending on the inoculation route and the MHV-CoV strain, different CNS regions are affected. Inoculation with experimental neurotropic strains, especially MHV-A59, induces a biphasic disease of acute meningoencephalitis at 10–14 days after inoculation, followed by a disease causing subacute, chronic inflammatory demyelination in the brain; spinal cord involvement is more pronounced (150). Virus translocation from the initial site of inoculation in the brain to the spinal cord is caused by the transit of virus particles in neural and glial cells, as well as mechanisms that involve the fusion of lipid membranes, probably during the virus internalization step (151). Intranasal and intracranial inoculation of JHM-CoV induces similar symptoms in BALB/c mice to those caused by MHV-A59. After intranasal inoculation of mice, MHV-CoV accesses the CNS through the olfactory nerve and propagates from the olfactory system to limbic system structures and their connections with the brainstem (152).

In order to study the immune system role in demyelination induction caused by MHV infection, Wang et al. (153) treated infected animals with gamma radiation to cause immunosuppression and, subsequently, reconstituted immunity by transferring cells from other immunocompetent animals. The results showed that demyelination was prevented by radiation and was present again when the immunity was restored, indicating that immunity is directly involved in the demyelination process (153). Moreover, CD4 and CD8 T cells have been observed to play a critical role in the development of the demyelinating process, with γδ T cells being the most important for this process (154, 155). In contrast, B cells, the most abundant cell type in the spleen, and NK cells are not involved in demyelination as nude animals without spleen do not present demyelination (154), MHV offers a unique model for studying host defense-mediated demyelination during chronic infection in a phase acute viral infection and immune response (156).

CONCLUSION

Research on SARS-CoV-2 has become a necessity due to the magnitude of its spread worldwide. Such aspects as infection rate and mechanisms, the possibility of reinfection, and possible therapeutic approaches make it necessary not only to use the experimental models previously employed to study the SARS-CoV and MERS-CoV coronaviruses, but also to generate new models to respond to urgent questions. The potential involvement of the CNS due to SARS-CoV-2 infection should be studied specifically, and research efforts must focus on obtaining information with cellular or animal models to expand our understanding of the virus.

AUTHOR CONTRIBUTIONS

JM-G and UG-P: lead researchers. All authors: research project group, manuscript drafting, and critical review of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.02163/full#supplementary-material

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REVIEW
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Neurological Aspects of SARS-CoV-2 Infection: Mechanisms and Manifestations

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The human infection of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a public health emergency of international concern that has caused more than 16.8 million new cases and 662,000 deaths as of July 30, 2020. Although coronavirus disease 2019 (COVID-19), which is associated with this virus, mainly affects the lungs, recent evidence from clinical and pathological studies indicates that this pathogen has a broad infective ability to spread to extrapulmonary tissues, causing multiorgan failure in severely ill patients. In this regard, there is increasing preoccupation with the neuroinvasive potential of SARS-CoV-2 due to the observation of neurological manifestations in COVID-19 patients. This concern is also supported by the neurotropism previously documented in other human coronaviruses, including the 2002–2003 SARS-CoV-1 outbreak. Hence, in the current review article, we aimed to summarize the spectrum of neurological findings associated with COVID-19, which include signs of peripheral neuropathy, myopathy, olfactory dysfunction, meningoencephalitis, Guillain-Barré syndrome, and neuropsychiatric disorders. Furthermore, we analyze the mechanisms underlying such neurological sequela and discuss possible therapeutics for patients with neurological findings associated with COVID-19. Finally, we describe the host- and pathogen-specific factors that determine the tissue tropism of SARS-CoV-2 and possible routes employed by the virus to invade the nervous system from a pathophysiological and molecular perspective. In this manner, the current manuscript contributes to increasing the current understanding of the neurological aspects of COVID-19 and the impact of the current pandemic on the neurology field.

Keywords: coronavirus, COVID-19, SARS-CoV-2, severe acute respiratory syndrome, pneumonia

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in Wuhan, China (1), in December 2019, and it has engulfed the world in an unprecedented global pandemic. The coronavirus disease 2019 (COVID-19) associated with this virus has caused more than 16.8 million new cases and 662,000 deaths as of July 30, 2020 (2), generating a high burden of disease that has exceeded the assistance capacities of several healthcare systems

around the world. The clinical characteristics of patients with COVID-19 are similar to those observed during the outbreak of SARS-CoV-1, which emerged in 2002–2003, causing more than 8,000 confirmed cases and ∼800 deaths (3). As such, the human infection with SARS-CoV-2 mainly affects the lower respiratory tract, causing mild to moderate respiratory symptoms in about 85% of patients with COVID-19 (4–6), including fever, headache, fatigue, myalgia, dry cough, and diarrhea. Most symptomatic individuals are men in their fifth and sixth decades of life that attend medical centers after an incubation period of about 4 to 5 days (4–7). Another 15% of patients present moderately severe forms of the disease manifested as pneumonia of atypical features in radiological studies of the lung, such as bilateral multi-lobe consolidations and ground-glass opacities (8). Finally, in 5 to 30% of COVID-19 patients, the virus causes severe acute respiratory syndrome (SARS), which is characterized by profound respiratory distress that obligates the establishment of intensive life support interventions, such as intubation and mechanical ventilation (4–6). Initial reports estimated mortality rates of about 2 to 4%. However, new analyses indicate a higher lethality of COVID-19 (9), especially among individuals older than 65 years, and patients with comorbidities (4–6). Unfortunately, the risk of a fatal outcome is disproportionally higher among patients requiring mechanical ventilation, with a mortality rate close to 80% (7).

The rapid transmission of SARS-CoV-2 and the increasing number of positive cases reported around the world have overcome the resources of the healthcare systems in different regions. This has significantly impacted all areas of medicine, especially those directly related to the management of severe respiratory infections, such as pneumology and critical care medicine. Nonetheless, SARS-CoV-2 has the potential to spread to different extrapulmonary tissues, and, in some of the most severe cases, the infection can progress to multiorgan failure (5). Therefore, all healthcare providers from any area of medicine must acquire adequate knowledge of the principal characteristics of COVID-19. Currently, there is increasing preoccupation about the potential capacity of SARS-CoV-2 to invade the central nervous system (CNS) and the peripheral nervous system (PNS). These concerns are based on recent observations in individuals infected with SARS-CoV-2 that present neurological findings (10). A better understanding of the mechanisms underlying the neurologic sequela of patients with COVID-19 is urgent to discover novel targets for therapeutics development. In the current review article, we therefore provide an overview of the spectrum of neurological manifestations of COVID-19. Additionally, we analyze host- and pathogen-specific factors that determine the tissue tropism of SARS-CoV-2 and discuss the possible routes employed by the virus to invade the nervous system. Furthermore, we propose possible therapeutics for the neurologic complications of COVID-19.

SARS-COV-2 BIOLOGY

Virology

SARS-CoV-2 is a novel member of the group of human coronaviruses (HCoVs), which is constituted of HCoV-229E, HCoV-NL63, HCoV-HKU1, HCoV-OC43, SARS-CoV, and MERS-CoV (11). These are RNA single-stranded viruses belonging to the Coronaviridae family. Some of these pathogens have caused a variety of respiratory diseases in the past. For instance, SARS-CoV-1 infected more than 8,000 individuals around the world (3). Also, the human coronavirus related to the respiratory syndrome of the Middle East (MERS-CoV), which emerged in Saudi Arabia in 2012 and caused high mortality rates among infected people (12, 13).

Based on sequence comparisons of viral genomes, HCoVs are grouped into four genera: alpha, beta, gamma, and delta coronaviruses. SARS-CoV-2 is a beta coronavirus genetically related to another bat coronavirus named BatCoV RaTG13 as well as SARS-CoV-1 (14, 15). Furthermore, SARS-CoV-2 also shares its genetic identity with coronaviruses isolated from pangolins (16, 17). Hence, it is believed that COVID-19 is a zoonotic disease that originated from bats or pangolins. The genome of SARS-CoV-2 consists of a single RNA strand of 29.903 bp that codifies for the replicase-transcriptase, as well as for the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) (15).

Mechanism of Infection and Determinants of SARS-CoV-2 Tropism

The initial step of SARS-CoV-2 infection is the recognition of its receptors on the surface of host cells. This step is mediated by the viral spike (S) protein, which recognizes the human receptor angiotensin I-converting enzyme 2 (ACE2), the same receptor for the S protein of SARS-CoV-1 (18–20). This protein owns two functional domains: the S1 domain contains the receptor-binding domain (RBD), which attaches to ACE2, whereas the S2 domain mediates the fusion of the viral and host cell membranes (20). Therefore, the organ distribution of the ACE2 receptor is a crucial determinant of the virus infectivity and tropism. A second step determinant in the infection process of SARS-CoV-2 is the activation of the S protein. This process is mediated by different host proteases, which execute the cleavage of the molecule at the S1/S2 and S'2 sites. This protein processing allows the complete activity of the S2 domain and the fusion of the viral and cellular membranes. For this purpose, and as in the case of SARS-CoV-1, SARS-CoV-2 uses the transmembrane serine protease 2 (TMPRSS2) (19, 21, 22). Interestingly, the proteases TMPRSS4 and cathepsin L also promote SARS-CoV-2 infection of human small intestinal enterocytes and 293/hACE2 cells (23, 24). Hence, the tissue patterns of expression of TMPRSS2, TMPRSS4, and cathepsin L is another decisive factor that determines the tropism of the virus, and, indeed, some drugs that inhibit the activity of these proteases are now proposed as potential therapeutic agents to prevent and treat COVID-19 (19, 23).

Other factors implicated in the process of SARS-CoV-2 infection include the phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) (23). This enzyme mediates the production of phosphatidylinositol-3,5-bisphosphate [PI(3,5)P2], a phosphoinositide that participates in the maturation process of endosomes. Treatment with apilimod, a potent inhibitor for

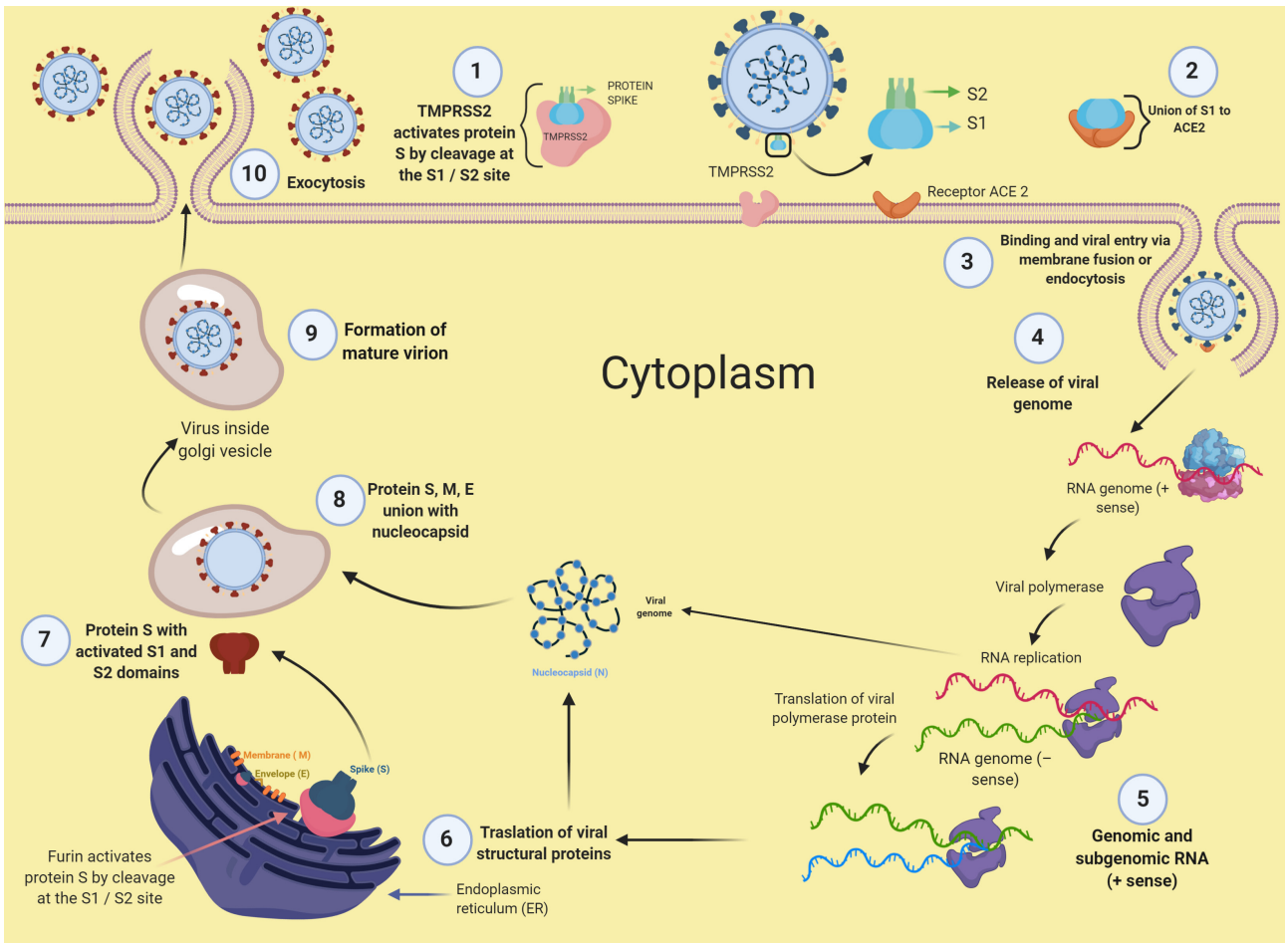

FIGURE 1 | Mechanisms of infection of SARS-CoV-2. This figure was created with *BioRender*.

PIKfyve, reduced the infectivity of SARS-CoV-2 and could be a novel candidate for therapeutic applications. After the entry into host cells, viral replication begins with the translation of the replicase-polymerase gene and the assembly of the replication-transcription complex. This complex subsequently transcribes the genomic regions that codify for structural proteins. New virions are assembled in the endoplasmic reticulum and Golgi apparatus to finally egress from the cell (11). A particular feature of SARS-CoV-2 is that it possesses a polybasic furin cleavage sequence (PRRA) in the S1/S2 site, which is absent in other close related coronaviruses (14, 20). This inserted furin cleavage sequence is processed at the Golgi apparatus during the biosynthesis of the S protein of novel virions inside the host infected cells (20). The novel virions of SARS-CoV-2 may thus contain an S protein primed and ready to infect any other cells expressing the ACE2 receptor, with no further requirement of TMPRSS2 activity. As virtually all cells express furin under normal conditions, the inserted furin cleavage sequence may expand the transmissibility and tissue tropism of SARS-CoV-2. **Figure 1** illustrates the process of SARS-CoV-2 infection.

The Immune Response Against SARS-CoV-2

SARS-CoV-2 elicits an exuberant immune response characterized by a dysregulated production of soluble immune mediators. This phenomenon has been called a "cytokine storm" and is responsible for mediating tissue damage in patients with COVID-19 that progress to severe illness (25–28). The immune receptors that recognize the viral infection and initiate the immune responses against SARS-CoV-2 are unknown. As this virus is genetically related to SARS-CoV-1, it is presumed that both viruses share mechanisms of infection. In this sense, SARS-CoV-1 is recognized by the toll-like receptors (TLR) TLR3 and TLR4, which induce an immune reaction via MyD88 and TRIF pathways (29, 30). Furthermore, SARS-CoV-1 triggers the production of IL-1β through the activation of the inflammasome (31). In this regard, the activation of the inflammasome is also possible to occur during SARS-CoV-2 infection, as high levels of IL-1β have been observed in COVID-19 patients (32). Other immune mediators that are exaggeratedly produced in response to SARS-CoV-2 include IL2, IL-6, IL7, IL10, G-SCF, CXCL10, MCP-1, MIP-1A, and TNFα (5, 28, 33). From these, IL-1β, IL-6,

CXCL10, and TNFα are the cytokines with a higher capacity to generate tissue damage in several organs, including the CNS, due to their pro-inflammatory properties. For instance, IL-1β and IL-6 have been implicated in neurotoxicity associated with chimeric antigen receptor (CAR) T cell therapy in patients with hematological malignancies (34, 35). These cytokines possess detrimental effects on endothelial function at several vascular niches, which may be implicated in the pathophysiology of the neurological complications of COVID-19, as discussed later.

Notably, despite the dysregulated production of immune mediators, an ample range of immune cell subtypes are depleted from the circulation of patients with severe SARS-CoV-2 infection. These cells include monocytes, dendritic cells, CD4+ and CD8+ T cells, and NK cells (36). Furthermore, the few adaptive lymphocytes that remain in the blood express markers of functional exhaustion (37). These data suggest that severe COVID-19 is a state of immunosuppression similar to the known sepsis-induced immunosuppression (38). However, it is also possible that the robust recruitment of functional immune cells to the sites of SARS-CoV-2 infection may explain the leukopenia observed during COVID-19.

MECHANISM OF SARS-COV-2 ENTRY INTO THE CNS

Expression of SARS-CoV-2 Viral Entry Factors in the CNS

Several HCoVs, including SARS-CoV-1, have the potential to infect different human tissues and organs (39, 40). Similarly, SARS-CoV-2 may also spread to several extrapulmonary tissues, including the CNS. Although recent analyses of autopsy specimens from patients with COVID-19 have not explored the presence of this virus in the brain (41), the neurological manifestations observed among individuals with COVID-19 (10) and the isolation of other human coronaviruses from neurological specimens support the notion of a possible neurotropism of SARS-CoV-2 (42–47). The neurotropism of other HCoVs has been extensively revised elsewhere (48).

As mentioned before, the patterns of expression of ACE2, TMPRSS2, TMPRSS4, furin, cathepsin L, and other entry factors used by SARS-CoV-2 for infection determine the tropism of the virus. The ACE2 receptor is highly expressed in cells of the alveolar epithelium (49, 50), which explains the vulnerability of the lungs to infection. The expression of ACE2 has also been found in other tissues, including the oral mucosa, endothelium, heart, kidney, lymphoid organs, testis, gut, and urinary tract (49–52). Nonetheless, distribution of TMPRSS2 has been assessed in a limited variety of human tissues, showing high expression in prostate cells, respiratory epithelial cells, salivary gland, kidney, liver, stomach, small intestine, and colon (53–55). TMPRSS2 is regulated by androgens (53), which may explain the higher susceptibility of men to suffer from severe forms of COVID-19. Few studies have analyzed the expression of TMPRSS4 and cathepsin L in healthy human organs. According to the Human Protein Atlas (https://www.proteinatlas.org/) dataset, TMPRSS4 is present in the cerebral cortex, hippocampus, caudate, thyroid gland, adrenal gland, nasopharynx, bronchi, lung, stomach, duodenum, colon, rectum, gallbladder, pancreas, and genitourinary tract. Meanwhile, cathepsin L shows medium expression in lung and liver, and low expression at bronchi, salivary gland, liver, kidney, pancreas, and genitourinary tract.

The expression in the human body of the known genes mediating the entry of SARS-CoV-2 into human cells coincides with the multiorgan pattern of COVID-19 manifestations. Nonetheless, the presence of such factors is low in the CNS under normal conditions. This may be in part because previous studies have only analyzed the bulk organ gene expression patterns of ACE2 and TMPRSS2. More recently, a comprehensive analysis of several single-cell RNA-seq databases showed that there are dual-positive ACE2+TMPRSS2+ cells in tissues beyond the respiratory system, including oligodendrocytes in the brain, and inhibitory enteric neurons (56). Furthermore, ACE2+CTSL+ cells were enriched in the olfactory epithelium. These data support some of the possible routes of SARS-CoV-2 entry into the CNS discussed below.

Routes of SARS-CoV-2 Entry Into the CNS

The mechanisms employed by SARS-CoV-2 to infect the nervous system are unknown. Currently, the hypotheses about the routes of viral entry into the CNS rely on previous observations made in experimental studies of SARS-CoV-1 infection. One hypothesis proposes that SARS-CoV-2 invades the brain by breaching the blood-brain barrier (BBB). Evidence in favor of this infection mechanism includes the high expression of the ACE2 receptor in endothelial cells of blood vessels (49, 50). The virus may therefore infect endothelial cells of the brain vasculature in the first term, and it may then spread to the surrounding ACE2+TMPRSS2+ oligodendrocytes and, finally, to the neurons. This would explain why SARS-CoV-1 has been observed inside neurons even when they have a mild expression of ACE2 under normal conditions (39, 40, 57). Despite this, SARS-CoV-1 has not been isolated from or observed inside endothelial cells (58). Conversely, the high concentrations of pro-inflammatory cytokines in the systemic circulation of patients with severe forms of COVID-19 might induce structural and functional alterations of the BBB (5, 28, 59). In this sense, it is well-known that different inflammatory mediators have detrimental effects on BBB integrity, increasing its permeability to neurotoxic molecules and immune cells (60). SARS-CoV-2 could thus gain access to the CNS directly through a paracellular route or within the immune cells, a mechanism that has been called a "trojan horse"(61). It might be feasible for this mechanism to occur during SARS-CoV-2 infection since the previous SARS-CoV-1 has also been observed inside different leukocyte subsets (40).

Secondly, the virus could enter the CNS through the olfactory epithelium, crossing the cribriform plate of the ethmoid bone and reaching the olfactory bulb from which it could spread to different areas of the brain (62). This route was demonstrated in mice intranasally inoculated with SARS-CoV-1, among which a rapid viral spread from the olfactory bulb to the brain stem was observed. This exposure to the virus caused high lethality among infected animals due to the occurrence of neuronal death in the respiratory centers of the brain stem (63).

Similar results were observed in another animal model of CNS infection with the HCoV-OC43 coronavirus (64). In humans, some studies demonstrated olfactory neuropathy in patients with SARS-CoV-1 infection (65). Likewise, recent investigations have shown that the olfactory epithelium is enriched with ACE2+CTSL+ cells (56). Moreover, hyposmia and anosmia are frequent manifestations of COVID-19 (10, 66), suggesting that SARS-CoV-2 might also infect the olfactory bulb in COVID-19 patients. In light of these findings, some researchers have proposed that the neuroinvasive potential of SARS-CoV-2 could contribute to the respiratory failure observed in patients with severe COVID-19 (67).

Finally, as in the case of other respiratory viruses with neurotropic potential, including the influenza A virus (68), SARS-CoV-2 could gain access to the CNS through the vagus nerve. The terminals of this nerve are located along the respiratory and gastrointestinal tracts, sites with high expression of ACE2 (49, 50) and enriched with ACE2+TMPRSS2+ enteric neurons (56). From these organs, the virus could gain access to the brain stem, taking advantage of the polarization of neurons and the machinery responsible for retrograde neuronal communication, or through endocytosis and clathrin-mediated exocytosis, as observed in the case of the transsynaptic transmission of the porcine coronavirus HEV 67N (69). The possible CNS invasion routes used by SARS-CoV-2 are illustrated in **Figure 2**.

NEUROLOGICAL MANIFESTATIONS OF COVID-19 DUE TO CNS INVOLVEMENT

Non-specific Neurological Symptoms

Some of the initial descriptions of the clinical phenotype of patients infected with SARS-CoV-2 showed that up to 10% of individuals with COVID-19 manifested non-specific neurological symptoms such as headache and dizziness (5, 6, 70). In a more recent report by Mao et al., a third of patients with COVID-19 presented non-specific neurological manifestations, including dizziness (16.8%), headache (13.1%), loss of consciousness (7.5%), and seizures (0.5%) (10). Two recent systematic reviews and meta-analyses showed that headache and dizziness are among the most frequent neurological symptoms affecting COVID-19 patients (71, 72). Interestingly, headache can occur even in the absence of fever and can be manifested as a migraine, tension-type, or cluster headache (73).

These non-specific neurological symptoms may reflect the neurotoxic effect of hypoxemia and the cytokine storm observed in patients with severe COVID-19. However, some of these manifestations must be differentiated from delirium, and other secondary causes, such as metabolic, gastrointestinal, renal, and hematological complications, must be ruled out, particularly in aged patients with underlying comorbidities. Interestingly, non-specific neurological manifestations are more frequent in patients who progress to respiratory failure, which supports the hypothesis about a possible contribution of the CNS infection to the respiratory failure caused by SARS-CoV-2 (67). Furthermore, patients with unspecific neurological symptoms have shown higher degrees of leukopenia, thrombocytopenia, and elevated blood urea nitrogen levels (BUN) (10). These data suggest that some of these findings might have certain prognostic value to predict the occurrence of more severe neurological complications. However, the predictive potential of non-specific neurological symptoms needs to be further evaluated in prospective studies. This would be of great help for the timely detection of patients at risk of neurologic sequela.

COVID-19 Associated Meningitis, Encephalitis, and Acute Necrotizing Encephalopathy

Acute meningitis and encephalitis are dramatic complications of various viral infections that often result in high morbidity and mortality rates due to their severity. Coronaviruses have also been associated with these neurological complications in humans. For instance, the HCoV-OC43 coronavirus was isolated from the brain of a patient who died of viral encephalitis (74). Likewise, both SARS-CoV-1 (46) and MERS-CoV have been reported to cause encephalitis (75).

In this context, SARS-CoV-2 can also cause viral meningitis and encephalitis, as demonstrated by a recent report of a 64-year-old patient with laboratory-confirmed COVID-19 who presented neurologic manifestations during the infection, including lethargy, clonus, and pyramidal signs in the lower limbs as well as stiff neck and Brudzinski sign (76). The cerebrospinal fluid (CSF) study revealed high protein levels and hypoglycorrhachia, although the virus could not be isolated from CSF in this case. The patient received antiviral drugs and symptomatic measures, progressing favorably without neurological complications. Similarly, in another report, a 24-year-old man with a 3-day history of headache developed fever, loss of consciousness, and seizures (77). Upon hospital admission, he presented signs of meningeal irritation and a low Glasgow Coma Scale (GCS) score, requiring mechanical ventilation. During his medical follow-up, he continued presenting seizures resistant to pharmacological treatment. His laboratory studies revealed high protein levels and pleocytosis in the CSF, and the MRI showed hyperintensities in the mesial temporal lobe. The patient tested negative for COVID-19 when his nasopharyngeal swab specimen was analyzed. Nonetheless, the result of the test was positive in the CSF sample. This finding was the first demonstration of the presence of SARS-CoV-2 in the CNS. The occurrence of encephalitis as the initial and even only manifestation of COVID-19 has latter been reported in three additional cases from China and the United States (78–80). Finally, another case report showed that SARS-CoV-2 infection could cause acute necrotizing encephalopathy (81). This entity is a severe neurological complication resulting from the disruption of the BBB associated with the cytokine storm observed in individuals with a critical illness.

Together, these cases illustrate the development of severe acute neurological complications in patients with COVID-19, confirming the neurotropism of SARS-CoV-2. These findings justify the intentional search for SARS-CoV-2 infection in patients attending with acute neurological manifestations and

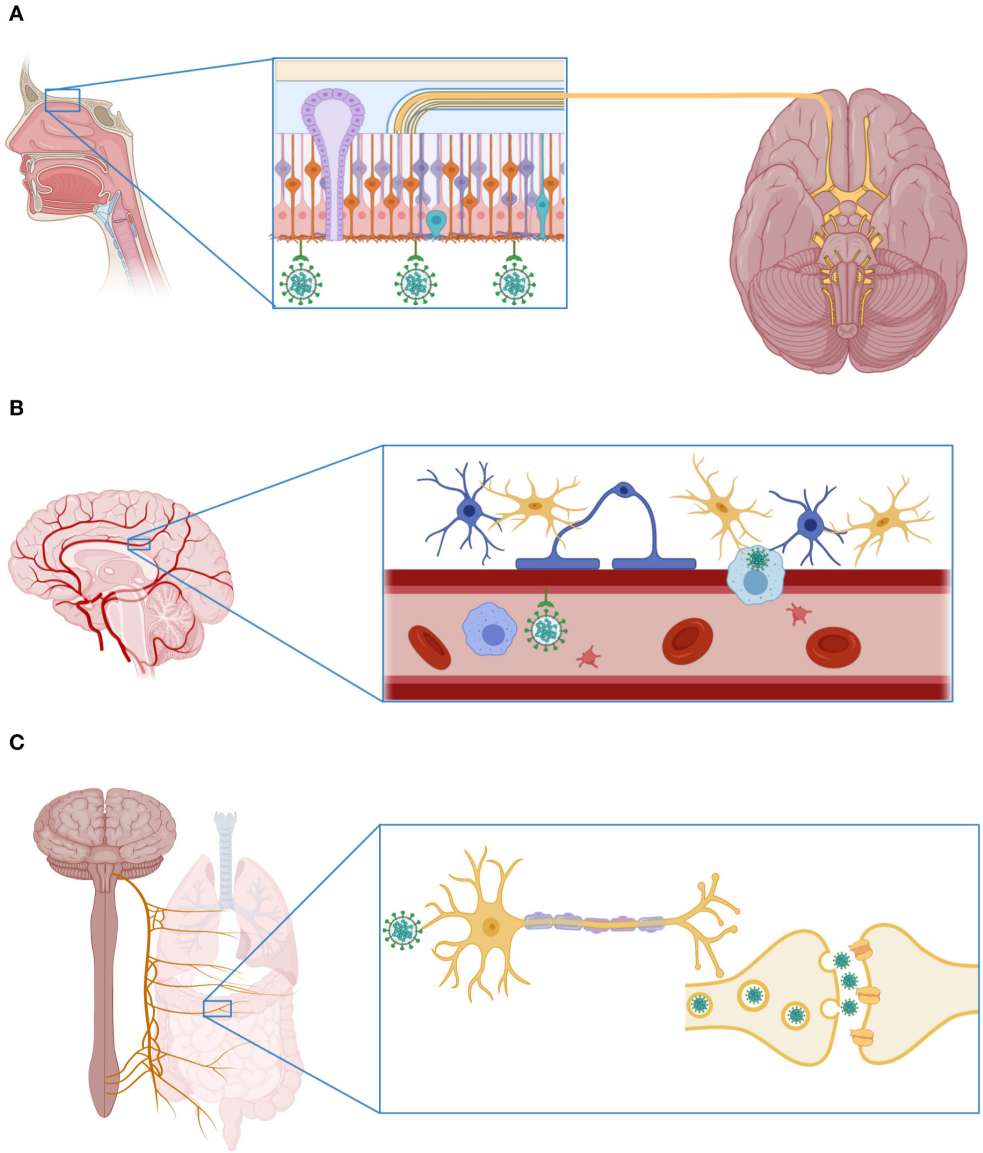

FIGURE 2 | Mechanisms of SARS-CoV-2 invasion to the central nervous system. **(A)** SARS-CoV-2 can enter the CNS through the olfactory bulb. Indeed, recent investigations have shown that the olfactory epithelium is enriched with cells that express the receptor ACE2 and the protease cathepsin L. **(B)** The virus can also infect the CNS via the hematogenous route, attaching to the ACE2 receptor expressed in endothelial cells of the cerebral blood vessels, or inside an immune cell. **(C)** Finally, SARS-CoV-2 can infect the nerve terminals of the vagus nerve located in the respiratory system and the gastrointestinal tract. From these sites, the virus can reach the CNS using the mechanisms of retrograde neuronal protein transport and transsynaptic transmission. This figure was created with *BioRender*.

febrile illness even in the absence of respiratory symptoms. The frequency and factors related to the severity of these complications need to be extensively investigated in future studies. This would require the conduction of comprehensive characterizations of genetic and immunological characteristics associated with the risk of severe neurological complications in COVID-19 patients. In addition, future studies evaluating the relationship between the temporal dynamics of neurological findings, the kinetics of viral loads in the circulation and CNS, and distinctive patterns of circulating immune mediators are warranted. This might inform about the potential of contagiousness of COVID-19 patients according to their neurological symptoms and reveal possible candidate biomarkers to distinguish individuals at high risk of severe neurologic sequela.

Cerebrovascular Disease

The antecedent of a recent respiratory infection, such as influenza, or influenza-like illness, has been related to acute cardiovascular complications, including acute myocardial infarction and stroke (82). Among infectious causes of vascular events, the infection with the varicella-zoster virus (VSV) is

the most frequent viral cause of stroke (83). The study by Mao et al. revealed that 3% of patients with COVID-19 also present stroke as their only neurological manifestation of the infection. From these, the majority were affected by ischemic strokes (six ischemic vs. one hemorrhagic from a total of 214 patients included in the study) (10). Stroke in individuals with COVID-19 occurs late during the disease and is more frequently observed among patients with severe respiratory failure. Interestingly, some COVID-19 patients were admitted to medical centers with hemiplegia and no history of respiratory symptoms (10). Other studies have also reported stroke in patients with severe COVID-19, even among young individuals (84, 85). This might indicate that stroke could be a result of vascular alterations directly driven by the virus, although the presence of cardiovascular risk factors can increase the incidence of this complication. A recent meta-analysis found that stroke, apart from being a frequent complication of COVID-19, has a considerable prognostic value to predict the risk of mortality. As such, patients with stroke have a 3-fold increase in the risk of death due to COVID-19 (86).

The association between stroke and COVID-19 might result from the critical condition and the pro-inflammatory state that prevails in most severely ill patients. The increased levels of pro-inflammatory cytokines in these individuals could alter the normal function of the cerebral vessels. These factors, along with the high prevalence of cardiovascular risk factors among patients with COVID-19, can trigger cerebrovascular complications. The endothelial infection of the cerebral vasculature due to its high expression of the ACE2 receptor might further contribute to these complications (49). In fact, the incidence of stroke in patients with VSV infection is a consequence of the invasion of the cerebral arteries by the virus (83). Other mechanisms by which SARS-CoV-2 could cause stroke include coagulation disorders. The abnormalities in the coagulation of severe COVID-19 patients have unique characteristics that partially resemble disseminated intravascular coagulation (DIC) or thrombotic microangiopathy. These alterations increase the risk of thrombotic complications and death due to COVID-19 (87). Acute myocarditis has been reported in patients with SARS-CoV-2 infection (88, 89). This cardiac complication could trigger events of brain embolization and stroke, which might explain the incidence of cerebral infarctions in young patients with COVID-19 in the absence of cardiovascular risk factors.

NEUROLOGICAL MANIFESTATIONS OF COVID-19 DUE TO PNS INVOLVEMENT

Peripheral Neuropathy and Myopathy Associated With SARS-CoV-2 Infection

During the outbreak caused by SARS-CoV-1, some studies described the occurrence of peripheral motor neuropathy and myopathy among infected individuals, mostly as part of the spectrum of manifestations of the critical illness polyneuropathy and myopathy disorders (90). Similarly, 2.3% of patients with COVID-19 have presented neuropathic pain, probably associated with peripheral neuropathy, whereas 10.7% of the cases showed data of skeletal muscle injury with elevated serum levels of creatine phosphokinase (CPK) (10). As in the case of other neurological manifestations, neuropathic pain and myopathy have been more frequently observed in patients with severe forms of COVID-19. Notably, these symptoms occur earlier during the disease in individuals with SARS-CoV-2 infection as compared to patients affected by SARS-CoV-1 (10). This suggests that the neuropathy and myopathy of COVID-19 are directly associated with the injury of peripheral nerves and striated muscles driven by the virus. COVID-19 patients with neuropathy and myopathy, however, exhibit higher levels of neutrophils and acute phase reactants than individuals without neurological manifestations (10). The nerve and muscle disorders observed during SARS-CoV-2 infection might thus be associated with a critical illness polyneuropathy of rapid development due to the more deteriorated clinical condition of patients with COVID-19 as compared to cases of SARS-CoV-1 infection. Vascular, thrombotic, ischemic, and direct nerve and muscle alterations driven by SARS-CoV-2 can contribute to neuropathy and myopathy of COVID-19 patients.

Cranial Neuropathies in Patients With COVID-19

A wide range of infectious diseases can cause complicate within cranial neuropathies like facial palsy and ophthalmoplegia. Among the pathogens that cause these manifestations with more frequency are HIV and VSV (91). Cranial nerves might also be susceptible to a direct or indirect injury caused by SARS-CoV-2. In fact, according to a recent study, about 85% and 88% of patients with COVID-19 develop olfactory and gustatory dysfunction (66). Similarly, in another investigation conducted in Italy, about 33% of patients with COVID-19 reported taste and/or olfactory disorders (92). These findings might be specific for SARS-CoV-2 infection and could predict the causative pathogen in patients with acute respiratory illness. Indeed, smell and/or taste disorders were more frequently observed among COVID-19 patients as compared to individuals with laboratory-confirmed influenza infection in a case-control study conducted in Spain (93). These observations might be of particular relevance for the upcoming flu season, which is predicted to be historically unique due to the convergence of influenza and COVID-19. During such an envisioned scenario, the differentiation of these diseases by clinical manifestations could be complicated. Nonetheless, the correct identification of the causative pathogen have therapeutic implications, such as the selection of adequate antiviral treatment. The presence of smell and taste dysfunction could thus be useful to distinguish COVID-19 from influenza. Furthermore, these symptoms can precede the onset of respiratory symptoms (94, 95), and their presence may predict a milder clinical course of the disease (96). Notably, COVID-19-related olfactory dysfunction has not been associated with rhinorrhea or nasal congestion, and more than half of the affected patients did not recover the function of the olfactory nerve (66). This suggests direct damage to the olfactory epithelium, which further reaffirms the possibility of an entry route of SARS-CoV-2 through the cribriform plate, reaching

the olfactory bulb from where it spreads to other parts of the CNS (62).

AUTOIMMUNE NEUROLOGICAL SYNDROMES ASSOCIATED WITH COVID-19

Guillain-Barré Syndrome

The association between viral infections and Guillain-Barré syndrome has been largely described in the past (97). Infection with *Campylobacter jejuni*, cytomegalovirus, or *Mycoplasma pneumoniae* precedes Guillain-Barré syndrome in up to two-thirds of affected individuals (98). More recently, several viral emerging diseases have shown this syndrome as one of its more common and severe complications, as is the case of the infection with the Zika virus (99). Although this association was not described in patients infected with SARS-CoV-1, cases of Guillain-Barré syndrome were observed during the outbreak of MERS-CoV (100). In this context, the infection with SARS-CoV-2 could also complicate with or manifests as Guillain-Barré syndrome. In a recent report, Zhao et al. described the case of a woman that developed asymmetric and progressive muscle weakness in the lower limbs, associated with leukopenia and high protein levels in the CSF without pleocytosis, accompanied by data of demyelinating neuropathy in studies of nerve conduction (101). The patient had a history of a recent trip to the city of Wuhan, and she showed no evidence of respiratory system involvement at the time of symptom onset. A total of 8-days later, the appearance of fever, respiratory symptoms, and radiological data compatible with pneumonia was documented, confirming the SARS-CoV-2 infection by laboratory testing in a nasopharyngeal swab sample. The patient received intravenous immunoglobulin treatment recovering the neurological function without sequel. Similarly, in another case from Italy, a 71-year-old male also developed Guillain-Barré syndrome before the onset of any respiratory symptom (102).

These reports suggest that Guillain-Barré syndrome may be the first manifestation of COVID-19, presenting as a para-infectious phenomenon. This is due to the parallel occurrence of neurological and respiratory symptoms, instead of the postinfectious pattern observed in other infections, including the MERS-CoV (100). Nonetheless, two new case reports of six COVID-19 patients demonstrated that Guillain-Barré syndrome could occur a few days after the onset of respiratory symptoms (103, 104). Collectively, these findings obligate physicians to continuously monitor the neurological condition of patients with suspected or confirmed SARS-CoV-2 infection to identify any sign of demyelinating neuropathy. Furthermore, the occurrence of Guillain-Barré syndrome associated with COVID-19 must be carefully distinguished from the myopathy and neuropathy disorders of the critically ill patient.

Miller Fisher Syndrome

Miller Fisher syndrome is a rare neurological disease that is considered a variant of the Guillain-Barré syndrome. This disorder is characterized by the triad of abnormal muscle coordination, paralysis of the eye muscles, and the absence of tendon reflexes. Miller Fisher syndrome can be associated with a history of recent viral illness; however, no evidence exists of its association with human coronavirus infections. Interestingly, a recent paper has reported the occurrence of the triad of ataxia, areflexia, and ophthalmoplegia in a 50-year-old man that presented cough, fever, anosmia, and hypogeusia some days before the onset of the neurological symptoms. The infection with the SARS-CoV-2 infection was confirmed in an oropharyngeal swab sample, and blood tests showed lymphopenia, elevated C-reactive protein levels, and anti-GD1b-IgG antibodies. He received treatment with intravenous immunoglobulin, which caused significant improvement in his neurological functions. Collectively, the literature summarized here indicates that neurological syndromes caused by aberrant immune responses, such as Guillain-Barré and Miller Fisher syndromes, can occur as part of the clinical spectrum of SARS-CoV-2 infection. Despite this, the immune mechanisms implicated in these phenomena, and the possibility of a direct neuropathic effect driven by the virus are unknown.

Molecular mimicry has been proposed as the primary immune alteration underlying the development of Guillain-Barré syndrome during infections (105). This phenomenon is related to the presence of carbohydrates in infectious agents of similar structural characteristics as compared to carbohydrates expressed on neuronal membrane ganglioside and galactocerebrosides. For instance, the lipopolysaccharides of *Campylobacter jejuni* share ganglioside-like epitopes with peripheral nerves (106), whereas galactocerebroside-like structures are present in glycolipids of *Mycoplasma pneumoniae* (107). These molecular similarities trigger the production of anti-glycolipid antibodies, which mediate autoimmune damage to peripheral nerves. Ganglioside- or galactocerebroside-like epitopes have not been described in SARS-CoV-2. However, the S protein of this virus possesses 22 N-linked glycan sequons per promoter (20), that could potentially share certain similitude with carbohydrates localized on the surface of the host nerve cells. Future studies are required to evaluate the serologic features of anti-glycolipid antibodies in patients with COVID-19 to elucidate possible mechanisms underlying the association between SARS-CoV-2 infection and Guillain-Barré syndrome.

NEUROPSYCHIATRIC MANIFESTATIONS OF COVID-19

Several viruses affecting humans have been implicated in the development of psychiatric symptoms due to their neurotropism. The immediate antecedent for the global transmission of a respiratory pathogen was the 2009 pandemic associated with the emergence of a novel influenza A (H1N1) virus. Several neuropsychiatric manifestations during the outbreak of influenza were observed among infected patients, including fear and behavioral changes (108). Similarly, during the SARS-CoV-1, a range of psychiatric disorders was identified, including anxiety, depression, suicidal ideation, and hallucinations (109, 110). A recent systematic review found a high incidence of confusion,

depression, anxiety, memory impairment, insomnia, and steroid-induced psychosis among patients with SARS-CoV-1 or MERS-CoV infection (111). The mechanisms underlying psychiatric disorders in patients with viral infections are not precise, but they might be related to the structural and functional disruption of the BBB mediated by circulating inflammatory cytokines produced in response to viruses. These mediators might also alter neuronal networks implicated in cognitive functions. Indeed, several psychiatric illnesses, including schizophrenia, have been proposed to result from immune-mediated pathogenic mechanisms (112).

In this sense, it is clear that the current pandemic can cause indirect effects on the mental health of infected and non-infected people due to quarantine and social distancing measures, which constitute sources of distress additional to the daily life problems (113). At this moment, however, there is little literature about neuropsychiatric disorders directly associated with the infection with SARS-CoV-2. In a case series of three patients with laboratory-confirmed COVID-19 and no evidence of respiratory symptoms, it was found that such individuals presented anxiety, agitation, paranoid behavior, disorganized thinking, and auditory hallucinations (114). Other authors have also reported that delirium can be present in a high percentage of COVID-19 patients (111). Thus, as mentioned before, delirium must also be considered in the differential diagnosis of individuals with acute neuropsychiatric manifestations associated with SARS-CoV-2 infection. Finally, the virus could lead to long-term neuropsychiatric and cognitive sequels. In fact, survivors of SARS-CoV-1 and MERS-CoV have been found to present depression, anxiety, and post-traumatic distress syndrome several months after the diagnosis (111). Therefore, it is essential to conduct long prospective observational studies to estimate the incidence of psychiatric disorders in the post-illness stage of COVID-19. Future studies must address possible links between the antecedent of SARS-CoV-2 infection and the incidence of chronic neurodegenerative disorders. The studies about the spectrum of neurological and psychiatric manifestations of COVID-19 are summarized in **Table 1**.

TABLE 1 | The spectrum of neurological manifestations of SARS-CoV-2 infection.

Clinical finding	No. of patients	Author	Country	References
Headache	3/39	Huang et al.	China	(5)
	9/138	Wang et al.	China	(6)
	8/99	Chen et al.	China	(70)
	28/214	Mao et al.	China	(10)
	29/112	Porta-Etessam et al.	Spain	(73)
Dizziness	13/138	Wang et al.	China	(6)
	36/214	Mao et al.	China	(10)
Loss of consciousness/ altered mental status	9/99	Chen et al.	China	(70)
	16/214	Mao et al.	China	(10)
Seizures	1/214	Mao et al.	China	(10)
Cerebrovascular disease	6/214	Mao et al.	China	(10)
	3/58	Helms et al.	France	(84)
	5 cases	Oxley et al.	United States	(85)
Meningitis/ encephalitis	1 case report	Moriguchi et al.	Japan	(77)
	1 case report	Yin et al.	China	(76)
	1 case report	Duong et al.	United States	(78)
	1 case report	Filatov et al.	United States	(79)
	1 case report	Ye et al.	China	(80)
Olfactory and/or taste dysfunction	20/59	Giacomelli et al.	Italy	(92)
	31/79	Beltrán-Corbellini et al.	Spain	(93)
	128/169	Yan et al.	United States	(96)
	130/374	Spinato et al.	Italy	(95)
	357/417	Lechien et al.	Belgium	(66)
	23/214	Mao et al.	China	(10)
	2 cases	Lorenzo Villalba et al.	Spain	(94)
Peripheral neuropathy/nerve pain	5/214	Mao et al.	China	(10)
Myopathy	23/214	Mao et al.	China	(10)
Guillain-Barre syndrome	1 case report	Zhao et al.	China	(101)
	1 case report	Alberti et al.	Italy	(102)
	1 case report	Sedaghat et al.	Iran	(103)
	5 cases	Toscano et al.	Italy	(104)
Miller-Fisher syndrome	1 case report	Gutierrez-Ortiz et al.	Spain	(115)
Neuropsychiatric manifestations	3 cases	Ferrando et al.	United States	(114)

Other neurological manifestations reported in COVID-19 patients include ataxia, acute hemorrhagic necrotizing encephalopathy, polyneuritis cranialis, and neuralgia (10, 81).

POTENTIAL THERAPEUTICS FOR THE NEUROLOGICAL COMPLICATIONS OF SARS-COV-2 INFECTION

Supportive measures, along with strict control and prevention of fever, high blood pressure, elevated glucose, and seizures, may ameliorate the neurotoxic potential of SARS-CoV-2 infection. Currently, there are no mechanism-based therapeutics specific for neurological complications of COVID-19. The evidence curated in this review suggests possible pathologic processes underlying the involvement of the CNS/PNS during SARS-CoV-2 infection. A better understanding of these mechanisms may reveal targets for therapeutic interventions. Direct effects driven by the virus, such as the infection of brain blood vessels and nerve cells, are proposed to play a role in neurologic manifestations of COVID-19. Although studies addressing the relationship between viral loads in the CNS and the severity of such manifestations are required, reducing the number of copies of SARS-CoV-2 in the circulation and tissues might be a useful strategy. Remdesivir is the only antiviral drug with demonstrated capacity to blocking SARS-CoV-2 replication in pre-clinical trials approved for usage in humans. This antiviral drug reduces the time of clinical recovery in patients with COVID-19 (116). The benefits of remdesivir for patients with neurological complications have not been evaluated. Other

candidate agents that antagonize the activity of the host proteases implicated in the infection process of SARS-CoV-2 may be useful to limit the infective capacity of this virus. These include the camostat mesylate and nafamostat mesylate, two compounds that block the activity of TMPRSS2 (19, 117). The effects of these drugs on the severity and recovery of neurologic sequela associated with COVID-19 must be evaluated in future clinical trials. Passive immunization by transfer of convalescent human plasma may also contribute to reduce the viral loads and prevent or reverse the development of neurological symptoms. This assumption is supported by recent systematic reviews that found that convalescent plasma therapy improves symptoms, reduce viral loads, and diminishes mortality in COVID-19 patients (118). The time at which these proposed interventions would be more useful to counteract neurologic sequela of COVID-19 is unknown.

Immune-mediated neurotoxicity is an obvious therapeutic target for individuals with SARS-CoV-2 infection (59). The immune profile of patients with severe COVID-19 resembles the cytokine release syndrome (CRS) observed after CAR-T-cell therapy (34, 35). As aforementioned, individuals receiving CAR-T cells who develop CRS are at risk of injury to the nervous system. Therefore, lessons from the treatment of patients with CAR-T-cell-therapy-associated neurotoxicity might be applicable to COVID-19 patients. Tocilizumab, an anti-IL-6 monoclonal antibody, is the primary treatment for CRS (119), and is currently being used to ameliorate inflammatory manifestations caused by SARS-CoV-2 infection (120). In patients with severe COVID-19, tocilizumab declined cytokine production and reduced the risk of intubation requirement (120). However, the utility of tocilizumab for management of CRS-associated neurotoxicity is controversial (121). Interestingly, mouse models have revealed that the antagonist of the receptor of IL-1β anakinra might be a better option than tocilizumab for the treatment of CRS and neurotoxicity after CAR-T-cell therapy (122). As a strong induction of IL-1β has been observed in severely ill COVID-19 patients (32), the potential use of anakinra deserves further investigation. Monocytes and macrophages also contribute to the development of CRS and neurotoxicity after CAR-T-cell therapy. As such, inhibition of GM-CSF with monoclonal antibodies has shown to reduce neuroinflammation in Phase I studies of patients receiving CAR-T-cell therapy (123). Interestingly, in a recent study conducted in patients with COVID-19, the GM-CSF blockade with mavrilimumab improved clinical symptoms, survival, and reduced intubation requirement (124). Inhibition of GM-CSF is thus a potential therapeutic for patients with COVID-19 that develop neurological complications. Short courses of steroids are safe and provide some benefits for the treatment of immune-mediated neurotoxicity. Specifically, dexamethasone may constitute a good candidate due to its excellent CNS penetration and beneficial effect on the integrity of the BBB. Dexamethasone has been safely used in patients with acute respiratory distress syndrome, reducing the requirement of mechanical ventilation and mortality (125). These data may support the use of dexamethasone for the management of neurological manifestations of SARS-CoV-2 infection, although possible consequences of the dexamethasone-induced immunosuppression need to be evaluated.

The breaching of the BBB is an important event for the development of neurotoxicity in COVID-19 patients and individuals under CAR-T-cell therapy. Pharmacological agents targeting the BBB may thus be useful to treat severe neurological manifestations of COVID-19. The sonic hedgehog (SHH) signaling pathway is essential for the maintenance of integrity and immune homeostasis of the BBB (126). Agonists of SHH, such as purmorphamine, a compound that activates the SHH by promoting smoothened protein (127), reduce BBB damage in animal models of infection and ischemic stroke (128–130). Although little evidence exists of the safety of purmorphamine in humans, this agent warrants further exploration for the management of neurotoxicity associated with severe infections, including COVID-19.

IMPLICATIONS OF COVID-19 PANDEMIC FOR THE NEUROLOGY FIELD

The magnitude of the current pandemic has generated concerns among professionals from various areas of medicine. The evidence summarized in this review shows that neurology is an active area of medicine at the frontline of the current pandemic. Actually, due to the increasing number of confirmed cases around the world and the neurotropic potential of SARS-CoV-2, it is highly likely that neurologists would have to provide medical care to patients with COVID-19, some of which might present neurological manifestations. The first and most important precautionary measure that must be taken by neurological centers around the world is to improve the knowledge of the disease among health care professionals. Obviously, measures of personal protection and social distancing should be extreme in neurology and neurosurgery clinics since it is possible that some COVID-19 patients attending with neurological symptoms with a not yet confirmed SARS-CoV-2 infection may constitute potential foci of inadvertent contagion for doctors, especially if they do not manifest respiratory symptoms initially. To prevent contagions, it is important to investigate previous contact with people with laboratory-confirmed COVID-19 in patients attending to neurological centers. Similarly, it is crucial to maintain a high degree of clinical suspicion about possible SARS-CoV-2 infection in patients presenting an acute neurological condition. Furthermore, the continuous surveillance and intentional search for neurological complications in patients with confirmed SARS-CoV-2 infection are necessary and even mandatory because these measures could allow the timely establishment of therapeutic strategies for limiting neurologic sequelae.

Finally, the current pandemic may obligate some changes in normal care to patients with neurologic disorders at medical centers receiving many COVID-19 cases. Of particular importance is the possible impact of this pandemic in the triage and management of patients with stroke and other acute neurological emergencies due to resource re-allocation. Also, the interruption of activities at many neurology outpatient clinics

may affect the management of chronic neurological conditions, requiring increased usage of tools such as telemedicine and e-care.

CONCLUSIONS

The clinical phenotype of COVID-19 encompasses a spectrum of neurological manifestations of varying severity that, besides respiratory symptoms, can cause high morbidity and mortality rates in individuals with SARS-CoV-2 infection. The current review constitutes a useful reference to improve our understanding of the pathophysiological mechanisms of SARS-CoV-2 infection and should motivate further studies about novel strategies to mitigate the impact of the current pandemic on the field of neurology.

AUTHOR CONTRIBUTIONS

PG-O, JC-P, and CS-M drafted the manuscript. FP-S, AR-N, and GG-Q are medical students from the Centro Especializado en Neurocirugía y Neurociencias México (CENNM) and the Escuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional in Mexico City. They participated in the search for scientific literature and revised the paper for intellectual content. All authors read and approved the final version of the manuscript.

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COVID-19-Related Anosmia: The Olfactory Pathway Hypothesis and Early Intervention

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Anosmia is a well-described symptom of Corona Virus Disease 2019 (COVID-19). Several respiratory viruses are able to cause post-viral olfactory dysfunction, suggesting a sensorineural damage. Since the olfactory bulb is considered an immunological organ contributing to prevent the invasion of viruses, it could have a role in host defense. The inflammatory products locally released in COVID-19, leading to a local damage and causing olfactory loss, simultaneously may interfere with the viral spread into the central nervous system. In this context, olfactory receptors could play a role as an alternative way of SARS-CoV-2 entry into cells locally, in the central nervous system, and systemically. Differences in olfactory bulb due to sex and age may contribute to clarify the different susceptibility to infection and understand the role of age in transmission and disease severity. Finally, evaluation of the degree of functional impairment (grading), central/peripheral anosmia (localization), and the temporal course (evolution) may be useful tools to counteract COVID-19.

Keywords: COVID-19, anosmia, SARS-CoV-2, olfactory receptors, olfactory bulb

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INTRODUCTION

A wide spectrum of symptoms characterizes SARS-CoV-2 infection, ranging from serious conditions, including acute respiratory distress syndrome (ARDS), to mild/moderate and also asymptomatic forms of the disease, contributing to the spread of the viral infection.

The Corona Virus Disease 2019 (COVID-19) rapid worldwide spread has led to characterization of "minor" symptoms, such as anosmia (1).

Anosmia was underestimated in the early stages of pandemic emergency, and most of the patients who needed hospitalization were not consistently investigated for this symptom that gradually emerged as a spy feature of infection.

Several respiratory viruses may cause post-viral olfactory dysfunction (PVOD), in most cases, reversible. Seldom, this dysfunction may persist, suggesting a sensorineural central damage (2). One of the COVID-19 clinical problems is the concomitant lack of prognostic indexes that may predict the need for early intervention and preventative therapies in patients with mild symptoms.

Since the olfactory bulb (OB) is considered an immunological organ (3), its involvement could provide information on host's immunological competence in the fight against the virus entrance and the virus spread into the central nervous system.

As members of Coronaviridiae family are known to cause CNS dysfunction, it appears mandatory to understand the role of SARS-CoV-2 neurotropism in the development of clinical manifestations.

EPIDEMIOLOGY

The first symptomatic characterization of COVID-19 evolved over the past months adding to major symptoms (fever, coughing, fatigue, and shortness of breath) a broad spectrum of minor symptoms. Among those, increasing olfaction disturbance (OD) observations have made that anosmia was identified as an emerging symptom and subsequently as a marker of SARS-CoV-2 infection (1, 4, 5).

Anosmia had attracted the most public interest between physicians and the general population both because of media coverage in an atmosphere of increasing and constant alarm and concern and for its potential capability of early identification of infection. For instance, in our country, after journalistic and media announcement of anosmia as a symptom of COVID-19 in March 2020, this term had a peak in search volumes on Google (6).

In scientific literature, after the first reports of olfactory and taste disorders (OTDs) in COVID-19, an increasing and rapidly evolving detailed analysis of this symptom was progressively collected to evaluate the prevalence and patterns of anosmia and its significance in the context of COVID-19.

In February 2020, a retrospective study of 214 COVID-19 patients in Wuhan (7) reported that 11 patients (5.1%) presented hyposmia and 12 patients (5.6%) presented hypogeusia. This study was an early report derived from the analysis of medical records without information on the timing of chemosensory dysfunction onset and patient conditions. When Italy became the new pandemic epicenter, these disorders appeared to be more common, particularly in the early stages of the disease, in paucisymptomatic patients and mild to moderate COVID 19 patients.

Giacomelli et al. from Sacco Hospital in Milan, in March 2020, highlight the prevalence of chemosensory dysfunction in 59 patients with laboratory-confirmed SARS-CoV-2 infection through a verbal interview. Of these, 20 (33.9%) reported at least one taste or olfactory disorder and 11 (18.6%) reported both; 20.3% presented the symptoms before hospital admission, whereas 13.5% presented during the hospital stay. Females reported OTDs more frequently than males (8).

More in-depth, a following Italian multicenter study on olfactory and gustatory function impairment in COVID-19 shows more objective data. The study cohort was composed of 161 patients in home quarantine and 184 hospitalized patients in all disease stages from asymptomatic to severe disease. Chemosensory dysfunctions have been self-reported by 74.2% of COVID-19 patients; 79.3% of these patients reported combined chemosensitive disturbances, 8.6% reported isolated olfactory disorders, and 12.1% reported isolated taste disorders. At the test time, the condition was self-reported as completely regressed in 31.3% of the patients concerning the sense of smell and in 50.4% for the taste. More interestingly, functional evaluation of patients who reported only gustatory disorders or without chemosensory dysfunction highlights mild hyposmia in an additional 10.7% of patients. Furthermore, 70% of patients who reported complete resolution proved hyposmic to an objective test. Also, the study population subgroup analysis showed a high frequency of olfactory disorders throughout the observation period, ranging between 77.4% (days 1–4) and 69.2% (days 25–35). Anosmia or severe hyposmia affected 70.9% of patients in the early stages; they improved after the first 10 days, reaching moderate hyposmia values. Despite a more effective recovery of taste with back to normal range after 15 days, the olfactory score improved significantly in the first 2 weeks without returning to average values but always remaining in the range of hyposmia, even in the group of patients evaluated in the 3rd and 4th week from the clinical onset. Interestingly, chemosensitive symptoms were the first symptom of COVID-19 in 29.2% of patients and the only one in 9.5% of the cases. In this study, according to other previous studies, no correlation was found between olfactory and gustatory disorders and nasal obstruction or rhinitis symptoms. No significant correlation was found between the gustatory and olfactory scores and the patients' gender and age (9). In April 2020, a multicenter European study reported olfactory dysfunction (OD) in 85.6% of patients; 11.8% of these cases presented with OD onset before other symptoms. In contrast with the previous data, this study showed that males were significantly less affected by OD than females ($p = 0.001$). In addition, among the 18.2% of patients without nasal obstruction or rhinorrhea, 79.7% were hyposmic or anosmic (10).

Another cross-sectional survey on OTDs that showed a prevalence of 91% in the context of SARS-CoV-2 infection reported that females presented OTDs more frequently than males (52.6 vs. 25%, $P = 0.036$). Moreover, patients who did present at least one OTD were younger than those without OTDs (11). More recently, Chung et al. performed an observational cohort study using questionnaires and smell tests, sinus imaging, nasendoscopy, and nasal biopsies in selected patients. This study showed olfactory symptoms in 12 of 18 (67%) COVID-19 patients and OD was confirmed in six patients by BTT smell test. Computed tomography sinus, performed for the six patients with OD, found radiological evidence of sinusitis, and nasendoscopy did not find any olfactory cleft obstruction, nasal polyps, or active sinusitis. Interestingly, nasal biopsies, performed in three patients, showed minimal inflammatory changes represented by mild infiltrations of lymphocytes, plasma cells, and rare neutrophils in the stroma.

Immunofluorescence staining for CD68 established the presence of macrophages within the epithelium and the stroma. Follow-up evaluation by Butanol Threshold Test Assessment for the six patients with OD shows that OD can persist even after viral clearance in a subset of patients (12).

In conclusion, the systematic reviews show a wide variability of olfactory impairment prevalence according to the relief method of anosmia, subjective reports or objective testing or combination, and geographic location. The prevalence varies from 5.1% (7) to 98.0% (4). Interestingly, other recent reviews and studies highlighted ethnic differences in the frequency and

prevalence of this chemosensory dysfunction, lower in Chinese COVID-19 patients than in Western cohorts of comparable size, attributing them to the variants of virus entry protein across populations (11, 12).

All these epidemiological data, even if conflicting due to the different assessment methods and geographic areas, could improve our knowledge of anosmia prevalence and patterns. It would be even better if methods of anosmia relief and follow-up were standardized. As noted above and in a recent case series of 86 COVID-19 patients (13), questionnaires and studies on clinical records could lead to an erroneous assessment of the prevalence of anosmia and recovery time and its subclinical persistence (9, 13).

PHYSIOPATHOLOGY

Understanding the underlying mechanism of anosmia linked to the olfactory pathway's anatomy and physiology could help distinguish olfactory disorders into a conductive/peripheral or sensorineural/central. Conductive olfactory dysfunctions occur when mechanical barriers prevent proximity between odorants and receptors on the olfactory epithelium. On the contrary, sensorineural disorders occur due to deficient processing of odorant stimulus by the olfactory receptors (ORs), olfactory neurons, and olfactory pathways, up to the CNS (OB and olfactory brain areas). Common conductive disorders arise from obstructive nasal diseases, such as chronic rhinosinusitis, nasal polyposis, allergic rhinitis, and nasal masses, characterized by a combination of obstruction to nasal airflow transporting odorant and mucosal edema that is inflammation related (13). On the contrary, viral infections are considered the most common cause of sensorineural olfactory dysfunction. Indeed, from 20–30 to 42.5% of adult patients with acquired sensorineural olfactory dysfunction reported a recent history of upper respiratory infection (13, 14), while the cumulative frequency of olfactory loss associated with sinonasal disorders or acute infections of the upper airways was 6% in the pediatric population (15). A recent study showed that olfactory dysfunction's etiologies changed with age: the frequency of congenital causes of anosmia decreased while upper respiratory tract infection-related anosmia frequency and idiopathic causes increased (15).

It was estimated that children suffer from 6 to 10 colds per year, whereas adults from 2 to 4 per year. More than 200 different viruses can cause cold symptoms, but the great majority are not associated with anosmia, hyposmia, and CNS involvement. Besides, most previous reviews, similar to what is observed in the context of the COVID-19 pandemic, have found that POVD is more common in women. Still, olfactory dysfunction could occur only after infection by a specific virus with peculiar neurotrophic proprieties, and when the host had some predisposing factors, virus may invade the CNS (16, 17). However, the pathogenesis of sensory loss and associated predisposing factors after viral infections are not well-characterized, and unfortunately, most patients were investigated when the virus was no longer detectable (17). The SARS-CoV-2 pandemic has turned our attention on POVD again.

Now, we know the straight association between anosmia and SARS-CoV-2. Thus, we should take advantage to better understand the pathogenesis of POVD and the neuroinvasive potential of the virus through the olfactory neuroepithelium (ONE) and olfactory pathway.

After the intranasal inoculation of several viruses, previous animal studies have shown central olfactory damage and damage to deeper areas of CNS (18, 19).

Therefore, the question is whether the olfactory dysfunction in COVID-19 and other viral infection arise from peripheral OR damage as a result of local inflammation or involvement of central olfactory pathways, or a combination of both (17).

Previous studies on POVD highlight direct evidence of a broad spectrum of epithelial damage, from the reduced number of ORs to abnormal dendrites that did not reach the epithelial surface or that were lacking sensory cilia to decreased nerve bundles or substitution of ONE with metaplastic squamous epithelium (20–23).

In contrast with these studies, Chung et al., analyzing the nasal mucosae of patients affected by SARS-CoV-2, showed minimal inflammatory changes represented by mild infiltrations of lymphocytes, plasma cells, and occasional neutrophils localized in the stroma but without detailed characterization of neuroepithelium alterations (12).

In a recent study, local TNF-α and IL-1β levels were assessed in COVID-19 patients. TNF-α was significantly increased in the olfactory epithelium of the COVID-19 group compared to the control group. However, no differences in IL-1β were seen between groups. In the authors' opinion, this evidence implies that inflammation can lead to OR impairment, and according to a previous study, this impairment arises from inflammation, which can damage olfactory neurons (24).

On the contrary, Kim and Hong demonstrated that persistent PVOD is associated with decreased metabolism in specific brain regions where the olfactory stimuli are processed and integrated, suggesting that anosmia is, in some cases, caused by a central injury mechanism (25). Virally induced damage of OB and other brain areas was highlighted through magnetic resonance imaging correlating olfactory function with OB volume (26).

Retrograde transport from the nasal mucosa to the brain has been recently hypothesized for SARS-CoV-2 (27–30) and previously described for SARS-CoV1 and HCoV-OC43 found in specific brain areas of infected patients (31–33) probably climbing *via* the olfactory nerves, as already shown in mice (34). Furthermore, once in the brain, HCoV-OC43 can disseminate from the OB to other regions of the brain, including the cortex and the hippocampus, from which it appears to spread by a trans-neuronal route before it eventually reaches the brainstem and spinal cord. These results suggest that coronaviruses may also invade the human CNS from the external environment through the neuroepithelium of the olfactory nerve and OB, before infecting the resident cells of the brain, and potentially the spinal cord. Mori et al. also reviewed these same observations for some neuroinvasive human viruses such as influenza virus and Herpes simplex virus (HSV) (35). Presumably, SARS-CoV-2 can involve the olfaction through a central mechanism also.

However, a recent study highlighted that olfactory epithelial cells, but not OR neurons (e.g., horizontal basal cells and supporting cells), express ACE2, the primary SARS-CoV-2 receptors (36). Therefore, virus could use alternative receptors to directly enter into OR neurons or an alternative pathway for OB involvement. The OB may be the first site of CNS involvement by neurotropic viruses.

IMMUNOLOGICAL ROLE OF THE OB

Olfaction, although not indispensable to the survival, is a crucial sense that induces several feedback processes, also unconscious in response to the molecular sampling of the environment. These processes are very complex, as well as the anatomical substrates that allow them. From odor receptors, the stimuli converge in the OB and then, through a multitude of projections, reach the higher brain regions, including the amygdala, septal nuclei, pre-pyriform cortex, the entorhinal cortex, hippocampus and the subiculum, thalamus, and the frontal cortex. These bidirectional connections provide a unique dynamic system (37). Considering the intricate interactions between the immune system and the CNS and the complexity of the relationship between CNS and the olfactory system, it does not surprise the relevance of smell for immunological investigation. Since OB, regularly exposed to the external environment, is considered an immune organ that prevents the invasion of viruses into the CNS (3), its dysfunction may be a concomitant factor that predisposes to a worse outcome in respiratory virus infections when his immunological function is impaired or disrupted as a result of aging or some pathological processes. In many animal studies concerning depression, olfactory bulbectomy is commonly used. Unexpectedly, a variety of immune abnormalities may be observed in the olfactory bulbectomized mice: reduced neutrophil phagocytosis, lymphocyte mitogenesis, lymphocyte number, and negative acute-phase proteins and increased leukocyte adhesiveness/aggregation, monocyte phagocytosis, neutrophil number, and positive acute-phase proteins. In addition, after bulbectomy, increases in serum IL-1β concentration and PGE2, while basal anti-inflammatory cytokine IL-10 concentration is suppressed, may be observed (38, 39).

These observations suggest that the olfactory system is intricately related to immunological function, and perturbations in the immunological system and in the olfactory system may be significant to each other (37).

Viral infection of the CNS is a rare condition and efficient mechanisms must be in place in the OB to protect the CNS from viral spread: Kalinke et al. showed that after intranasal instillation of VSV in mice with selective type I interferon receptor depletion only in neuroectodermal cells, the virus moved *via* the olfactory nerves to the OB and further spread over the whole CNS. On the contrary, control mice infected with the same virus showed infection of olfactory nerves, but within the OB, the virus was arrested in the glomerular layer (40).

This experiment highlighted in the OB a type I IFN-dependent mechanism that efficiently inhibited virus spread. After exposure to viruses, expression of MHC I and II, pattern recognition receptors (PRRs), and type 1 interferon (IFN-I) is increased in astrocytes, microglia, and ONE. The increase in INF-I and the rapid infiltration of both CD4+ and CD8+ T cells decrease viral load in the OB (3). Therefore, inflammatory cell infiltration in OB, while possibly involved in the development of anosmia, contributes to the viral spread arrest. Unfortunately, it is unknown whether type I IFN stimulation of olfactory neurons affects axonal virus spread and infection of the CNS.

In an interesting recent work, according to a previous study that demonstrated selective expression of interferon-gamma in sustentacular cells inducing anosmia without damage to the neuroepithelia, the authors hypothesized that IFNs, or other cytokines, can activate an antiviral response within the OR neurons that suppress OR expression. They also demonstrated that interferon signaling correlates with OR neuron dysfunction (41). If this IFN-I antiviral response model is confirmed in patients with COVID-19 infection, ORs may have an essential role in the virus mechanisms of cell infection.

The OR neuron cell body is located in the olfactory epithelium, whereas their axons project into the OB, and the virus can readily spread within the OB in an anterograde manner. To further move to other brain areas, the virus can spread trans-synaptically using retrograde and anterograde transport. Considering the capability of some coronaviruses to spread from the lower respiratory tract by a synapse connection to the medullary cardiorespiratory center (partially responsible for the acute respiratory failure of COVID-19 patients) (34), it is possible to hypothesize an inverse path: SARS-CoV-2 could spread from OB to the CNS to periphery through nerve endings of the lower respiratory tract. Obviously, the virus can enter the CNS also *via* non-olfactory paths. It was shown that, after intranasal VSV instillation, the olfactory route is preferentially used for CNS entry. Only if OR neurons are destroyed, are alternative entry paths, such as *via* the cerebrospinal fluid or the trigeminal nerve or blood, used (27). The local microenvironment of distinct brain regions may be critical to determine virus permissiveness. The neurological involvement can occur independently of the respiratory system and coronaviruses could infect brainstem neurons responsible for the cardiorespiratory regulation, resulting in hypoxia (42). It would be interesting to know if OR neurons are infected, microscopic alteration of OB, and grading of corresponding olfaction alteration, under conditions of subclinical infections vs. severe disease. Unfortunately, to date, only a few studies documented OB involvement. Politi et al. show a first report of *in vivo* human brain involved in a patient with COVID-19 showing an MRI signal alteration compatible with viral brain invasion in the OB and cortical region (i.e., posterior gyrus rectus) associated with anosmia 3 days later his onset. No brain abnormalities were seen in other patients with COVID-19 presenting anosmia who underwent brain MRI in this and other studies (43). Nevertheless, COVID-19 patients in need of admission in an intensive care unit, not investigated adequately for anosmia, could have direct involvement of higher CNS center detectable by MRI. Li et al. reported a 21-years-old male with a 5-days loss of smell, initially without other symptoms and respiratory tract involvement. On the day of discharge, after

23 days of hospitalization with partial recovery of the sense of smell, brain magnetic resonance imaging (MRI) showed smaller right olfactory blub and linear hyperintensities inside bilateral olfactory nerves, suggestive of bilateral olfactory neuropathy (44). A recent case series highlighted the abnormal intensity of the OBs in five COVID-19 adult patients, three of whom had anosmia, maybe due to abnormal enhancement or microbleeding because they only underwent the sequence after injection of gadolinium in fat-suppressed T1WI (45).

SARS-COV-2–HOST INTERACTION AND ORS

Virus entry into specific cells and virus spread in different organs depend on virus–receptor interaction and the involvement of coreceptors. Using multiple receptors might be advantageous for virus spread to various organs. Some viruses can use more than one receptor or mutate their envelope proteins by acquiring the ability to bind different receptors or coreceptors. Thus, in the beginning, infection usually has a minor impact on the host, while the subsequent replication of the virus may significantly damage secondary organs (46, 47). Admittedly, SARS-CoV-2 causes a broad spectrum of clinical manifestations characterized in most cases in significant pulmonary damage. Still, other organs may be involved, including the heart, kidney, liver, gastrointestinal tract, gonadal function in males, and, as recently emphasized, the CNS (7).

The angiotensin-converting enzyme 2 (ACE2) is considered the primary receptor for cellular entry for SARS-CoV-2 (29). This knowledge has driven researchers to investigate the expression of ACE2 in different tissues to relate it to the clinical phenotypes of the disease. More specifically, regarding the neurological involvement, glial cells and neurons have been reported to express ACE2 receptors, and previous studies have shown that SARS-CoV may cause neuronal death in mice by invading the brain *via* the olfactory epithelium (48). Autopsy findings in humans have also demonstrated the presence of SARS-CoV by electron microscopy, immunohistochemistry, and real-time reverse transcription-PCR into the CNS (48). The confirmed entry mechanism of coronaviruses (SARS-CoV, MERS-CoV, and hCoVs) is mediated by the Spike protein of the virus that directly binds cell receptors (ACE2) and, after being cleaved by a protease (TMPRSS2), allows membrane fusion (49–52). Thus, the coronavirus entry into cells seems to be conditioned not only by the expression of the receptor but also by the protease expression. Therefore, several studies have performed *ACE2* and *TMPRSS2* co-expression profiles into healthy human tissues to identify possible target organs. However, a recent literature review has underlined several limitations of most studies, highlighting that these two proteins alone cannot explain all the clinical observations. Indeed, it was noted that several cell lines, which do not express *ACE2* RNA, can be infected by SARS-CoV-2 and that *ACE2* expression could not be detected in healthy individual organs, including lung, bronchus, nasopharynx, esophagus, liver, and stomach, contrasting to clinical data of SARS-CoV-2 infection. It was also observed, for instance, that cardiomyocytes express ACE2 but do not appear to express *TMPRSS2/4*, and some studies reported *ACE2* expression in various brain cells, but *ACE2* and *TMPRSS2/4* were rarely co-expressed within the same cells. Besides, a recent study failed to detect ACE2 expression in mature OR neurons at either the transcript or protein levels and in neurons in the OB. A detailed survey of nasal epithelium did not detect TMPRSS2 in the neuronal component (53, 54). Together, these observations suggest that our understanding of SARS-CoV-2 cellular tropism is still insufficient, and maybe SARS-CoV-2 could bind alternative receptors to enter into several cells. We have hypothesized that these alternative receptors need to have specific properties, including being highly conserved between species, presenting polymorphism, ubiquitous expression in human organs, and altered expression depending on age, sex, and comorbidity.

Our attention was focused on a family of receptors, "sensory G-protein coupled receptors (GPCRs)" (55), still poorly understood, suffering from the bias due to their discovery as specialized receptors expressed on sensory neurons only in the nose: ORs.

ORs represent the largest gene family in the human genome (418 genes classified into 18 families). ORs are divided into two classes, class I receptors and class II receptors, based on the species they were initially identified: aquatic and terrestrial animals, respectively. In humans, all class I genes are located on chromosome 1, while class II genes are located on all chromosomes except chromosomes 20 and Y (40, 56).

ORs are expressed throughout the body, and their expression in non-olfactory tissues has been documented for more than 20 years, but the most significant part is still "orphans" of ligands. Their functional roles were unknown, but many studies have demonstrated that these G-protein-coupled receptors are involved in various cellular processes (40, 57).

Highly Conserved Receptors

Olfaction is one of the most developed senses in animals [including bats, the natural reservoir of SARS-CoV-2 (58)], and evolutionary conservation was also demonstrated for ectopic OR between mouse, rat, and humans (40, 59–61).

OR proteins are composed of highly conserved (each family having >40% sequence identity) amino acid motifs that distinguish them from other GPCRs and some highly conserved motifs with other non-OR GPCRs. These OR residue sequences seem to have specific functional activities. In analogy to other Class A GPCRs, each OR has seven transmembrane domains (TM1–TM7) connected by extracellular and intracellular loops. Additionally, there is an extracellular N-terminal chain and an intracellular C-terminal chain that, together with TM4, TM5, and the central region of TM3, are highly variable, participating in ligand binding. The fact that some amino acid sequences have been evolutionarily conserved across species implies that they may have critical roles (61).

Polymorphism

There is a wide variability of functional OR genes among different people. Recent studies, genotyping 51 odor receptor

loci in 189 individuals of several ethnic origins, found 178 functionally different genomes. Additional variation in the population may come from differences in gene expression. Indeed, other experiments have found that the expressed OR repertoire of any pair of individuals differs by at least 14%, suggesting that polymorphisms also exist in the promoter and other regulatory regions. Furthermore, variation in the copy number of OR genes contributes significantly to individual olfactory abilities (55, 61, 62).

Ubiquitous Expression in Human Organs

ORs are detected in migrating neural crest, smooth muscle, endothelial precursors and vascular endothelium, endocardial cells, neuroepithelium, and ocular tissues. ORs were found in various additional non-olfactory tissues, including the prostate, tongue, erythroid cells, heart, skeletal muscle, skin, lung, testis, placenta, embryo, kidney, liver, brain, and gut (40). Moreover, ORs are detected in cancerous tissues of the liver, prostate, and intestine (63, 64). In non-olfactory tissues, several ORs are co-expressed in the same cell, as demonstrated for the B- and T-lymphocytes, polymorphonuclear leukocytes, and human sperm cells, while in the olfactory system, only one allele of OR gene is expressed in each olfactory neuron (40).

Physiological functions of non-olfactory ORs are not entirely understood and seem to be unrelated to the olfactory system in diverse cell types. For instance, renal and cardiovascular ORs regulate blood pressure, ORs on airway smooth muscle decrease remodeling and proliferation, while exposure of the airways to γ/LPS resulted in markedly increased OR expression (65). ORs on pulmonary macrophages, induced by IFN-γ and LPS, may contribute to innate immune response (66).

Altered Expression Depends on Age, Sex, and Comorbidity

A large number of OR genes appear to be detectable only after birth. In mice, experiments demonstrated that the expression level of ORs could be classified into different patterns that reach a peak at different ages. For example, some ORs reach a height of expression between the 10 and 20th day of life and then reduce to a low level while other ORs reach a peak at the 10th day of life and continue to be expressed at a high level until 18 months. In the authors' opinion, these expression patterns may correlate with their functions in each life stage, such as nursing or reproductive cycle (61). Additionally, there is a high incidence of age-related olfactory dysfunction supported by histological evidence in the olfactory epithelium (67).

Interestingly, sex differences in olfaction are highlighted in a meta-analysis: the female OB presents more dense microcircuits and slower aging than males (68). Another study reveals that mRNA levels of sex steroid, GnRH receptor, and aromatase in the OB vary with sex, social status in males, and females' reproductive condition. In the authors' opinion, these observation highlights that during the reproductive cycle, OR expression level may change to fine-tune the olfactory system, suggesting the hypothesis that the changes in receptor levels could be an essential mechanism for regulating reproductive, social, and seasonal plasticity in olfactory perception (69). In another work, a sex difference in the absolute number of total, neuronal, and non-neuronal cells was demonstrated, favoring women by 40–50%, also after correction for mass. Thus, it was hypothesized that quantitative cellular differences may have functional impact (70). ORs in non-olfactory tissue are correlated with the development of several diseases such as glucose homeostasis in diabetes, tumor cell proliferation, apoptosis, metastasis, and invasiveness. Some ORs seem to accelerate obesity development, angiogenesis, and tissue regeneration and to initiate hypoxic ventilatory responses (71).

Concerning the CNS, in the adult human brain, several ORs are expressed in neurons of the neocortex, hippocampus, dentate gyrus, striatum, thalamus, nuclei of the basal forebrain, hypothalamus, nuclei of the brainstem, cerebellar cortex, dentate nucleus, and neurons of the spinal cord. ORs have also been reported in the autonomic nervous system and murine sensory ganglia. Their functions and kinetic expressions are still unknown (65). Interestingly, OR gene expression into the CNS is altered in several neurodegenerative diseases, including Parkinson's disease (PD) and Alzheimer's disease (AD). For example, it was demonstrated that the mRNA encoding for some ORs increases with age in the cortex and hippocampus of wild-type and transgenic Alzheimer's disease-like mice; nonetheless, transcript expression of the same ORs is impaired in the brain of Alzheimer's disease-like mice. Besides, in transgenic Alzheimer's disease-like mice, ORs are observed near amyloid plaques (65, 72).

All these features make ORs ideal viral receptors and could also contribute to explain the broad spectrum and wide interindividual variability of clinical manifestations in COVID-19.

We have no data to support our hypothesis. Our knowledge of the ORs is insufficient in terms of physiological and pathological functions, intra-individual diversity during life, epigenetic processes acting on ORs expression, and above all ORs ligands within the different cell types. Identifying these cell-surface receptors as required for viral infection, given their peculiar characteristics, may be necessary for developing antiviral therapies and effective vaccines. To our knowledge, the only literature data supporting the possible involvement of ORs in virus entry into a cell is on OR14I1 as a receptor for HCMV infection. This OR is required for HCMV attachment, entry, and infection of epithelial cells, revealing previously neglected targets for vaccines and antiviral therapies (73, 74). Unfortunately, like many others, OR14I1 is an "orphan receptor" without known ligand. As noted concerning HCMV, these findings do not exclude roles for other receptors and coreceptors during infection but answer questions regarding epithelial tropism of HCMV and offer alternative opportunity to develop antiviral strategies for the management and transmission of the disease. The same could happen in COVID-19 and other infectious diseases if only ORs were considered.

HYPOTHESIS STATEMENTS AND DATA DISCUSSION

Understanding the mechanisms behind COVID-19-related olfactory dysfunction will require further investigation to delineate his prognostic value concerning coronavirus neuroinvasion, immune reaction, and virus spread from the nasal cavity to other distant organs. However, considering all the data above described, it is possible to propose several hypotheses.

Since anosmia has been observed generally in the absence of cold and rhinosinusitis and considering the reported persistent hyposmia also detected after clinical recovery, we may hypothesize the prevalence of sensorineural dysfunction.

Defining the role of local inflammatory mediators in host defense and tissue damage of ONE may explain the mechanism of COVID-19-related anosmia. Indeed, in non-infected cells, the interaction between virus and receptor may induce defense mechanisms resulting in cytokine secretion (i.e., interferons), apoptosis, and innate immune response, which can have a significant impact on the development of disease both locally and at the systemic level.

Adults with severe disease have a depletion of the B-cell compartment (75), and levels of serum IL-2R, IL-6, IL-10, and TNF-α are higher than in moderate cases. The absolute number of CD4+ T and CD8+ T cells decreased in nearly all the patients and were markedly lower in severe cases than moderate ones. The expression of IFN-γ by CD4+ T cells tended to be lower in severe cases (14.1%) than in moderate ones (22.8%) (76).

On the contrary, in the pediatric population, recent studies showed that an early polyclonal B-cell response (75) augmented percentage of CD3+, CD4+, and CD8+ lymphocytes related to increased levels of IL-6, IL-10, and IFN-γ (76). These data observed at the systemic level may reflect what locally happens when, in the elderly, IFN-dependent OB defense mechanism is not efficient, causing OB barrier to overcome, subsequently, virus spread and worse outcome.

It may be suggested, considering the above-discussed interaction between olfactory and immunological systems, that the nasal epithelium and OB may be one of the first battlefields between SARS-CoV-2 and host; the outcome of this battle may be critical for the pathological development of COVID-19. Considering the OB as an immune organ, if the local fight against SARS-CoV-2 is successful, the damage remains localized, leading to anosmia, as in the case of women and younger patients; conversely, the virus can spread and replicate in the upper olfactory sites causing central anosmia or directly invading the CNS. The prevalence of chronic olfactory impairment increases with age. Olfactory deficit affects up to 50% of people ages ≥65 years and >80% of people ages ≥80 (77). It is clear that COVID-19 causes more severe complications in patients with advanced chronological age. The age-related dysregulation of ONE and OB homeostasis might contribute to more severe manifestations of COVID-19 in the elderly, likely due to immunosenescence. The spread of the virus and the neuroinvasive potential have been proposed according to the known routes of SARS-CoV and a growing body of findings specific for SARS-CoV-2. Since the basal expression level of receptors determines, at least in part, the tropism of the virus, identifying the kind of receptor involved is crucial to predict which tissues are probably involved in infection and to guide research toward new prevention and therapeutic strategies. In this context, considering the contrasting data concerning ACE2 expression in human tissue that cannot entirely explain the wide spectrum of clinical manifestation, we hypothesize that SARS-CoV-2 could use ORs as ideal alternative entrance receptors as already demonstrated for HCMV.

This hypothesis may have the power to attract the attention of the broader community of scientists and neuroscientists on the olfactory system to investigate the biological significance of these neglected receptors in sickness and health.

Besides the acute neurological involvement of SARS CoV-2 infection, there are many overlaps between SARS-CoV-2-related manifestations and OR-related disease. For instance, it has been shown in animal and human studies that coronaviruses could be implicated in the pathogenesis of Parkinson's disease, acute disseminated encephalomyelitis, multiple sclerosis, and other neurodegenerative diseases, as well as for ORs. Further monitoring for long-term sequelae may reveal viral contribution in pathophysiology or increased risk for neuroinflammatory and neurodegenerative diseases and the possible link with OR dysregulation or damage.

In light of these observations, the role of ORs and OB in COVID-19 infection could be significant, explaining at least in part the age- and sex-related differences in the clinical course.

In conclusion, from anosmia onset in SARS-CoV-2-positive patients, a precise timing for the olfactory route climbing by the virus can be speculated. In this window period, a potential early intervention could change the disease's course, supporting natural defenses when they lack, as a result of age, sex, or other genetic backgrounds. Since PAMPs (pathogen-associated molecular patterns) can improve the up-regulation of IFN, it could be hypnotized to use immune-stimulatory molecules to increase the ability to fight the infection. Simultaneously, the use of other topical pharmacological agents (i.e., antiviral drugs and "molecular competitor binding ORs") could be helpful.

CONCLUSION

The evidence of OB involvement in COVID-19 remains scarce, but the knowledge of this different way of spreading could lead to significant developments in the management of SARS-CoV-2.

Magnetic resonance imaging cannot be an early detector tool in all COVID-19 patients with anosmia. However, the latest evidence on the CNS involvement, beyond the anosmia, could justify it as a valid indication in high-risk patients. Autopsies of the COVID-19 patients, detailed neurological investigation, and attempts to isolate SARS-CoV-2 from OB and neuronal tissue can clarify the role of this novel coronavirus in the mortality linked to neurological involvement. Existing studies to assess the incidence of anosmia and related immunological patterns are limited; therefore, investigating the local cytokine composition at the onset of symptoms could be useful.

In conclusion, we invite to focus on anosmia in each patient suspected of infection or with a positive swab for SARS-CoV-2. Future studies should evaluate the degree of functional impairment (grading), central/peripheral anosmia (localization), and the temporal course (evolution) through MRI and olfactory tests, perhaps through standardized workup protocol to explore this issue better.

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

All persons who meet authorship ICMJE criteria are listed as authors, and all authors certify that they have participated equally in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication.

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CASE REPORT
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Acute Seizures Occurring in Association With SARS-CoV-2

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Seizures are an infrequent and serious neurological complication of SARS-CoV-2 infection, with limited data describing the etiology and the clinical context in which these occur or the associated electrographic and imaging findings. This series details four cases of seizures occurring in patients with COVID-19 with distinct time points, underlying pathology, and proposed physiological mechanisms. An enhanced understanding of seizure manifestations in COVID-19 and their clinical course may allow for earlier detection and improved patient management.

Keywords: seizures, SARS-CoV-2, COVID-19, status epilepticus, neurological complication

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) associated with severe respiratory syndrome coronavirus 2 (SARS-CoV-2) presents with cough, fever, fatigue, and gastrointestinal symptoms. However, neurologic manifestations are reported in a third of patients and include dizziness, headaches, impaired consciousness, neuromuscular injury, changes in taste or smell, stroke, ataxia, or seizures (1).

Two early multicenter studies from China reported seizures in 0.47–0.66% of patients with COVID-19 (1, 2). Subsequent individual cases of seizures associated with COVID-19 were described, both as a presenting feature of COVID-19 and as a complication emerging later in the course of critically ill patients (3–12). Although seizures are uncommon, their occurrence is not unexpected given their association with other human coronavirus infections such as SARS-CoV and MERS reported in the literature (13, 14). There is increasing appreciation of the SARS-CoV-2 pathogenic mechanisms causing neurological injury and seizures.

This paper presents the clinical course and electroencephalographic (EEG) and neuroradiographic features of four cases of acute symptomatic seizure in the setting of SARS-COV-2 infection, highlighting distinct pathological mechanisms by which seizures may occur in the context of the unique and formidable illness of COVID-19.

CASE PRESENTATIONS

Case 1

A 48-year-old woman presented to urgent care complaining of fever, cough, vomiting, and malaise. Pneumonia was confirmed on chest X-ray, and she was prescribed with doxycycline and azithromycin. Three days later, she was brought in emergently for progressive confusion. She was febrile and tachypneic, with a blood pressure of 179/87 mmHg. She was alert, though aphasic, with impaired verbal output, naming, comprehension, and repetition. Babinski reflex was positive bilaterally.

The patient then experienced three consecutive generalized tonic–clonic seizures (GTCS) associated with full body stiffening, dilated pupils, foaming at the mouth, and tongue bite up to 6 min apart, concerning for status epilepticus for which she was administered intravenous (IV) lorazepam and levetiracetam with resolution.

Initial laboratories were notable for mild transaminitis (AST 34 μ/L, ALT 53 μ/L) and elevated inflammatory markers (D-dimer 279 ng/ml, ferritin 357 ng/ml, and C-reactive protein 32 mg/ml). The toxicology result was negative. The chest X-ray result revealed bilateral interstitial infiltrates, and non-contrast computed tomography (CT) of the head was unrevealing. The patient was empirically treated for encephalitis with acyclovir and ceftriaxone. Lumbar puncture was traumatic, with the white blood cell count corrected to five per cubic millimeter and elevated protein of 125 mg/dl. The results of cerebrospinal fluid (CSF) gram stain, culture, and polymerase chain reaction (PCR) for herpes were subsequently negative. The nasopharyngeal COVID-19 PCR test result was positive. CSF COVID-19 PCR was unavailable. Continuous EEG was performed postictally, showing severe generalized slowing but no epileptiform activity.

The patient remained febrile, tachycardic, and tachypneic, requiring intubation for hypoxemic respiratory failure. A 5-day course of high-dose IV methylprednisolone was given for suspected COVID-19-associated central nervous system (CNS) involvement, as well hydroxychloroquine and a single 400-mg dose of tocilizumab. On day 13, the patient was extubated, with significant neurological improvement and resolution of her aphasia. MRI of the brain on day 19 demonstrated T2-weighted (T2) and fluid-attenuated inversion recovery (FLAIR) hyperintensity in the medial temporal lobes bilaterally without restricted diffusion (**Figure 1**). The patient gradually improved and was discharged on day 25.

Case 2

A 29-year-old woman with iron deficiency anemia and menorrhagia presented after two GTCS with postictal confusion in the context of preceding fever, cough, and headache for 2 weeks. The initial vital signs were normal, except for tachycardia. While under observation, she suffered another GTCS with right gaze deviation, forced eye opening, tonic limb movements, and loss of awareness. This was aborted with IV lorazepam and levetiracetam. Postictal right-sided paralysis was noted.

The nasopharyngeal COVID-19 PCR test result was positive. Prolactin and D-dimer were notably elevated. Non-contrast CT of the head revealed edema, mass effect, and subcortical hemorrhages in the left temporal and parietal lobes as well as a hyperdense left transverse sigmoid sinus (**Figure 1**). A CT venogram confirmed left sigmoid and transverse cortical venous thrombosis (CVT), for which IV heparin was commenced. Pulmonary ground-glass opacities were noted on imaging.

EEG demonstrated asynchronous slowing and attenuation over the left hemisphere. CT of the head on day 2 showed evolving left temporal–parietal hemorrhagic infarction with midline shift and partial effacement of the right lateral and third ventricles. The patient developed bilateral abducens palsies and papilledema and was given acetazolamide for increased intracranial pressure. Mentation improved significantly by day 9 as she was able to respond to questions and moved all limbs antigravity, with a mild right facial droop. Hypercoagulable workup revealed anticardiolipin IgM antibodies. The patient was discharged on enoxaparin and levetiracetam.

Case 3

A 64-year-old woman with a history of hypertension, stage 4 chronic kidney disease, and non-insulin-dependent type 2 diabetes presented after two GTCS, lasting 2 min each, with postictal unresponsiveness. There had been 3 days of preceding

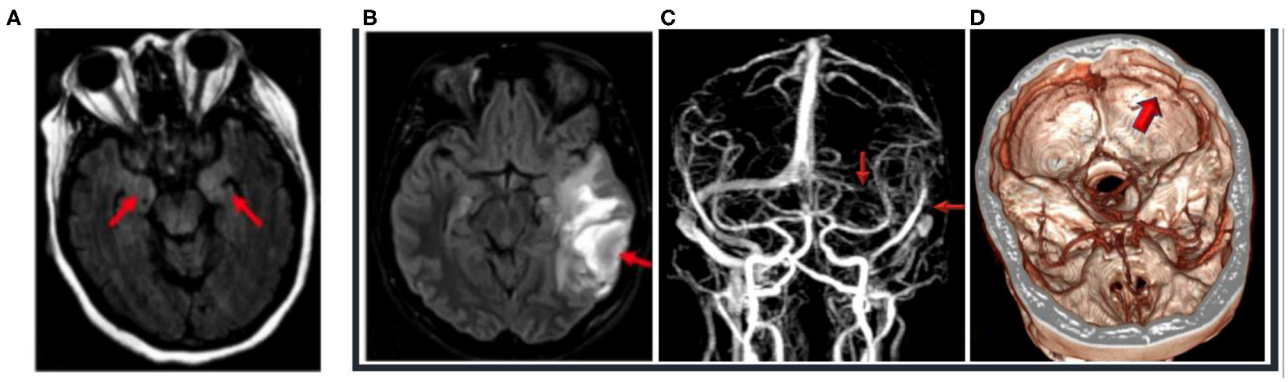

FIGURE 1 | (A) Case 1: 48-year-old, COVID-19-positive female with altered mental status; 3-T MRI axial FLAIR image (red arrows) of hyperintense FLAIR in the medial temporal lobe because of altered mental status and seizures, unable to hold still for scan, with resultant motion artifact. **(B–D)** Case 2: 29-year-old, COVID-19-positive female who presented with seizures. **(B)** 1.5-T MRI axial FLAIR (red arrow) of the left temporal lobe with hemorrhagic venous infarction, with the FLAIR hyperintensity consistent with edema and mass effect effacing the left ambient cistern. **(C)** 1.5-T MRI—time-of-flight MRI—with red arrows noting the absent flow void with venous thrombosis of the left transverse and sigmoid sinus and the left internal jugular vein. **(D)** CT venogram with the purple arrow demonstrating absent contrast enhancement and thrombosis of the left transverse and sigmoid sinus.

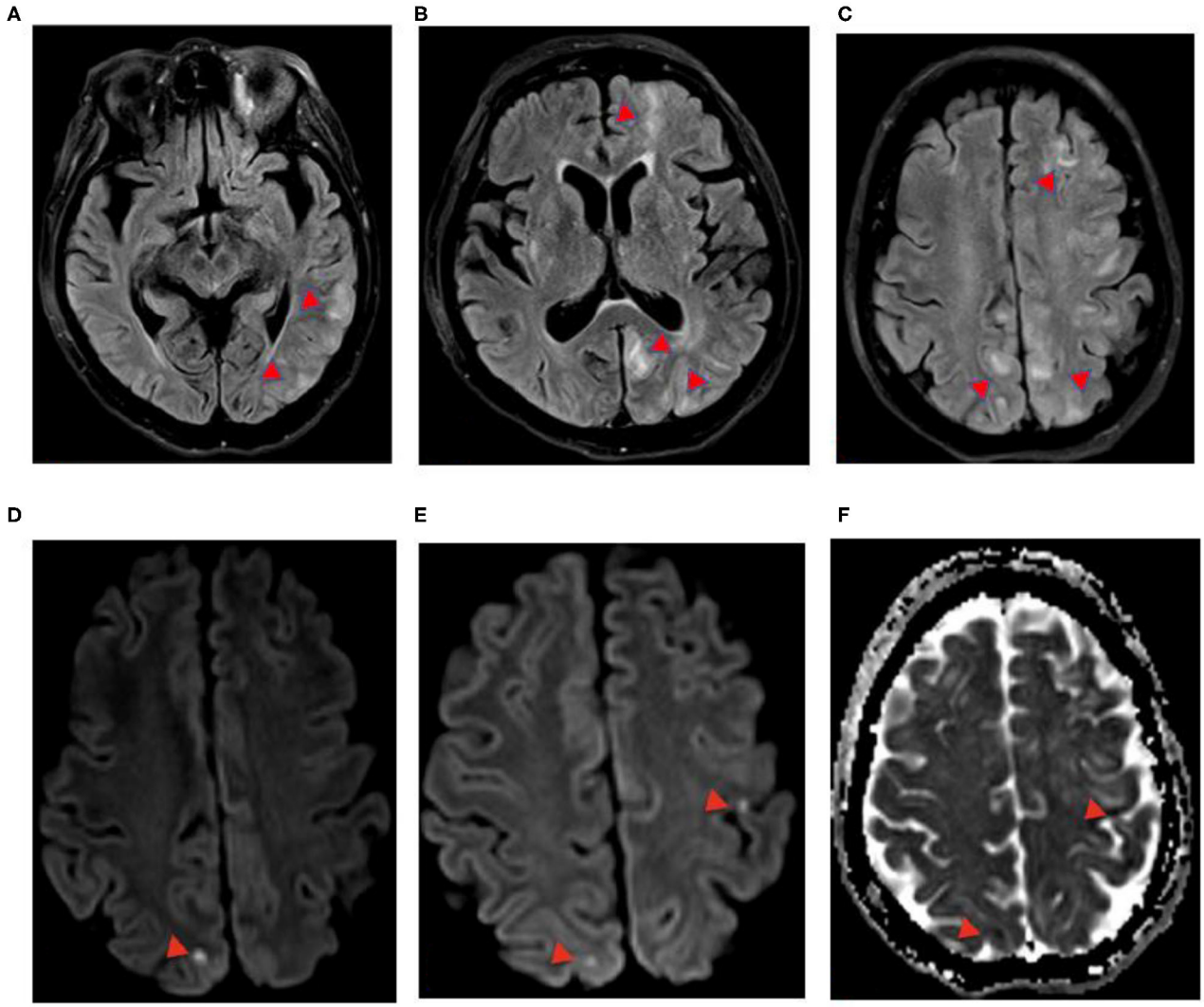

FIGURE 2 | (A–F) Case 3: 64-year-old COVID-positive female, axial 3-T MRI. **(A–C)** Axial FLAIR. **(D,E)** Axial DWI. **(F)** Axial apparent diffusion coefficient (ADC). FLAIR hyperintensity in the left was greater than that in the right cerebral hemispheres, with punctate DWI hyperintensity (red arrowheads) with ADC restricted diffusion. **(F)** Left precentral sulcus and right superior medial right parietal lobe, foci of FLAIR, DWI hyperintensity without restricted diffusion, and ADC T2-shine through, concerning posterior reversible encephalopathy in a patient with elevated blood pressure.

headache, vomiting, cough, and malaise. The patient was febrile, tachycardic, and tachypneic, with a blood pressure of 213/100 mmHG. She appeared obtunded with dilated and poorly responsive pupils, right gaze preference, and minimal withdrawal in all limbs to painful stimuli. Under observation, she suffered two additional GTCS and was treated with IV lorazepam, levetiracetam, and fosphenytoin with clinical cessation. Labetalol was given for hypertension.

The laboratory results were significant for thrombocytopenia (77 K/μl), hyponatremia (126 mmol/L), elevated creatinine (2.7 mg/dl), blood urea nitrogen (40 mg/dl), and elevated ferritin. The non-contrast CT of the head and the chest radiograph were unrevealing. The CSF showed four nucleated cells with lymphocytic predominance, while gram stain, culture, and herpes testing were negative. The nasopharyngeal COVID-19 PCR was positive.

EEG was notable for generalized periodic discharges and slowing. Brain MRI without gadolinium, due to kidney dysfunction, showed punctate diffusion positive lesions in the left precentral sulcus and superior right medial parietal subcortical regions as well as patchy T2 prolongation in the left frontal, occipital, and bilateral parietal and cerebellar regions, most consistent with posterior reversible encephalopathy syndrome (PRES) (**Figure 2**).

The patient remained obtunded without seizures on continuous EEG, with steady improvement of her hyponatremia and blood pressure. Though PRES was of primary concern, a 5-day course of high-dose IV methylprednisolone was given due

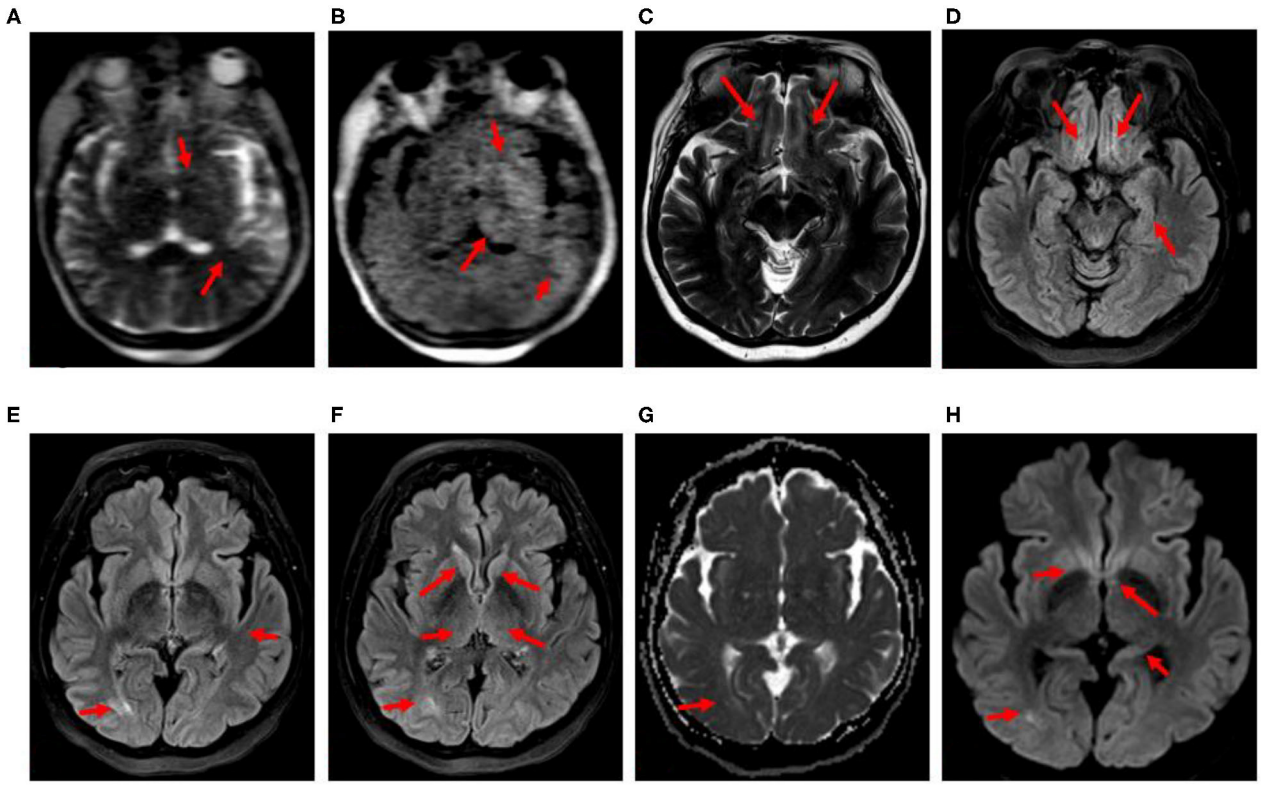

FIGURE 3 | (A–H) Case 4: 68-year-old, COVID-positive female. **(A,B)** Hyperfine 0.064-T portable MRI obtained 26 days after admission at the patient's bedside showing focal T2 and FLAIR hyperintensities (red arrows). **(C–H)** 3-T MRI obtained 39 days after admission. **(C)** Axial T2 and **(D)** axial FLAIR hyperintensities (red arrows): left subinsular cortex, lentiform nuclei, thalami, and left temporal lobe. **(E,F)** Axial FLAIR, **(G)** axial ADC, **(H)** axial DWI, T2, FLAIR, and DWI hyperintensities (red arrows): bilateral olfactory tracts, medial temporal lobes including hippocampal tails, caudate heads, fornices and posterior commissural fibers, right posterior lateral temporal lobe without restricted diffusion.

to consideration of inflammation-mediated seizures associated with COVID-19. The respiratory function was stabilized, and mentation improved by day 10. On day 21, she was discharged home at clinical baseline.

Case 4

A 68-year-old woman presented for diarrhea, fever, malaise, cough, and shortness of breath and found to be positive for COVID-19 3 days prior. She was febrile, tachycardic, tachypneic, and hypoxemic upon arrival. The chest X-ray showed bilateral lung infiltrates for which she was treated with azithromycin, ceftriaxone, and hydroxychloroquine. The patient decompensated, requiring intubation due to acute respiratory distress syndrome, and was given methylprednisolone and anakinra.

At 2 weeks into her course, the patient remained obtunded off of sedation. The head CT obtained on days 16 and 25 after admission demonstrated decreased attenuation in the left subinsular cortex and the thalamic region. EEG on day 17 showed severe generalized slowing. On day 26, portable 0.06-T MRI (Hyperfine) of the brain without contrast was performed, showing areas of T2 and FLAIR hyperintensity in the left thalamus and lentiform nuclei, though technically limited (**Figure 3**).

By day 27, she was extubated but remained confused, with the psychomotor slowed, and unable to follow commands. EEG on day 33 revealed unremitting high-amplitude centrotemporal sharp rhythmic 3–5-Hz activity concerning non-convulsive status epilepticus (NCSE) (**Figure 4**). Clinical improvement was noted on the following day after the administration of IV lorazepam, levetiracetam, and lacosamide, with EEG transitioning to moderate background, slowing with intermittent left temporal sharp waves. The CSF results on day 36 were normal, including for COVID-19 PCR. A follow-up 3-T contrast-enhanced brain MRI on day 39 showed T2, FLAIR, and diffusion-weighted imaging (DWI) hyperintensity without restricted diffusion along the bilateral olfactory tracts, caudate heads, posterior commissural fibers of the fornices, hippocampal tails, and right temporal lobe (**Figure 3**). By day 49, the patient was discharged to rehabilitation, alert and capable of conversation, though with moderate persistent cognitive and bilateral proximal weakness.

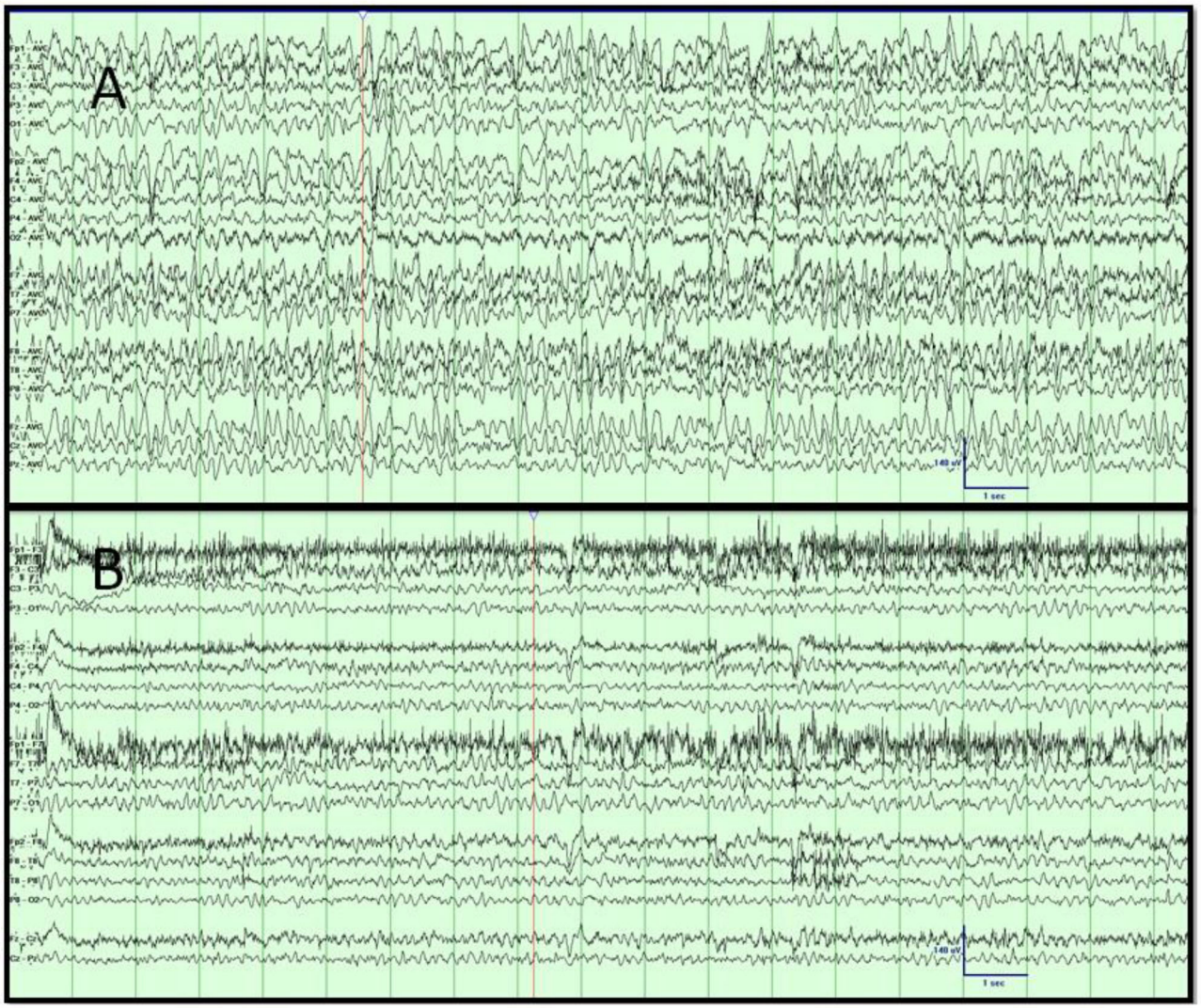

FIGURE 4 | (A,B) Case 4. Video EEG results. **(A)** Day 1 showing the high-amplitude rhythmic sharply contoured; intermixed 3- and 6-Hz activity concerning non-convulsive status epilepticus. **(B)** Day 2 with resolution of rhythmic slowing after the administration of lorazepam and levetiracetam, corresponding with improved mentation. Low-frequency filter, 1 Hz; high-frequency filter, 70 Hz; sensitivity, 7 μV/mm; timebase, 30 mm/s.

Summaries of the pertinent clinical details of each case are presented in **Table 1**.

DISCUSSION

The cases above describe four scenarios of seizures occurring in association with COVID-19, illustrating the remarkable variety of neurological complications accompanying this disease. Seizures in this report may occur by differing mechanisms, including in the context of inflammatory changes in the brain as demonstrated by neuroimaging or vascular abnormalities related to the effects of SARS-CoV-2 on hypercoagulability and endothelial cell dysfunction.

In summary, the first case presented with seizures during the early acute phase of infection, with elevated inflammatory markers, and FLAIR changes in the temporal region on MRI indicative of focal inflammation (**Figure 1A**). Lumbar puncture did not reveal an evidence for encephalitis. In addition to antiseizure medications, the patient responded well to immunotherapy, with good neurological outcome, supporting a hypothesis of excessive innate immune activation. In case 2, the seizures resulted from acute CVT in the left transverse and sigmoid sinus, resulting in a left temporal lobe venous infarct (**Figures 1B,D**), highlighting a coagulopathic state associated with COVID-19. The seizures observed in case 3 manifested in the setting of a patient with elevated blood pressure and end-stage kidney disease with PRES, raising concern for COVID-19 effects on endothelial cell function and vascular permeability.

This patient demonstrated classic findings of increased T2, DWI, and FLAIR hyperintensity without restricted diffusion in the setting of elevated blood pressure (**Figures 2A–F**) and with a few punctate foci of restricted diffusion and ischemic change. The association of PRES as a neurologic complication of COVID-19 has been recently documented in the literature (15–18). The final patient was determined to have seizures later in her course, along with persistent altered mentation and radiographic findings suggestive of inflammatory changes and concern for immune-mediated necrotizing encephalopathy. Early portable magnetic resonance imaging using a Hyperfine MRI at the bedside, as the patient was too unstable to come down to the scanner (**Figures 3A,B**), demonstrated thalamic T2 and FLAIR hyperintensity, with follow-up conventional magnetic resonance imaging 2 weeks later showing bilateral hippocampal, forniceal, sub-insular, and orbitofrontal T2 and FLAIR abnormalities. Initiating antiseizure medications led to significant electrographic improvements and eventual delayed clinical improvement.

A few recent case reports are published in the literature describing acute symptomatic repetitive seizures in the setting of COVID-19. In an early report, the patient had positive CSF COVID-19 PCR and mesial temporal and lateral ventricular signal hyperintensities on MRI indicative of encephalitis (3). Additional rare cases may present with lymphocytic pleocytosis (19).

De novo focal seizures including status epilepticus in the absence of other medical comorbidities, neuroimaging, or lumbar puncture abnormalities are also described (7, 9–12, 20). New-onset seizures are reported in medically complex elderly patients with COVID-19; however, these were in the setting of multiorgan failure, metabolic derangements, and, in certain patients, superimposed sepsis or hypotension as complicating factors, and unfortunately, MRI or lumbar puncture results were often unavailable (4, 7, 8). Seizure exacerbation and status epilepticus may also be exacerbated in patients with preexisting epilepsy and focal lesions, where COVID-19 infection may have lowered the seizure threshold (5, 21).

In a series of 214 consecutive patients with COVID-19 from China, only one individual experienced a convulsion, although 16 additional patients were reported with unspecified impaired consciousness (1). An additional retrospective multicenter study of 304 consecutive patients with COVID-19 reported only two cases of seizure-like events (2). One patient presented with facial deviation and acute anxiety, while the other experienced myoclonus with electrolyte imbalance. Eight additional patients were described as encephalopathic or comatose. Notably, in both publications, EEG was not performed due to concerns regarding infection control.

Although clinical seizures are infrequently encountered with COVID-19, NCSE may still need to be excluded by EEG in patients with unexplained altered mentation. Investigators recognize encephalopathy as a common occurrence among severely ill patients with COVID-19; however, concurrent EEG data are often unavailable, and further research is required to understand the true incidence of electrographic seizures in patients with SARS-CoV-2 infection. Relatively small cohorts of patients with COVID-19 and EEG are published to date, with the patients in these studies only undergoing routine duration or reduced montage EEG recording, for the reasons discussed above, and less frequently continuous EEG monitoring. Several authors observed sporadic epileptiform abnormalities and periodic patterns of concern, including generalized periodic discharges, along with more expected patterns of generalized slowing (22–26). Due to centers taking precautions to avoid transmission risk by reducing staffing and utilization of EEG, as well as obtaining imaging, cases of electrographic seizures and NCSE may be less likely to be diagnosed and adequately imaged. While the safety and risks of infection must be weighed carefully, patients may potentially benefit from the use of EEG and neuroimaging to recognize potentially treatable seizures.

The speculative mechanisms responsible for the neurological manifestations of SARS-CoV-2 are grouped broadly into direct viral invasion and indirect effects *via* systemic inflammation, coagulopathic states, endotheliopathy, and homeostatic disruptions, including metabolic derangements due to hypoxia and organ failure.

Given the genetic semblance between SARS-CoV and SARS-CoV-2 and the shared manner of cellular entry *via* receptors for angiotensin-converting enzyme II (ACE2), neurotropism may be similar (27). Previous studies have demonstrated SARS-CoV antigens and RNA within human neurons, supporting a hypothesis of neuroinvasion in SARS-CoV-2 (28). One proposed route of direct cell invasion involves receptors for ACE2, found on the surface of glia and endothelial cells, allowing for distinct ports of CNS entry (29, 30). CNS penetration may involve trans-axonal transport along adjacent glia or hematogenous spread. Invasion *via* cells expressing ACE2 in the olfactory tract may lead to encephalitis associated with seizures and MRI signal changes indicative of edema and inflammation (3).

The upregulation of proinflammatory cytokines may also play a role in para-infectious immune-mediated neurological complications, such as demyelinating disease, acute necrotizing encephalopathy, or Guillain–Barre syndrome in COVID-19 (31–36). Endothelial dysfunction and disruption of the integrity of the blood–brain barrier may result in cerebral antigen exposure and increased susceptibility to abnormal adaptive immunity. Similar neuroimmunological manifestations are reported with other coronaviruses, and seizures have been reported in this context (HCoV-OC43, SARS CoV, and MERS) (14, 30). These neurological presentations highlight the need for brain imaging in encephalopathic patients with COVID-19, particularly those with focal neurological deficits or seizures, which may reflect aberrant and excessive autoimmune phenomena. The neuroimaging findings in COVID-19 are extremely heterogenous and may vary based on the severity of infection (37). Immunotherapy, such as corticosteroids, targeted molecular therapies such as monoclonal antibodies, IV immunoglobulins, or convalescent plasma for patients with these manifestations may be potentially beneficial, though more systematic research is required (36, 38–40). During their course of treatment early on in the New York area pandemic, three patients in our series received methylprednisolone, two received hydroxychloroquine, one received tocilizumab,

TABLE 1 | Case summaries.

	Clinical presentation	Pertinent neurological findings	Seizure semiology	Intubated	Serum inflammatory markers	CSF results	EEG findings	Radiological findings	Possible mechanism of neuronal injury
CASE # 1	Fever, cough, vomiting, malaise, fever, tachycardia	Global aphasia, bilateral babinski signs	GTCS	Yes	ESR = 9; CRP = 32.6; D-dimer = 279; Fibrinogen = 352; Ferritin= 357.4; Procalcitonin = 1.23	WBCs = 5/uL; Protein = 125 mg/dL; Glucose = 117 mg/dL; Gram Stain, culture, and HSV PCR negative; COVID-19 PCR unavailable	Severe generalized slowing	MRI brain: T2 hyperintensities in the medial temporal lobes	Viral invasion vs. secondary inflammation
CASE # 2	Fever, cough, dyspnea, headache, tachycardia	Encephalopathy, right hemiparesis	FBTCS	No	ESR = 30; CRP = 111.7; D-dimer = 2,876; Fibrinogen = N/A Ferritin= 10.4; Procalcitonin = 0.06	N/A	Asynchronous slowing and left sided attenuation	CT head: edema, mass effect and subcortical hemorrhages in the left temporal and parietal lobes. CTV: left sigmoid and transverse cortical venous thrombosis	CVT
CASE # 3	Headache, vomiting, cough, malaise, hypertension	Encephalopathy	FBTCS	No	ESR = 22; CRP = < 0.1; D-dimer = < 150; Fibrinogen = 588; Ferritin= 3,647; Procalcitonin = 0.45	WBCs = 4/uL; Protein = 43 mg/dL; Gram Stain, culture, and HSV PCR negative; COVID-19 PCR unavailable	GPDs	MRI brain: multiple diffusion positive lesions in the left precentral sulcus and right medial parietal regions; patchy T2 prolongation in the left frontal, occipital, and bilateral parietal and cerebellar regions	PRES
CASE # 4	Fever, diarrhea, malaise, cough, dyspnea	Coma, subsequent prolonged encephalopathy	NCSE	Yes	ESR = 77; CRP = 22.8; D-dimer = 1,137; Fibrinogen = 747; Ferritin= 22.8; Procalcitonin = 0.29	WBCs = 1/uL; Protein = 30 mg/dL; Glucose = 73 mg/dL; Gram Stain, culture, and HSV PCR negative; COVID-19 PCR unavailable	High amplitude rhythmic frontotemporal theta, intermixed left temporal sharp waves	MRI Brain = T2, FLAIR and DWI hyperintensity without restricted diffusion along the bilateral olfactory tracts, caudate heads, fornices, hippocampi and right temporal lobe	Secondary inflammation

Key: GTCS, Generalized tonic-clonic seizures; GPDs, Generalized periodic discharges; FBTCS, Focal to bilateral tonic-clonic seizures; NCSE, Non-convulsive status epilepticus; PRES, Posterior reversible encephalopathy syndrome; MRI, Magnetic resonance imaging; ESR, Erythrocyte sedimentation rate; CRP, C-Reactive protein; WBCs, White blood cells; HSV, Herpes simplex virus; PCR, Polymerase chain reaction; COVID-19, SARS-CoV-2; CVT, Cortical venous thrombosis; CT, Computed tomography; N/A, Not available. Normal laboratory values: ESR = 4–25 mm/h; CRP < 5 mg/L; D-dimer < 230 ng/mL; Fibrinogen = 300–500 mg/dL; Ferritin = 15–150 ng/mL; Procalcitonin = 0.02–0.1 ng/mL; CSF Protein = 15–40 mg/dL; CSF Glucose = 40–70 mg/dL; CSF WBCs = 0–5 cell/uL.

and one was administered anakinra. The treatments for COVID-19 remain highly variable at this time, and therapies specifically for neurological complications lack consensus. While potentially helpful for cytokine storming, data have subsequently called into question the early recommendations to utilize hydroxychloroquine in hospitalized COVID-19 patients due to safety concerns (36). While corticosteroids have significant anti-inflammatory effects, caution is likewise advised to avoid the potential delay of viral clearance or other systemic complications, and timing and dosing of steroids may need to be considered carefully on a case-by-case basis for more severe cases of neuroinflammation (41). Research continues in support of drugs targeting cytokines *via* the IL-6 receptor, such as tocilizumab, and IL-1 receptor, such as anakinra (36).

Endothelial dysfunction may contribute to vascular events such as stroke (35). Viral cellular invasion *via* ACE2 expressed on the surface of glia, endothelial cells, myocardium, and arterial smooth muscle may predispose patients to thromboinflammation (42). Increases in systemic proinflammatory cytokines observed in COVID-19 such as IL-6, IL-10, and TNF-α are associated with hypercoagulability and microthrombi (43, 44). Elevated D-dimer levels are associated with thrombus formation and more severe infection and mortality (42). In one study, 6% of patients with COVID-19 were found to have acute strokes, theoretically predisposing them to a higher incidence of seizures (1). Nearly 3% of non-COVID-19 adults with ischemic stroke suffer early seizures, while the rates of seizures in CVT are as high as 34% (45, 46). CVT, as described in case 2, in COVID-19 has been reported, and given seizures as a common presenting feature, one should remain vigilant for this entity in the appropriate clinical context (47).

PRES in the setting of COVID-19 may similarly involve elevated cytokines due to systemic hyperinflammation, leading to endothelial activation and increased cerebrovascular permeability and edema. In the patient presented, underlying hypertension and renal disease may have been predisposing factors exacerbated by COVID-19, with additional cases of PRES in COVID-19 also reported (15, 16, 18, 48).

Acute seizures may also occur as a result of other systemic complications of COVID-19 resulting from hypoxia and inflammation, including multiorgan failure and associated metabolic disorders such as uremia, electrolyte abnormalities, and potential drug toxicity (14).

Seizures are a serious neurological manifestation of COVID-19. Their occurrence may be a concerning indicator of direct viral CNS involvement, autoimmune-mediated inflammation of the brain, neurovascular compromise, or cerebral autoregulatory abnormalities for which clinicians may need greater awareness of as potentially grave complications of SARS-CoV-2. Appropriate early diagnosis and treatment, with interventions such as antiseizure medications, immunotherapy, or modulation of blood pressure, may improve the outcome in these cases. Continued research is required to understand the true prevalence of electrographic seizures in a COVID-19-related neurological illness in a larger case series and to correlate those findings with radiographic abnormalities and effects on clinical outcome.

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

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SH, AB, UM, AS, and AH were responsible for the initial conception and draft of this manuscript. CK was responsible for neuroimaging descriptions and image preparation. CK, RK, and SN were responsible for guidance and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Neurological Associations of COVID-19—Do We Know Enough: A Tertiary Care Hospital Based Study

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The neurotrophic potential of SARS-CoV-2 virus is manifesting as various neurological disorders in the present pandemic. Nervous system involvement can be due to the direct action of the virus on the brain tissue or due to an indirect action through the activation of immune-mediated mechanisms. This study will discuss the detailed systematically evaluated clinical profile and relevant investigations and outcome of 14 laboratory confirmed SARS-CoV-2 positive patients presenting with neurological signs and symptoms. The patients were further categorized into confirmed, probable, and possible neurological associations. The probable association was found in meningoencephalitis ($n = 4$), stroke ($n = 2$), Guillain-Barré syndrome ($n = 1$), and anosmia ($n = 1$). The other six patients had coexisting neurological diseases with SARS-CoV-2. One patient with a large artery stroke succumbed to the illness due to respiratory complication. Memory impairment as a sequela is present during follow up of one encephalitis patient. Presently the early recognition and diagnosis of neurological manifestations remains a challenge for clinicians as the SARS-CoV-2 related neurological manifestations are in evolution. A long-term correlation study of clinical profile, radiological and laboratory investigations, along with neuropathological studies is needed to further understand the pathophysiology behind the SARS-CoV-2 neurological manifestations. Further understanding will facilitate timely recognition, therapeutic intervention, and possible prevention of long-term sequalae.

Keywords: SARS-CoV-2, neurological manifestation, stroke, meningoencephalitis, GBS, neurotrophic potential

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INTRODUCTION

The pandemic due to COVID-19 is growing exponentially worldwide after first being notified from Wuhan, China, in December 2019. The virus that causes COVID-19 is designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Primarily this virus affects the respiratory system and thereby initiates the cascade of sequences that involves multiple organs. There are evidences of involvement of other systems like nervous system, cardiovascular system, and gastrointestinal system, which are a matter of scientific study and research. Six months into this pandemic, the understanding and recognition of neurological manifestations and complications has increased as evident from the cases series and reports in literature (1). There are reports of

COVID-19 patients presenting with various neurological manifestations, and in others there is exacerbation of underlying neurological illness. The neurological presentations include dizziness, headache, anosmia, ageusia, seizure, meningitis, encephalitis, stroke, and acute inflammatory demyelinating polyneuropathy.

The neurotrophic potential of SARS-CoV-2 is being recognized and well-established in some studies by detection of SARS-CoV-2 in CSF by RT-PCR and its culture from brain tissue biopsy (2–4). The limited available data on neurological manifestations raises important concerns regarding the extent of nervous system involvement and the pathogenesis causing these manifestations. There is need for an early recognition and diagnosis as shown in a few studies (5).

In our clinical practice during the pandemic we have systematically evaluated and investigated patients for evidence of this new emergent virus and its associated neurological manifestations. This clinical study will facilitate in understanding the connection between SARS-CoV-2 and different clinical presentation. Early identification of the various probable neurological manifestations of COVID-19 will be instrumental in deciding the available therapeutic options and reducing the possible long-term complications.

MATERIALS AND METHODS

A cross-sectional study came from April 2020 to July 2020 at Institute of Human Behavior and Allied Sciences, a tertiary care neuropsychiatry center in North India.

This Institute caters to acute and chronic neurology and psychiatry patients in emergency and outpatient departments. The study was conducted in Department of Neurology.

All the admitted neurological patients during the study period had been evaluated for neurological symptoms and signs and carefully assessed and investigated for any atypical neurological finding. Patients were also monitored for the presence of influenza like illness (ILI) considering the current pandemic.

Routine hematological (Complete Blood Count, ESR), biochemical parameters (blood sugar, LFT, KFT, electrolytes, thyroid functions, CRP, serum ferritin) were done. X ray chest, ultrasound abdomen, and neuroimaging–brain MRI and CT head was done in all patients.

Autoimmune Profile (NMDA, LGi1, AMPA, CASPR-2) was done in selected patients.

CSF examination was selectively done for cytology and biochemistry.

Neuroviral panel (herpes simplex virus1 & 2, mumps virus, varicella-zoster virus, enterovirus, parechovirus) was done in all CSF samples of patients suspected of having meningoencephalitis. CSF PCR for SARS-CoV-2 was done in all patients of meningoencephalitis.

The neurological diagnosis was made after evaluating the clinical history, examination, and relevant investigations. The patients were critically evaluated and investigated for atypical neurological presentation.

Patients with atypical neurological presentation and with influenza like illness (ILI) in the recent past or during hospitalization were categorized into suspected COVID-19.

The real-time reverse-transcription polymerase chain reaction assay using a SARS-CoV-2 nucleic acid was done from oral and nasopharyngeal sample in all patients, from CSF in selected patients by the below mentioned method.

Nucleic acid extraction was done using chemagic Viral DNA/RNA 300 Kit H96 in Chemagic 360 instrument from Perkin Elmer as per the manufacturer's instruction. The sample processing was carried out in BSL-2 following standard precautions. An amount of 315 μl of lysis buffer was added to 300 μl of sample volume for extraction. Internal reference of 5 μl as provided by the FOSUN COVID-19 PCR kit was added to each sample to monitor the efficiency of sample preparation and to differentiate between true negative and false negative. A qualitative PCR test was done subsequently with the FOSUN COVID-19 PCR detection kit using primer-probe mixture for detection of N gene, E gene, and ORF1ab gene. The results were interpreted as per the kit insert.

The laboratory confirmed cases of COVID-19 with neurological manifestations are included in study. The demographic, clinical, laboratory investigations, follow up, and outcome of the patients is presented.

We obtained written consent from patients or their relatives for publication of the data including images and any identifiable data that might reveal a patient's identity.

RESULTS

A total of 358 patients with neurological diagnosis were admitted in the hospital during the study period. Out of these, 69 cases were suspected of having COVID-19 disease and there were 14 laboratory confirmed cases.

The detailed demographic, clinical profile, investigations, and outcome has been summarized in **Table 1**.

Among 14 positive SARS-CoV-2 infection patients, there were seven male and female patients, respectively. Median age at onset of symptoms was 41.5 years (range 15–70). The presenting neurological symptoms were altered sensorium ($n = 6$), headache ($n = 4$), seizures ($n = 5$), limb weakness ($n = 3$), anosmia ($n = 2$), while ophthalmoplegia and memory impairment occurred in one patient, respectively.

Influenza like illness (ILI) was present in 10 patients. Nine had fever; sore throat and shortness of breath were present in two patients, diarrhea and myalgia were seen in one patient, respectively. The ILI symptoms were present in seven patients on an average of 1 week prior to admission. One patient developed ILI on admission while other two developed these symptoms after 7–14 days of hospitalization. Four patients were asymptomatic.

Abnormal laboratory parameters included leucocytosis ($n = 6$), hyperglycemia ($n = 1$), deranged INR ($n = 1$), and hyponatremia (n=3). Complete blood counts were normal in eight patients. CRP was raised in two patients. D dimer was raised in one patient. Serum ferritin was found to be normal in all patients.

TABLE 1 | Clinical features, investigations, treatment, and outcome of 14 positive SARS-CoV-2 cases.

S. No.	Patient demographic	Neurological presentation	Associated COVID-19 symptoms	Positive SARS-CoV-2 test type	Relevant blood investigations and radiologic findings	Neurological investigations (CSF findings, neuroimaging)	Treatment and outcome
		Cerebrovascular accidents					
1	55 yr F	Patient presented with left hemiparesis with global aphasia.	Two weeks after the stroke developed fever with dyspnoea.	Upper Respiratory swab PCR	Increased total cell count with neutrophilic leucocytosis. Mildly deranged transaminases with deranged INR (2.54). CRP was raised. D-dimer was raised. Chest X ray showed an opaque left hemi thorax suggestive of a collapse/consolidation.	CT brain showed right malignant MCA infarct. MRA showed MCA main stem occlusion	Treated conservatively for stroke. After development of COVID-19 symptoms required intubation and mechanical ventilator. Died within 2 days of diagnosis of COVID-19.
2	70 yr F	Patient presented with sudden onset left hemiparesis (lower limb more than upper limb), NIHSS 6 at the time of admission with a window period of 3.5 h.	Cough and sore throat at the time of admission.	Upper Respiratory swab PCR	Normal blood counts and other parameters. CRP was raised. Ferritin Normal.	Infarct in right centrum semiovale. Left CCA showing 30% stenosis.	Was thrombolysed with alteplase. Post-thrombolysis her NIHSS improved from 6 to 4. She was treated with azithromycin, hydroxychloroquine, and was discharged on day 15 post-admission.
		Meningoencephalitis					
3	15 yr M	Patient presented with fever and headache from 5 days prior to admission.	Sore throat, diarrhea, and fever 5 days prior to admission.	Upper Respiratory swab PCR positivity, negative CSF PCR	Routine investigations were normal.	CSF study revealed an opening pressure of 30 cm of water, 12 cells (60% lymphocytes, and 40% neutrophils) with normal sugar, protein levels. Negative culture and Virology results with a negative TB PCR. MRI brain was normal.	Empirically started on acyclovir but had disabling headache. Put on dexamethasone, topiramate, acetazolamide. Required a repeat lumbar puncture for therapeutic purpose. Discharged on tapering dose of dexamethasone, acetazolamide, and topiramate. One month into follow up patient is symptom free and not on any medication.
4	35 yr F	Presented with new onset focal seizures with impaired awareness, acute onset memory impairment.	Fever 7 days prior to presentation.	Upper Respiratory swab PCR positivity, negative CSF PCR	Routine investigations normal.	CSF study 100 cells with 90% lymphocytes and mildly raised protein (56mg/dl). Negative cultures and virology panel. MRI showed T2/Flair hyperintensity in left temporo-occipital lobe, hippocampus with diffusion restriction, and right frontal periventricular white matter T2 flair hyperintensity (**Figure 1**). EEG showed generalized slowing.	Empirically started on acyclovir and levetiracetam. Then put on dexamethasone. Discharged after 14 days of inpatient stay with a diagnosis of probable COVID-19 encephalitis.
5	38 yr M	Presented with fever, headache, altered behavior.	Fever 5 days prior to admission.	Upper Respiratory swab PCR positivity, negative CSF PCR	Routine investigations normal.	Lumbar puncture showed 200 cells with 90% lymphocytes with increased protein. Negative cultures and virology pattern. Negative TB PCR. MRI brain with contrast normal.	Treated empirically with acyclovir but gradual improvement in symptoms, no other treatment given.

(Continued)

TABLE 1 | Continued

S. No.	Patient demographic	Neurological presentation	Associated COVID-19 symptoms	Positive SARS-CoV-2 test type	Relevant blood investigations and radiologic findings	Neurological investigations (CSF findings, neuroimaging)	Treatment and outcome
6	23 yr M	Presented with headache, fever, altered sensorium.	Fever, myalgia, vomiting, abdominal pain five days prior to admission.	Upper Respiratory swab PCR positivity, negative CSF PCR	Normal counts. Deranged liver function Tests, hyponatremia. CXR showed opacities (**Figure 2**).	CSF showed 94 cells 80% neutrophils and normal sugar and protein. MRI brain normal/CT Normal. Negative culture and viral serology. TB PCR negative.	Treated with anti tubercular drugs, acyclovir and dexamethasone.
		Other neurological diseases with COVID-19					
7	70 yr F	Patient diagnosed case of tubercular meningitis presented with altered sensorium.	Fever Shortness of breath	Upper Respiratory swab PCR. Initial test was Negative	Normal counts with hyponatremia. Rest investigations within normal limit. CT chest showed consolidation in bilateral upper zone and right lower zone.	CSF study showed 140 cells with 90% lymphocytes with normal sugar and increased protein (112 mg/dl). Neuroimaging consistent with TBM with hydrocephalus.	Treated with dexamethasone, anti-tubercular drugs, mannitol, and acetazolamide. She was referred for neurosurgical intervention.
8	25 yr F	Diagnosed case of Tubercular Meningitis with CNS Tuberculoma on treatment presented with status epilepticus.	Fever, Myalgia, Dyspnea 4 days prior to admission.	Upper Respiratory swab PCR	Neutrophilic leucocytosis with hypokalemia, CXR showing right lower zone opacities.	MRI brain with contrast suggestive of Tuberculoma. EEG suggestive of generalized epileptiform discharges. CSF normal study.	Treated for status epilepticus, Anti tubercular drugs, recovered and discharged.
9	15 yr F	Seizures and myoclonus	Asymptomatic	Upper Respiratory swab PCR	Normal investigations.	CSF showed 2 cells with normal sugar and protein. EEG showed slow periodic 2-3Hz discharges. CSF IgG positive for measles antibody	Diagnosed as SSPE - Treated with valproate, levetiracetam. She was asymptomatic. Discharged after monitoring.
10	53 yr M	Presented with status epilepticus and altered mental status.	Asymptomatic	Upper Respiratory swab PCR	Increased counts. Deranged Liver function test. CXR showed Bilateral middle zone opacities.	Gliosis in left fronto parietal lobes. CSF normal study.	Treated for status epilepticus with IV antibiotics and hydroxychloroquine, recovered well and discharged.
11	45 yr M	Diabetic patient presented with right eye ptosis, complete ophthalmoplegia, anosmia, ageusia with headache.	Fever and running nose 10 days prior to admission.	Upper Respiratory swab PCR positivity, negative CSF PCR	Leucocytosis with other normal blood parameters.	Neuroimaging revealed right side cavernous sinus thrombosis with pansinusitis. CSF study showed 35 cells with 90% lymphocytes and normal sugar and protein. Negative for culture and virology. TB-PCR negative.	Treated with IV antibiotics and IV amphoterecin B on suspicion of fungal cavernous sinus thrombosis.
12	48 yr F	Diabetic patient presented with altered sensorium and non-convulsive status.	Asymptomatic	Upper Respiratory swab PCR	Leucocytosis with raised blood sugar and serum osmolality. CXR was normal.	Neuroimaging showed bilateral caudate hyperdensities with hypodensity in left basal ganglia. EEG showing generalized epileptiform discharges. CSF study was normal.	On treatment with IV anti epileptics, insulin infusion.
13	65 yr M	Peripheral nervous system manifestation Presented with paraparesis with progressing weakness to upper limb and dysphagia.	Fever, ageusia five days prior to presentation. Cough present at the time of admission.	Upper Respiratory swab PCR	Neutrophilic Leucocytosis with Thrombocytosis. Hyponatremia. CXR Normal.	Demyelination with secondary axonal changes in nerve conduction studies.	On Intravenous immunoglobulin.
14	30 yr M	Loss of smell and taste.	Asymptomatic	Upper Respiratory swab PCR	Normal	Normal	Isolation and hydroxychloroquine for 5 days.

CSF was abnormal in four patients. Pleocytosis was a significant finding. High opening pressure was seen in one patient. Neuroviral panel for other virus was negative in all patients. Chest X ray was abnormal in four patients. Neuroimaging was normal in six patients. EEG abnormality were seen in four patients.

Patients were categorized into probable SARS-CoV-2 meningoencephalitis in four, stroke associated SARS-CoV-2 in two, GBS in one, and anosmia with ageusia in one. The other six patients had coexisting neurological disease with SARS-CoV-2 infection.

There was mortality in one patient with large artery stroke due to respiratory involvement on day 21 after admission. Eleven patients were discharged who are doing well on follow up with no complications, except one patient who has memory impairment, while two are still admitted.

DISCUSSION

The neurological manifestations are now being increasingly recognized and reported in different case series from the centers exclusively managing COVID-19 patients. This clinical cross-sectional study presents the different probable neurological manifestations of SARS CoV-2 from a tertiary care neuropsychiatry center. The patients are primarily presenting to us with neurological signs and symptoms attributing to the known neurological disorders. Amid the ongoing pandemic, we have carefully assessed all the patients for the typical COVID-19 symptoms.

The great heterogeneity of neurological presentation for individual patients with viral illnesses has been witnessed in past pandemics. The experience with other earlier pandemics of respiratory pathogens SARS, MERS, and H1N1 influenza and the neurological complications associated with them has given an insight into the present pandemic (6, 7).

The spectrum of neurological presentations has a wide array as reported in recent literature. Anosmia, ageusia, encephalitis, post-infectious acute disseminated encephalomyelitis, cerebrovascular accidents, and Guillain-Barré syndrome are common among the reported manifestations (8). It is important to understand the timing and relation of neurological manifestations and complications associated with SARS-CoV-2, a new emergent virus.

Different mechanisms have been proposed for the entry of the virus into the brain in research studies. Hyposmia and anosmia, the common symptoms, have been explained by the entry of SARS-CoV-2 into brain tissues via dissemination and spread from the cribriform plate, which is in close proximity to the olfactory bulb (9). This neurotrophic virus causes damage by a surge of inflammatory cytokines, mainly Interleukin-6.

Initially, in the largest retrospective study of 1,099 patients with laboratory-confirmed COVID-19 in China, Guan et al. reported respiratory and generalized symptoms as the presenting complaints. The definite neurological symptoms were not reported in this study (10). Later in the recent study from Wuhan, it has been described that 74% of COVID-19 patients can develop neurological symptoms in addition to common respiratory symptoms. Impaired consciousness and stroke were two common symptoms reported in critically ill patients (11).

Recently, a cross-specialty surveillance study from UK reported 62% of patients presented with stroke. Altered mental status was the second most common presentation, comprising encephalopathy or encephalitis and primary psychiatric diagnoses, often occurring in younger patients (12). The neuropsychiatry presentation has also been reported.

In our single center study, 14 patients have fulfilled the WHO confirmed case definition of COVID-19 (13). The detailed clinical characteristics of the 14 patients has been described in **Table 1**. Accordingly the patients were primarily categorized into probable and possible neurological manifestations due to SARS-CoV-2.

In probable SARS-CoV-2 meningoencephalitis patients, altered sensorium, irritability, and headache were present in all patients. These patients were relatively young (age range 14–50 years). Contrary to our study, eight adults patients aged 24–78 years (median 62 IQR 40–70), four women and men each, have been described with encephalitis associated with COVID-19 (14). Patient 3 has presented with acute onset disabling headache. Mao et al. have reported headache and encephalopathy in 40% of patients in their cohort (15). The brain imaging was unremarkable. His CSF examination had high opening CSF pressure of 30 cm of water. The CSF cytology and biochemical parameters were normal. Subsequently he required repeat therapeutic lumbar puncture as his headache was resistant to other therapeutic options. He was given steroids for 2 weeks and was symptom free on follow up. There is a report of steroid responsive encephalitis in coronavirus disease (16). Raised opening pressure has been described in a patient of COVID-19 related encephalitis by Moriguchi et al. (14).

CSF pleocytosis was a predominant finding indicating brain inflammation in patients with meningoencephalitis. The neuroviral panel was negative for all patients. CSF PCR for SARS-CoV-2 done in four patients was found to be negative. Recently the presence of SARS-CoV-2 RNA in the cerebrospinal fluid has been detected by genome sequencing in a patient with clinically proved meningoencephalitis in Japan (14).

In one study of 183 hospitalized children with clinically suspected acute encephalitis, 22 (12%) had coronavirus infection, the type was not specified by detection of anti-CoVIgM (17). Neuroimaging plays an important role in diagnosing meningoencephalitis.

Figure 1 of patient 4 who presented with seizures and altered sensorium is consistent with meningoencephalitis. On follow up after 3 weeks, she has memory impairment as a sequela.

Poyiadji et al. described a report where haemorrhagic ring enhancing lesions consistent with acute necrotizing encephalitis were seen in the bilateral thalami, medial temporal lobes, and sub-insular regions on brain MRI (18). They have proposed that the virus does not directly invade the blood-brain-barrier and acute necrotizing encephalitis is caused by SARS-CoV-2 via cytokine storm (18). In the French series of 58 intensive care patients with COVID-19, the neurological complications were present in 49 (84%) and 40 (69%) had encephalopathy while 39

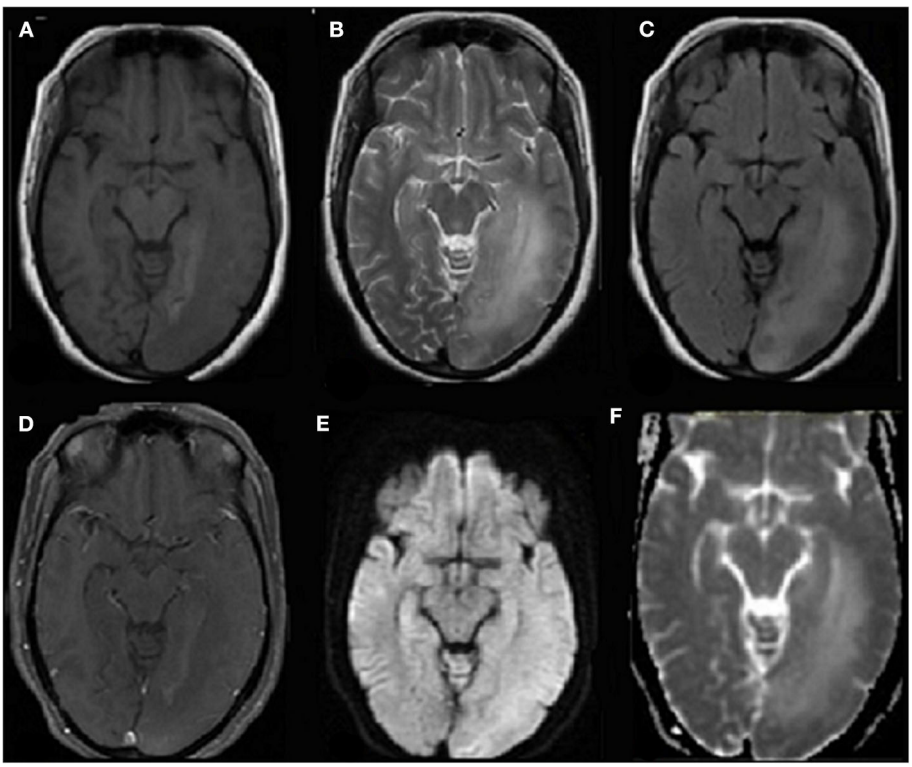

FIGURE 1 | Axial T1 weighted **(A)** MR image shows hypointensity in left parieto-occipital region in subcortical and deep white matter with overlying sulcal effacement and corresponding hyperintensity on T2 weighted image **(B)** and FLAIR **(C)** images. No associated contrast enhancement seen on contrast-enhanced T1-weighted MR image **(D)** with no restricted diffusion on diffusion-weighted MR image **(E)**, and apparent diffusion coefficient (ADC) map **(F)**.

(67%) had cortico-spinal tract signs. MRI findings in 13 patients showed leptomeningeal enhancement and eight patients had acute ischaemic change (5).

According to the provisional case definition for the association of COVID-19 with neurological disease, the probable case is defined as SARS-CoV-2 detected in respiratory or other non-CNS sample, or evidence of SARS-CoV-2 specific antibody in serum indicating acute infection and no other explanatory pathogen or cause found (1, 13). Accordingly our four cases are categorized into probable meningoencephalitis due to SARS CoV-2 infection.

The possibility of stroke associated with COVID-19 has increased as reported from publications from around the world. In the pooled analysis of four studies on cerebrovascular disease (CVD) with COVID-19, it is concluded that there is 2.5-fold increase in odds of severe COVID-19 illness with a history of stroke and trend toward increased mortality rate (19). The incidence of ∼2% is being reported for CVD among patients infected with SARS-CoV-2 admitted to the hospital (11).

SARS-CoV-2 infection is associated with hypercoagulable state and the depletion of angiotensin-converting enzyme 2 (ACE2) that results in tissue damage, including stroke. The binding of SARS-CoV-2 with ACE2 (a cardio-cerebro vascular factor) damages ACE2 and can lead to strokes. The cytokinin related injury also plays a role in causing stroke (20, 21).

There is a predilection for large vessel involvement and male preponderance. Avula et al. reported four patients who initially presented with computed tomography (CT) proven stroke and later tested positive for COVID-19 (22). Patient 1, admitted with right middle cerebral artery infarct, was managed conservatively. She developed fever and breathlessness and was found to be positive for SARS-CoV-2 on day 14 and succumbed to her illness due to respiratory insufficiency. Her X-ray showed an atypical finding of unilateral consolidation and collapse. Both of our stroke patients do not have the conventional risk factors for stroke. Li et al. has described that the median durations from first symptoms of COVID-19 to cerebrovascular disease was 10 days (23).

In the case series from Turkey, four patients with COVID-19 were reported to be simultaneously accompanied by acute ischemic stroke. The average time from COVID-19 symptom onset to the diagnosis of stroke was 2 days in this series (24).

Patient 2 presented in a late window period with NIHSS score of 6. CT head scan was normal. She was thrombolyzed with alteplase. She had a developed fever the next day. Her nasopharyngeal swab PCR was positive for SARS-CoV-2. Her NIHSS improved to 4 in 2 days. Her post-thrombolysis CT scan showed hypodensity in right centrum semiovale. She was managed with antiplatelets and neurorehabilitation.

Intravenous thrombolysis in acute stroke during this pandemic is a bigger challenge. The patients are reaching emergency care late due to several reasons. At large patients with minor strokes are also avoiding attending hospitals for the fear of infection. There is possibility of neurological symptoms being masked by the infection itself, or a delay in access to neuroimaging or revascularization techniques could be responsible as reported by Oxley et al. (25).

The single center retrospective study describes 13 of 221 patients diagnosed with SARS-CoV-2 virus to have ischemic stroke (23). Thrombocytopenia with elevated D-dimer and C-reactive protein in severe COVID-19 and stroke are consistent with a virus-associated microangiopathic process. Inflammatory response along with coagulopathies play an important role in the COVID-19 related stroke. The inflammatory marker, CRP levels were raised in both the patients while D-Dimer was raised in one patient with large artery thrombosis. The treatment comprises of anticoagulation with low-molecular-weight heparin for patients with COVID-19, to reduce the risk of thrombotic disease (26).

Hyposmia, anosmia, and dysgeusia are commonly reported symptoms seen in COVID-19 pausisymptomatic or asymptomatic patients. In a retrospective study by Klopfenstein et al. on analysis of patients with anosmia, he concluded that 47% (54 out of 114) of COVID-19 patients reported anosmia (27). Anosmia began 4.4 [±1.9 (1–8)] days after the onset of infection and the mean duration of anosmia was 8.9 [±6.3 (1–21)] days (27).

Patient 14 had only subacute onset of anosmia and ageusia for around 2 weeks. He was followed up in isolation for development of any other symptoms. No further symptoms developed and he was SARS-CoV-2 negative after 15 days. This is contrary to a patient reported by Eliezer et al. who reported a woman in her 40s presented with hyposmia with a history of dry cough along with headache and generalized fatigue a few days before presentation (28).

The pathophysiologic mechanism underlying the smell and taste impairment needs to be understood; the neurotrophic potential of the SARS-CoV-2 virus is known and it is hypothesized that the virus spread through olfactory bulbs into the central nervous system (29). Screening for SARS-CoV-2 should be done in patients presenting with isolated anosmia.

Neuromuscular disorder has been reported with SARS-CoV-2 by Tsai et al. (30). Guillain-Barré syndrome is an acute polyradiculopathy characterized by rapidly progressive, symmetrical limb weakness, areflexia on examination, sensory symptoms, and facial weakness in some patients. Patient 13 had anosmia and ageusia 5 days prior to the onset of classic symptom of GBS. He developed fever and sore throat on the day of admission. Electrodiagnostic findings confirmed demyelinating neuropathy. Besides the typical GBS, Miller Fisher variant of Guillain-Barré syndrome with ophthalmoplegia, ataxia, and areflexia is also being reported (31, 32).

The common antecedent infections related with GBS are Campylobacter jejuni, Zika virus, and influenza virus (33–35).The SARS-CoV-2 infection stimulates inflammatory cells and produces various inflammatory cytokines and, as a result, it creates immune-mediated damage (36). To our understanding, GBS being an autoimmune disorder, the hypothesis for it as a manifestation of COVID-19 remains unclear.

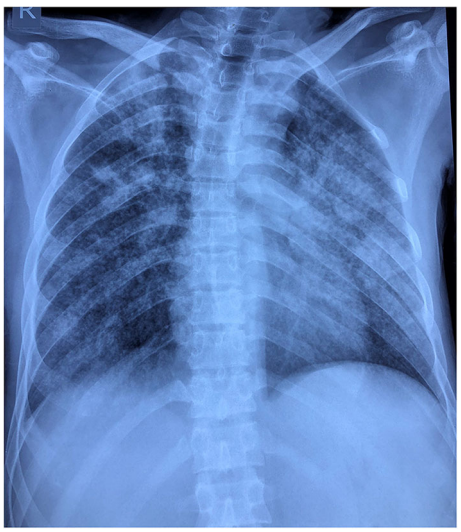

FIGURE 2 | Chest radiograph reveals multifocal patchy peripheral areas of air space opacification scattered in right lung field with right upper zone predominance. Similar confluent opacities with ground glass shadows are evident in the left middle and lower zone with relative sparing of left upper zone.

In our clinical practice we have seen the concomitant infection of tubercular meningitis and SARS-CoV-2 in Patients 7 and 8. The tubercular meningitis is diagnosed at the presentation and simultaneously she was positive for SARS-Co-V-2. Another 6 months follow up stable case of tubercular meningitis with tuberculoma presented in status epilepticus. She was asymptomatic and found to be SARS-CoV-2 positive on day 2 of hospitalization. There are reports wherein the new onset of seizure or status epilepticus can be a presenting symptom of COVID-19 (37). To explore the association of tuberculosis/tubercular meningitis and COVID-19 the global tuberculosis network cohort study is underway. The preliminary analysis suggests that 38.8% of patients had COVID-19 while on antitubercular treatment. There is limited or no protection against COVID-19 in patients of tuberculosis (38).

Patient 6 presented with acute onset encephalopathy. The CSF showed mild pleocytosis with normal sugar and protein, TB PCR and neuroviral panel were negative. Contrast brain MRI was normal. His chest radiograph showed opacities (**Figure 2**). Tubercular meningitis as a cause of encephalopathy was ruled out. The diagnosis of pulmonary tuberculosis was particularly challenging in this patient in presence of overlapping SARS-CoV-2 symptoms and positive PCR. CT chest was not possible as our hospital does not have a dedicated CT facility for COVID-19 patients. He was started on anti-tubercular drug along with other supportive treatments. In the tuberculosis endemic country of India, during the current COVID-19 pandemic it is challenging to differentiate the acute meningoencephalitis presentation of SARS-CoV-2 from tubercular meningoencephalitis.

An observational study has explored the relationship between MTB infection and COVID-19 pneumonia, suggesting that individuals with latent or active TB may be more susceptible to SARS-CoV-2 infection, and that COVID-19 disease progression may be more rapid and severe (39). A chronic respiratory disease like pulmonary tuberculosis is susceptible to this virus. The treatment for both coexistent diseases should be tailored accordingly, as use of an immunosuppressant will exacerbate tuberculosis.

There is evidence that SARS-CoV-2 virus may penetrate the brainstem, aggravating respiratory impairment (40). Seizures in severe/end-stage disease are likely to be due to COVID-19 related hypoxia, encephalopathy, or encephalitis rather than lowered seizure threshold in susceptible individuals with pre-existent neurologic disease.

There is limited information in literature that suggests that individuals with epilepsy are more likely to be infected by the virus. Giovanni et al. reported worsening of seizures in 18% of people with epilepsy (PwE) during the COVID-19 period, mostly seen in patients who were having poor sleep quality and on multiple antiepileptics. PwE were not found to be at increased risk of acquiring SARS-CoV-2 infection (41). New onset seizures and status epilepticus has been described as a presentation of COVID-19 (42). Musolino et al. investigated preliminary COVID-19 findings and found one out of 10 infected children with seizures, while others presented predominantly with fever, cough, and diarrhea (43).

In our study, five patients presented with seizures and were concomitantly diagnosed with SARS-CoV-2. Patient 9 with history of 1 week duration of myoclonic jerks and generalized seizures was diagnosed with subacute sclerosing panencephalitis (SSPE). Her CSF examination showed 2 cells with normal sugar and protein. CSF IgG measles for antibody was positive. EEG showed slow periodic 2–3 Hz discharges. Patient 10 with post-traumatic seizure disorder, who was well-controlled for 2 years, presented in status epilepticus.

Diabetic patients are considered to be at risk for SARS-CoV-2 infection. Patient 12, a middle aged female with uncontrolled diabetes mellitus, presented in non-convulsive status epilepticus. She was asymptomatic for COVID-19 symptoms and was in non-ketotic hyperosmolar state. Patient 11 presented with right eye ptosis, complete ophthalmoplegia, anosmia, and ageusia with headache. His neuroimaging revealed cavernous sinus thrombosis. In diabetes, due to hyperglycaemic state there is high risk of acquiring infection and progression to severe-stage of COVID-19 leading to immune dysfunction (44). The patients with diabetes are vulnerable to nosocomial infection, which can deteriorate their general condition and aggravate COVID-19 symptoms (45). In this patient we have suspected fungal infection causing cavernous sinus thrombosis due to complicated pan sinusitis with bony destruction on brain imaging.

The presentation of patients with seizures raises the question of whether epilepsy patients are more prone to SARS-CoV-2 infection. COVID-19 may exacerbate epileptic seizures from associated systemic effects not directly related to SARS-CoV-2 CNS infection. The reasonable current understanding remains that in some patients with COVID-19, seizures develop as a consequence of hypoxia, metabolic derangements, organ failure, or even cerebral damage. The association of COVID-19 with the patients of epilepsy needs to be explored further in well-planned studies.

This study gives an insight into the neurological associations of SARS-CoV-2, the new emergent virus in the current pandemic. The infection itself might be a source of neurological manifestations, and patients with neurological disorders might be at risk of the most severe complications of this infection. The effect of SARS-CoV-2 on pulmonary tuberculosis and tubercular meningitis in endemic areas needs to be closely monitored for increased risk of occurrence and its appropriate management.

Our limitations include the spectrum of neurological manifestations from a single center study may not be complete. The long term follow up for possible sequalae is essential to elucidate the pathophysiological mechanism.

Absent of the dedicated CT facility for COVID-19 patients, the respiratory involvement at the early stage may have been missed.

As we are still evolving with our knowledge in the pandemic, identification and reporting of neurological manifestations of SARS-CoV-2 will help in formulating the guidelines and protocol for early diagnosis and management.

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. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

VS, PB, and KR were involved in patient management and care. SK, VS, PB, and KR summarized and conceptualized the data and drafted the initial manuscript. SK, VS, SM, and AA revised the final manuscript. RG and SB collected the laboratory data including RT PCR for COVID-19. Radiological images have been described by MC. All authors agreed upon the final form of the manuscript before submission.

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OPINION
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Loss of Smell in COVID-19 Patients: Lessons and Opportunities

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Keywords: SARS-CoV-2, COVID-19, olfaction, diabetes, ACE2, insulin

INTRODUCTION

When Rebecca Medrano, a Washington D.C. resident who tested positive for SARS-CoV-2 infection, first started feeling unwell with viral symptoms, she noticed an additional symptom—she had lost her sense of smell (NPR.org, 2020). Worldwide, doctors have reported similar cases of smell and taste loss in up to 60% of COVID-19 patients, now confirmed in a peer-reviewed study (Yan et al., 2020). A second unexpected observation was that infected patients with diabetes had higher rates of serious complications and death (ADA, 2020; Barron et al., 2020).

Underlying mechanisms for these observations remain unclear. In addition, they raise important questions: Does SARS-CoV-2 infect olfactory tissue and if so, which olfactory cell types do they infect and how do viral mechanisms in these cells modulate smell function? Does diabetes exacerbate viral infection or does virus-mediated dysregulation of diabetic mechanisms further aggravate symptoms in diabetics? These are critical questions to focus on amidst the tremendous increase in research efforts related to this pandemic.

Within just few months, the number of SARS-CoV-2 cases has crossed 40 million with over one million deaths reported around the world (Johns Hopkins Coronavirus Resource Center, 2020). A major focus of current pandemic-related research efforts seems to be on virus entry and host immunity mechanisms, and on developing vaccines. While these are important research goals, unless we develop effective drugs that are disease specific, this virus is likely to persist and mutate causing more harm to human life and economies around the world. There is thus an urgent need for basic research into viral mechanisms and to generate innovative strategies to slow down disease progression, especially in diabetics.

The cases of Rebecca Medrano and many like her losing their sense of smell highlight unique opportunities to study SARS-CoV-2 mechanisms. Such an opportunity to better understand SARS-CoV-2 mechanisms and its relationship to diabetes might be found in our nose. Here, we argue that our communal fight against this dreaded disease would benefit greatly from an additional focus on studying viral mechanisms in olfactory tissue. Olfactory tissue offers a convenient venue to explore interactions between viral and diabetic mechanisms and evaluate them at the level of function (loss of smell).

How Does SARS-CoV-2 Infection Lead to Smell Loss?

While many COVID-19 patients have reported a loss in their ability to smell, it is unclear how this virus mediates smell loss. Is there SARS-CoV-2 activity in olfactory tissue or could loss in smell simply be a byproduct of an overactive host immune response to the virus? Ample recent evidence supports the idea that viral mechanisms play a role in olfactory tissue.

SARS-CoV-2 uses the receptor, Angiotensin-converting enzyme 2 (ACE2) to attach to a host cell. ACE2, a metalloprotease is found in cells of several tissues including olfactory tissue (Hamming et al., 2004; Sungnak et al., 2020). Within olfactory tissue, ACE2 and TMPRSS2 (a priming protease that facilitates viral uptake) express in several cell types (Hamming et al., 2004; Baig et al., 2020; Bilinska et al., 2020; Brann et al., 2020; Sungnak et al., 2020). Expression of these genes in olfactory

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cell types suggests that olfactory tissue may be sensitive to SARS-CoV-2 infections. It may also explain why FDA approved ACE inhibitors such as Captopril are associated with loss in smell and taste (Doty and Bromley, 2004).

However, early studies are unresolved as to whether infection of olfactory sensory neurons (OSNs) or of neighboring cells is responsible for smell loss. One possibility is that smell loss may be due to death of OSNs (Netland et al., 2008; Baig et al., 2020). Immunostainings of SARS-Co-V N protein revealed high abundance of this antigen in olfactory bulb of infected mice. This study suggested SARS-virus entry via olfactory nerve with subsequent transneural spread into brain regions. The olfactory nerve, which consists mainly of OSNs and directly connects nasal cavity with the central brain, is considered a shortcut for several viruses including the influenza virus to gain entry into the brain (van Riel et al., 2015). Viral infections of olfactory nerve are usually accompanied by concomitant (non-apoptotic) neuronal death (Netland et al., 2008).

Another possibility is that infection of non-neural olfactory cells and not OSNs may be responsible (Bilinska et al., 2020; Brann et al., 2020). Bilinska et al. report high expression of ACE2 and TMPRSS2 in sustentacular cells of the olfactory epithelium and less expression in OSNs (Bilinska et al., 2020). Since sustentacular cells play key roles in supporting OSN metabolism and odor sensing, any virus-mediated damage to these cells could lead to olfactory deficits (Heydel et al., 2013). Brann et al. also report that ACE2 and TMPRSS genes are expressed in sustentacular cells, HBCs, microvillar cells, and Bowman's gland cells of the olfactory epithelium but not in OSNs. They argue that viral infection of sustentacular cells may be enough to cause a pathophysiological cascade that culminates in damage to OSNs and thereby cause smell loss. They also found ACE2 expression in vascular pericytes, which are involved in inflammatory response (Brown et al., 2019; Brann et al., 2020).

Overall, these studies offer several hypotheses as to how viral mechanisms could lead to smell loss in COVID-19 patients: *one* ACE2 mechanisms in olfactory neurons lead to a direct modulation of olfactory sensitivities or even cell death; *two* ACE2 mechanisms in non-neural olfactory cells lead to indirect modulation of OSN function and; *three* ACE2 mechanisms in neighboring glial cells lead to increased inflammatory response, whose downstream effects could alter OSN function and reduce olfactory sensitivities. While this is not an exhaustive list of possible hypotheses, it's a start. Each hypothesis needs to be rigorously assessed so that we may better understand why many COVID-19 patients lose their sense of smell.

Why Do Diabetics Have Worse Outcomes After SARS-CoV-2 Infections?

SARS-CoV-2-infected patients with diabetes face higher rates of serious complications and even death (ADA, 2020). Type 1 and type 2 diabetics have up to 3.5 times the odds of dying with COVID-19 (Barron et al., 2020). A recent meta-analysis study of data collected from 1,527 patients in China revealed that diabetes was among the most prevalent comorbidities associated with COVID-19 (Li et al., 2020). These observations raise important questions: Could diabetes-associated symptoms aggravate viral infection? Conversely, could viral infection aggravate diabetes symptoms?

A relationship between various infections and diabetes has long been debated. Population-based studies have revealed that infections such as influenza and pneumonia are common and more serious among older people with type 2 diabetes (McDonald et al., 2014; Pearson-Stuttard et al., 2016; Li et al., 2019). While diabetes may predispose individuals to certain infections, it is less clear how (Knapp, 2013). There are several theories as to how diabetes and SARS-CoV-2 associate to influence symptom severity and mortality. A simple explanation is that high levels of inflammation, coagulation, immune response impairment, etc. in diabetics could aggravate viral infection and symptoms (Hussain et al., 2020).

However, there is also evidence to support a case for complex interactions between viral and diabetic mechanisms within cells. For instance, upon being fed a high calorie diet, ACE2 knockout mice had impaired glucose tolerance compared to their wild type littermates (Takeda et al., 2013). This result suggests that ACE2 expressed in insulin-sensitive tissues plays a critical role in maintaining glucose homeostasis and insulin sensitivity. Thus, a SARS-CoV-2/ACE2-mediated dysregulation of insulin signaling could further aggravate symptoms in diabetics.

Searching for Insights in the Nose

The olfactory tissue offers a convenient venue to research interactions between viral and diabetic mechanisms and gain fundamental insights about SARS-CoV-2 mechanisms. As described above, several olfactory cell types express ACE2 and TMPRSS, likely rendering them sensitive to viral infections. It turns out that olfactory tissue is also sensitive to insulin. In fact, highest density of central insulin receptors and highest concentration of insulin in mammalian brain are found in the olfactory bulb (Havrankova et al., 1981). Insulin signaling plays a significant role in satiety-dependent modulation of smell sensitivities (Bargmann, 2012; Taghert and Nitabach, 2012; Ko et al., 2015; Slankster et al., 2020). Like ACE2, insulin receptors are expressed on surface of both OSNs and glia (Nassel et al., 2013; Musashe et al., 2016). Using the olfactory system, we could ask whether ACE2 and insulin mechanisms interact and, if they do, identify downstream molecules that link these mechanisms. Identifying downstream molecules that link ACE2 and insulin mechanisms will expand our understanding of how viral and diabetic mechanisms interact to impact cell function. Additionally, these results may inform studies in other tissues and generate drug targets with relevance for disease progression in diabetics.

Olfactory systems, especially in genetically tractable model organisms like mice and *Drosophila* may aid in our fight against this virus. These systems have been commonly used as convenient *in vivo* model systems for disease research (Steuer et al., 2014; Franks et al., 2015). Previous studies have characterized *Drosophila*-orthologs of human ACE (*AnCE* and *ACER*) (Cornell et al., 1995). Notably, the same drugs that inhibit human ACE also inhibit ACE orthologs in *Drosophila* (Kim et al., 2003). Genetic tools to manipulate ACE receptor and

insulin signaling in mice and *Drosophila* olfactory cells have been described and are available in public collections. Considering olfactory tissue offers a convenient venue to explore interactions between viral and diabetic mechanisms as well as to evaluate them at the level of function, we cannot ignore the possibility that olfactory research will lead to insights that may aid our fight against this virus. Few other tissues harbor both ACE2 and insulin mechanisms and even when they do, such as in lungs and liver, studying functional interactions in these tissues can be challenging. We therefore urge research agencies to consider funding basic research focused on viral mechanisms in olfactory tissue.

DISCUSSION

SARS-CoV-2 has negatively impacted lives around the world. Amidst growing concerns of its impact, federal and private agencies are rushing to develop effective cures and vaccines. While there is a need for such solutions, there is also a need for basic research into underlying viral mechanisms and thereby generate more innovative solutions. One area that needs more basic research is the relationship between viral and diabetic mechanisms. Could abnormal insulin signaling in olfactory neurons of diabetics exacerbate SARS-CoV-2-mediated mechanisms and lead to loss in smell in Covid-19 patients? This relationship can no longer be ignored, especially since diabetics infected with SARS-CoV-2 have up to 3.5 times the odds of serious complications and death. Fortunately, stories of Rebecca Medrano and others offer us an opportunity to tackle this enemy from a different perspective and eventually defeat it. Studying interactions between viral and diabetic mechanisms in olfactory tissue of genetically tractable model organisms such as mice and *Drosophila* offers a different approach. Such an approach will not only lead to new insights about SARS-CoV-2 mechanisms but may also help identify potential drug targets primarily relevant to disease progression in diabetics. Identifying viable drug candidates will greatly impact our collective capability of fighting this disease over the next 5–10 years. If scientists and funding agencies consider novel but thoughtful actions right now, they will help change course of events and thus make a significant impact in the long term.

AUTHOR CONTRIBUTIONS

DM conceived and wrote this opinion piece.

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BRIEF RESEARCH REPORT
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SARS-CoV-2 Is Not Detected in the Cerebrospinal Fluid of Encephalopathic COVID-19 Patients

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Neurologic manifestations of the novel coronavirus SARS-CoV-2 infection have received wide attention, but the mechanisms remain uncertain. Here, we describe computational data from public domain RNA-seq datasets and cerebrospinal fluid data from adult patients with severe COVID-19 pneumonia that suggest that SARS-CoV-2 infection of the central nervous system is unlikely. We found that the mRNAs encoding the ACE2 receptor and the TMPRSS2 transmembrane serine protease, both of which are required for viral entry into host cells, are minimally expressed in the major cell types of the brain. In addition, CSF samples from 13 adult encephalopathic COVID-19 patients diagnosed with the viral infection via nasopharyngeal swab RT-PCR did not show evidence for the virus. This particular finding is robust for two reasons. First, the RT-PCR diagnostic was validated for CSF studies using stringent criteria; and second, 61% of these patients had CSF testing within 1 week of a positive nasopharyngeal diagnostic test. We propose that neurologic sequelae of COVID-19 are not due to SARS-CoV-2 meningoencephalitis and that other etiologies are more likely mechanisms.

Keywords: SARS-CoV-2, COVID-19, CSF, encephalopathy, cerebrospinal fluid

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INTRODUCTION

The novel coronavirus SARS-CoV-2, responsible for the COVID-19 pandemic, principally affects the respiratory system and, in severe cases, leads to pneumonia complicated by acute respiratory distress syndrome (ARDS). The viral infection impacts other organ systems as well and can cause gastrointestinal symptoms, renal insufficiency, and thrombosis among other complications. However, little is known about the neurologic manifestations and related mechanisms of COVID-19.

A common observation is that patients that develop severe COVID-19 pneumonia and ARDS manifest encephalopathy during the course of the disease and their recovery (1, 2). Possible etiologies for such central nervous system (CNS) impairment include direct infection of neural tissue, hypoxic ischemic injury, ischemic infarcts due to hypercoagulability, metabolic encephalopathy (including renal and hepatic etiologies), or effects of massive systemic cytokine release. A recent autopsy study of COVID-19 patients based on detection of viral genome by *in situ* hybridization suggested the brain abundance of the virus is approximately 10^6-fold less than in the lung and 10^3-fold less than in the kidneys, liver and heart (3). However, this low-level presence of viral genome in CNS tissue does not really address the question of whether the neurologic

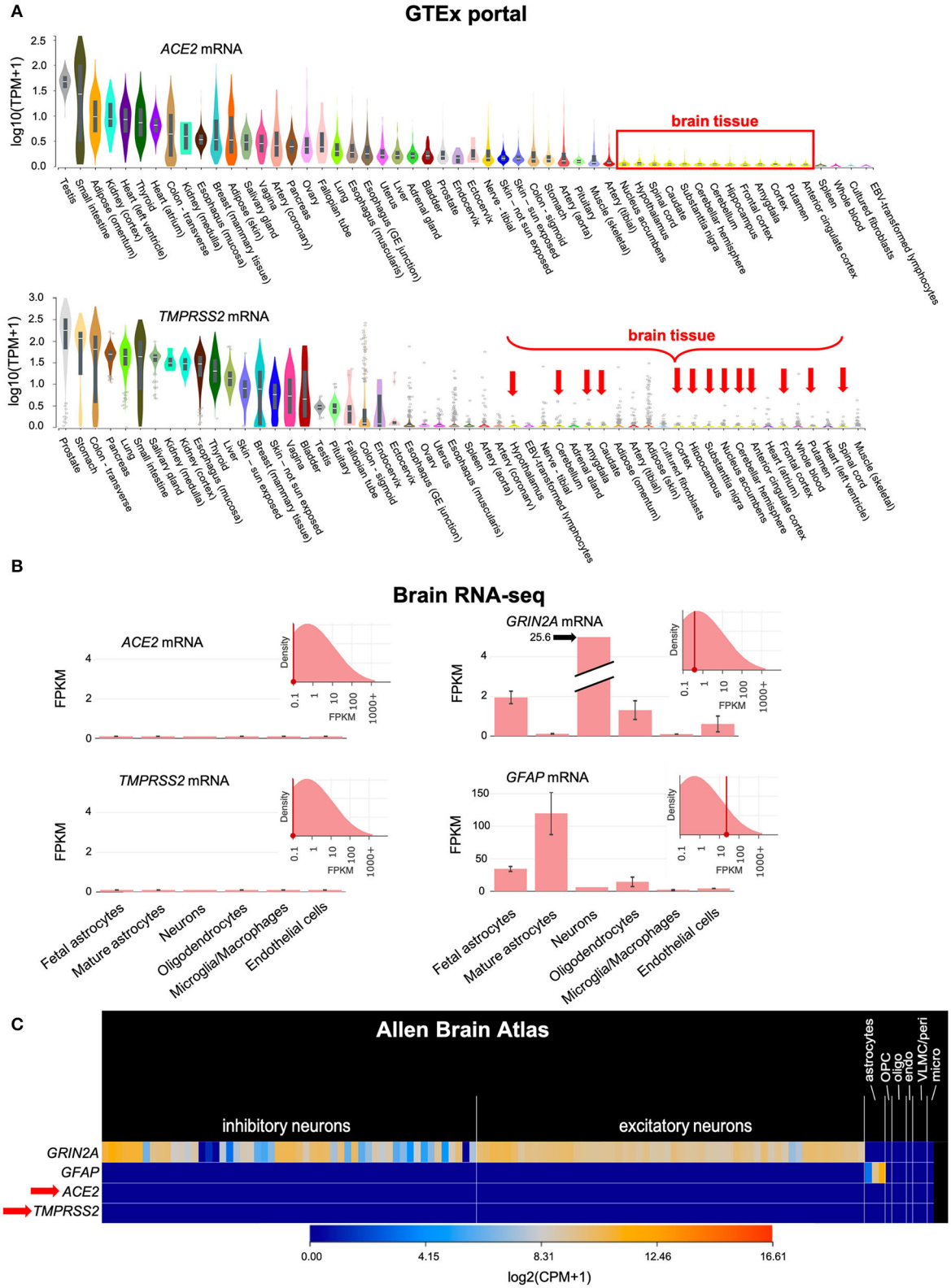

FIGURE 1 | *ACE2* and *TMPRSS2* mRNAs are only minimally expressed in the brain. **(A)** Bulk RNA sequencing of normal human tissues in the GTEx portal shows that *ACE2* and *TMPRSS2* mRNAs are minimally expressed in the human brain (red box and arrows). **(B)** The Brain RNA-seq database, which is based on RNA-seq of

(Continued)

FIGURE 1 | sorted cellular populations, shows minimal expression of *ACE2* and *TMPRSS2* in brain cells, including neurons, glia, microglia and endothelial cells. The expression pattern of *GRIN2A*, which encodes the NMDA receptor 2A subunit in neurons, and *GFAP*, which encodes an intracellular filament found in astrocytes, are shown for comparison. **(C)** Similar information is found in the Allen Brain Atlas, which is based on single-cell RNA-seq of normal brain specimens. *ACE2* and *TMPRSS2* (red arrow) are minimally expressed. *GRIN2A* and *GFAP* are again shown for comparison. TPM, Transcripts Per Million mapped reads; FPKM, Fragments Per Kilobase of transcript per Million mapped reads; CPM, Counts Per Million mapped reads; OPC, Oligodendrocyte Precursor Cells; oligo, oligodendrocytes; endo, endothelial cells; VLMC/peri, Vascular and LeptoMeningeal Cells/pericytes; micro, microglia.

sequelae of COVID-19 are related to SARS-CoV-2 encephalitis/meningitis. Furthermore, it is not clear whether the patients in this autopsy study had any neurologic manifestations.

To help answer the question of whether SARS-CoV-2 causes meningoencephalitis in encephalopathic COVID-19 patients, we utilized a two-pronged approach. First, we asked whether brain tissue expresses the cell surface proteins required for viral entry into host cells, ACE2 and TMPRSS2 (4). And second, we tested whether SARS-CoV-2 RNA is detected in the cerebrospinal fluid (CSF) of encephalopathic COVID-19 patients.

METHODS

Computational Analysis of RNA-seq Data

We analyzed public-domain RNA-seq data (both bulk and single-cell) from normal brain tissue in the GTEx, Brain RNA-seq and Allen Brain Atlas databases [www.brainrnaseq.org; celltypes.brain-map.org/rnaseq/human/cortex; (5)] for expression of *ACE2* and *TMPRSS2* mRNA. We used *GRIN2A*, which encodes the 2A subunit of the N-methyl-D-aspartate (NMDA) receptor and *GFAP* (glial fibrillary acidic protein) mRNAs as positive controls for expression in neurons and astrocytes, respectively.

Validation of CSF Testing by SARS-CoV-2 RT-PCR

Current diagnostic modalities for COVID-19 rely predominantly on the detection of SARS-CoV-2 RNA using RT-PCR. Most clinical laboratories have implemented commercial platforms that have received Emergency Use Authorization (EUA) by the FDA. The advantage of commercial platforms is their reliability, well-standardized reagents, automation and high throughput. The extent of the pandemic and particularly its impact in New York City made these platforms a highly desirable option. Our institution used the Cepheid (Sunnyvale, CA) Xpert® Xpress, as well as the Roche (Basel, Switzerland) Cobas®, platforms for SARS-Cov-2 RT-PCR by nasopharyngeal swab. Both systems have comparable performance with similar limits of detection (LOD), 250 and 100–200 RNA copies/mL, respectively, which is important for consistency of results.

As clinical demands for CSF testing for SARS-CoV-2 RT-PCR testing in our institution increased, we planned the validation of this sample type according to the New York State Wadsworth Center procedure for SARS-CoV-2 Laboratory Developed Test (LDT). The Food and Drug Administration (FDA) has given the NYS Wadsworth Center the authority to approve SARS-CoV-2 LDT tests to qualified laboratories during the COVID-19 pandemic.

We chose the Cepheid Xpert® Xpress platform, which targets the *N2* (nucleocapsid) and *E* (envelope) sequences of the SARS-CoV-2 genome, to validate SARS-CoV-2 RT-PCR in CSF specimens. Detection of the SARS-CoV-2-specific *N2* sequence results in a positive diagnostic test. Refrigerated CSF samples, obtained from patients without COVID-19 and previously analyzed for other tests in the laboratory, were pooled and used as the sample matrix for validation studies. The pooled CSF tested negative by SARS-CoV-2 RT-PCR. To determine the limit of detection (LOD), we added to the pooled CSF serial 2-fold dilutions of SeraCare AccuPlex™ Reference Material (Cat # 0505-0126), which contains 5,803 copies/mL of SARS-CoV-2 RNA, including the target *N2* sequence detected by the Cepheid Xpert Xpress platform. The lowest dilution of reference material that we tested was 90 copies/mL. All dilutions were tested in triplicates. We determined the LOD in CSF to be 181 copies/mL, comparable to the platform's LOD in nasopharyngeal swab specimens. This LOD was 100% reproducible in 20 replicate experiments.

To determine the specificity of the assay, we spiked our pooled CSF with control material containing viral genomic sequences of cytomegalovirus (CMV), herpes simplex virus 1 (HSV1), herpes simplex virus 2 (HSV2), parechovirus type 3, enteric cytopathic human orphan (ECHO) virus, and human herpesvirus 6 (HHV6). We also included CSF proficiency panel samples containing HSV1 and CMV, as well as one clinical CSF sample known to contain HSV1. All these samples tested negative for SARS-CoV-2 by RT-PCR.

To further evaluate the assay for clinical use, we used contrived individual CSF samples spiked with Seracare AccuPlex™ Reference Material at six different increments of the LOD (2X, 4X, 6X, 8X, 10X, and 25X). We included five CSF samples per condition. Ten unspiked CSF samples were used as negative controls. All 30 contrived samples spiked with different LOD increments tested positive by SARS-CoV-2 RT-PCR, while all 10 controls tested negative.

Retrospective Analysis

To test if SARS-CoV-2 can be detected in the CSF of COVID-19 patients with neurologic complications, we retrospectively mined data from our COVID-19 adult patient cohort in the NYU Langone Health System in New York City (NYC) and searched for SARS-CoV-2 RT-PCR tests in CSF.

RESULTS

SARS-CoV-2 requires the cellular receptor ACE2 and the transmembrane serine protease TMPRSS2 to enter cells (4).

TABLE 1 | Patient demographics and clinical information.

Patient number	Sex	Age (years)	CSF test date	Outcome	Method	Swab test date	Outcome	Interval (days)	Indication for CSF test	CSF glucose	CSF protein	CSF RBC	CSF WBC	CSF differential	Brain imaging	Admission date	Discharge date	Clinical outcome	Repeat swab test date	Outcome
1	F	68	12/04/2020	Neg	LP	05/04/2020	Pos	7	Encephalopathy	65	37	0	3	80% lymphs	CT 4/5/20: normal	05/04/2020	30/04/2020	Skilled nursing facility	28/04/2020	Neg
2	F	66	27/03/2020	Neg	LP	20/03/2020	Pos	7	Encephalopathy	93	80	1	9	57% lymphs	No	20/03/2020	09/04/2020	Death		
3	M	88	20/04/2020	Neg	LP	14/04/2020	Pos	6	Seizure	64	62	0	14	99% lymphs	CT 4/14/20: abnormal hypodensities	14/04/2020	28/04/2020	Recovered		
4	M	69	25/03/2020	Neg	LP	24/03/2020	Pos	1	Seizure	81	86	14,000	24	75% PMNs	CT, CTA 3/24/20, 3/26/20: lacunar infarct	24/03/2020	01/04/2020	Death		
5	M	38	26/04/2020	Neg	LP	24/03/2020	Pos	33	Encephalopathy	125	16	1	0		MRI 4/21/20, 4/29/20: hypoxic injury/infarcts	23/03/2020	10/06/2020	Rehabilitation		
6	F	22	20/04/2020	Neg	Shunt tap	01/04/2020	Pos	19	Encephalopathy	146	128	0	2		No	01/04/2020	12/05/2020	Death		
7	M	38	29/04/2020	Neg	LP	29/04/2020	Pos	0	Seizure	75	60	0	5	60% lymphs	CT 4/29/20: normal; MRI 5/1/20: normal	29/04/2020	08/05/2020	Recovered		
8	F	60	31/03/2020	Neg	LP	30/03/2020	Pos	1	Encephalopathy	34	85	13,000	31	78% lymphs	MRI brain 3/17/20, 3/27/20, 4/6/20: FLAIR hyperintensities	15/03/2020	10/04/2020	Recovered	06/04/2020	Pos
9	F	63	30/04/2020	Neg	LP	30/03/2020	Pos	31	Encephalopathy	62	25	1	0		MRI 4/29/20: FLAIR hyperintensities	30/03/2020	03/06/2020	Recovered		
10	M	64	05/05/2020	Neg	LP	26/03/2020	Pos	40	Encephalopathy	70	49	900	2		CT 4/23/20: normal; MRI 5/1/20: diffusion restriction and FLAIR hyperinsities	26/03/2020	22/05/2020	Recovered		
11	F	34	14/05/2020	Neg	LP	09/05/2020	Pos	5	Seizure	114	21	40	1	100% lymphs	CT 4/12: pons hypodensities	09/05/2020	30/06/2020	Recovered		
12	F	36	15/05/2020	Neg	EVD	07/05/2020	Pos	8	Hemorrhage/ encephalopathy	62	219	46,000	50	69% PMNs	CT 5/13: hemicraniectomy for ICH, IVH	07/05/2020	08/07/2020	Hospice care		
13	F	67	12/05/2020	Neg	LP	25/04/2020	Pos	17 (3)	Hemorrhage/ encephalopathy	53	153	2,000	402	78% PMNs	CT 5/12: ICH, SAH	25/04/2020	24/05/2020	Death	09/05/2020	Pos

F, female; M, male; neg, negative; pos, positive; LP, lumbar puncture; EVD, external ventricular drain; RBC, red blood cells; WBC, white blood cells; lymphs, lymphocytes; PMNs, polymorphonuclear cells; ICH, intracranial hemorrhage; IVH, intraventricular hemorrhage; SAH, subarachnoid hemorrhage.

Our analysis of public-domain RNA-seq data indicates that only minimal amounts of *ACE2* and *TMPRSS2* mRNAs are expressed in human brain cells, including neurons, glia, microglia and endothelial cells (**Figure 1**). This suggested that the brain may be less susceptible to infection than other tissues with higher expression of ACE2 and TMPRSS2.

We retrospectively identified 13 adult patients with severe COVID-19 pneumonia (**Table 1**) who had CSF tested for SARS-CoV-2 by RT-PCR, due to concern for meningoencephalitis. CSF sampling was obtained via lumbar puncture ($n = 11$), by tapping the reservoir of a ventriculoperitoneal shunt valve (n=1), and by aspirating CSF through an external ventricular drain (EVD) ($n = 1$). Indications included encephalopathy ($n = 7$), seizures ($n = 4$), and encephalopathy associated with known intracranial hemorrhage ($n = 2$). Encephalopathic symptoms preceded CSF sampling in all cases. All patients had SARS-CoV-2 infection confirmed by nasopharyngeal swab testing, but, importantly, none showed evidence of the virus in the CSF by RT-PCR. There was no pleocytosis in the CSF, with the exception of 1 patient with known subarachnoid hemorrhage (**Table 1**). **Figure 2A** shows the time interval between the nasopharyngeal and CSF tests in these patients. Of particular interest are three patients (23.1%) whose CSF was tested the same day ($n = 1$) and the day after ($n = 2$) the nasopharyngeal swab that indicated SARS-CoV-2 infection (**Figure 2B**). In one of these cases, a second nasopharyngeal swab 8 days after the CSF test remained positive for the virus. Overall, 53.8% of patients in this cohort had their CSF tested within 1 week after initial diagnosis; and 61.5% had the CSF test within 1 week of a positive nasopharyngeal swab test (**Figure 2B**). Eleven of the patients (84.6%) had brain imaging (MRI or CT) at the time of the CNS workup, with nine of those (81.8%) showing abnormal findings. These imaging abnormalities included, in at least three cases, evidence for subcortical hypoxic ischemic injury and infarcts.

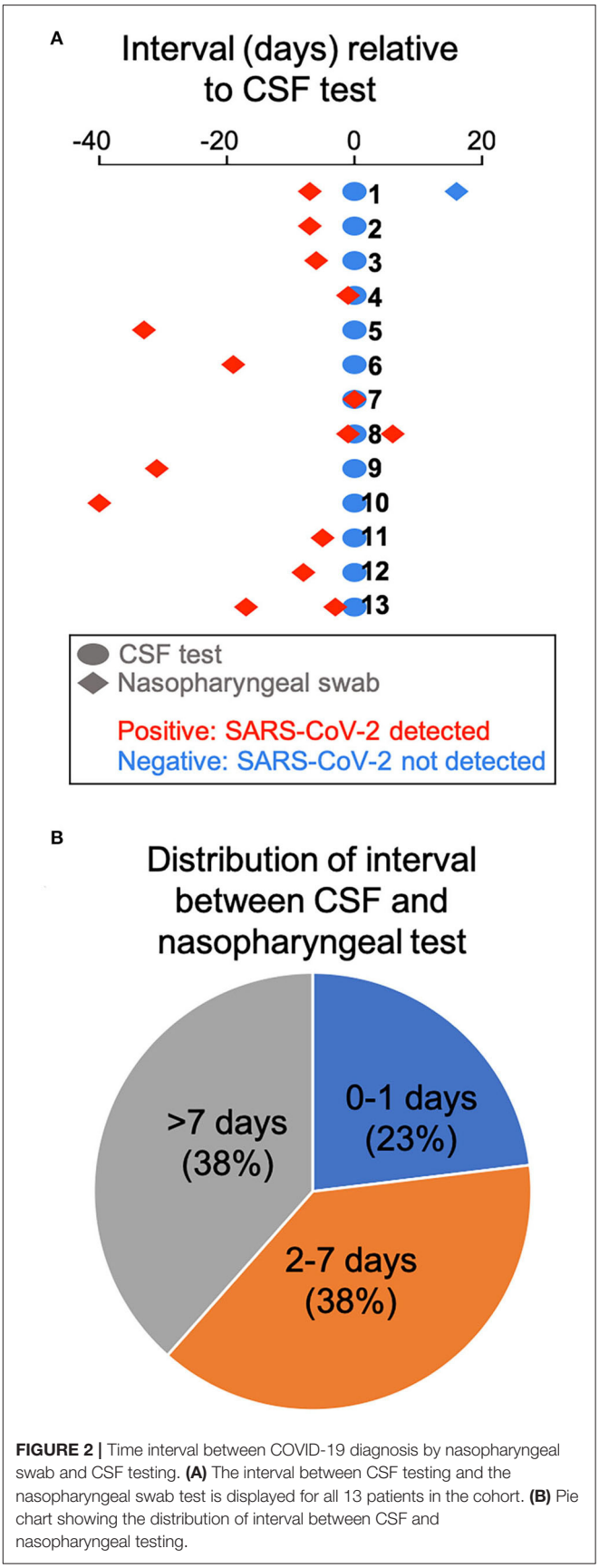

FIGURE 2 | Time interval between COVID-19 diagnosis by nasopharyngeal swab and CSF testing. **(A)** The interval between CSF testing and the nasopharyngeal swab test is displayed for all 13 patients in the cohort. **(B)** Pie chart showing the distribution of interval between CSF and nasopharyngeal testing.

DISCUSSION

Although this is a small cohort of patients and the data were analyzed retrospectively, our negative CSF findings are consistent with the observation that *ACE2* and *TMPRSS2* mRNAs are minimally expressed in the brain. We postulate that SARS-CoV-2 is not likely to establish persistent or severe meningoencephalitis that can account for the neurologic sequelae of COVID-19, a hypothesis that will have to be tested in larger studies in the future. In theory, possible routes of spread of the novel coronavirus to the brain could include: viremic spread and infection of cerebrovascular endothelium or diapedesis through the vasculature or blood-brain barrier; the choroid plexus, the tissue responsible for CSF production; transsynaptic spread to the brain via the olfactory epithelium in the nasal cavity; or introduction of the virus by infected peripheral leukocytes, in a "trojan horse" scenario. However, we believe that, even if SARS-CoV-2 were to reach the brain via one of these mechanisms, it would nonetheless be unable to establish a clinically significant, persistent infection due to

minimal expression of ACE2 cellular receptor and TMPRSS2 transmembrane protease, both necessary for viral entry into cells (4). Ultimately, autopsy studies of such encephalopathic COVID-19 patients will be required to determine whether SARS-CoV-2 infects brain tissue, using techniques such as electron microscopy, which identifies viral particles; *in situ* hybridization for the viral genome; or immunohistochemistry for viral proteins (3).

These observations need to be considered in the context of an isolated report of SARS-CoV-2 meningitis/cerebritis (albeit with a negative nasopharyngeal test result) (6), which suggests that CNS infection may be possible. However, recent studies corroborate our findings that SARS-CoV-2 is not detected in the CSF of COVID-19 patients (2, 7). Our case series, one of the largest so far on the status of CSF in COVID-19 patients, builds on the prior reports to more definitively argue against SARS-CoV-2 meningoencephalitis, because it utilizes a CSF-validated RT-PCR diagnostic assay, and, equally importantly, because it reports negative CSF testing within 0–1 days from COVID-19 diagnosis in 23.1% of patients, and within 1 week of positive nasopharyngeal testing in 61.5% of the subjects. The results of early CSF testing in these patients essentially fulfill the criterion for near-simultaneous nasopharyngeal swab and CSF assays during the critical window of active infection, in order to safeguard against the concern that a long delay in CSF testing after initial diagnosis may be negative anyway due to viral clearance.

Limitations of our study include the small sample size, as well as the possibility that low-grade CSF infection with SARS-CoV-2 may fall below the limit of detection of our diagnostic test. Additional patients tested after this manuscript was written also tested negative for SARS-CoV-2 in the CSF. Larger studies will be required to ascertain our findings in this small cohort.

Overall, our study suggests that SARS-CoV-2 infection of the CNS is unlikely to account for neurologic symptoms of the COVID-19 syndrome. In our opinion, the CNS manifestations of COVID-19 are more likely the result of a coagulation disorder leading to multiple ischemic infarcts; hypoxic ischemic injury; toxic metabolic effects of prolonged critical illness, residual sedation and uremic encephalopathy; or CNS effects of the cytokine storm that characterizes severe COVID-19 cases (1, 2, 8).

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: www.brainrnaseq.org and celltypes.brain-map.org/rnaseq/human/cortex.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NYU School of Medicine IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

DP, EL, JF, and AL provided clinical data. MA-R, AF, JC, KI, DZ, and MS validated the SARS-CoV-2 PCR assay in CSF. DP wrote the manuscript, which was edited by all other authors. All authors contributed to the article and approved the submitted version.

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REVIEW
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Progress in Research on SARS-CoV-2 Infection Causing Neurological Diseases and Its Infection Mechanism

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COVID-19 has spread rapidly worldwide since its outbreak and has now become a major public health problem. More and more evidence indicates that SARS-CoV-2 may not only affect the respiratory system but also cause great harm to the central nervous system. Therefore, it is extremely important to explore in-depth the impact of SARS-CoV-2 infection on the nervous system. In this paper, the possible mechanisms of SARS-CoV-2 invading the central nervous system during COVID-19, and the neurological complications caused by SARS-CoV-2 infection were reviewed.

Keywords: neurological complications, olfactorial nerve, ACE2, central nervous system, SARS-CoV-2, COVID-19

INTRODUCTION

The novel coronavirus is a previously unknown β-coronavirus, which is a single-stranded positive-strand RNA virus. The World Health Organization named it 2019-nCoV and the International Committee on Virus Taxonomy named it SARS-CoV-2. The virus belongs to a branch of the sarcoma virus subfamily of the coronavirus subfamily. SARS-CoV-2 is the seventh member of the coronavirus family that infects humans (1, 2).

SARS-CoV-2 is the virus that caused COVID-19 in 2019. SARS-CoV-2 infection can cause severe acute respiratory syndrome. SARS-CoV-2 has a high potential to spread and infect humans all over the world (3). Since the first case of COVID-19 was diagnosed in Wuhan, the number of SARS-CoV-2 infections worldwide has increased exponentially in the past few months. COVID-19 was originally described as a respiratory infection, but now it is increasingly regarded as a multi-organ disease, including nervous system manifestations. An updated version of the new guidelines for the diagnosis and treatment of coronary pneumonia issued by the National Health Council of China (China NHCotPsRo, 2020) points out that histopathological samples from some COVID-19 patients showed that SARS-CoV-2 invasion involved multiple organs, including lung, spleen and hilar lymph nodes, heart and blood vessels, liver and gallbladder, kidney, brain, adrenal gland, esophagus, stomach, and intestine. In particular, edema, and partial neuronal degeneration were observed in brain tissue (China NHCotPsRo, 2020) Neurodegenerative changes observed in cells infected with SARS-CoV-2, including cell death and hyperphosphorylation, as well as dislocation of Tau

protein, these changes can be observed in conditions such as hyperthyroidism or Alzheimer's disease (4) However, the specific mechanism of neurodegenerative changes induced by SARS-CoV-2 remains to be further studied in the future. The central nervous system may serve as a reservoir for SARS-CoV-2, some groups observed that viral particles gradually accumulated within the neuronal cells of the brain organs from 6 to 72 h after SARS-CoV-2 infection, indicating that the virus replicated actively and effectively in the neuronal cells within the first few days of infection. However, some groups observed that viral infection did not replicate effectively in the first few days and suggested that the central nervous system might serve as a long-term reservoir of the virus (4). More and more evidence shows that SARS-CoV-2 has a potential neuroinvasive effect (5). It is estimated that more than 1/3 of COVID-19 patients will have nervous system symptoms, including central nervous system symptoms (dizziness, headache, disturbance of consciousness, acute cerebrovascular disease, ataxia, epilepsy). Peripheral nervous system symptoms (taste disorder, olfactory disorder, visual impairment, neuralgia) (6).

So far, although the epidemic in China has been effectively controlled, the COVID-19 epidemic is still very serious worldwide. According to statistics, there are more than 54 million confirmed cases worldwide, and more than 15 million existing confirmed cases. In this global public health emergency, we are still facing a very serious situation. In the face of SARS-CoV-2, understanding the impact of SARS-CoV-2 infection on the nervous system and its invasion mechanism is of great significance for the reasonable treatment of patients. In this paper, the effects of SARS-CoV-2 on nervous system are systematically analyzed and reviewed (**Figure 1**).

THREE MECHANISMS OF SARS-COV-2 INVADING THE NERVOUS SYSTEM

The central nervous system is protected by a highly complex brain barrier system, which is the first line of defense against virus invasion. The brain barrier is composed of the blood-brain barrier, blood-cerebrospinal fluid barrier, and brain-cerebrospinal fluid barrier. The blood-brain barrier has a maximum surface area that can be used for communication between the brain and blood. It consists of cerebral capillary endothelial cells, extracellular matrix, and astrocyte podocytes. The blood-cerebrospinal fluid barrier is located in the choroid plexus of the ventricle of the brain. The epithelial cells of the choroid plexus are mainly responsible for the barrier function of the blood-cerebrospinal fluid barrier. The blood-brain barrier and the blood-cerebrospinal fluid barrier can inhibit paracellular diffusion, protect the central nervous system from the influence of the constantly changing blood environment, infections and toxins, and are crucial for maintaining the homeostasis of the central nervous system (7–9).

To cause a central nervous system infection, the virus must first successfully cross the protective barrier of the brain. Crossing the blood-brain barrier or the blood-cerebrospinal fluid barrier requires special adaptation of the virus. Despite this, SARS-COV-2 can still enter the nervous system rapidly in some special ways after infection (10–13).

The following three ways may be the main ways for SARS-COV-2 to invade the central nervous system: (1) SARS-CoV-2 directly infects vascular endothelial cells by means of angiotensin converting enzyme 2, thus directly crosses the blood-brain barrier. (2) SARS-CoV-2 enters the central nervous system through synaptic connections via the olfactory nerve. (3) SARS-CoV-2 Induces Inflammation to destroy the Brain Barrier System and enter the central nervous system.

SARS-CoV-2 Directly Infects Vascular Endothelial Cells and Crosses the Blood-Brain Barrier

Studies have shown that similar to SARS-COV, SARS-COV-2 can use ACE2 to enter the cell interior (14, 15). The spike protein (S protein) in SARS-CoV-2 is a trimer projecting from the viral membrane and contains a receptor binding domain (RBD) in each monomer. Through it, the viral protein can directly interact with ACE2 receptors on the surface of many host cells. S protein is the main tool for SARS-CoV-2 to bind to the ACE2 receptor (infect cells), and it can strongly bind to ACE2 (16). Therefore, the S protein may be used as a key target for the treatment of COVID-19 and vaccine development.

In addition, host cell protease also plays an important role in virus entry and infect cells (17, 18). The S1 subunit of S protein on the surface of SARS-CoV-2 first binds to neuron ACE2 receptor and adheres to the surface of target cells; then, the S2 subunit of the virus is activated by the serine protease TMPRSS2 of host cells, and the virus can enter the nerve cells (19). Therefore, TMPRSS2 activity is very important for the infection and transmission of SARS-CoV-2 in host cells and is important pathogenesis of neurological complications (20–22).

Although the S protein activity of SARS-CoV-2 is weaker than that of previous coronaviruses (23). However, the binding affinity of SARS-CoV-2 S protein to ACE2 is 10–20 times higher than that of SARS-CoV S protein to ACE2 (15). This is due to the fact that the receptor binding domain of SARS-CoV2 is different from that of previous coronaviruses on several key amino acid residues (17). It contains 4 positively charged residues and five negatively charged residues, so SARS-CoV-2 S protein is slightly more positively charged than SARS-CoV S protein. Although the charge difference between the S proteins of SARS-CoV-2 and SARS-CoV is quite small, this effect can be amplified by the presence of a large number of S proteins on the virus particles (16).

At the interface between the SARS-CoV-2 RBD and the cellular ACE2 receptor, the difference in the amino acid content of the S protein can lead to a more specific interaction between the S protein and the host cell receptor. Therefore, compared with SARS-CoV, SARS-CoV-2 is more likely to establish interactions with different targets in the human body through non-specific and specific interactions. All of these can ultimately increase the ability of SARS-CoV-2 to enter human

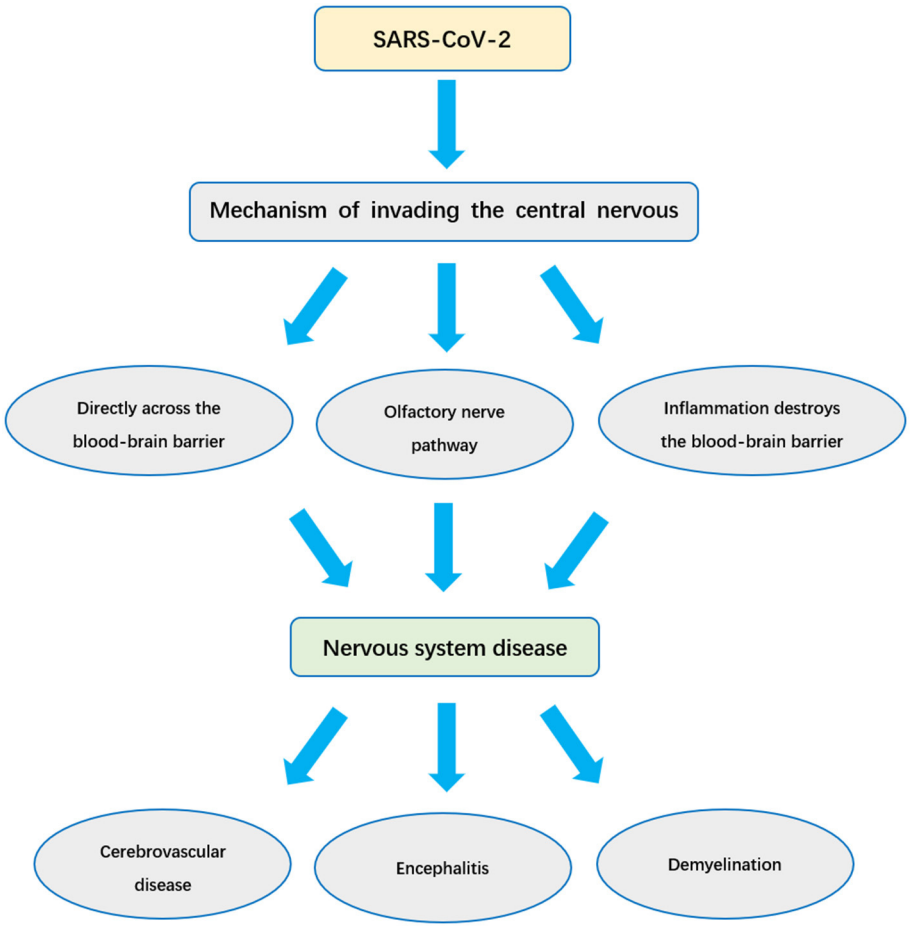

FIGURE 1 | The mechanism of SARS-CoV-2 causing nervous system infection and nervous system diseases.

cells (16), which may explain why COVID-19 has stronger pathogenicity, transmissibility, and greater global influence (17).

This charge difference between SARS-CoV-2 and SARS-CoV S proteins can have a significant impact on endothelial cell adhesion and crossing the blood-brain barrier (16) so that SARS-CoV-2 infected with vascular endothelial cells of the blood-brain barrier has a higher efficiency of reaching the brain and can cross the blood-brain barrier directly into the central nervous system (16).

Through the study of the distribution of ACE2 in the nervous system, it was found that ACE2 was expressed in different brain regions, such as subfornical organs, nucleus tractus solitarius and ventrolateral region of the medullary head, as well as regions such as motor cortex and raphe. According to spatial distribution analysis, ACE2 was also expressed in the substantia nigra (24–26). Due to the existence of ACE2 receptors in glial cells and neurons, it has become a potential target for SARS-CoV-2 causing brain injury and neurological symptoms (27).

In addition, ACE2 is widely attached to the extracellular surfaces of the lungs, arteries, heart, kidneys, intestines, and brain (cell membranes) (16, 28, 29). In addition to respiratory system involvement, SARS-COV-2 infection may also cause multi-organ dysfunction. Despite the predominance of respiratory symptoms, there is post-infection damage to the myocardium, kidneys, intestines, and liver, perhaps ACE2 provides a crucial link between immunity, inflammation, and cardiovascular disease (17).

The autopsy of novel coronavirus pneumonia showed brain edema and partial degeneration of neurons (30). However, there is not enough autopsy evidence to prove that SARS-CoV-2 exists in neurons and glial cells. Further studies are needed to prove this.

Olfactory Nerve Pathway

Among the 12 pairs of cranial nerves, the olfactory nerve is not a real nerve but a conduction bundle of the central nervous system. It can directly contact the brain (31), coupled with the special location and structure of the olfactory nerve itself, we speculate that perhaps in the mechanism of SARS-CoV-2 invading the central nervous system, the virus entering the central nervous system through the olfactory nerve is also one of the main ways (32, 33). And the olfactory nerve provides this way to enter

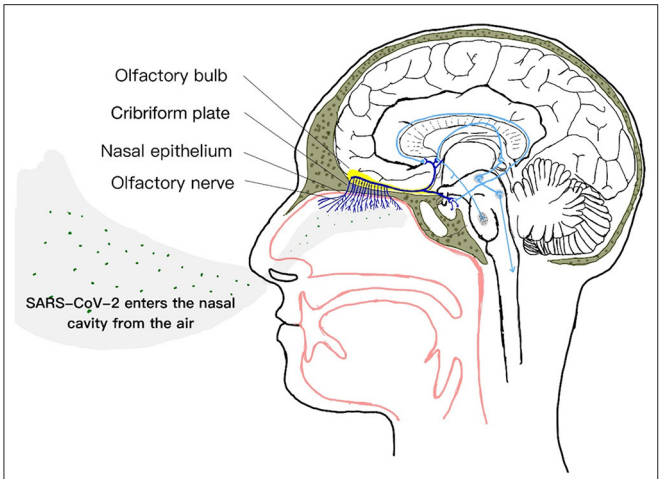

FIGURE 2 | SARS-CoV-2 invades the brain through the olfactory nerve.

the central nervous system, successfully bypassing the blood-brain barrier (34–36), effectively making it a channel between nasal epithelium and central nervous system. In the early stage of respiratory transmission, SARS-CoV-2 can enter the brain through the olfactory nerve (37) (**Figure 2**).

In the nasal cavity, the special olfactory neuroepithelium has an apical surface mainly composed of support cells, supporting the dendritic processes of neurons containing olfactory cilia (38). The dendrites of olfactory neurons are directly exposed in the airway of the nose (10). Although the olfactory system is very effective in controlling viral nerve invasion under normal conditions (39), however, data from several studies indicate that nasal respiratory epithelial cells express ACE2 and TMPRSS2 (31, 38, 40–42). As mentioned above, SARS-CoV-2 can enter the cell with the help of ACE2 and the serine protease TMPRSS2 of the host cell.

The expression of ACE2 and TMPRSS2 located in the olfactory neuroepithelium indicates a potential entry point for SARS-CoV-2 into the central nervous system (31), so that the virus can invade the olfactory nerve. The retrograde or anterograde transport of neurons is realized by kinesin and kinesin (37, 43, 44). During the infection process, SARS-CoV-2 uses the olfactory nerve to pass through the cribriform plate of the ethmoid bone to cause brain invasion (45, 46), which results in a rapid, transneuronal spread of the SARS-CoV-2 to relevant regions of the brain, which in turn interacts with ACE2 expressed on the surface of brain neurons (47–49). This cell tropism may be the reason why SARS-CoV-2 is highly infectious and related to olfactory dysfunction (38).

Because SARS-CoV-2 can directly act on nasal respiratory epithelial cells in the nasal cavity, olfactory dysfunction often occurs in the early stages of the disease. In mild to moderate cases, sudden loss of smell and taste is considered to be the strongest predictive symptom of early infection with the SARS-CoV-2 virus (50), and this mild, non-specific symptom can become asymptomatic. Or the only manifestation of a mildly infected person. The report of symptoms related to anosmia should be regarded as a sign of SARS-CoV-2 infection and a sign of COVID-19 (51, 52). If it is accompanied by transient brain edema and other neurological diseases, the first consideration should be the neuroinvasiveness of SARS-CoV-2 (38).

The olfactory dysfunction caused by SARS-CoV-2 may be explained by the following four mechanisms: ① Viral infections of the nasal mucosa can trigger inflammation of the nasal tissue, including the olfactory mucosa, thereby creating an obstructive barrier between odor chemicals and olfactory receptors; ② direct damage to olfactory receptors could prevent odor signals from being transmitted; ③ the virus, being neurotropic, can attack the area of the brain responsible for smell along the path of the olfactory nerve; ④ Loss of sense of smell may actually be a sequela of brain edema and partial neurodegeneration. Any or all of these four mechanisms may lead to loss of sense of smell in COVID-19 (53). Therefore, exploring the relationship between early loss of sense of smell and a long-term sense of smell has special clinical and prognostic value (54).

Although there have been many studies proving that the olfactory neuroepithelium expresses ACE2 and TMPRSS2, there are no sufficient and strong studies to prove that ACE2, TMPRSS2, and SARS-CoV-2 are widely present in the transmission bundle from the olfactory bulb to the CNS. In the future, further research is needed to resolve these inconsistencies, and more autopsy reports are needed to prove the presence of SARS-CoV-2 in the olfactory tract. Finally, the distribution of SARS-CoV-2 infected cells in human olfactory nerve conduction tracts was determined.

Coronavirus Induces Inflammatory Responses to Disrupt the Blood-Brain Barrier System

The destruction of the blood-brain barrier through inflammation is also one of the ways for SARS-CoV-2 to enter the central nervous system. Infection with SARS-CoV-2 can destroy the blood-brain barrier by producing a large number of inflammatory mediators in the following three ways.

SARS-CoV-2 Directly Induces the Release of Cytokines by Immune Cells

When SARS-CoV-2 enters the human body, the virus activates immune cells, such as monocytes/macrophages, neutrophils, T cells, natural killer cells, and mast cells. The activated immune cells kill the virus by synthesizing and releasing cytokines (55–59). These cytokines mainly include interferon (IFN), interleukin (IL), chemokine, and tumor necrosis factor (TNF) (60). Some of their functions are to promote the inflammatory response and some to inhibit the inflammatory response. These cytokines are maintained in a balanced state in the healthy human body. Among them, pro-inflammatory factors can activate and recruit other immune cells, immune cells can secrete more cytokines, activate, and recruit more immune cells, thus forming a positive feedback cycle (55).

SARS-CoV-2 can cause immune cells to produce excessive immunity, cytokines are uncontrolled, a large number of

cytokines are released, amplifying positive feedback, breaking the balance, marking an uncontrolled and dysfunctional immune response, leading to systemic inflammation, further aggravating the inflammatory response and increasing the severity of the disease. Although this excessive immune response can kill the virus, it can also cause some additional damage. Among them, the blood vessels suffered the most damage. Cytokine storms make the vessel wall more easily penetrated, and the blood-brain barrier is disrupted (61), causing neocoronavirus to enter the brain, inducing corresponding central nervous system symptoms.

Activation of Glial Cells Releases Proinflammatory Cytokines

Some neurotropic viruses can induce the pro-inflammatory state of glial cells and make them secrete cytokines (62). As mentioned earlier, glial cells express ACE2, We speculate that SARS-CoV-2, which enters the central nervous system through olfactory nerve, blood-derived, and other pathways, may also activate glial cells and induce a pro-inflammatory state (37, 63). In addition, experiments have confirmed that glial cells secrete a large number of inflammatory factors after being infected with coronavirus, such as interleukin-6, interleukin-12, interleukin-15, and tumor necrosis factor α, etc. (52). These cytokines can also damage the blood-brain barrier, further promote coronavirus to enter the brain, causing symptoms of central nervous system disease.

So far, the autopsy report on the glial cells of SARS-CoV-2 patients is still insufficient. We propose this possible mechanism based on the existing literature. In future studies, more autopsy reports are needed to prove whether SARS-CoV-2 infects glial cells through ACE2, or whether there are other receptors that can bind to SARS-CoV-2 in glial cells.

Vascular Endothelial Growth Factor Induces Inflammation

Vascular endothelial growth factor (VEGF) is widely distributed in the central nervous system (64). In addition, the combination of SARS-CoV-2 and ACE2 can activate the renin-angiotensin system which is involved in inflammation response, and then further promote the synthesis of VEGF through the binding of angiotensin II (AngII) and angiotensin II type 1 receptor (AT1R). In fact, in brain diseases, VEGF not only promotes angiogenesis but also destroys the blood-brain barrier by inducing inflammatory responses (64, 65).

Inflammation is the precursor and companion of blood vessel formation, manifested by increased vascular permeability and recruitment of inflammatory cells (65). ACE2 is a key enzyme that catalyzes Ang I and Ang II to Ang 1-9 and Ang 1-7, respectively (66). When SARS-CoV-2 attacks ACE2, the inactivation of this enzyme can lead to the enhancement of the ACE/AngII/AT1R axis signal, followed by excessive AngII production. In the brain infected with SARS-CoV-2, the cumulative feedback of Ang II promoted the increase of ACE2. VEGF in turn reversely enhances Ang II, thus forming a vicious cycle in the release of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-8, and ICAM-1 (64, 67). In addition, among these cytokines, interleukin-6 (IL-6) is an important member of pro-inflammatory cytokines, which is positively correlated with the severity of COVID-19 symptoms. It may be used as one of the indicators of the severity of COVID-19 (37, 58) (**Figure 3**).

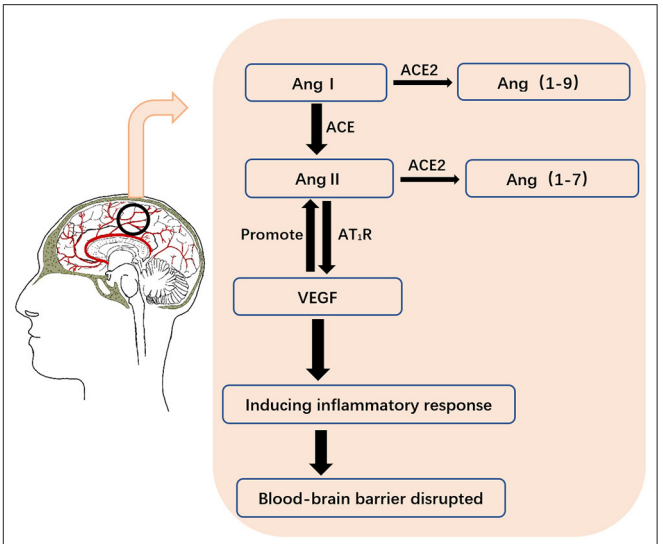

FIGURE 3 | Inflammation induced by VEGF destroys the blood-brain barrier.

CLINICAL MANIFESTATIONS OF NERVOUS SYSTEM DISEASES CAUSED BY SARS-COV-2

The neurological diseases possibly caused by SARS-CoV-2 can be divided into three major categories: ① Nervous system consequences of related lung and systemic diseases, such as cerebrovascular disease; ② The virus directly invades the central nervous system, such as encephalitis; ③ Potential immune-mediated complications after infection, such as Guillain-Barre syndrome (GBS) and other types of demyelinating diseases.

Cerebrovascular disease refers to a group of diseases that occur in the blood vessels of the brain and cause brain tissue damage due to the disturbance of intracranial blood circulation.

Although the main manifestation of patients infected with SARS-CoV-2 is a lung disease, there are also cerebrovascular diseases (68). When the virus proliferates in lung tissue, it causes diffuse alveolar and interstitial inflammatory exudates, and even hyaline membrane formation. This will lead to abnormal alveolar gas exchange, hypoxia of the central nervous system, an increase of anaerobic metabolism of brain tissue, induction of intercellular edema, obstruction of cerebral blood flow, causing ischemia of cerebral circulation, with the increase of intracranial pressure, the brain function deteriorated gradually. It may even induce the occurrence of acute cerebrovascular disease, such as acute ischemic stroke (3, 37).

On the other hand, cerebral hemorrhage caused by elevated blood pressure may also be the result of the expression of ACE2 receptor (69, 70). ACE2 is one of the cardio-cerebrovascular

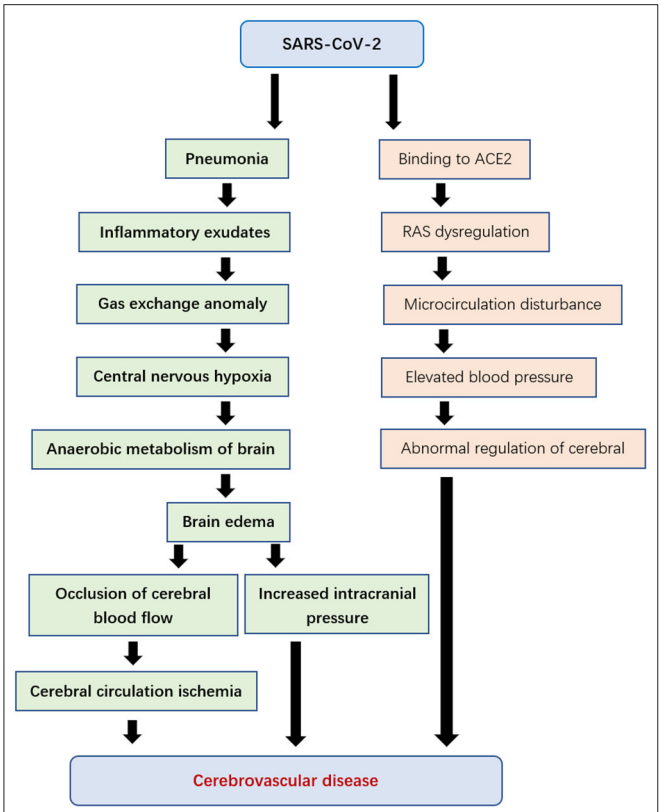

FIGURE 4 | Cerebrovascular disease caused by SARS-CoV-2.

protective factors, which plays an important role in regulating blood pressure and anti-atherosclerosis mechanism (71). SARS-CoV-2 may cause an imbalance of the renin-angiotensin system (RAS) by acting on the ACE2 receptor, leading to microcirculation disorder, affects cerebral blood flow regulation (72, 73), leading to an abnormal increase of blood pressure, and increases the risk of cerebral hemorrhage and ischemic stroke (**Figure 4**).

Encephalitis refers to the inflammatory lesions of brain parenchyma caused by pathogens, including neuronal damage and nerve tissue damage. It is characterized by acute episodes and common symptoms include headache, fever, nausea, vomiting, fatigue, convulsions, and disturbance of consciousness (37). At present, the presence of viral encephalitis in many patients infected with SARS-CoV-2 further speculated the existence of this neurological complication (74–77).

The treatment team of Beijing Ditan Hospital confirmed the presence of SARS-CoV-2 in the cerebrospinal fluid of patients infected with SARS-CoV-2 through genome sequencing, thereby clinically confirming viral encephalitis (37). This provides a solid foundation for SARS-CoV-2 to cause encephalitis. However, no signs of inflammation were found in brain tissue images of patients infected with SARS-CoV-2 (78). We guess that SARS-CoV-2 will produce virion vacuoles like MERS-CoV and SARS-CoV, if this hypothesis holds, then vacuolation may be a defense against infection. This problem needs further study and more pathological cases to clarify.

In patients with SARS-CoV-2, SARS-CoV-2 can stimulate immune cells to produce a variety of cytokines, resulting in an immune response process that causes nerve demyelination (79). For example, SARS-CoV-2 can cause Guillain-Barré syndrome (80, 81). Guillain-Barre syndrome, also known as acute idiopathic polyneuritis or symmetrical polyradiculitis, is an acute polyradiculoneuropathy (82). Clinical manifestations are progressive ascending symmetrical paralysis, quadriplegia, and varying degrees of sensory disorders (83). The exact pathogenesis of nerve demyelination caused by SARS-CoV-2 is not clear and remains to be further studied.

FUTURE PROSPECTS

COVID-19 is a challenge to the world. At present, there is sufficient evidence that SARS-CoV-2 can invade the central nervous system and induce nervous system diseases. The possible pathways of SARS-CoV-2 invasion into the central nervous system include direct invasion of infected endothelial cells, invasion through the olfactory nerve, and invasion by inducing inflammation to destroy the brain barrier system. These pathways are all related to ACE2 receptors, so the relationship between SARS-CoV-2 and ACE2 should be further studied so as to take better measures to protect the central nervous system in patients with COVID-19. In fact, human respiratory viruses may also enter the central nervous system through other different ways, including the trigeminal nerve, cerebrospinal fluid, lymphatic system, and so on. The three mechanisms discussed in this article may be applicable to SARS-CoV-2, but we must be alert to other invasion mechanisms of SARS-CoV-2 until there is conclusive pathological evidence. In addition, it can be inferred from the existing data that encephalitis, cerebrovascular disease, nerve demyelination symptoms, and olfactory changes in COVID-19 patients are all likely to be related to SARS-CoV2 infection. These symptoms can be used as potential indicators of patient severity and prognosis. Understanding these knowledge is very important for the prevention and treatment of central nervous system symptoms and the rehabilitation of COVID-19 patients.

In addition, recent studies point out that other proteins expressed in nerve cells, such as Nrp1, may also become receptors for SARS-CoV-2. Future work needs to verify whether SARS-CoV-2 can invade the central nervous system using alternative receptors.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Infection Mechanism of SARS-COV-2 and Its Implication on the Nervous System

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In late December 2019, multiple atypical pneumonia cases resulted in severe acute respiratory syndrome caused by a pathogen identified as a novel coronavirus SARS-CoV-2. The most common coronavirus disease 2019 (COVID-19) symptoms are pneumonia, fever, dry cough, and fatigue. However, some neurological complications following SARS-CoV-2 infection include confusion, cerebrovascular diseases, ataxia, hypogeusia, hyposmia, neuralgia, and seizures. Indeed, a growing literature demonstrates that neurotropism is a common feature of coronaviruses; therefore, the infection mechanisms already described in other coronaviruses may also be applicable for SARS-CoV-2. Understanding the underlying pathogenetic mechanisms in the nervous system infection and the neurological involvement is essential to assess possible long-term neurological alteration of COVID-19. Here, we provide an overview of associated literature regarding possible routes of COVID-19 neuroinvasion, such as the trans-synapse-connected route in the olfactory pathway and peripheral nerve terminals and its neurological implications in the central nervous system.

Keywords: severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, central nervous system, neurotropism, neuroinvasion, neurological alterations, long-term sequelae

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a positive single-stranded RNA coronavirus responsible for the severe pneumonia coronavirus disease 2019 (COVID-19), as the World Health Organization named in February 2020. As of December 2020, the World Health Organization reported more than 71 million confirmed cases and more than 1.6 million deaths worldwide. SARS-CoV-2 is the seventh member of the coronavirus family that infect humans. Among them, NL63-CoV, HKU1-CoV, 229E-CoV, and OC43-CoV, typically cause common cold symptoms, while SARS-CoV, MERS-CoV, and now the SARS-CoV-2 are responsible for the SARS pandemic in 2002 and 2003, MERS in 2012 and the current COVID-19 pandemic, respectively (1).

SARS-CoV-2 is a betacoronavirus that shares almost 80% sequence identity with SARS-CoV and 50% sequence identity with MERS-CoV (2). Similar to SARS-CoV, SARS-CoV-2 binds to the enzymatic domain of the angiotensin-converting enzyme 2 (ACE-2) receptor exposed on the surface of several cell types, including alveolar cells, intestinal epithelial cells, endothelial cells, kidney cells, monocytes/macrophages, as well as neuroepithelial cells and neurons (3, 4). After spike (S) protein binding to ACE-2 receptor, a subsequent cleavage by transmembrane protease serine 2, cathepsin, or furin, probably induces the endocytosis and translocation of SARS-CoV-2 into endosomes (5, 6), or a direct viral envelope fusion with host cell membrane for cell entry (7). Interestingly, SARS-CoV-2 *in silico* modeling shows a highly structural sequence similarity of 74-79% with SARS-CoV; however, SARS-CoV-2 exhibits some differences associated with a higher binding affinity ACE-2 receptor (8). It has been suggested that the SARS-CoV-2 S protein is slightly more positively charged than SARS-CoV, and the ACE-2 binding interface has a negative electrostatic potential. This electrostatic difference allows a stronger interaction between these two proteins (9, 10). Therefore, this increased binding affinity may promote high virulence of SARS-CoV-2 (11, 12).

COVID-19 symptoms include mild-to-medium fever, cough, diarrhea, fatigue, and dyspnea, progressing to acute respiratory distress syndrome (13). In addition to systemic symptoms, patients also can experience neurological affectation, including headache, dizziness, hypogeusia, hyposmia, myalgia, ataxia, and seizures (14, 15). There are reports of brain edema, partial neurodegeneration, even encephalitis in severe cases of COVID-19 (13, 16, 17). To date, there is no described direct mechanism of SARS-CoV-2 neuroinvasiveness (18). However, it is known that coronaviruses are not always limited to the respiratory system, but they can reach the central nervous system (CNS), inducing neurological impairments (19). This neuroinvasive capacity has been well demonstrated for most beta coronaviruses, including SARS-CoV (20), MERS-CoV (21), 229E-CoV (22), OC43-CoV (23), and HEV (24). Although the SARS-CoV-2 neuroinvasion mechanism remains unknown, considering the high similar viral sequence and infection pathways reported from other betacoronavirus (i.e., SARS-CoV), a similar pathogenic process may be applicable for SARS-CoV-2 (19).

NEUROINVASIVENESS PATHWAYS

Despite being a highly protected system with multilayer barriers, the CNS can be reached by some viruses that can infect neurons and glial cells (25). A significant evidence body demonstrates that coronaviruses may reach the CNS inducing neurovirulence (26). Nonetheless, coronaviruses' determinant route to infect the CNS has not been fully described (27). Usually, the viral infection begins in the peripheral tissues with a subsequent spreading to peripheral nerves and, finally, the CNS (28). This process may explain neurological lesions' presence, particularly demyelination (29). Moreover, bypasses peripheral barriers, such as the blood-brain barrier (BBB), would be another mechanism (28). In the case of SARS-CoV-2 infection, hyposmia is denoted as one frequent symptom (16), indicating an olfactory dysfunction probably due to neuronal and non-neuronal cells infection in the olfactory system and the involvement of cranial nerves (30, 31). In this way, the CNS invasion, specifically the respiratory center in the medulla and pons, may promote acute respiratory distress in patients with COVID-19 (32).

Although experimental evidence regarding SARS-CoV-2 neuroinvasiveness is still lacking (33), *post-mortem* studies evidenced the virus's presence in the brain microvasculature, cerebrospinal fluid, even neurons (4, 26, 34). Also, studies demonstrated that the ACE-2 receptor is expressed on neuron and glial cells of structures such as the olfactory epithelium, cortex, striatum, substantia nigra, and the brain stem (35), supporting the SARS-CoV-2 potential to infect cells throughout the CNS. Therefore, there are suggested mechanisms for coronaviruses neuroinvasion (**Figure 1**), including the neuronal anterograde and retrograde spreading in the transcribial route (8, 16) and (19, 33) the hematogenous route (36). The neuronal retrograde/anterograde transport and the trans-synaptic transfer are supported by *in vitro* studies where the SARS-CoV-2 is detected within neuronal soma and neurites using human brain organoids (31, 37).

TRANSCRIBIAL ROUTE AND NEURONAL TRANSPORT DISSEMINATION

Growing evidence shows that some coronaviruses first invade peripheral nerve terminals, then are anterograde/retrograde spread throughout the CNS *via* synapses (19, 24, 38), a well-documented neuroinvasive route for coronaviruses such as HEV67 (24, 39) and OC43-CoV (23). Among the peripheral nerves, the olfactory nerve is considered one of the strongest candidates for SARS-CoV-2 dissemination into CNS because of its close localization to olfactory epithelium (27). The olfactory epithelium cells highly express the ACE-2 receptor and the TMPRSS2, necessary for viral binding, replication, and accumulation (40). Recent studies found that neuropilins also have an essential role in cell infectivity. Interestingly, while neuropilin-1 alone promotes SARS-CoV-2 entry and infection, its coexpression with ACE-2 and TMPRSS2 markedly potentiates this effect (41). Similar to ACE-2 and TMPRSS2, the neuropilins are expressed abundantly in the respiratory and olfactory epithelium, becoming the nasal cavity epithelium, a key infection site for CNS infection (8, 41). A recent study presented evidence of SARS-CoV-2 entrance to CNS by crossing the neural-mucosal interface with subsequent penetration of defined neuroanatomical areas receiving olfactory tract projections, including the primary respiratory and cardiovascular control center in the medulla (42). Here, SARS-CoV-2 RNA and characteristic CoV substructures were detectable in the olfactory epithelium and olfactory mucus cells. A subsequent colocalization study within the olfactory mucosa using neuronal markers revealed a perinuclear S protein immunoreactivity in $TuJ1^+$, $NF200^+$, and OMP^+ neural cells (42). In this way, SARS-CoV-2 may penetrate the CNS throughout the olfactory receptor cells that pass the cribriform plate contacting second-order neurons of the spherical glomeruli (32, 43). This passage of the olfactory nerve

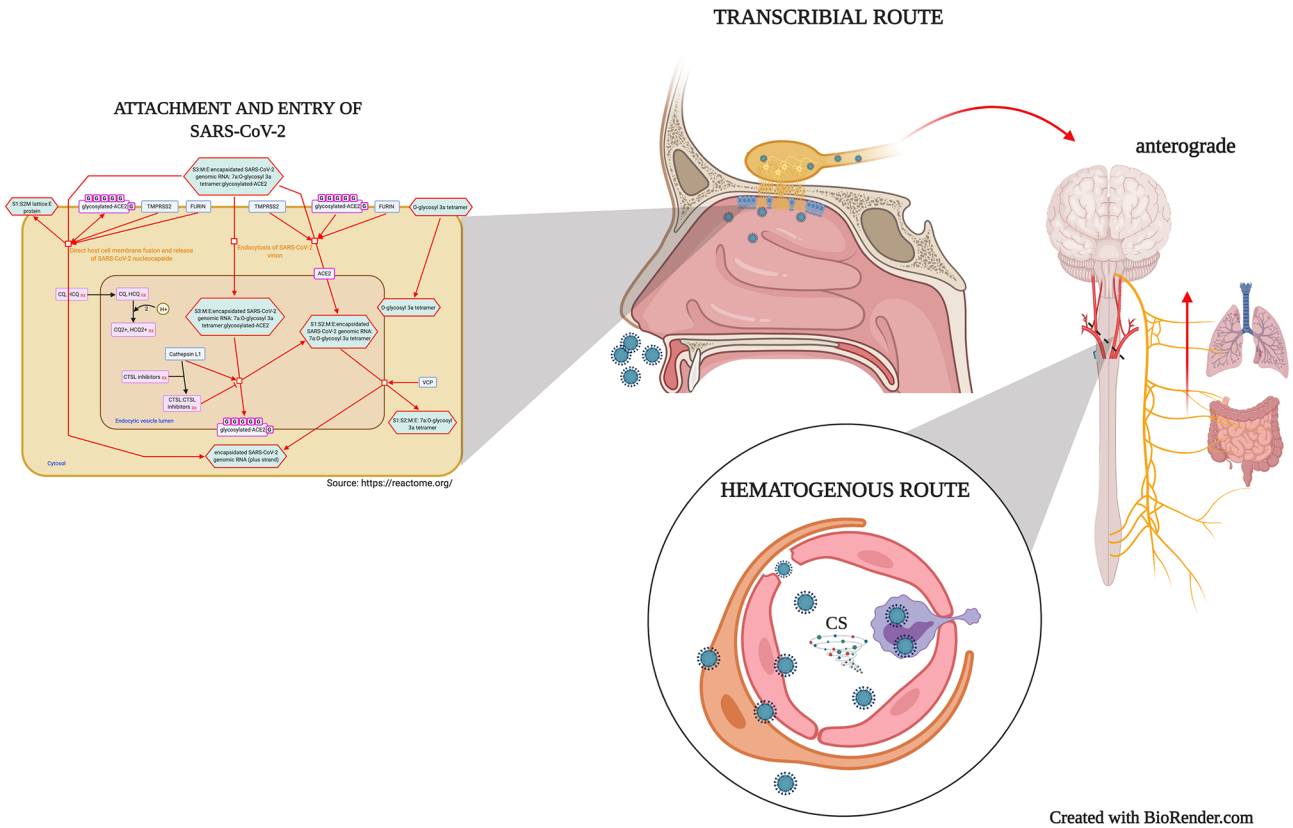

FIGURE 1 | Mechanisms of virion attachment/infection and possibles routes of SARS-CoV-2 neuroinvasiveness. SARS-CoV-2 spike protein interaction with the ACE-2 receptor may promote a direct host cell membrane fusion and the virion nucleocapsid release or induce a Furin/TMPRSS2-mediated endocytosis. Once in the epithelial cells, SARS-CoV-2 spreads to CNS through peripheral neurons in the olfactory epithelium and the second-order neurons along the olfactory nerve (Transcribial route). Similarly, the virus may use different peripheral nerves such as the vagus nerve, which afferences from the lungs and gut reach the brainstem. In the hematogenous route, the viremia induces the infection and viral transcytosis across vascular endothelial cells, as well as leukocytes infection and mobilization towards the BBB, and in several cases, the BBB disruption. CS, Cytokine storm.

via the cribriform plate of the ethmoidal bone was termed transcribial route.

During HEV67 infection, the first coronavirus reported invading porcine brains; the nasal mucosa, lung, and small intestine are first infected. Then is spread to medullary neurons *via* peripheral nerves using the clathrin-mediated endocytic/exocytic system (24, 44). In OC43-CoV infection, a human coronavirus sharing more than 91% homology with HEV67 (45), once in the olfactory bulb, this coronavirus can disseminate to the cortex and other regions including the hippocampus and spinal cord (23, 46). In MHV-CoV infection, the MHV-CoV also accesses the CNS through the olfactory nerve and disseminates it to the limbic system and brainstem (7, 47). The cortical areas with MHV-CoV persistence are associated with demyelinating lesions due to the trafficking and accumulation of T cells and macrophages that participate in myelin destruction (48).

In the intranasal administration of SARS-CoV and MERS-CoV into transgenic mice, the viral CNS invasion is possible through the transcribial route, gaining direct access to the olfactory bulb, and then spreading to the thalamus and brainstem (21, 49). However, the exact mechanism of early CNS access is still unclear. In this context, it has been suggested that SARS-CoV-2 might spread from the olfactory epithelium to the olfactory bulb towards the olfactory nerve, employing the endocytosis/exocytosis system for transsynaptic transfer (34, 50).

In addition to the transcribial route and the olfactory nerve, the virus may use other peripheral nerves such as the vagus nerve, which lungs and gut afferents reach the brainstem (32, 51). The gut-brain axis is a key component involved in disorders that affect the CNS (52). Interestingly, SARS-CoV-2 was detected in COVID-19 patient feces (53). A recent *in vitro* study demonstrated the SARS-CoV-2 capacity to infect human intestinal epithelium (54). Moreover, it has been reported the anterograde and retrograde viral transmission from duodenal cells to brainstem neurons (55). Therefore, it is possible that upon enterocyte SARS-CoV-2 infection, a further transmission to glial and neuronal cells within the enteric nervous system could reach the CNS *via* the vagus nerve (27, 51). In this line, different sets of data demonstrated that initial lung SARS-CoV infection leads to a secondary viral spreading to the brain, particularly thalamus and brainstem regions such as medullary nuclei of the dorsal vagal complex (56). Similarly, Matsuda et al., 2004 reported the influenza A virus spreading

from the respiratory tract to the vagal ganglia through the vagus nerve, suggesting a possible transmission from the respiratory mucosa to the nucleus of the solitary tract and the nucleus ambiguous in the brain stem by vagal dissemination (57). However, evidence regarding the enteric nervous system and the SARS-CoV-2 vagus nerve dissemination is almost null, and further research is required.

HEMATOGENOUS ROUTE

The infection and damage of cells of epithelial barriers allow the virus entrance to the bloodstream and lymphatic system, spreading to multiple organs, including the brain (50). Specifically, the BBB is one of the most frequent viral entry routes to the CNS (58). In this way, there are two possible mechanisms for SARS-CoV-2 spreading, which involves the circulation of viral particles into the bloodstream (25, 33): the infection and viral transcytosis across vascular endothelial cells, and the leukocytes infection and mobilization towards the BBB, a well-described mechanism termed Trojan horse (59).

In the first scenario, the virus in peripheral circulation and the sluggish blood flow within the microvasculature appear to be responsible for the binding enhancement of S viral protein and ACE-2 receptor in the capillary endothelium, promoting the viral transport across the basolateral membrane (8, 60). A structural analysis reported that viral-like particles were actively budding across brain capillary endothelial cells, suggesting the hematogenous route as the most probable pathway for SARS-CoV-2 entry (4). Moreover, an *in vivo* research of MERS-CoV tropism demonstrated a virus's bloodstream circulation followed the endothelial infection (61). Tseng et al. reported a low-level viremia and brain detection of high viral titers two days after intranasal inoculation of SARS-CoV, supporting the virus transmission through the hematogenous route (62). Additionally, SARS-CoV-2 triggers a systemic inflammatory response due to the cytokine storm, with remarkable BBB permeability effects (58, 63). The disruption of this barrier may result in the viral and infected immune cell entry, promoting a further inflammatory response enhancement (64).

In contrast, peripheral lymphocytes and macrophages' possible infection allows their use as dissemination vehicles facilitating the pass across BBB, meninges, and choroid plexus (58, 65). Interestingly, the coronavirus ability to infect leukocytes (mainly monocytes/macrophages) has been reported in the 229E-CoV and SARS-CoV (66, 67), but only in 229E-CoV has reported the activation of chemokine secretion (67). This trojan horse mechanism generally involves the extravasation of infected leukocytes into meninges and the cerebrospinal fluid (68). However, compelling evidence for immune cell infection by SARS-CoV-2 is still unclear so far.

SHORT- AND LONG-TERM NEUROLOGICAL MANIFESTATION OF COVID-19

To date, it has been widely described that a broad spectrum of virus infection can spread through the body and eventually reach and affect the mammalian peripheral nervous system (PNS) and CNS when optimal conditions exist (28). Though coronaviruses are mainly associated with upper and lower respiratory disease, their particular neuroinvasive potential is associated with remarkably neurological affections. The hypoxia promoted by respiratory distress has been associated with disturbed brain metabolism and a subsequent neurological manifestation (36). Notwithstanding, there is still a debate regarding if the neurological manifestations are a primary neurologic symptom or secondary consequences of acute respiratory distress syndrome. The evidence supports the neuroinvasive and neurotropism and possible long-term neurological sequelae of coronaviruses, including SARS-CoV and MERS-CoV (69).

Regardless of neuroinvasiveness mechanisms, emergent data from case reports and clinical studies demonstrated that COVID-19 patients exhibit some CNS and PNS complications, ranging from mild to fatal incomes. The most frequent neurological symptoms are mostly nonspecific in the short-term, such as loss of smell and taste, headache, malaise, myalgias, and dizziness. In contrast, moderate-to-severe cases developed acute cerebrovascular diseases, impaired consciousness, and skeletal muscle injury (70). Indeed, these manifestations can be considered a direct virus effect in the CNS (19, 71).

Unfortunately, recovery of the acute infection does not promise a full viral clearance, and if the infection becomes chronic, it may result in long-term sequelae, including chronic neurological impairment (36). Some studies reported the coronavirus persistence in the CNS and some neurologic and tissular affections (69). In mice surviving acute encephalitis caused by OC43-CoV, the viral RNA could be detected even six months post-infection; in correlation with the viral persistence, these mice also display a reduced locomotor activity, subjacent decreased density of hippocampal layers and gliosis (72). Similarly, the RNA of MHV-CoV is detectable in the brain, even 10–12-month post-infection. Surprisingly, the chronic-CNS demyelination persists as late as 90 days post-infection to scattered demyelinated axons at 16 months after infection (73). Interestingly, case reports support that neurotropic viral infection promotes an exacerbated inflammatory response leading to encephalitis or CNS-target autoimmune (i.e., demyelination) response in COVID-19 patients (29, 58).

Guillain-Barre and Miller-Fisher cases are reported without SARS-CoV-2 detection in cerebrospinal samples, supporting the inflammatory response's role in neurological manifestations (47, 74). Whether the dysregulated immune response remains after the illness resolution, neurological disorders can be developed, including dementia, depression, and anxiety (56, 75). Furthermore, the hypoxia and cerebrovascular diseases reported in COVID-19 patients, particularly encephalitis and stroke, are expected to produce permanent or at least long-term neurological impairments (76).

Although a direct association between SARS-CoV-2 and cognitive impairment is still not correlated, the viral neurotropism and the already reported neurological manifestations support this possible association. A cohort study reported cognitive complaints after SARS-CoV-2 infection, specifically between 10 and 35 days after hospital discharge (71). Here, oxygen therapy and headache were the main variables strongly related to poor performance in neuropsychological tests, indicating cognitive deficits related to

attention, memory, and executive function. A case series report evidenced a marked cognitive impairment independent of delirium (4AT score for delirium was unobtrusive) in severely affected patients after 4 to 5 weeks of acute disease onset (47). Another cohort study reported an altered mental status, reflecting neurological and psychiatric, such as encephalopathy, encephalitis, psychosis, and dementia-like syndrome in patients from 23–94 years old; however, cerebrovascular events predominated in older patients (77).

Currently, additional neurological complications reported in other coronaviruses infections may be applicable for SARS-CoV-2. However, the precise and well documentation of neurological symptoms, comprehend the immune response, and the direct impact of brain infection is still needed to better prognosis and prevent long-term effects of SARS-CoV-2 infection.

CONCLUSION

Regardless of the different routes of neuroinvasion, it has been demonstrated that SARS-CoV-2 affects the CNS. As overwhelming proof, there is the isolation of SARS-CoV-2 from cerebrospinal fluid, the colocalization within the olfactory system, the ultrastructural evidence of frontal lobe budding SARS-CoV-2, and the list of neurologic manifestations in the COVID-19 patients. Unfortunately, it is unclear if the neurological symptoms of COVID-19 result from cytokine storm-induced neuroinflammation or some brain areas' infection. Nevertheless, the CNS and immune system involvement might have remarkably neurologic long-term consequences, including the development of neuropsychiatric disorders. Therefore, the awareness of the CNS invasion pathways, the degree of CNS and PNS involvement, and the time course of the viral spreads throughout the nervous system will help comprehend the pathological consequences better and improve the treatment's diagnostic criteria of possible neurological sequelae.

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BRIEF RESEARCH REPORT
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Incidence of Electrographic Seizures in Patients With COVID-19

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Critical illness and sepsis are commonly associated with subclinical seizures. COVID-19 frequently causes severe critical illness, but the incidence of electrographic seizures in patients with COVID-19 has been reported to be low. This retrospective case series assessed the incidence of and risks for electrographic seizures in patients hospitalized with COVID-19 who underwent continuous video electroencephalography monitoring (cvEEG) between March 1st, 2020 and June 30th, 2020. One hundred and twenty-two patients were initially identified who resulted SARS-CoV-2 nasopharyngeal RT-PCR swab positivity with any electroencephalography order placed in the EMR. Seventy-nine patients met study inclusion criteria: age ≥18 years, >1 h of cvEEG monitoring, and positive SARS-CoV-2 nasopharyngeal swab PCR. Six (8%) of the 79 patients suffered electrographic seizures (ES), three of whom suffered non-convulsive status epilepticus. Acute hyperkinetic movements were the most common reason for cvEEG in patients with ES (84%). None of the patients undergoing cvEEG for persistent coma (29% of all patients) had ES. Focal slowing (67 vs. 10%), sporadic interictal epileptiform discharges (EDs; 33 vs. 6%), and periodic/rhythmic EDs (67 vs. 1%) were proportionally more frequent among patients with electrographic seizures than those without these seizures. While 15% of patients without ES had generalized periodic discharges (GPDs) with triphasic morphology on EEG, none of the patients with ES had this pattern. Further study is required to assess the predictive values of these risk factors on electrographic seizure incidence and subsequent outcomes.

Keywords: COVID-19, coronavirus, EEG, seizure, status epilepticus

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) has been associated with several neurological syndromes (1). The existing literature suggests that the incidence of seizures in COVID-19 patients is relatively low. Small case series have described new-onset electrographic seizures (2, 3)—both clinical and subclinical—and status epilepticus (4) in patients with COVID-19, but a larger retrospective review including 304 COVID-19 patients did not find any cases of clinical seizures (5). The patients in the larger study were not monitored with continuous video electroencephalography (cvEEG), so it is possible that these patients had electrographic subclinical seizures. Electrographic subclinical seizures are of particular concern in COVID-19 patient since up to one fifth of patients with COVID-19 are critically ill (6);

subclinical seizure activity can be seen on cvEEG in as many as 19% of critically ill patients in medical intensive care units [ICUs; (7, 8)]. The goal of this study is to describe the results of 257 EEG days across 79 patients as well as to identify the incidence of, and risk factors for, detecting seizures on cvEEG.

METHODS

Subjects

In this retrospective case series, we evaluated patients who were hospitalized at four New York Presbyterian Hospitals—Columbia University Irving Medical Center (CUIMC), Morgan Stanley Children's Hospital, Allen Hospital, or Lawrence Hospital—from March 1st 2020 to June 30th 2020 and fulfilled the following inclusion criteria: (1) age ≥18 years old; (2) a positive SARS-CoV-2 nasopharyngeal Real-Time Reverse Transcriptase PCR swab; and (3) connection to cvEEG during the same hospitalization for COVID-19 for longer than 1 h. Patients were excluded if the cvEEG was performed during a separate hospitalization. Patient information and cvEEG results were obtained from review of the electronic medical record. This study was approved by the institutional review board at CUIMC.

EEG Placement and Monitoring

Electrode placement followed the international 10–20 system. EEG was recorded using a digital video EEG bedside monitoring system (Xltek; Natus Medical) EEGs were interpreted by board-certified clinical neurophysiologists/epileptologists and reported using the American Clinical Neurophysiology Society's Standardized Critical Care EEG Terminology (9).

Procedures and Statistics

Major indications for EEG monitoring were acute hyperkinetic movements, altered mental status, and persistent coma. Acute hyperkinetic movements were either focal or generalized. Altered mental status was defined as acute encephalopathy, either fluctuating, declining or stable, with level of alertness higher than coma. Patients with persistent coma connected to cvEEG were typically those who remained clinically comatose despite the withdrawal of sedating or anesthetic medications. Other indications for EEG monitoring were acute therapeutic temperature management protocol in post-cardiac arrest patients. All cases of acute brain injury were diagnosed clinically or radiographically. The Salzburg criteria were used for classifying non-convulsive status epilepticus, as applied ultimately by fellowship-trained epilepsy faculty at Columbia University Medical Center (10). R version 3.6.1 was used to calculate descriptive statistics, including frequencies, median, interquartile range (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 79 patients met the inclusion criteria comprising 257 days of EEG recording (**Table 1**). The most common indication for cvEEG was hyperkinetic movements, followed by altered mental status and persistent coma. Six (8%) patients had seizures captured on EEG, with 3 of these meeting criteria for non-convulsive status epilepticus. Five of the 6 patients with electrographic seizures were monitored due to concern for seizure in the setting of hyperkinetic movements; the sixth was monitored for fluctuating mental status in the setting of acute intracranial hemorrhage. None of the patients monitored for persistent coma or post-cardiac arrest ($N = 27$) had electrographic seizures (**Table 1**).

Seventy-six percent of the patients in our cohort were admitted primarily for SARS-CoV-2 PCR positivity and/or suspicion with associated symptoms, including acute respiratory distress syndrome, hypoxemia, cough, fever, and gastrointestinal illness. Two of these patients subsequently developed electrographic seizures. The other 4 patients with electrographic seizures were primarily admitted for acute neurologic illness (including refractory seizures and intracranial hemorrhage),

TABLE 1 | Cohort characteristics.

Clinical characteristics	All patients ($n = 79$)	Seizures on EEG ($n = 6$)	No seizures on EEG ($n = 73$)
Median age (IQR)	64 (57, 70)	61 (57, 66)	64 (57, 71)
Number of female patients (%)	25 (31.6)	4 (66.7)	21 (28.8)
Total days of recording	257	47	210
Median days of recording per patient (IQR)	2 (1, 3)	6 (5, 12)	2 (1, 3)
Number of seizure days (percentage of total days of recording)	11 (4.3)	11 (23.4)	x
History of seizure (%)	7 (8.9)	3 (50.0)	4 (5.5)
History of chronic brain disease (%)	24 (30.4)	4 (66.7)	20 (27.4)
ICU admission (%)	64 (81.0)	4 (66.7)	60 (82.2)
Acute brain injury (%)	27 (34.2)	3 (50.0)	24 (32.9)
Anoxic/Hypoxemic injury	4 (5.1)	0 (0)	4 (5.5)
Acute ischemic stroke	11 (13.9)	0 (0)	11 (15.1)
Acute intracranial hemorrhage	12 (15.2)	2 (33.3)	10 (13.7)
PRES	1 (1.3)	0 (0)	1 (1.4)
Worsening vasogenic edema	1 (1.3)	1 (16.7)	0 (0)
Primary reason for admission (%)			
COVID-19 only	60 (75.9)	2 (33.3)	58 (79.5)
Neurologic disease	7 (8.8)	2 (33.3)	5 (6.8)
COVID-19 + Neurologic disease	6 (7.6)	2 (33.3)	4 (5.5)
Other (or Other +COVID-19)	6 (7.6)	0	6 (8.2)
Disposition			
Discharged	56 (70.8)	4 (66.6)	56 (76.7)
Deceased	21(26.5)	2 (33.3)	19 (26.0)
Still in hospital	2 (2.5)	0	2 (2.7)

Acute intracranial hemorrhage includes subarachnoid, lobar, subdural, and subcortical/brainstem hemorrhages.
PRES, Posterior Reversible Encephalopathy Syndrome.
Worsening vasogenic edema occurred in the setting of multifocal metastatic disease.
"Other" reasons for admission: hip fracture (x2) +/– COVID-19; undifferentiated encephalopathy (COVID-19 negative on admission), falls in setting of decompensation, gastrointestinal cancer, and cardiac arrest.

TABLE 2 | EEG: Indication and findings.

Indication for EEG	All patients ($n = 79$)	Seizures on EEG ($n = 6$)	No seizures on EEG ($n = 73$)
Altered mental status	22 (27.8)	1 (16.7)	21 (28.8)
Acute hyperkinetic movements	30 (40.0)	5 (83.3)	25 (34.2)
Persistent coma	23 (29.1)	0	23 (31.5)
Other (including TTM)	4 (5.1)	0	4 (5.5)
Findings on EEG			
Diffuse slowing or attenuation	78 (98.7)	6 (100.0)	72 (98.6)
Focal slowing (not including bitemporal slowing)	11 (13.9)	4 (66.7)	7 (9.6)
Sporadic interictal ED	6 (7.6)	2 (33.3)	4 (5.5)
Periodic/Rhythmic ED	5 (6.3)	4 (66.7)	1 (1.4)
GPDs with triphasic morphology	11 (13.9)	0	11 (15.1)
NCSE	3 (3.8)	3 (50.0)	x

ED, Epileptiform Discharges (not including GPDs with Triphasic Morphology); GPD, Generalized Periodic Discharges; NCSE, Non-Convulsive Status Epilepticus; TTM, Targeted Temperature Management.

three of whom had systemic COVID-19 symptoms or signs on admission (**Table 1**). Interestingly, one of these four patients was coincidentally found to have asymptomatic SARS-CoV-2 PCR positivity on admission with electrographic seizures observed within 24 h of admission.

Twenty-seven patients experienced acute neurological injury (ANI) either upon presentation or during hospitalization, with the most common causes being ischemic and hemorrhagic strokes (13.9 and 15.2% of study population, respectively; see **Table 1**). Only 7 patients in our study had a documented history of prior seizures, 3 of whom were subsequently found to have electrographic seizures. Four of the 6 patients with electrographic seizures in our cohort were discharged from the hospital while the other two suffered severe comorbid, fatal, neurologic disease (**Table 1**).

In patients who had seizures recorded on EEG, the interictal background was more likely to show focal slowing, sporadic interictal epileptiform discharges, and periodic epileptiform discharges. Specifically, four of the six patients with electrographic seizures had lateralized, generalized, or bilateral independent periodic epileptiform discharges. Each of these four patients had underlying brain pathology: multifocal brain metastases with worsening vasogenic edema, acute subarachnoid & intraparenchymal hemorrhage, prior subarachnoid hemorrhage complicated by epilepsy, and prior PRES with associated prior seizures. Of the other two patients with electrographic seizures, one had subdural and subarachnoid hemorrhage. The other had de Novo seizures without clear underlying brain pathology despite neurologic investigation (**Tables 2**, **3**).

Conversely, only one patient without electrographic seizures had periodic or rhythmic epileptiform activity; specifically, this patient developed lateralized periodic discharges over the left posterior head region in the setting of a poorly differentiated intraparenchymal process favored to be reversible posterior leukoencephalopathy over another inflammatory process.

Furthermore, the EEG was less likely to show generalized periodic discharges with triphasic morphology in patients who suffered electrographic seizures (**Table 2**).

DISCUSSION

We detected electrographic seizures in six of 79 patients with COVID-19 that underwent EEG monitoring, three of whom had new-onset seizures. This is a small percentage of patients suffering from electrographic seizures, and most were in the setting of hyperkinetic movements, acute neurologic disease on admission, and prior history of epilepsy or neurologic disease.

Specifically, three of the six patients with seizures on EEG had a prior history of seizures (see **Table 3**). Of these, one (patient B) had epilepsy secondary to brain metastases requiring dual anti-seizure therapy and had a breakthrough seizure 1 month prior to admission. A second patient (patient C) had epilepsy secondary to reversible posterior leukoencephalopathy related to liver transplantation and immunosuppression in 2017, which resulted in seizures and non-convulsive status epilepticus during the current admission. This patient continued to have breakthrough seizures every 3 months on triple therapy. A third patient (patient D) had epilepsy secondary to an ischemic infarct in 2012 and was seizure free on dual therapy prior to admission. All three patients initially had seizures similar to their known semiology, but all progressed to non-convulsive status epilepticus.

Of the remaining three patients (see **Table 3**), two (patients E & F) had concurrent intracranial hemorrhages—including subarachnoid hemorrhage; the latter is a known risk factor for seizures (11, 12). The final patient (patient A) had renal cell carcinoma which was widely metastatic, including metastases to the spine. A non-contrast head CT did not disclose any obvious intracranial abnormalities, but further workup for intracranial disease was not completed before discharge. He was transitioned to palliative care shortly after discharge due to systemic progression of malignancy. Although the patient was mildly hyponatremic on admission, the clinical suspicion upon discharge was that unrecognized intracranial metastatic disease was the most likely etiology for clinical and electrographic seizures (see **Table 3**).

In addition to history of seizures and brain injury/disease, we found that hyperkinetic movements and periodic or rhythmic epileptiform activity on EEG were more common in patients with electrographic seizures; no patients with persistent coma or GPDs with triphasic morphology suffered electrographic seizures. Of our patients connected to cvEEG for persistent coma in COVID-19, none experienced seizures. As such, the diagnostic yield of cvEEG in comatose COVID patients—without the mitigating risk factors discussed above—appears to be low.

TABLE 3 | Characteristics of patients with electrographic seizures.

Patient #	Age	Gender	Primary indication for admission	Chronic neurological history	Acute neurological insult on admission or during hospital stay prior to seizures	Acute systemic COVID-19 symptoms on admission or during hospital stay prior to seizure	Reason for EEG connection
A	51	M	Symptomatic COVID-19 infection (dyspnea and myocarditis) + witnessed new- onset seizures	N	N*	Y (dyspnea & myocarditis)	Hyperkinetic movements (left sided shaking)
B	57	F	Witnessed seizures + symptomatic COVID-19 & influenza infection	Y (multifocal metastatic lesions)	Y (worsening vasogenic edema on neuroimaging)	Y (febrile)	Hyperkinetic movements (clinical seizures)
C	61	F	Symptomatic COVID-19 infection	Y (PRES c/b focal epilepsy)	N	Y (severe hypoxia)	Hyperkinetic movements (myoclonic jerks)
D	68	F	Clinical status epilepticus	Y (ischemic stroke c/b focal epilepsy)	N	Y (febrile)	Hyperkinetic movements (clinical SE at OSH)
E	66	M	Symptomatic COVID-19 infection	N	Y (SDH & SAH)	Y (severe hypoxia)	Hyperkinetic movements (left arm/face shaking)
F	83	F	Acute intracranial hemorrhages with emergent craniotomy	N	Y (SAH, ICH, SDH)	N	Fluctuating mental status

**Patient A was admitted for hyperkinetic movements in the setting of acute COVID-19 symptoms including dyspnea, myocarditis, and fever. Initial head CT scan did not show acute intracranial abnormality. However, subsequent imaging was not completed. This patient had widespread metastatic renal cell carcinoma, although there was no acute or prior evidence of intracranial metastases. Still, clinical suspicion was that electrographic seizures during this admission were triggered by unrecognized intracranial metastatic disease.*

There were several strengths to the study. First, cases were drawn from 4 hospitals within the same hospital network, which yielded a fairly large number of patients included in study. Moreover, all patients included in the study underwent EEG for a median of 2 days per patient. ACNS and Salzburg criteria were systematically applied to all EEGs reviewed, thus yielding standardized and relatively generalizable results across institutions.

The main limitation of this study is the lack of a SARS-CoV-2 negative comparator group to which these results could be evaluated. However, this case series was in part inspired by a similar study investigating the occurrence of electrographic seizures in critically ill patients at the same institution (8). Specifically, those investigators evaluated 98 patients admitted to Medical ICU for severe sepsis secondary to non-COVID-19 infections. They found 14 episodes of periodic discharges without non-convulsive seizures and 11 episodes of periodic discharges plus non-convulsive seizures. Direct comparison of these two studies is clearly limited, particularly given that 20% of the patients in this case series did not require intensive care. Still, in the cohort analysis by Gilmore and colleagues, they found prior history of neurologic disease to be a significant predictor of periodic discharges and non-convulsive seizures in their patients (8). Similarly, we found five of the 6 patients with electrographic seizures in this study to have chronic and/or new-onset neurologic disease. Another significant limitation of this study is the potential for under sampling given the need to reduce exposure to our EEG technicians.

Ultimately, we found a relatively low incidence of electrographic seizures (8%) in SARS-CoV-2 positive patients undergoing cvEEG monitoring. Five of the six patients with electrographic seizures had history of chronic and/or acute neurologic disease otherwise sufficient to cause or trigger seizures. As discussed above, one of the six patients with electrographic seizures had no clear history of chronic or acute neurological disease; however, the leading clinical suspicion was that unrecognized intracranial metastases were the most likely etiology for these seizures.

This adds to evolving literature indicating that seizures comprise a relatively small percentage of patients undergoing cvEEG monitoring in the setting of COVID-19 illness (13, 14). Moreover, findings on interictal EEG such as focal slowing and epileptiform discharges appear to increase the likelihood of also recording electrographic seizures in this population. If other studies support these findings, routine EEG may be sufficient to identify COVID-19 patients at risk of electrographic seizures without the mitigating factors discussed above. Given risk to staff and limited resources in COVID-19 pandemic, using the aforementioned risk factors—including hyperkinetic movements, chronic and/or acute intracranial disease, history of epilepsy, and epileptiform discharges or focal slowing on routine EEG—may help identify patients more appropriately of cvEEG monitoring.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Columbia University Medical Center, Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

BW, AM, and DB contributed to drafting the manuscript. BW, AM, DB, and AB contributed to data acquisition. BW, AM, and MB contributed to data analysis and preparing the table. All authors contributed to study design, critical review, and manuscript revision.

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BRIEF RESEARCH REPORT
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TaSCA, an Agile Survey on Chemosensory Impairments for Self-Monitoring of COVID-19 Patients: A Pilot Study

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Background/Objective: During the COVID-19 pandemic, smell and taste disorders emerged as key non-respiratory symptoms. Due to widespread presence of the disease and to difficult objective testing of positive persons, the use of short surveys became mandatory. Most of the existing resources are focused on smell, very few on taste or trigeminal chemosensation called chemesthesis. However, it is possible that the three submodalities are affected differently by COVID-19.

Methods: We prepared a short survey (TaSCA) that can be administered at the telephone or through online resources to explore chemosensation. It is composed of 11 items on olfaction, taste, and chemesthesis, in order to discriminate the three modalities. We avoided abstract terms, and the use of semiquantitative scales because older patients may be less engaged. Statistical handling included descriptive statistics, Pearson's chi-squared test and cluster analysis.

Results: The survey was completed by 83 persons (60 females and 23 males), which reported diagnosis of COVID-19 by clinical ($n = 7$) or molecular ($n = 18$) means, the others being non-COVID subjects. Cluster analysis depicted the existence of two groups, one containing mostly asymptomatic and one mostly symptomatic subjects. All swab-positive persons fell within this second group. Only one item, related to trigeminal temperature perception, did not discriminate between the two groups.

Conclusions: These preliminary results indicate that TaSCA may be used to easily track chemosensory symptoms related to COVID-19 in an agile way, giving a picture of three different chemosensory modalities.

Keywords: COVID-19, olfaction, taste, chemesthesis, trigeminus, self-assessment, chemical senses, recovery

INTRODUCTION

After the first weeks of SARS-CoV2 spreading (1) and the worldwide dissemination of COVID-19 pandemic, it became clear that the virus is able to affect different organs, while giving the most dismal outcomes in the case of respiratory tract infection (2). In addition to severe respiratory symptoms, rather non-specific signs were reported as initial manifestation of infection, like fever, myalgia, and headache[1]. However, other symptoms emerged as associated to the infection, including the loss of smell termed anosmia (3–5), whose sudden onset appeared as a typical feature of the COVID-19 disease (6, 7). Actually, other viruses may affect the ability to smell, however in these cases the loss of olfactory function is smoother and associated to various degree of nasal symptoms, like running nose (8), while in the case of COVID-19, the loss of smell may appear also in the absence of any other symptom, often in a sudden and dramatic way. The consequences of olfactory loss, whatever the cause, may be life-threatening, by reducing the awareness of potentially dangerous stimuli like gas or rotten food (9).

While the loss of smell is the most apparent sign of chemosensory involvement and can be regarded as a predictor of COVID-19 (10–14) also taste loss (ageusia) may be present, and in some cases the trigeminal chemical sense (15) called chemesthesis is involved (14, 16).

During the initial phase of the pandemic, we followed some mildly symptomatic COVID-19 patients experiencing symptoms related to the chemical senses. However, most of the existing survey at that time were focused on olfaction, and none put together olfaction, taste and chemesthesis. We observed that patients could not easily discriminate between the three different chemosensory modalities, which are sensitive to different stimuli and use a variety of transduction pathways and central connections to reach specific sensory areas in the brain. Hence, we felt compelled to create an agile tool to collect information on these three chemosensory modalities, that could be used either online or as direct or telephone interview, to allow the widest collection of data and reach even persons in remote areas.

The need for a tool easy to use and manage, that allows patients to respond and follow the evolution of their symptoms, prompted us to create a very short online survey on the three chemosensory modalities, focusing on items which are part of everyday life for most people. We referred to sensory experiences related to specific objects, instead of using more generic terms (like "smell") and tried to discriminate between different odorant sources, in case the subject has no relevant experience: for example, one person in isolation may not have a direct contact with perfumes, yet may still retrieve some soap to smell. We also avoided the use of semi-structured scales, since these may be difficult to be adopted by older persons. Taste-Smell-Chemesthesis Agile (TaSCA) survey is presented here along with the responses collected online from April 30 to the end of May 2020, from asymptomatic, non-affected persons and COVID-19 positive patients. The aim of the present work is to show that this tool may discriminate between persons showing chemosensory systems involvement or not, and may serve as a proof-of-principle for the use of TaSCA in conditions in which direct testing is prevented.

[1]https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html (accessed November 23, 2020).

METHODS

The survey was created to collect data for an observational, prospective single center study aimed at determining medium/long term consequences of COVID-19 on neurological status (NEUROCOVID, Ethical Committee Prot. 056881). Data herein presented were collected online in May 2020, during the first COVID-19 outbreak, by soliciting patients and non-affected persons to participate. No exclusion criteria were set, since we aimed at establishing differences between groups of patients and healthy subjects and explore the viability of TaSCA as a tool for COVID-19 patients.

The questionnaire is composed of three sheets implemented in Google Forms. One introductory sheet is entitled "COVID-19 and taste and smell disturbances" and shows an introductory statement. It presents one mandatory question, on the willingness to participate, and 3 more questions (name or nickname, gender, and age). The following sheet is the actual survey. The subject should answer to 12 questions. The first 11 questions take in consideration the sensory experience in the last days, with respect to the usual sensitivity. One of four responses is possible: (1) No change in the last period (I sense as always), (2) Moderate change (I sense less than usual),[3] Loss of function (I don't sense those items), (3) Don't know/don't remember, in the case the person did not had the chance to sense the item in the last period, because of physical constraints. The questions explore smell (questions 1, 3, 5), nasal and oral chemesthesis (questions 2, 4, 10, 11), and taste (questions 6, 7, 8, 9). The last question refers to the presence of symptoms related to COVID-19, with the possibility to add some notes. The third and last sheet is salutation and thanks. The questionnaire in the original language is available at the link:

https://docs.google.com/forms/d/1yvCBD8QBWTv1dOahpYWVhMRfS-8WaESCx80jhDIBW_Q/viewform?ts=5ea2c776&edit_requested=true

The printout is presented in the **Supplementary Material 1**, while the English translation is shown in **Figure 1**.

Data Analysis

Data were analyzed using IBM SPSS Statistics v26 (RRID:SCR_019096) to produce descriptive statistics, Pearson's chi-squared analysis and cluster analysis. Descriptive statistics were calculated for each item.

As a first step, we asked whether the items were able to indicate a difference among persons experiencing a change in sensitivity and COVID-19 status. Hence, the 4 types of responses were coded as 0 (no change in sensitivity, answer 1) or 1 (there was a change in the last period, answers 2 and 3). Concerning answer 4, we manually coded it as 0 if all the other responses indicated no change.

Please answer thinking about your sensations when you smell or eat something, referring to today or the last days in comparison to the usual intensity of your sensations.
How do you perceive:

	No change	Less than usual	Don't feel	Don't know
1. Odors like flowers and fruits	☐	☐	☐	☐
2. Odors like garlic and mint	☐	☐	☐	☐
3. Soaps and perfumes	☐	☐	☐	☐
4. Odors like acetone or ammonia	☐	☐	☐	☐
5. The odor of gas	☐	☐	☐	☐
6. The sweet taste (sugar, sweeteners…)	☐	☐	☐	☐
7. The salty taste (table salt)	☐	☐	☐	☐
8. The bitter taste (coffee, chicory…)	☐	☐	☐	☐
9. The sour taste (lemon, vinegar…)	☐	☐	☐	☐
10. Piquant (chili, pepper…)	☐	☐	☐	☐
11. Hot and cold in your mouth	☐	☐	☐	☐

FIGURE 1 | TaSCA questions on taste, smell, and chemesthesis impairment.

The hierarchical agglomerative algorithm according to Ward's method was applied for clustering (17), since this warrants robust results (18), using squared Euclidean distance for assessing similarity and Pearson's chi-square to test for difference between the clusters for each item. Significance level was set at $p < 0.0045$ after Bonferroni correction for multiple comparisons.

RESULTS

Eighty-three persons completed the survey: 60 females and 23 males. 56 were asymptomatic, 7 had a COVID-19 clinical diagnosis based on symptoms (because during the first pandemic, access to naso-pharyngeal swab was restricted to patients with severe symptoms), 18 had a SARS-CoV2 positive nasopharyngeal swab and 2 self-reported sudden olfactory loss but when the swab test become available, it turned out to be negative. Age ranged from 19 to 73 years old (mean ± SD: 35.98 ± 16.61). All subjects presented valid data: percentages of response for each item are presented in **Table 1**.

In the open question, 42 subjects (50.6%) reported no symptoms related to COVID-19, 27 (32.5%) declared some symptoms and 14 (16.9) answered "I don't know."

Several items had 5 cases or less for the responses 0-1-2 (**Table 1**): 6 for response 0, 5 for response 1, and 4 for response 2. Due to the low frequencies, the responses were categorized in 2 classes namely, 0: No change compared to previous sensitivity, 1: Change compared to previous sensitivity (see **Table 1**, last column).

Cluster analysis showed that two main groups emerged from data, one composed of 53 subjects (63.8%) and the other of 30 subjects (36.2%). The relative dendrogram is shown in **Figure 2**.

Apparently, group 1 mostly collects asymptomatic subjects (92.5%), while 73% persons in group 2 received a diagnosis of COVID-19 (**Supplementary Material 2**).

Supplementary Material 3 reports the distribution of subjects in the two groups emerged from the cluster analysis with respect to the absence or presence of modification in sensitivity: for items 1 to 10, the percentage of subjects reporting a change in sensitivity is significantly different in the two groups. Only for item 11, related to temperature perception, the reported change in sensitivity is not significantly different between the two groups (**Supplementary Material 3**).

Supplementary Material 4 reports the raw data for the cluster analysis.

The mean responses in the two groups are shown in **Figure 3**.

Concerning the last item, on the presence of COVID-19-related symptoms, 3 responses were analyzed: (1) Yes, (2) No, (3) I don't know. The two groups emerged from cluster analysis significantly differed between each other (Pearsons' chi-squared = 42.344, df = 2, $p < 0.0001$). In group 1, 71.7% (38 subjects) reported no symptoms, 7.5% ($n = 4$) reported symptoms and 20.8% ($n = 11$) did not know, while in group 2 13.3% ($n = 4$) did not report symptoms, 76.7% reported symptoms ($n = 23$), and 10% ($n = 3$) did not know.

DISCUSSION

Often regarded as an ancillary sense, olfaction has a great role in our everyday life for its involvement in lifeguarding processes (e.g., avoiding dangerous chemicals) as well in food evaluation.

The COVID-19 pandemic brought to the attention of clinicians an astonishing number of persons infected with mild symptoms, the most intriguing being a sudden and complete

TABLE 1 | Percentage (%) of response and number (N) of persons reporting a modification in their sensitivity.

	0—Don't Know	1—Don't Feel	2—Moderate Change	3—No Change	No. of persons reporting a change
ITEM 1	1.2%	19.3	9.6	69.9	24
Flowers	*N* = 1	16	8	58	
ITEM 2	12.0	15.7	8.4	63.9	20
Mint	10	13	7	53	
ITEM 3	0	19.3	8.4	72.3	23
Perfumes	0	16	7	60	
ITEM 4	13.3	13.3	3.6	69.9	14
Acetone	11	11	3	58	
ITEM 5	26.5	8.4	4.8	60.2	11
Gas	22	7	4	50	
ITEM 6	1.2	7.2	10.8	80.7	15
Sweet taste	1	6	9	67	
ITEM 7	2.4	9.6	12.0	75.9	18
Salty taste	2	8	10	63	
ITEM 8	8.4	7.2	12.0	71.1	17*
Bitter taste	7	6	10	59	
ITEM 9	6.0	6.0	8.4	79.5	12
Sour taste	5	5	7	66	
ITEM 10	21.7	4.8	4.8	68.7	8
Piquant	18	4	4	57	
ITEM 11	2.4	0	3.6	94.0	3
Temperature	2	0	3	78	

**One person reported hypersensitivity.*

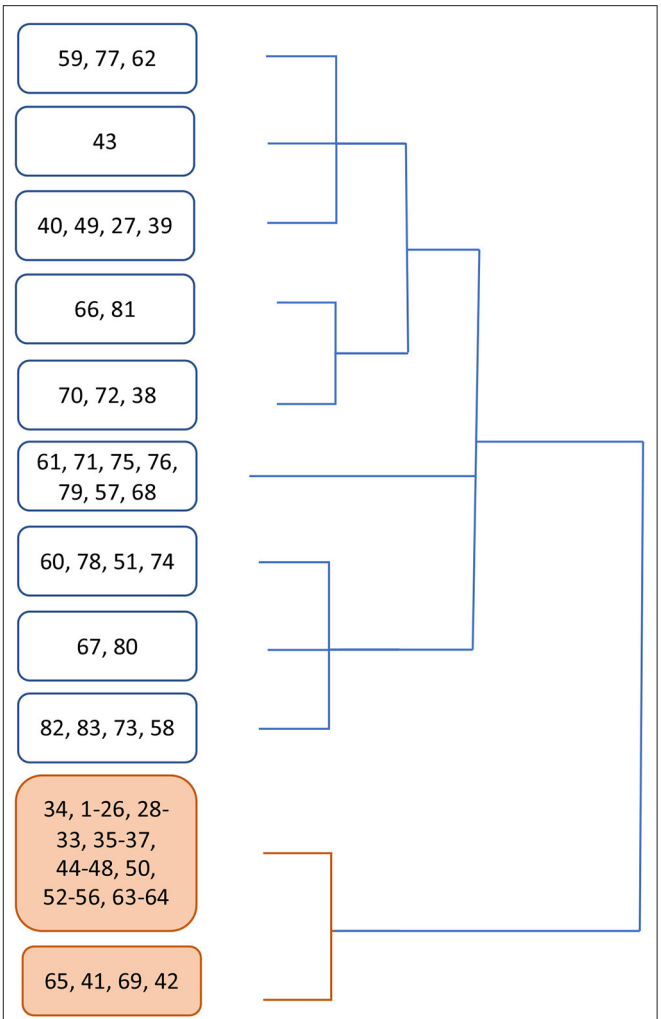

FIGURE 2 | Dendrogram showing clustering emerged from cluster analysis. In white boxes, the branch containing mostly symptomatic patients, in orange the branch containing no swab-positive subject.

loss of olfaction (6), which may last for a variable period of time, often accompanied by taste and chemesthesis impairment (14). While anecdotical self-reports are intriguing, to pose a diagnosis and follow the course of the disease, it is necessary to develop instruments that allow a reliable quantification of the functional impairment.

In the case of chemical senses, two main objective tests have been developed and used over the years in the clinic, namely the Sniffin' Sticks (19) and the University of Pennsylvania Smell Inventory Test (UPSIT) (20), while others are being developed. Their use is mandatory to have an objective evaluation of the impairment, since the subjective report is often misleading (21). However, they both require the direct testing of the patient, which may be difficult or impossible in the case of COVID-19 infected persons. On the contrary, collecting data from patients may be of paramount importance to follow the disease. Sadly, the utility of self-reporting about chemical sensitivity has been repeatedly questioned (22).

Many questionnaires are available to test olfactory function and some of them are widely used (23–25). However, in the case of COVID-19 disease some caution should be warranted. First, most of the currently available surveys are focused on either smell or taste and almost none take into consideration the trigeminal chemical sensitivity. However, one of the most intriguing feature of COVID-19-related chemical senses impairment is the involvement of multiple chemosensory modalities in the disease, as well as their sudden loss of function. Hence, the development of surveys specifically targeting all three chemical sensitivity is long needed. Another feature of COVID-19 disease is the wide range of age of affected persons, yet the diagnostic tools should be easily accessible to most of them: hence, we avoided the use of visual-analog scales, which may be more sensitive than other descriptors but less flexible in terms of accessibility.

Other tools have been developed, including the 40-items GCCR multi-lingual online questionnaire (14), however this requires the access to the web and a certain degree of confidence in using online surveys, which may prevent older persons to access it. Moreover, given the worldwide diffusion of the pandemic, it may be useful to create agile instruments that are easily administered using different tools, including self-administration, direct interviews or over the phone, besides online presentation. Lastly, while most available tools may be complete and hence rather long, we focused the questions on

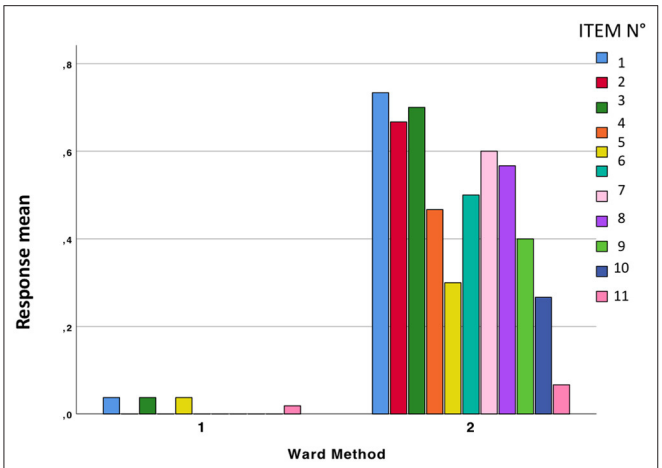

FIGURE 3 | The two groups emerging from cluster analysis present a different response pattern.

the three chemical modalities to have a glimpse of their relative degree of impairment. By keeping the questions tied to real objects that could be sensed and avoiding abstract terms, we tried to grasp the sensory experience in a more faithful way.

Present data show that mostly asymptomatic patients cluster together, while most patients experiencing chemosensory impairment, with particular involvement of olfaction, cluster together, suggesting that TaSCA survey may be a valuable tool in case of impossibility to administer more objective tests. It may also help in collecting critical information on the objective sensitivity in a fast and easy way.

The present work is limited to the initial collections of a case-series to test whether it could discriminate between symptomatic and non-symptomatic patients. Since it relied on the voluntary participation of subjects, we did not attempt to refine sampling, to stay closer to the real-life use: hence, the sex balance is skewed toward females, probably because females are more compliant and willing to take part in preliminary testing. Similarly, we did not take any action on age balance, leaving this for future investigations.

Some initial speculations are possible on the present data. Whether items concerning olfaction are the most discriminative indexes, compared to taste and trigeminal will be explored in future work. Also, with a larger sample it will be possible to fully assess the psychometric and statistical properties of the questionnaire, including for example case-control approaches and cutoff point determination. Interestingly, item 11 on thermal sensitivity does not appear to discriminate between the 2 groups that emerged from cluster analysis. This suggests that the trigeminus nerve may be differentially affected in its different sensory components. Chemical sensitivity in trigeminal afferents involves receptors which are also temperature-gated (26, 27), but cold receptors also exist (28–30), and functional specializations have been reported for trigeminal receptors (31). It is worth to include item 11 because it may discriminate among persons experiencing true chemosensory deficit from deceitful answers.

While obtained in a limited number of subjects, these data show that TaSCA is a short survey available in the same form online, on paper or for oral interview that may be used to screen chemosensory deficits in COVID-19 patients. It remains to be determined its possible use in other conditions where these sensory systems may be involved.

In COVID-19 patients which are still positive, isolated at home or when direct objective testing is not feasible or recommendable, TaSCA could be easily administered. It is fast, yet complete in exploring all three chemosensory modalities and could be used also with patients still presenting annoying symptoms, which may prevent them from extensive sessions of online or live testing.

Possibly, it could be useful in the future for repeated self-checking with substances commonly available in most houses, given the necessity of long-term monitoring for possible adverse outcomes (32).

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 per la Sperimentazione Clinica della Provincia di Padova (affiliation: Padova province). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CM-C conceived the questionnaire. CM-C and PB analyzed the data. GM prepared the online material. CM-C, GM, AC, and CP participated in data collection. AA provided financial support. All authors drafted the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.633574/full#supplementary-material

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COVID-19-Related Neuropsychiatric Symptoms in Patients With Alcohol Abuse Conditions During the SARS-CoV-2 Pandemic: A Retrospective Cohort Study Using Real World Data From Electronic Health Records of a Tertiary Hospital

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Patients with an alcohol abuse disorder exhibit several medical characteristics and social determinants, which suggest a greater vulnerability to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and a worse course of the coronavirus disease 2019 (COVID-19) once infected. During the first wave of the COVID-19, most of the countries have register an increase in alcohol consumption. However, studies on the impact of alcohol addiction on the risk of COVID-19 infection are very scarce and inconclusive. This research offers a descriptive observational retrospective cohort study using real world data obtained from the Electronic Health Records. We found that patients with a personal history of alcohol abuse were 8% more likely to extend their hospitalization length of stay for 1 day (95% CI = 1.04–1.12) and 15% more likely to extend their Intensive Care Unit (ICU) length of stay (95% CI = 1.01–1.30). They were also 5.47 times more at risk of needing an ICU admission (95% CI = 1.61–18.57) and 3.54 times (95% CI = 1.51–8.30) more at risk of needing a respirator. Regarding COVID-19 symptoms, patients with a personal history of alcohol abuse were 91% more likely of exhibiting dyspnea (95% CI = 1.03–3.55) and 3.15 times more at risk of showing at least one neuropsychiatric symptom (95% CI = 1.61–6.17). In addition, they showed statistically significant differences in the number of neuropsychiatric symptoms developed during the COVID-19 infection. Therefore, we strongly recommend to warn of the negative consequences of alcohol abuse over COVID-19 complications. For this purpose. Clinicians should systematically assess history of alcohol issues and drinking

habits in all patients, especially for those who seek medical advice regarding COVID-19 infection, in order to predict its severity of symptoms and potential complications. Moreover, this information should be included, in a structured field, into the Electronic Health Record to facilitate the automatic extraction of data, in real time, useful to evaluate the decision-making process in a dynamic context.

Keywords: alcohol abuse (AA), COVID-19, neuropsychiatric symptoms, real world data, electronic health record

INTRODUCTION

Patients with a substance abuse condition (SAC), such as alcoholic patients, exhibit several medical characteristicsm and social determinants that suggest a greater vulnerability to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and, even more, a worse course of the coronavirus disease (2019) COVID-19 once infected.

According to the European Monitoring Center for Drugs and Drug Addiction and several studies (1, 2) on the implications of COVID-19 for illegal drugs users, they may suffer from more cardiovascular and respiratory pathologies, being also more vulnerable to damage from COVID-19 infection (3, 4). In addition to this cardiopulmonary comorbidity, the fact that immune response may be compromised in this population and that they may lack health-seeking behavior could increase the severity of the COVID-19 infection (5). Moreover, medications used for COVID-19 may be less effective and worse tolerated by drug users, who are also at an increased risk for pharmacological agent–drug of abuse interactions (6, 7).

An independent effect of "chronic alcohol abuse" on acute respiratory distress syndrome (ARDS) in critically ill patients has been demonstrated in a prospective cohort study (8). A recent systematic review and meta-analysis found that any measure of high relative to low alcohol consumption was associated with a significantly increased risk of acute respiratory distress syndrome (ARDS) [odds ratio (OR) 1.89; 95% CI, 1.45–2.48] (9). Alcohol can increase the risk of developing ARDS through various mechanisms including alveolar epithelium dysfunction, alcohol-induced oxidative stress, and interference on alveolar macrophage function (9). In hospitalized patients with pneumonia, having an alcohol-related diagnosis was associated with a greater likelihood of admission to the Intensive Care Unit (ICU) (OR, 1.63) and a longer length of stay (adding extra 0.6 days) (10).

Although an increase in alcohol consumption has been a constant in most of the countries that have gone through the first wave of COVID-19 (1), studies on the impact of alcohol addiction on the risk of COVID-19 infection are very scarce and inconclusive (11). In the 760 cases of COVID-19 detected in a cohort of 387,109 adults followed up in the United Kingdom, obesity and smoking increased the risk of infection but not heavy drinking, although the impact of alcohol abuse was not studied (11). Understanding heavy drinking for men as consuming 15 drinks or more per week, and for women 8 drinks or more per week, the average profile of patients with an alcohol abuse condition attended at the Hospital 12 de Octubre is of a heavy drinker with a daily basis and a systematic alcohol consumption. It is worth to note that the binge drinking is usual in Spain, but the person does not usually seek medical advice neither at the hospital nor at the Primary Care System; since asking for toxic habits is not systematically explored during the medical intervention unless directly related medical issues related to them are observed, these patients have no alcohol abuse condition diagnosis noted in their medical records.

Considering that alcohol-dependent patients associate multiple medical comorbidities and immune system disorders, in both phases, active consumption and withdrawal, it is foreseeable to assume that this population is more prone to get infected and suffer more severe complications from COVID-19 (2, 12). Hence, our aim is to assess whether alcohol consumption is associated with an increased severity of COVID-19 infection in hospitalized patients and an increased presence of neurological symptoms, considering the co-occurrence of other possible medical comorbidities.

MATERIALS AND METHODS

Study Design

Descriptive, observational, retrospective cohort study in which data were obtained from Electronic Health Records (EHRs), admission information, and *ad-hoc* fulfillment of some variables of interest. The inclusion criteria were as follows: having at least one episode of hospitalization at the Hospital Universitario 12 de Octubre between February 25th and September 4th, 2020 and a diagnosis of COVID-19. The exposition criterion was having at least one diagnosis related to past or present alcoholism. The exclusion criteria were as follows: patients with incomplete data and patients with confidential occupational health data or errors due to low data quality. A match was performed using the propensity score matching with a replacement method (13, 14). Each alcoholic individual was paired with two non-alcoholic individuals who had the closest propensity score to minimize bias and improve the quality of matching; to compensate for the difficulty in identify confounding factors, indirect methods were used, and some of the alcoholic patients were paired with more than one control (60:128). Possible confounding factors or observable covariates were sex, age, obesity, vital state, pulmonary thromboembolism, ischemic temporal accident or stroke, confusion, diabetes, chronic obstructive pulmonary disease (COPD), arterial hypertension, anxiety, depression, asthma, liver disease (hepatitis or cirrhosis), smoking, human immunodeficiency virus (HIV), transplantation, cancer, cognitive impairment or dementia, and psychosis.

EHR Semiautomatic Extracted Variables

The variables considered in the study were as follows: demographics (age and sex); personal health history (neuropsychiatric history and hepatic damage); severity risk for COVID-19 comorbidities (diabetes, arterial hypertension, obesity and overweight, chronic obstructive pulmonary disease); COVID-19 diagnosis (clinical diagnosis and PCR result); admission variables (length of stay and ICU admission).

Outcomes

Vital state at hospital discharge, neuropsychiatric COVID-19 symptoms (psychosis, depression, anxiety, posttraumatic syndrome, cognitive impairment, confusion, transient ischemic attack, stroke, anosmia, ageusia).

Ad-hoc Follow-Up Variables

Result variables: COVID-related chief complaint, vital state, length of stay, ICU admission, ICU length of stay, and complications. Adjustment variables: age, sex, personal history of alcoholism, diabetes, arterial hypertension, obesity and overweight, COPD, asthma, hepatitis, cirrhosis, toxic habits, smoking, human immunodeficiency virus (HIV), transplant, psychosis, depression, cognitive impairment or dementia, and anxiety. COVID-19 signs: levels of alkaline phosphatase (ALP), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), reactive C-protein, D dimer, and fibrinogen at diagnosis. COVID-19 symptoms: pneumonia, type of pneumonia (unilateral, bilateral), fever, cough, asthenia, degree of asthenia, diarrhea, vomit, dyspnea, degree of dyspnea (low, moderate, severe), anosmia, ageusia, confusion, memory loss, psychotic crisis, delirium, depression, anxiety, sleep disorders, attention and concentration disorders, emotional lability, speech impairment, euphoria, aggressiveness, irritability, hallucinations, suicidal tendencies, posttraumatic syndrome, and consciousness level.

Statistical Analysis

The variables were summarized by means and standard deviation or median (p50) and the interquartile range (p25-p75), according to the normality distribution. To check the normality distribution, the Shapiro–Wilk test was used. Qualitative variables were expressed in absolute numbers (number of cases) and in relative frequencies (percentage). To compare alcohol-related variables between groups, we used the Student's *T*-test or the non-parametric Mann–Whitney *U*-test, chi-square test (X^2), or Fisher's exact test, depending on the nature of the variables.

A logistic regression model was created to study the association between possible risk factors for alcoholism adjusted for age, sex, obesity, cognitive impairment–dementia, HIV, smoking, transplantation, diabetes, anxiety, liver disease, depression, and psychosis. The results of the model are presented in the form of OR together with the 95% confidence interval (95% CI) (**Table 1**). The statistical study was completed with a multivariate analysis, considering both risk factors with a significant result in the univariate analysis and those that had some relevance ($p < 0.1$). The use of a selection by steps was used to highlight the most relevant factors, identify those significant sets among the possible variables, and avoid confusion in the model due to possible relationships between them since this method determines the significance of the effect of a variable in the presence of those already in the model. The Hosmer and Lemeshow test was used to evaluate the goodness of fit of the model.

All analysis were performed using the STATA version 16 statistical software. In all cases, the level of confidence was 95%, considering that there was statistical significance when $p \leq 0.05$.

RESULTS

During the study period, the Hospital Universitario 12 de Octubre offered health assistance (**Figure 1**) in 72,364 episodes to patients either at the emergency department, as inpatients or as a reference laboratory for the PCR technique from Primary Care. Fourteen thousand seven hundred eight had a diagnosis of COVID-19; 847 had at least one diagnosis or reference to a personal history of alcoholism (1.17% of the total episodes) of whom 88 had COVID-19 (10.39% of the alcoholic patients; 0.6% of the COVID-19 patients). **Table 1** summarizes the descriptive analysis of demographic-related variables. The cohort of patients diagnosed with alcohol abuse had a higher proportion of male patients ($p = 0.032$).

Twenty-eight patients who met the exclusion criteria were removed from the cohort. Six of the patients were hospital workers, and their data were confidential occupational health data. Fifteen were misclassified since they had no hospital admission, having only a positive PCR demanded from the Primary Care. Lately, seven patients were misclassified as "confirmed COVID-19," but they had no COVID-19 diagnosis or it was finally ruled out. Thus, the final cohort was composed of 60 patients with a confirmed personal record of alcoholism, a diagnosis of COVID-19, and an EHR episode registered as "alcoholic." Alcoholic patients were paired 2:1 with 128 non-alcoholic patients. We considered non-alcoholic patients as those who had an explicit comment of "no alcohol consumption" or the absence of a diagnosis of alcohol abuse in the medical records.

In the descriptive analysis of the historic cohorts (see **Table 1**), there were statistically significant differences in the length of inpatient stay ($p < 0.001$), being longer for alcoholic patients with a mean of 9 days compared to non-alcoholics in which the mean was 5 days. There were also differences in the frequency of ICU admission ($p = 0.005$) and in the length of stay in ICU ($p < 0.001$), as well as in the need of respirator ($p = 0.0049$). However, it is important to highlight that the variables of cirrhosis ($p < 0.001$) and other toxic habits such as smoking ($p < 0.001$) and drug addiction ($p < 0.001$) were significantly higher in the alcoholic cohort, which could be related to the severity of the disease.

As for the COVID-19 symptoms, the univariate analysis showed differences in several result variables. The final multivariable model showed no significant differences for respiratory or digestive symptomatology with the exception of dyspnea ($p = 0.043$). There were, however, statistically significant differences in the neuropsychiatric symptoms ($p = 0.001$);

TABLE 1 | COHORT descriptive analysis.

	Total ***N* = 188**	**Non-alcoholic** ***N* = 128**	**Alcoholic** ***N* = 60**	***p*-value**
Age [mean (SD)]	60.45 (16.55)	59.93 (18.52)	61.56 (11.29)	0.53
Sex (% men)	134 (71.28%)	85 (66.41%)	49 (81.67%)	0.038
Non-COVID derivation (%)	39 (20.74%)	15 (11.71%)	24 (40.00%)	<0.001
COVID confirmed (%)	123 (65.43%)	72 (56.25%)	51 (85.00%)	<0.001
Health outcomes				
Vital state (%)	36 (19.15%)	23 (17.97%)	13 (21.67%)	0.56
Length of stay (IC95%)	5.00 (0.00–10.00)	2.00 (0.00–8.00)	9.00 (5.00–17.00)	<0.001
ICU admission	13 (6.91%)	4 (3.13%)	9 (15.00%)	0.005
ICU length of stay (95% CI)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	<0.001
Need for respirator	26 (13.83%)	11 (8.59%)	15 (25.00%)	0.0049
PCR	101 (53.72%)	58 (45.31%)	43 (71.67%)	<0.001
Complications	42 (22.34%)	23 (17.97%)	19 (31.67%)	0.040
Personal health history				
Diabetes	47 (25.00%)	29 (22.66%)	18 (30.00%)	0.279
Arterial hypertension	75 (39.89%)	48 (37.50%)	27 (45.00%)	0.339
Obesity and overweight	28 (14.89%)	15 (11.72%)	13 (21.67%)	0.082
OCPD	29 (15.43%)	20 (15.63%)	9 (15.00%)	0.999
Asthma	13 (6.91%)	7 (5.47%)	6 (10.00%)	0.351
Hepatitis	31 (16.49%)	15 (11.72%)	16 (26.67%)	0.019
Cirrhosis	27 (14.36%)	6 (4.69%)	21 (35.00%)	<0.001
Drug addiction	15 (7.98%)	4 (3.13%)	11 (18.33%)	<0.001
Smoker	44 (23.40%)	22 (17.19%)	22 (36.67%)	<0.001
Ex-smoker	21 (11.17%)	8 (6.25%)	13 (21.67%)	
HIV	6 (3.19%)	3 (2.34%)	3 (5.00%)	0.389
Transplant	13 (6.91%)	7 (5.47%)	6 (10.00%)	0.348
Psychosis	5 (2.66%)	3 (2.34%)	2 (3.33%)	0.651
Depression	38 (20.21%)	20 (15.63%)	18 (30.00%)	0.031
Suicidal attempts	6 (3.19%)	2 (1.56%)	4 (6.67%)	0.083
Cognitive impairment	20 (10.64%)	10 (7.81%)	10 (16.67%)	0.078
Anxiety	27 (14.36%)	20 (15.63%)	7 (11.67%)	0.509
Laboratory parameters				
AST	35.0 (24.0–51.0)	33.0 (24.0–44.0)	42.0 (26.0–70.0)	0.066
ALT	66.0 (27.0–131.5)	50.0 (24.0–99.0)	115.0 (49.0–255.0)	<0.001
Gama GT	133.53 (278.5)	72.74 (72.49)	236.33 (430.18)	<0.001
C-reactive protein	5.60 (1.43–12.82)	6.44 (1.34–12.49)	4.70 (1.62–16.56)	0.789
Fibrinogen	658.09 (207.17)	658.35 (194.77)	657.67 (229.24)	0.989
MCV	91.35 (7.03)	91.13 (6.57)	91.74 (7.83)	0.609
D-dimer	786 (414–1,463)	669 (381–1,488)	992 (600–1,463)	0.390
COVID-19 debut symptoms				
Pneumonia (yes/no)	103 (54.79%)	65 (50.78%)	38 (63.33%)	0.119
Unilateral pneumonia	22 (11.70%)	13 (10.16%)	9 (15.00%)	0.219
Bilateral pneumonia	81 (43.09%)	52 (40.63%)	29 (48.33%)	
Fever	114 (60.64%)	75 (58.59%)	39 (65.00%)	0.431
Cough	94 (50.00%)	64 (50.00%)	30 (50.00%)	1.000
Asthenia	41 (21.81%)	30 (23.44%)	11 (18.33%)	0.459
Diarrhea	46 (24.47%)	30 (23.44%)	16 (26.67%)	0.719
Vomits	15 (7.98%)	11 (8.59%)	4 (6.67%)	0.782
Dyspnea	86 (45.74%)	52 (40.63%)	34 (56.67%)	0.043
Anosmia	15 (7.98%)	13 (10.16%)	2 (3.33%)	0.149

(Continued)

TABLE 1 | Continued

	Total N = 188	Non-alcoholic N = 128	Alcoholic N = 60	*p*-value
Ageusia	15 (7.98%)	12 (9.38%)	3 (5.00%)	0.389
Anosmia and Ageusia	8 (4.26%)	6 (4.69%)	2 (3.33%)	1.000
Confusion	22 (11.70%)	9 (7.03%)	13 (21.67%)	0.006
Memory loss	7 (3.72%)	2 (1.56%)	5 (8.33%)	0.035
Psychotic crisis	2 (1.06%)	0 (0.00%)	2 (3.33%)	0.101
Depression	8 (4.26%)	5 (3.91%)	3 (5.00%)	0.711
Anxiety	12 (6.38%)	7 (5.47%)	5 (8.33%)	0.531
Sleeping problems	11 (5.85%)	7 (5.47%)	4 (6.67%)	0.749
Attention problems	12 (6.38%)	7 (5.47%)	5 (8.33%)	0.529
Emotional lability	8 (4.26%)	3 (2.34%)	5 (8.33%)	0.111
Disorders of consciousness	21 (11.17%)	12 (9.38%)	9 (15.00%)	0.319
Speech impairment	6 (3.19%)	5 (3.91%)	1 (1.67%)	0.67
Aggressiveness	3 (1.60%)	0 (0.00%)	3 (5.00%)	0.031
Irritability	2 (1.06%)	0 (0.00%)	2 (3.33%)	0.100
Hallucinations	4 (2.13%)	1 (0.78%)	3 (5.00%)	0.097
Other NP symptoms	19 (10.11%)	7 (5.47%)	12 (20.00%)	0.004
At least one NP symptom	51 (27.13%)	25 (19.53%)	26 (43.33%)	0.001
Number of NP symptoms (median)	0.00 (0.00–1.00)	0.00 (0.00–0.00)	0.00 (0.00–2.00)	<0.001

Frequency and statistical significance between variables in patients with any annotation in their medical record of alcohol abuse (alcoholic) vs. patients without a reference to alcohol abuse (non-alcoholic).

NP, neuropsychiatric; MCV, mean corpuscular volume; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ICU, intensive care unit; GGT, gamma-glutamyl transferase; GOT (AST), glutamyl oxaloacetic transaminase/aspartate aminotransferase; non-COVID derivation, patient who seek medical attention due to symptoms initially unrelated to COVID-19.

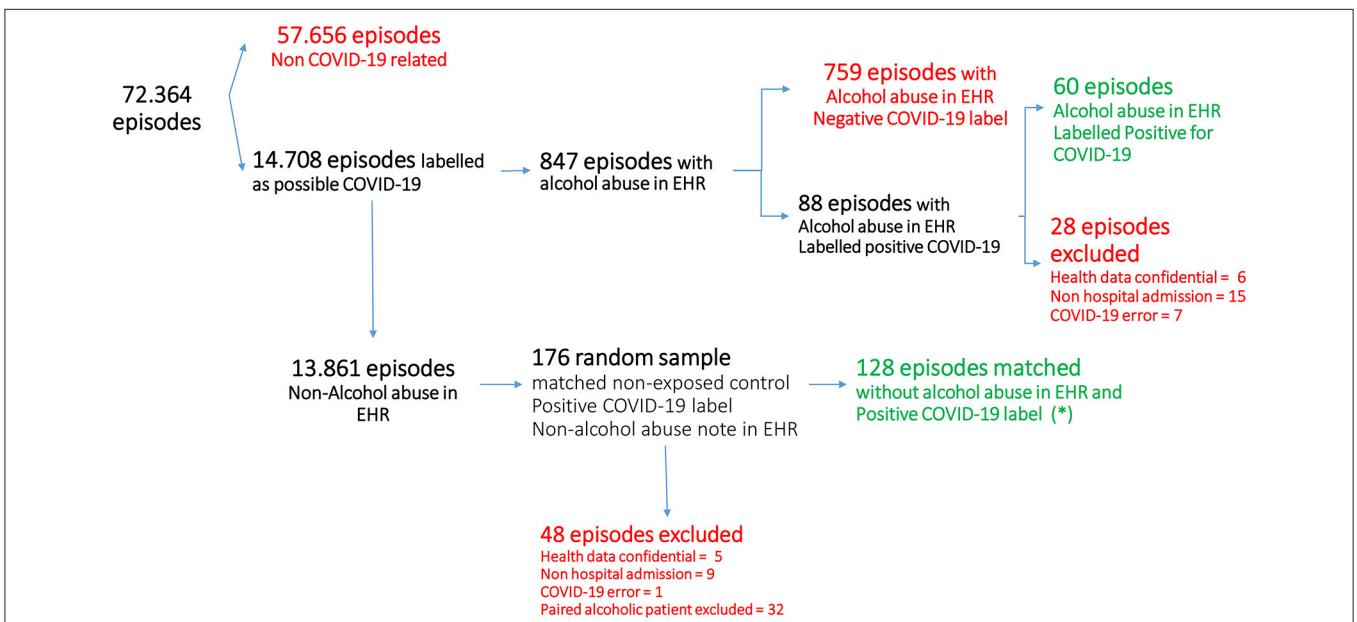

FIGURE 1 | Patients flow and final cohort identification. *During the matching process, to compensate for the difficulty in identify cofounding factors, some of the alcoholic patients were paired with more than two controls.

statistical significance was reached for confusion ($p = 0.006$), memory loss ($p = 0.035$), violent behavior ($p = 0.031$) and other neuropsychiatric symptoms ($p < 0.001$).

The variable "other neuropsychiatric symptoms" was diverse, including pain ($n = 4$), stroke ($n = 2$), agitation ($n = 2$), confusion and disorientation ($n = 2$), hepatic encephalopathy ($n = 2$), weakness and walking impairment ($n = 3$), bradypsychia ($n = 2$), dysarthria ($n = 2$), Guillain-Barre syndrome ($n = 1$), hemiparesis ($n = 1$), panic attack ($n = 1$), dementia ($n = 1$), diplopia ($n = 1$), and syncope ($n = 1$). Five of the patients showed

more than one symptoms. The neuropsychiatric symptoms (NP symptoms) variable included all the neuropsychiatric symptoms but not ageusia and anosmia, which were analyzed separately.

One hundred one (53.72%) patients of the global cohort were diagnosed with COVID-19 having a supporting positive PCR test for SARS-CoV-2, while the other 87 patients were diagnosed as COVID-19 without this diagnosis test, receiving only a clinical diagnosis, most probably due to the initial lack of test supplies. Patients with a personal history of alcohol abuse were more frequently tested as positive for SARS-CoV-2 than patients without personal records of alcoholism ($p = 0.003$) and more frequently (40.00 vs. 11.71%) requested medical assistance in the hospital setting with the suspicion of COVID-19 ($p < 0.001$).

Adjustment was incomplete due to certain characteristics of alcoholism, such as its association to other toxic habits, for instance drug abuse and smoking ($p < 0.001$), more frequent than in non-alcoholic patients, and the effects of alcohol over the liver function that can lead to cirrhosis ($p < 0.001$) and hepatitis ($p = 0.025$). In addition, depression ($p = 0.031$) was not completely paired in the non-alcoholic population. Thus, the analytical variables measuring liver function (ALT and GGT) showed significant differences ($p < 0.001$) between alcoholic and non-alcoholic patients. However, the median values in these variables were in the pathological range in both cohorts; besides, depression as a COVID-19 symptom had no significant differences between both cohorts.

In the univariate analysis of the paired cohorts, patients with a personal history of active alcohol abuse were 8% more likely to extend their length of stay for 1 day ($CI_{95\%} = 1.04–1.12$) and 15% more likely to extend their ICU length of stay ($CI_{95\%} = 1.01–1.30$). They were 5.47 times more at risk of needing ICU admission ($CI_{95\%} = 1.61–18.57$) and 3.54 times ($CI_{95\%} = 1.51–8.30$) more at risk of requiring a respirator. As for the symptoms of COVID-19, patients with a personal history of alcohol abuse showed 91% more frequency of dyspnea ($CI_{95\%} = 1.03–3.55$), they were 3.15 times more at risk of exhibiting at least one neuropsychiatric symptom ($CI_{95\%} = 1.61–6.17$), and they reached statistically significant differences in the number of NP symptoms developed during the COVID-19 infection. In the final model for the multivariate analysis (see **Table 2**), patients with a personal history of alcohol abuse were 2.38 times more at risk of developing at least one neuropsychiatric symptom ($CI_{95\%} = 1.01–5.59$). Through the Hosmer and Lemeshow test, we can verify that the goodness-of-fit test in the proposed multivariate model (**Annex 1**) is considered a good fit since the value of the Cg statistic was 0.8324.

TABLE 2 | Logistic regression model, odds ratios in univariate, and multivariate analysis.

	p-value	OR (95% CI)	Multivariate analysis (*) OR (95% CI)
Length of stay	<0.001	1.08 (1.04–1.12)	1.07 (1.03–1.12)
ICU admission	0.029	1.15 (1.01–1.30)	
AST	0.045	1.01 (1.00–1.02)	
GGT	<0.001	1.01 (1.00–1.01)	1.01 (1.00–1.01)
C-reactive protein	0.994	0.99 (0.96–1.03)	
Fibrinogen	0.987	0.99 (0.99–1.00)	
MCV	0.611	1.01 (0.96–1.06)	
D-dimer	0.350	0.99 (0.99–1.00)	
Number of NP symptoms	0.009	1.31 (1.07–1.61)	
Death	0.549	0.79 (0.37–1.69)	
ICU admission	0.006	5.47 (1.61–18.57)	
Need of respirator	0.004	3.54 (1.51–8.30)	
PCR	0.001	3.05 (1.58–5.91)	
Complications	0.038	2.11 (1.04–4.29)	
COPD	0.912	0.95 (0.40–2.24)	
Asthma	0.260	1.92 (0.62–5.98)	
Hepatitis	0.012	2.74 (1.25–6.01)	2.46 (0.85–7.08)
Drug addiction	0.001	6.96 (2.11–22.90)	
Suicidal attempts	0.088	4.50 (0.80–25.29)	
COVID	<0.001	4.41 (2.00–9.71)	3.22 (1.02–10.09)
Pneumonia	0.180	1.67 (0.89–3.14)	
Unilateral pneumonia	0.171	1.98 (0.74–5.27)	
Bilateral pneumonia	0.168	1.59 (0.82–3.10)	
Fever	0.403	1.31 (069–2.48)	
Cough	0.999	1.00 (0.54–1.85)	
Asthenia	0.431	0.73 (0.34–1.58)	
Diarrhea	0.631	1.19 (0.49–2.40)	
Vomits	0.650	0.76 (0.23–2.49)	
Dyspnea	0.041	1.91 (1.03–3.55)	
Anosmia and ageusia	0.670	0.70 (0.14–3.58)	
NP symptoms	0.001	3.15 (1.61–6.17)	2.38 (1.01–5.59)

NP, neuropsychiatric; MCV, mean corpuscular volume; COPD, chronic obstructive pulmonary disease; ICU, Intensive Care Unit; GGT, gamma-glutamyl transferase; GOT (AST), glutamyl oxaloacetic transaminase/aspartate aminotransferase.
**Drug addiction was excluded due to the ample range of the CI.*

DISCUSSION

The most relevant results of our study indicate that patients with COVID-19 infection and alcohol abuse had more complications as suggested by longer lengths of stays, greater need for ICU and ventilator support, and significantly more neuropsychiatric complications such as the presence of confusion, memory deficits, violent behavior, and other neuropsychiatric symptoms. However important for prognosis and management of patients, alcohol abuse conditions are neither properly identify on the EHR nor systematically screened while caring for a patient; and this can have important impact on the patient's healthcare.

Regarding COVID-19 complications, our findings are consistent with those of other studies that have also found that high-dose alcohol consumption increases the risk for ARDS (9), ICU admission, and longer hospital lengths of stay (10). Chronic alcohol consumption can induce ciliary dysfunction in the respiratory tract that reduces the capacity for bacterial and viral clearance (15).

Possibly the most suggestive finding in our study is the correlation between a personal history of alcoholism and neuropsychiatric complications, as evidenced by the presence of significant symptoms in that group such as confusion, memory loss, violent behavior, and other neuropsychiatric manifestations.

These manifestations may reflect the greater severity of the disease, and, in fact, in a study that included patients admitted to ICU, up to 84% presented neurological complications, indicating that these were more frequent in the most severe cases (16). It could also be due to the presence of the virus in the central nervous system (CNS), as it has been pointed out in a recent review of the neurological complications of COVID-19 (17).

There are multiple possible routes of entry of the virus into the CNS: through the olfactory bulb, through the blood–brain barrier (BBB) (18), through infected leukocytes, or through axonal transport in peripheral nerves (19). The enzyme angiotensin-converting enzyme 2 (ACE2), through which the virus enters into the cell, is found in the cerebral vascular endothelium (17). COVID infection can damage endothelial cells and activate proinflammatory and thrombotic pathways (17). Thus, the most severe symptoms may also be a consequence of the cytokine storm (16).

One of the plausible hypothesis to explain the increased CNS vulnerability to the COVID infection in patients with a personal history of alcohol abuse is that ethanol is able to interact forcefully at different levels, acting on both natural or innate immunity [phagocytosis, natural killer (NK) cells, complement] and on specific or acquired immunity (20, 21). In particular, the activity of NK cells is altered by the following actions of ethanol: interference of the bond between NK and the target cells, modification of the production and use of some cytokines, alteration of cytolytic activity, alteration of signal transduction, and a direct effect on the neuro-endocrine system (22). Another entry pathway could be related to an increased permeability of the BBB (23, 24), which would favor the proinflammatory cascade caused by the virus.

As for the strengths and limitations of the study, one of the main issues observed during the pandemic is the insufficient quality of clinical and epidemiological data to design observational studies and to elaborate hypothesis for future researches. Another key difficulty is to identify patients who meet clinical characteristics of interest, other than COVID-19-related information (currently well-recorded). Automatic extraction of information from EHR could be of great help in a pandemic. This study has been supported by real world data (RWD) semiautomatically extracted from EHR and compared with the current gold standard for retrospective studies, the manual EHR review. The iterative approach for patient cohort identification has been very specific in the variable's identification even though not very sensitive (<2% of patients with a personal record of alcohol abuse were identified, although within inpatients, it was expected to be identified around 20%). In this way, one supposedly alcoholic patient was considered as non-alcoholic and three non-alcoholic patients were identified as alcoholic by the reviewers. Thus, 98.3% of the patients identified as patients with a personal history of alcohol abuse were confirmed as so by individual and manual review of the health records of every single patient (gold standard), and 97.6% of the patients identified as non-alcoholic were confirmed as so by the gold standard.

Despite this strength, several limitations should be taken into account. First, identifying alcoholism exposure was done following medical records of EHR and not any other objective measures. For this reason, there could be a selection bias since alcoholism, among other substance abuse conditions, is a stigmatized disease that carry psychological and physical suffering to the patients who could minimize or deny their alcohol consumption; in addition, because alcohol consumption is considered part of a cultural legacy within our social context, it could be not systematically recorded in the patient's medical records, underdiagnosing alcohol abuse. Moreover, the chances of being codified as an alcoholic are higher when associating pathologies such as cirrhosis or a severe dependence. Finally, drug abuse and smoking habit are systematically explored in patients identified as alcoholics but not in non-alcoholics, and therefore, the significant differences observed in these variables could be due to an appropriate diagnosis in alcoholic and ex-alcoholic patients but a misdiagnosis in non-alcoholic patients.

On the other hand, medical history should have been structured in the "personal health history" section of EHR, and it has not, and in this way, the semiautomatic identification of variables could have been mistaken. Ideally, a multicentric observational prospective cohort study including the variables from our database and the systematic recording of toxic habits in all COVID-19 inpatients could greatly reinforce our conclusions regarding the impact of alcohol abuse in COVID-19 neuropsychiatric symptoms and prognosis. In fact, alcohol consumption has an important effect on the central nervous system and on mental health and, in COVID-19 patients, seems to increment significantly the probabilities of suffering at least one neuropsychiatric symptoms and frequently more than one. Unfortunately, the insufficient size of the cohort and the intrinsic limitations of this study do not allow a stronger assessment; however, it is a research topic worth exploring in depth.

In conclusion, considering the use and abuse of alcohol that is occurring in the general population as a consequence of the pandemic, it would be advisable to warn of the negative consequences that these habits may have on the complications of COVID-19 infected people.

Clinicians should systematically assess history of alcohol issues and drinking habits in all patients, especially for those who seek medical advice regarding COVID-19 infection in order to prevent symptoms severity and complications that results from this association.

Information regarding personal health records should be recorded in a structured field of EHR to facilitate the automatic extraction of data for observational studies in real time, useful to evaluate the decision-making process in a dynamic context.

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

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for

participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CV, FA, and GR contributed in the study desing, data recording, manuscript writing, and scientific discussion. CM-A did the statistical analysis and patient matching and propensity score adjustment. CC, AJ, ME, IR, LS, and ÁC participated in the data recording and colaborated in science discussion. BB and MM participated in the english translation. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.630566/full#supplementary-material

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REVIEW
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Elucidating the Neuropathologic Mechanisms of SARS-CoV-2 Infection

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The current pandemic caused by the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a public health emergency. To date, March 1, 2021, coronavirus disease 2019 (COVID-19) has caused about 114 million accumulated cases and 2.53 million deaths worldwide. Previous pieces of evidence suggest that SARS-CoV-2 may affect the central nervous system (CNS) and cause neurological symptoms in COVID-19 patients. It is also known that angiotensin-converting enzyme-2 (ACE2), the primary receptor for SARS-CoV-2 infection, is expressed in different brain areas and cell types. Thus, it is hypothesized that infection by this virus could generate or exacerbate neuropathological alterations. However, the molecular mechanisms that link COVID-19 disease and nerve damage are unclear. In this review, we describe the routes of SARS-CoV-2 invasion into the central nervous system. We also analyze the neuropathologic mechanisms underlying this viral infection, and their potential relationship with the neurological manifestations described in patients with COVID-19, and the appearance or exacerbation of some neurodegenerative diseases.

Keywords: SARS-CoV-2, storm cytokine syndrome, neuroinflammation, blood-brain barrier, neurological alterations, neurodegenerative diseases, Alzheimer's disease

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first reported in December 2019 in Wuhan, in Hubei province, China (1). In March 2020, the world health organization (WHO) declared the COVID-19 a pandemic. Almost a year later, on March 1, 2021, more than 114 million cases and 2.53 million deaths have been reported (2–4). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),

the causative agent of COVID-19, is 100 nm with an oval shape and covered with crown-shaped glycoprotein spikes (5). It is transmitted through respiratory droplets from infected individuals or contact with fomites. Once SARS-CoV-2 enters the body, the onset of symptoms ranges from 2 to 14 days. Patients can manifest clinically from asymptomatic or mild symptoms to moderate or severe symptoms (6, 7). Mild to moderate symptoms manifest as fever, dry cough, nasal congestion, sore throat, runny nose, fatigue, myalgias, diarrhea, anosmia, and ageusia as main symptoms (6, 7). The severe condition is characterized by atypical pneumonia, which can be observed as "ground-glass opacification" with bilateral multi-lobular consolidations by imaging studies (8). Between 5 and 30% of patients develop acute respiratory distress syndrome, characterized by rapid onset and with generalized inflammation in the lungs, requiring invasive life support therapy, such as mechanical ventilation (6, 7). There is increasing evidence that these critically ill COVID-19 patients suffer a so-called "cytokine storm syndrome," characterized by the release of many pro-inflammatory cytokines [interleukin (IL)-1β and IL-6] and a low number of T cells into the bloodstream (9). The mean period from the onset of the symptoms to death is around 13 days [interquartile range (IQR) 11–18 days], and this depends on advanced age (>65 years) and comorbidities such as diabetes mellitus (DM), hypertension, cardiovascular disease, or chronic obstructive pulmonary disease (COPD) (10). These comorbidities are also risk factors for severity and transfer to intensive care unit (ICU), endotracheal intubation, and death in patients with COVID-19 (11). However, there may be bias in the epidemiological data due to the in-hospital stay of the patient, as well as the human development index of each country. SARS-CoV-2 also may be able to invade multiple organs, including the nervous system, and thus cause multiple organ dysfunction syndrome (MODS) (12). The neurological manifestations are beginning to take on unquestionable importance, mainly in the critical patient (13, 14). Neurological manifestations of COVID-19 and other coronavirus infections involve febrile seizures, disorientation, difficulty in speaking, encephalitis, and stroke (15–18). The mechanisms by which SARS-CoV-2 can spread, infect, cause damage to nerve cells and finally affect both the central (CNS) and peripheral (PNS) nervous system, are not yet understood. This review will analyze the potential mechanisms by which SARS-CoV-2 can invade the CNS and PNS and generate a neurotoxic environment that may trigger or worsen neurological disorders.

SARS-CoV-2: Structure and Mechanism of Infection

The coronaviruses (CoVs) belong to the *Orthocoronaviridae* subfamily; order: *Nidovirales*; subordination: *Cornidovirineae*; family: Coronaviridae (19). They can be grouped into four genera, including α/β/γ/δ-CoV: α and β infect mammals and γ/δ infect birds (20). CoVs are large, positive-stranded RNA viruses, and they are enveloped with a lipid membrane derived from a host cell. The protein protruding from the virus membrane is the spike (S) protein, giving the virus the appearance of a solar corona (1) (**Figure 1A**). Coronaviruses have single-stranded RNA of between 26.4 and 31.7 kilobases, making them the largest of RNA viruses (21). CoVs have several main structural proteins (**Figure 1A**): nucleocapsid (N) proteins, which surround the RNA genome; membrane (M) proteins (also known as E1 membrane glycoprotein or matrix protein) (20); envelope (E) proteins, involved in virus assembly, and S protein, which mediates virus entry into host cells. Some CoVs also encode an envelope-associated hemaglutinin-esterase protein (HE) used as an invading mechanism (22).

The S protein is the main antigenic component of SARS-CoV-2 structural proteins and is comprised of two subunits, S1 and S2 (23). This protein is multifunctional, contributing to host receptor binding, pathogenesis, and cell tropism. The S protein binds to host receptors on target cells, inducing virion particle endocytosis, and then catalyzes the fusion between host and viral membranes, allowing the virus genome penetration into the host cytoplasm (24). The S1 domain has a high-affinity association with the host receptor angiotensin-converting enzyme 2 (ACE2) (25). The receptor-binding domain (RBD) of the S protein binds to the extracellular peptidase domain of ACE2, mediating cell entry (25, 26). SARS-CoV-2 uses the SARS-CoV receptor ACE2 for entry and the transmembrane serine protease 2 (TMPRSS2) for S protein priming. The endosomal cysteine proteases cathepsin B and L (CatB/L) can be used to mature the S protein (27, 28). However, while TMPRSS2 is indispensable for viral spread and pathogenesis, the CatB/L activity is not essential (**Figure 1B** step 1). Once the virus enters the host cell, viral replication begins with translation of the replicase-polymerase gene and assembly of the replication-transcription complex. This complex also transcribes the genomic regions to structural proteins. New virions are assembled in the endoplasmic reticulum and Golgi apparatus released from the cell (**Figure 1B** step 1) (29). Finally, the newly assembled SARS-CoV-2 virions possess protein S on the surface and are ready to infect any cell that expresses the ACE2 receptor with no further requirement for TMPRSS2 activity (30).

SARS-CoV2 Pathophysiology

The SARS-CoV-2 pathophysiology is not yet clear. It has been suggested that it can be similar to SARS-CoV (31, 32) with two possible responses (32):

1) After the viral infection occurs, active viral replication and dissemination through ACE2 receptors occurs with the associated host antiviral responses. SARS-CoV-2 downregulates ACE2 receptors, with loss of their catalytic effect at the membrane surface. Inflammation and thrombosis have been related to enhanced and unimpeded angiotensin II effects through the ACE-Angiotensin II-AT1 receptor axis (33) (**Figure 1B** step 2). The SARS-CoV-2 infection can lead to an acute immune response. This response is driven by inflammatory alveolar and monocyte-derived macrophages that can be activated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) released by infected pneumocytes (34–36). Subsequently, several pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α) and IL-1β, secreted

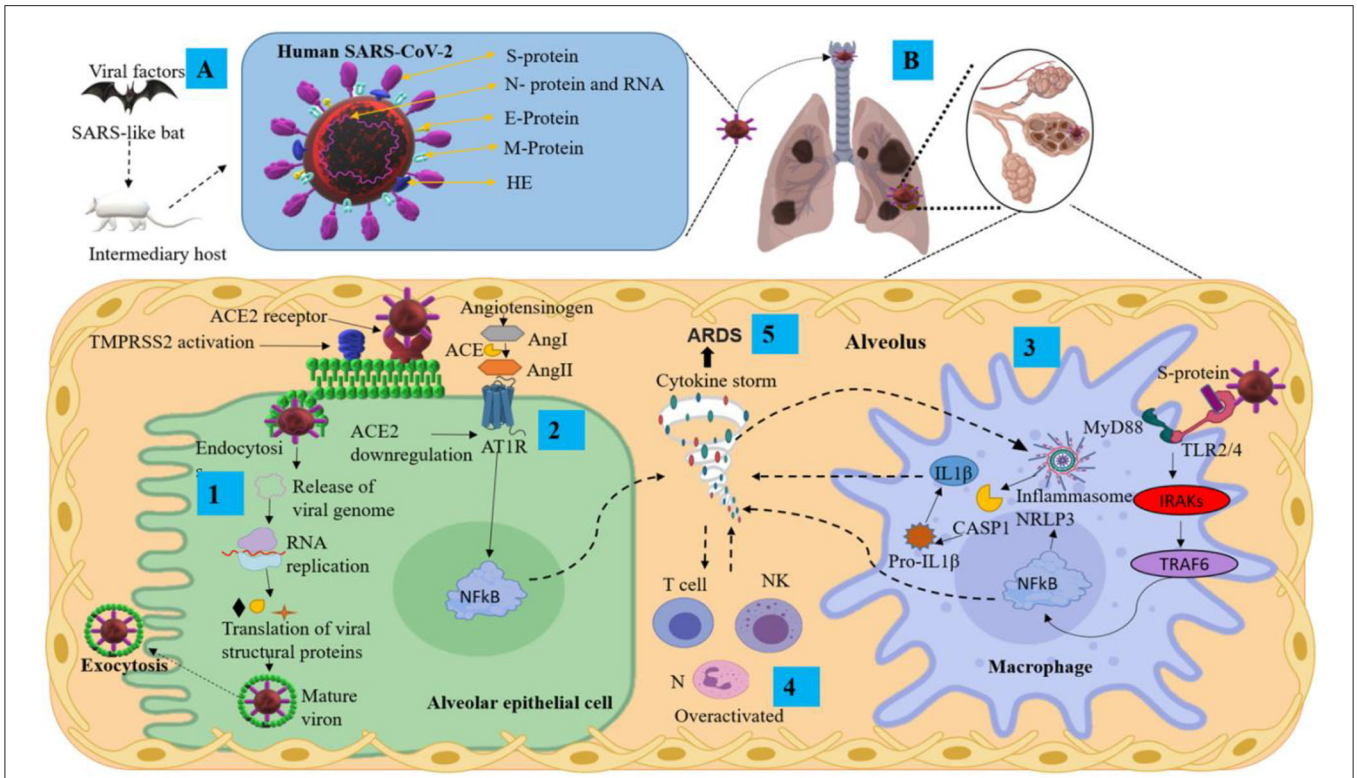

FIGURE 1 | Pathological mechanisms of SARS-CoV-2 in the pulmonary alveolus. **(A)** Mode of transmission and main structural proteins of SARS-CoV-2. **(B)** Mechanisms of SARS-CoV-2 infection and pulmonary inflammatory immune response. ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; ARDS, acute respiratory distress syndrome; AT1R, angiotensin II type I receptor; CASP1, aaspase 1; E protein, envelope small membrane protein; HE, hemagglutinin esterase; IL1β, interleukin 1 beta; IRAKs, interleukin-1 receptor-associated kinases; M protein, membrane protein; MyD88, myeloid differentiation primary response 88; N protein, nucleoprotein; N, neutrophils; NF-κB, nuclear factor Kappa B; NK, natural killer cells; NLRP3, nucleotide-binding domain-, leucine-rich repeat-containing receptor, pyrin domain-containing 3; RNA, ribonucleic acid; S protein, spike protein; TMPRSS2, transmembrane serine protease 2; TRAF6, tumor necrosis factor receptor-associated factor 6.

by alveolar macrophages, initiate the acute inflammatory cascade that triggers cell death and damage. Aside from PAMP/DAMP production, the recruitment of immune cells and activation of the nucleotide-binding domain leucine-rich repeat-containing receptor, pyrin domain-containing 3 (NLRP3), establish a pro-inflammatory positive feedback cascade (32, 34, 35) (**Figure 1B** steps 3 and 4). This localized inflammatory cell death could lead to a hyper-inflammatory microenvironment and spread to the vasculature, inducing leakage, edema, and pneumonia in COVID-19 patients (35, 37). Serum of COVID-19 patients is characterized by increased levels of the following: IL-2, IL-7, IL-10, TNF-α, protein monocyte chemoattractant-1 (MCP1; also known as C-C motif chemokine ligand 2 CCL2), granulocyte colony-stimulating factor (G-CSF), macrophage inflammatory protein 1 alpha (MIP1α; also known as CCL3), C-X-C motif chemokine ligand 10 (CXCL10), C-reactive protein (CRP), D-dimers and ferritin (19, 38–40).

2) The SARS-CoV-2 infection can also lead to the generation of adaptive immunity and neutralizing antibody (NAb). The virus-NAb complex can trigger Fc receptor (FcR)-mediated inflammatory response and acute lung injury. SARS-CoV-2 can infect cells that have FcRs, which provide the ability for antibody-mediated internalization. This mechanism can occur in macrophages, monocytes, or B cells even without ACE2 and TMPRSS2 expression, and especially during infection (41). The internalization of the virus–antibody immune complexes can also promote tissue damage and inflammation by activating myeloid cells *via* FcRs (42). Both primary and secondary responses culminate in the postulated pathogenesis of SARS-CoV-2 infection (32). Interestingly, it has been suggested that fatal COVID-19 is characterized as a cytokine release syndrome (CRS) induced by a cytokine storm and associated with adverse outcomes of acute respiratory distress syndrome (ARDS) (**Figure 1B** step 5) and high mortality rate (43, 44).

For mechanistic insights into the life cycle of SARS-CoV-2, the mouse hepatitis virus (MHV) represents a suitable comparator. MHV is a βCoV very similar to SARS-CoV, MERS-CoV, and SARS-CoV-2 (45). Therefore, an MHV animal model could contribute to the elucidation of the neuropathological mechanisms of SARS-CoV-2 in the following aspects: (1) MHV can invade and replicate in the CNS, triggering lesions

in the white matter (45); (2) Infection with MHV induces meningoencephalitis in an acute stage and subsequently subacute chronic inflammatory demyelination in the brain and spinal cord (46); (3) CD4 and CD8 T lymphocytes, especially γδ T cells, play an important role in the MHV-induced demyelination process (47); (4) MHV can be translocated from the initial inoculation brain area to the spinal cord through the transit of viral particles in glial and neural cells, as well as by mechanisms that involve the fusion of lipid membranes (48); (5) After intranasal MHV-CoV inoculation in mice, the virus can access the CNS through the olfactory nerve and spread from this area to neuroanatomically interconnected structures such as the limbic system and the brainstem (49).

Potential Neuroinvasive Pathways of SARS-CoV-2

There are four possible routes by which SARS-CoV-2 could enter the CNS: (1) the hematopoietic pathway and subsequent rupture of the blood-brain barrier (BBB); (2) *via* blood-cerebrospinal fluid (B-CSF); (3) transsynaptic viral spreading; (4) through the entry to circumventricular organs (CVO). In this section, we will discuss the four routes in greater detail.

1) Coronaviruses access the bloodstream *via* the airway and infect immune cells, which may cross BBB facilitated by pro-inflammatory cytokines and chemokines (**Figure 2B** step 1). The mechanism by which infected immune cells cross the BBB may occur *via* intercellular adhesion molecule 1 (ICAM-1) mediated transport that is upregulated by TNF-α, followed by activation of matrix metalloproteinases (MMPs) such as MMP9, which specifically influences cellular leakage and membrane degradation (50). Besides, SARS-CoV-2 tropism, toward the CNS endothelial cells (BECs) favors BBB disruption; by entering the cytosol of the astrocyte *via* the receptor; the virus increases the release of pro-inflammatory cytokines, such as IL-2, IL-6, IL-7, IL-8, TNFα, CCL2, CCL3, CCL7, and CXCL10. The reactive astrocyte could lead to activation of microglia and the peripheral immune infiltrate such as macrophages, neutrophils, and lymphocytes (**Figure 2B** step 2) (51–54), which could end in neurotoxicity. DM, hypertension, and metabolic syndrome are risk factors for both contracting COVID-19 and a poor prognosis in patients. These comorbidities also contribute to vascular and BBB alteration (55), increase neuroinflammation, and exacerbate neuropathology (56).
2) The CSF circulation comprises both a directional CSF flow and a pulsatile to and from movement throughout the entire brain and which involves a local fluid exchange between blood, interstitial fluid, and CSF (57). It has been suggested that viral infection may occur *via* B-CSF and alter gene expression in the choroid plexus. This process activates the nuclear factor kappa (NF-kB), upregulates MMP9, and affects B-CSF permeability and immune cell trafficking (MMP8, TNFα, IL6, IL1B, MCP1, intercellular adhesion molecule 1 (ICAM1) (58), leading to a neuroinflammatory environment.
3) Another entry route to the CNS for SARS-CoV-2 could be through axonal transport and transneuronal spread from olfactory, gustatory, trigeminal, and vagal nerves, allowing the virus to infect the brainstem in the early stages of infection (**Figures 3A–D**) (52, 59). The transneuronal pathway is one of the potential routes that would allow SARS-CoV-2 to enter through the primary sensory neurons, which communicate with the mitral cells. Mitral cells have projections toward the ventricle and the medulla, and this favors the transfer of the virus from the cerebrospinal fluid toward the lymphatic system within the CNS and toward the PNS (60). The virus could also enter the CNS following the transneuronal olfactory bulb pathway and is reflected by changes at the level of the olfactory nerve, bulb, and cortex (61–63). It has been proposed that SARS-CoV-2 could spread retrogradely through transsynaptic transfer, using an exocytosis/endocytosis mechanism or *via* rapid axonal transport, which would move the virus along the microtubules to the neuronal soma (64). Supporting this hypothesis, it has been shown that some CoVs and other viruses such as rabies and hemagglutinating encephalomyelitis can enter and spread to the CNS *via* retrograde transsynaptic pathways (65–67), from peripheral nerve endings through membranous-coating-mediated endocytosis and exocytosis (66). Mechanisms have also been described by which viruses can enter and leave axons, both retrograde and anterograde, through coupled transport mediated by vesicles or separate transport which is not mediated by vesicles (68). For this reason, it would be interesting in the future to know if SARS-CoV2 uses transsynaptic transport and to trace neural circuits, using specific labeling techniques.
4) Finally, we suggest that SARS-CoV-2 might enter the CNS through CVOs. CVOs include the subfornical organ, the paraventricular nucleus, the nucleus tractus solitarius (NTS), and the rostral ventrolateral medulla, all of which express ACE2. Besides, these CVOs are highly vascularized and lack a BBB (69). Therefore, these areas would be more susceptible to the virus, triggering neurovascular damage, as we have discussed previously.

Once SARS-CoV-2 enters the CNS, it could bind to CNS cells, such as neurons, astrocytes, oligodendrocytes, and microglia (70), due to the presence of ACE2 (28, 71) and TMPRSS2 (72) receptors and probably *via* binding to other receptors (**Table 1**). It is important to highlight that the expression of ACE2 is low in the human brain, with a higher expression in certain areas such as thalamus and choroid plexus. ACE2 also has access to peptides in the circulation in the cerebrospinal and interstitial fluid, and it is present in pericytes and smooth muscle cells of human brain vessels (95). ACE2 receptors have been reported in other organs, mainly enterocytes, renal tubules, gallbladder, cardiomyocytes, male reproductive cells, placental trophoblasts, ductal cells, eye, and vasculature. In the respiratory system, its expression is limited (96). ACE2 plays a role in attenuating microvascular pathology and protecting against atherogenesis, endothelial dysfunction, thrombus formation, oxidative stress,

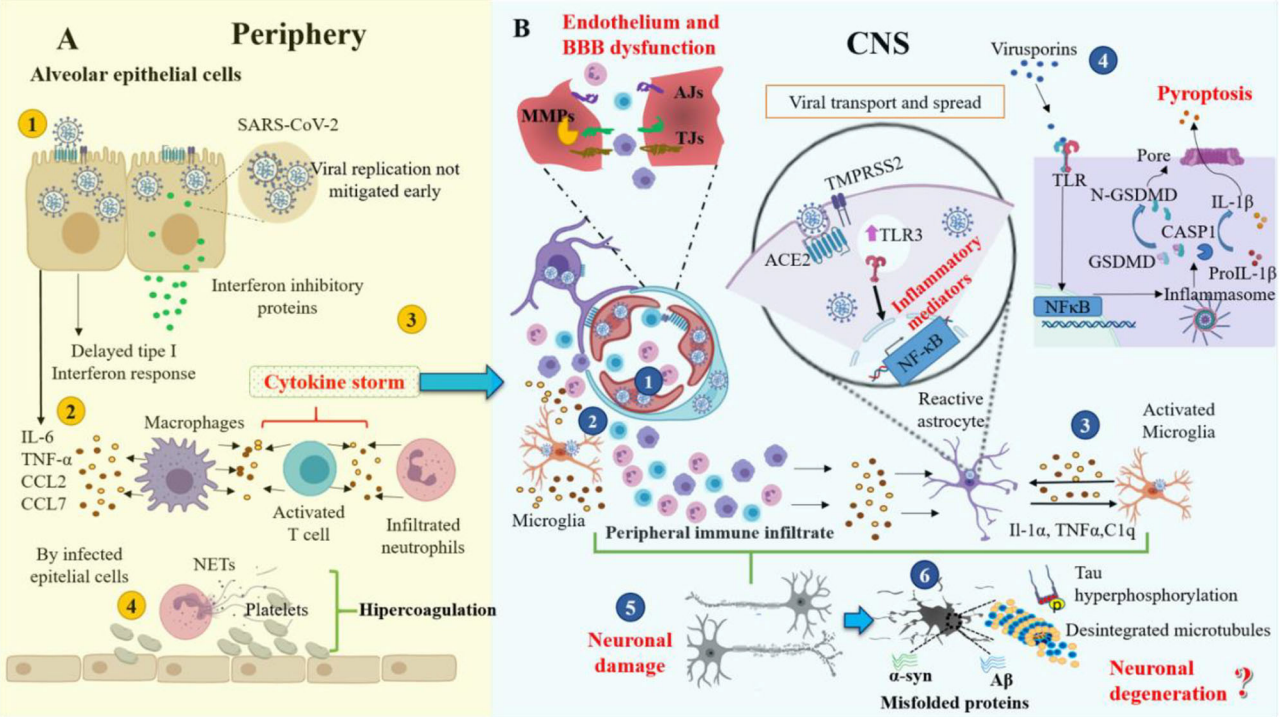

FIGURE 2 | Schematic representation of the pathophysiological mechanisms of SARS-CoV-2. **(A)** Peripheral pathological events triggered by SARS-CoV-2 infection. **(B)** Possible CNS pathological mechanisms caused by the severe peripheral hyperinflammation associated with COVID-19. ACE2, angiotensin-converting enzyme 2 receptor; AJs, adherent junctions; Aβ; amyloid-beta; BBB, blood-brain barrier; C1q, the complement component 1q; CASP1, caspase1; CCL, chemokine (C-C motif) ligand; CNS, central nervous system; CXCL10, C–X–C motif chemokine 10; GSDMD, gasdermin-D; IL, interleukin; MMPs, metalloproteinases; NETs, neutrophil extracellular traps; NF-κB, Nuclear factor Kappa B; N-GSDMD, N-terminal gasdermin; NLRP3, nucleotide-binding domain-, leucine-rich repeat-containing receptor, pyrin domain-containing 3; TJs, tight junctions; TLR3, toll-like receptor 3; TNF-α, tumor necrosis factor-alpha; α-syn, alpha-synuclein.

and inflammatory cascades responsible for monocyte-endothelial cell interaction (71, 97).

SARS-CoV-2 interaction with ACE2 could cause astrogliosis and microgliosis, increase BBB permeability, allowing monocyte and leukocyte infiltration to the CNS in multiple brain regions (98, 99). These areas include the olfactory bulb, choroid plexus, cerebral cortex, caudate/putamen, ventral striatum, thalamus, hypothalamus (paraventricular nuclei), spinal cord, hippocampus, frontal cortex (52, 95, 100), substantia nigra, middle temporal gyrus (64, 101), and other brain areas (**Table 1**). Since many viruses have neurotropic properties (102), SARS-CoV-2 could spread through neuroanatomically interconnected pathways (103) and lead to nerve cell dysfunction and neurodegeneration in the CNS.

Can the Systemic Inflammation by COVID-19 Trigger Neurovascular Disturbance?

In this section, we highlight evidence at the systemic level and locally in the respiratory tract tissue of patients with COVID-19. Since this virus induces lung pathology, the detailed information of other organs and systems such as the CNS has yet to be fully investigated. Therefore, the nerve signaling pathways proposed here are based on the systemic evidence from similar viruses.

Channappanavar and Perlman focused on the systemic immune response against pathogenic human coronaviruses such as SARS-CoV and Middle East respiratory syndrome CoV (MERS-CoV). They proposed that a dysregulated immune response in the host is responsible for triggering the pulmonary pathology and fatal clinical manifestations (104).

On the other hand, high levels of viral replication in the host could contribute to tissue damage. Two mechanisms might be responsible: first, the delayed induction of interferon responses and second, the production of interferon inhibitory proteins by the human CoVs. Therefore, early unmitigated viral replication could be responsible for the high and exaggerated production of cytokines and chemokines by infected alveolar epithelial cells, macrophages, and leukocytes infiltrated into the lung tissue, which leads to severe damage (**Figure 2A** steps 1–3) (104). Other investigators have concluded that COVID-19 is characterized by an extreme hyper-inflammatory process followed by hyper-coagulation (**Figure 2A** step 4) (105).

CRS is a systemic inflammatory response that can be triggered by SARS CoV-2 infection, characterized by a drastic increase in the levels of the pro-inflammatory cytokines (**Figure 2A**) (106). The CRS may induce a MODS in COVID-19 patients, which

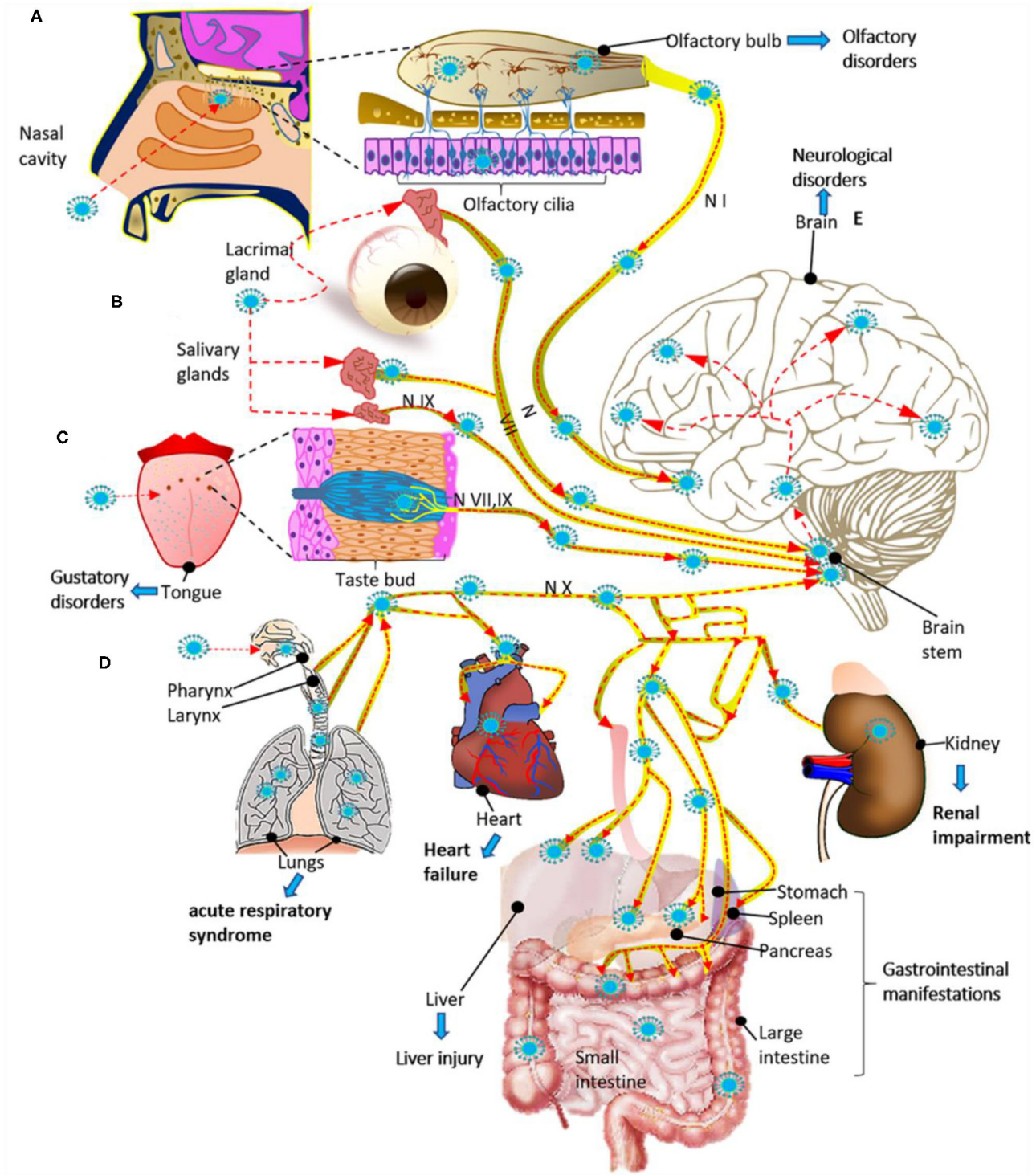

FIGURE 3 | Potential routes for infection and spread of SARS-CoV-2 to systemic organs and the central nervous system through the cranial nerves (N). **(A)** SARS-CoV-2 could enter through the olfactory mucosa (causing anosmia), spread through the olfactory nerve (N I) and end in the olfactory cortex. **(B)** SARS-CoV-2 could also enter through the lacrimal and salivary glands, spread through the facial (N VII) and glossopharyngeal (N IX) nerves, and end in their respective brain stem nuclei. **(C)** The infection could spread from the taste buds (triggering ageusia) through the N VII and N IX nerves ending in the NTS located in the brain stem. **(D)** SARS-CoV-2 could also enter through the respiratory tract, reach the respiratory system and *via* the vagus nerve (N X), spread to other systemic organs innervated by this nerve, and end in the brain stem. **(E)** Finally, once the virus reaches the brain stem, it can spread to the brain through neuroanatomically interconnected pathways. The SARS-Cov2 infection can cause multiple organ dysfunction syndrome **(A–E)**. The red dashed arrows indicate the possible dissemination route for SARS-CoV-2 through the cranial nerves.

TABLE 1 | Receptors or proteins related to SARS-CoV-2 infection in the nervous system.

Receptor or protein	Expression in nerve cells	Neuroanatomic areas of gene expression*	Pathological effects on the nervous system	References
ACE2	Neurons, astrocytes, microglia, BECs, OLGs	PG, Acb, Hy, SC, Cd, SN, Cb, HiF, FroCx, Amg, Pu and ACC	The direct binding of SARS-CoV-2 to the ACE2 receptor could trigger microvascular dysfunction, disrupt coagulation processes, cause neuronal depolarization, and increase expression of glutamate and MMPs, resulting in neuroinflammation, seizures, and hemorrhages.	(56, 58, 69, 73–75)
TMPRSS2		PG, Hy, Cb, Amg, Cd, HiF, SN, Acb, ACC, FroCx, Pu and SC.	It acts as a co-receptor for ACE2 and cleaves S protein, facilitating viral binding to the ACE2 receptor and its activation. Therefore, it promotes the same effects described for ACE-2.	(76–78)
DPP4	Astrocytes (in murine)	FroCx, SC, ACC, PG, SN, Hy, HiF, Amg, Cb, Acb, Cd and Pu.	It is strongly associated with MERS-CoV. The murine models for DPP4 receptor infected with MERS-CoV have shown neuronal damage and peripheral immune infiltrates.	(79–81)
TLR4	Astrocytes, microglia	Cd, SN, Acb, Amg, Pu, SC, ACC, FroCx, y, HiF and Cb.	Molecular docking studies have demonstrated the binding of the native S protein of SARS-CoV-2 to TLR1, TLR4, and TLR6. However, TLR4 is most likely to recognize molecular patterns from SARS-CoV-2 to induce inflammatory responses. In CNS, it could promote the neuroinflammation environment.	(82, 83)
ATR1	Neurons, astrocytes	PG, SN, Hy, Cb SC,HiF, Cd, Acb, Pu, Amg, FroCx and ACC	It has been suggested that SARS-CoV-2 causes lung damage by increasing Ang II production. The hyperactivation of Ang II/ATR1/ACE signaling results in increased expression of pro-inflammatory cytokines, macrophage activation, and possibly BBB dysfunction.	(84–87)
ITGB1	Microglia	SC, PG, SN, Hy, HiF,Pu, Cd, Amg, FroCx, Acb, ACC and Cb.	It has been suggested that ITGB1 could bind to S protein through the RGD or KGE motif. ITGB1 mainly activates the MI3K/MAPK pathways, inducing an inflammatory response.	(24, 88, 89)
CatB and CatL	Microglia, neurons, astrocytes.	Cat B: FroCx, PG, SC, Cb, Hy, Acb, ACC, Cd, SN, Pu, HiF and Amg Cat: PG, SC, FroCx, Cb, SN, Hy, Cd, Acb, Pu, ACC, HiF and Amg	It has been suggested that S protein priming is partly dependent on the endosomal proteases, CatB and CatL. Nevertheless, TMPRSS2 is essential for viral entry into primary target cells and viral spread in the infected host. Also, CatB and CatL can contribute to the neuroinflammatory process.	(90, 91)
NLRP3	Microglia, astrocytes, neurons	SC, FroCx, Acb, Hy, SN, ACC, HiF, Amg, Cd, PG, Pu, Cb	To date, it is unclear if SARS-CoV-2 activates the NLRP3 inflammasome. However, SARS-CoV expresses at least three proteins (viroporins) that activate the NLRP3 inflammasome: envelope (E), ORF3a, and ORF8b. The NLRP3 inflammasome activation could trigger inflammatory cell death.	(92–94)

Neuroanatomic areas and nerve cells in which these receptors or proteins are expressed and their possible neuropathological effects.
Acb, nucleus accumbens; ACC, anterior cingulate cortex; ACE2, angiotensin-converting enzyme 2; Amg, amygdala; ATR1, angiotensin receptor type 1; BBB, blood-brain barrier; BECs, brain endothelial cells; Cat, cathepsin; Cb, cerebellum; Cd, caudate nucleus; DPP4, dipeptidyl peptidase-4; FroCx, frontal cortex; HiF, hippocampal formation; Hy, hypothalamus; ITGB1, integrin subunit beta 1; KGE, Lys-Gly-Glu; MAPK, Mitogen-Activated Protein Kinases; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; MI3K, myo-inositol 3-kinase; MMPs, matrix metalloproteinases; NF-kB, nuclear factor kappa B; NLRP3, nucleotide-binding domain-, leucine-rich repeat-containing receptor, pyrin domain-containing 3; OLGs, oligodendrocytes; PG, pituitary gland; Pu, putamen; RGD, Arg-Gly-Asp; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SC, spinal cord (cervical c-1), SN, substantia nigra; TLR, Toll Like Receptor; TMPRSS2, transmembrane protease serine 2.
**The GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2) was used to obtain the gene expression data (from highest to lowest expression) in several brain areas.*

is characterized by acute failure of different organs such as the liver, kidney, heart, and as well as hematological, gastrointestinal, and neurological disorders (**Figure 3**) (107). Besides, it has been proposed that patients who died from severe COVID-19 have a significant endothelial affectation or "endothelitis," which may be associated with MODS. It has been suggested that endothelial dysfunction in several organs may be triggered by the interaction between SARS-CoV-2 with ACE2 receptors that express endothelial cells and the subsequent inflammatory response (108, 109). This inflammatory response can contribute to increased vascular permeability, edema, and the synthesis of coagulation factors (110). From a meta-analysis, it has been reported that levels of D-dimer, an indicator of fibrinolysis, have been reported following severe infection by COVID-19 (111). The formation of clots could result in the occlusion of blood vessels and cerebral arteries, which can lead to cerebral venous thrombosis. Therefore, we assume that some of the symptoms and even neurological complications may be caused by the systemic cytokine storm and subsequent endothelium and BBB dysfunction (**Figure 2**). In this way, systemic hyper-inflammation caused by maladaptive innate immunity may trigger neurovascular function damage, a BBB rupture, and activate the CNS innate immune signaling pathways (112). This BBB disruption could promote immune cell infiltration (113) (**Figure 2B** steps 1 and 2). The intracerebral cytokine storm also could contribute to the BBB rupture (114, 115), leading to a vicious cycle of increasing pathology. These events may also be responsible for developing other neuropathies such as necrotizing encephalopathy or Guillain-Barré syndrome (GBS) (116, 117). The coagulopathy observed in COVID-19 could make patients prone to thrombotic cerebrovascular or bleeding events (118).

On the other hand, microglia and astrocytes are the main cell lineages that mediate immunological processes within the CNS. Thus, microglia, the macrophage of the CNS par excellence, can also promote states of hyper-inflammation that exacerbate hypercoagulation by infiltration of professional immune cells and coagulation elements. Nevertheless, we hypothesize that SARS-CoV-2 could activate the microglia and, subsequently, induce the reactivation of A1 astrocytes *via* secreting IL-1α, TNF, and the complement component 1q (C1q), as occurs in other neurological diseases (119) (**Figure 2B** step 3). Besides, exposure to the viruses or their components promotes the expression and activation of Toll-like receptors (TLR) in astrocytes. This signaling promotes the production and release of pro-inflammatory mediators and induces inflammatory responses in the CNS (**Figure 2B** step 3), eliminating the pathogen as demonstrated for Flavivirus infections (82, 120). Therefore, this pathological signaling causes neuronal degeneration and dysfunction of the nerve cells short or long term.

Viroporins belong to a family of small transmembrane proteins that include CoV protein E (121, 122), which could generate neurotropism of SARS-CoV. According to previous studies, viroporins can promote the activation of the NLRP3 inflammasome (123, 124). The NLRP3 inflammasome is a subcellular multiprotein complex that is highly expressed in several nerve cells and CNS areas (**Table 1**). Activation of the NLRP3 occurs after infection by the influenza A virus and SARS-CoV (125) (**Figure 2B** step 4). After NLRP3 inflammasome activation, caspase-1 and other non-canonical inflammasome caspases (caspase-4, caspase-5, or caspase-11) activate gasdermin-D (GSDMD), which subsequently forms pores in the cell membrane. These pores facilitate the secretion of IL-1β and IL-18 and, importantly, they also enable the simultaneous influx of Na^+ and water molecules, facilitating neuroinvasion by causing excessive cellular swelling, membrane rupture, and subsequent pyroptosis, an inflammatory form of cell death (126, 127). Therefore, it is possible that pyroptosis may occur in nerve cells (**Figure 2B** step 4).

Neurological and Neuropsychiatric Manifestations, Diagnostic, and Treatment in Patients With COVID-19

The respiratory symptoms caused by the SARS-CoV-2 virus are still the most readily identified and studied. However, neurological manifestations are beginning to take on unquestionable importance, mainly in the critically affected patient. Our understanding of the long-term neurological symptoms is limited and presents a real challenge (13, 14, 62, 128). A physiopathological explanation for the neurological and neuropsychiatric manifestations of COVID-19 has yet to be found. Although there are hypotheses about the direct effects of SARS-CoV-2 on the CNS and PNS, evidence suggests that these effects can be attributed to other causes such as: (1) the impact of the systemic inflammatory response caused by the virus and (2) the underlying comorbidities of the patients (62, 129, 130). Patients with mild COVID-19 have been reported to have non-specific neurological disorders such as headache and myalgias, dizziness, dysgeusia, and anosmia with variations in their prevalence (**Table 2**) (13, 14).

Some studies point to headache as the most common neurological symptom and often as the only symptom of COVID-19 (129, 147). However, other authors have defined the headache as a consequence of systemic disease. It has been suggested that the chronic release or exposure of vasoactive peptides such as Calcitonin Gene-Related Peptide (CGRP; pain and migraine-related peptide) (148) can activate trigeminal sensory fibers and thus modulate the transmission of impulses related to headache (149). Besides, in COVID-19, a close link between cytokine storm and headache has been proposed, due to the release of the vasoactive peptides (150).

In contrast, for hospitalized patients, encephalopathy with neuropsychiatric manifestations such as delirium and agitation have been observed. There has also been a considerable increase in the reports of patients with neuromuscular diseases, among which are GBS with some of its variants and several rhabdomyolysis cases. However, it has not been possible to find a clear relationship between these manifestations and COVID-19. An increasing incidence of neurological manifestations has been observed in COVID-19-infected patients that have been associated with severe health conditions and prolonged hospital stays (129, 134). However, there are also reports of neurological manifestations in outpatients with COVID-19 infection (129, 138). Therefore, it is difficult to determine the incidence of each of the neurological manifestations due to the different screening methods applied for each reported case.

Neurological disorders such as multiple sclerosis (MS), encephalopathy, and GBS, have been associated with SARS-CoV2. In some cases, CNS demyelination has occurred shortly after SARS-CoV-2 infection, suggesting a causal relationship between these two pathologies (151). Viruses, such as the Epstein-Barr virus (EBV), have been linked to MS, with high titers of EBV antibodies found in MS patients. Viral induced demyelination could be a direct result of viral infection of oligodendrocytes, which leads to cell death and myelin degeneration, or to the exacerbated inflammatory response caused by virus replication (152, 153). The cytokine storm caused by SARS-CoV-2 may cause the activation of glial cells and the start of the demyelination process (154). Conversely, other studies suggest that SARS-CoV-2 could act as an accelerating factor for MS but not the trigger for the disaese (155). Likewise, several case reports have reported the appearance of GBS after SARS-CoV-2 infection (156–158). Although hypoxic/metabolic changes caused by intense inflammatory response against the virus together with the presence of comorbidities, may result in encephalopathy (159) there is still insufficient evidence to prove that SARS-CoV-2 virus infection invades the CNS directly to provoke encephalopathy (151). In the same way, despite the complications associated with SARS-CoV-2 infection in patients with GBS, there is no clear evidence yet that COVID-19 initiates GBS (151).

Cerebral events have been associated with SARS-CoV-2 patients, with cerebral ischemic events being the most frequent. Cerebral hemorrhages and microhemorrhages are also noticed (62, 144) (**Table 2**).

TABLE 2 | SARS-CoV-2 infection in the central and peripheral nervous system: clinical manifestations, mechanism of pathogenicity, laboratory, and clinical findings and suggested treatment.

Clinical manifestation	The probable mechanism of pathogenicity	Laboratory and/or clinical alterations	Treatment or recommendations	References
Central nervous system				
Headache: occurs in ∼70% of patients, with an average duration of 3 days.	(1) Direct viral invasion of the trigeminal nerve endings in the nasal or oral cavity. (2) An increase in the levels of peptides related to the circulating calcium gene has been linked to the trigeminal vascular activation.	The use of neurological and laboratory imaging techniques is only recommended if the headache is associated with focal neurological symptoms.	(1) NSAIDs and steroids are not recommended as they can exacerbate COVID-19 symptoms. (2) Anticonvulsants may offer benefits.	(131–133)
Delayed awakening: has been observed in some patients after ventilation for COVID-19-related ARDS.	A relationship between posterior circulation inflammation and brainstem function may be related to altered consciousness.	(1) Brain MRA: an increase in the abnormal contrast has been observed in the arterial wall associated with endotelialitis. (2) EEG: non-specific changes have been observed. (3) In serum and CSF: oligoclonal bands have been observed.	The use of IV methylprednisolone has been proposed for 5 days, followed by decreasing doses of prednisone.	(130)
Encephalopathy: One study Liotta et al. (134) determined that it was present in about a third of patients, and was associated with increased mortality.	It has been proposed that they may be involved in toxic-metabolic processes such as hypoxemia, ROS production, and organ failure.	MRI: intensity changes in the leptomeningeal spaces, in the mesial temporal lobe, and the hippocampus, as well as frontotemporal hypoperfusion.	The use of low potency antipsychotic agents and alpha-2 agonists has been proposed to control psychomotor agitation.	(134–136)
Ischemic stroke event: is a life-threatening complication and is associated with cardioembolic events.	(1) Elevated inflammation, DIC, and hypoxia have been associated with a state of hypercoagulability. (2) Complement activation is associated with microvascular damage leading to thrombotic injury.	(1) The neuroimaging patterns observed are extensive vessel thrombosis, embolism, or stenosis, followed by affected multiple vascular territories. (2) Laboratory studies have revealed an increase of D-dimer, fibrinogen, antiphospholipid antibody levels.	Prophylactic or therapeutic anticoagulation therapy, as well as thrombectomy, have been recommended.	(62, 137)
Hemorrhagic stroke: has been attributed to COVID-19 and risk factors as anticoagulation, trauma, and hypertension.	Lupus anticoagulant and antiphospholipid antibodies have been suggested to play a role in its pathophysiology.	Imaging studies have revealed microhemorrhage foci, hematomas larger than 5cm, surrounding edema, and even descending hernia.	Reduce risk factors that affect hypertension, aneurysm, and states of anticoagulation.	(62)
Peripheral nervous system				
Olfactory disorders: present around 86% of COVID-19 patients: anosmia (79%), hyposmia (20.4%), phantosmia (12%), and parosmia (32%) Lechien et al. (138).	(1) Nasal epithelial damage is characterized by a reduced number of ORs and abnormal dendrites that do not reach the epithelial surface or lack sensory cilia. (2) Substitution of ONE with metaplastic squamous epithelium. (3) Inflammation can lead to impairment of ORs and also damage of olfactory neurons.	MRI has shown abnormalities in the signaling of one or both olfactory bulbs, edema of the olfactory bulb, and microhemorrhage in one of the olfactory bulbs.	The most widely used treatments for olfactory dysfunction are saline nasal irrigations, nasal corticosteroids, oral corticosteroids, vitamins, and trace elements.	(138–141)
Gustatory disorders: present around 88% of COVID-19 patients: hypogeusia (79%) or dysgeusia (21%) Lechien et al. (138)	(1) Diffuse expression of ACE2 receptors (modulation of taste perception) in the oral mucosa, particularly in the tongue. (2) SARS-CoV-2 can bind to sialic acid receptors, accelerating the degradation of taste particles.	Recent evidence suggests that imaging or laboratory studies are not usually done on patients who only manifest gustatory disorders.	Treatment for these disorders has not been established; however, l-carnitine or trace elements and vitamins have been used.	(138, 142)
Neuromuscular disorders: myalgia and fatigue affect between 44 and 70% of patients. About 10% of patients have a skeletal muscle injury.	(1) SARS-Cov-2 could trigger viral myositis. (2) Alteration in the expression of ECA2 in skeletal muscle. (3) Skeletal muscle damage from cytokine storm.	(1) Elevated serum creatine kinase levels. (2) Muscle injury has been associated with multiple organ damage, such as liver dysfunction (increased levels of LDH, ALT, and AST) and kidney (increased levels of blood urea nitrogen and creatinine).	The use of corticosteroids has resulted in benefits.	(129, 143)

(Continued)

TABLE 2 | Continued

Clinical manifestation	The probable mechanism of pathogenicity	Laboratory and/or clinical alterations	Treatment or recommendations	References
Guillain-Barre Syndrome GBS: has been associated with COVID-19. Interestingly, the interval between the onset of COVID-19 symptoms and the first symptoms of GBS has ranged from 5 to 10 days.	(1) It has been proposed that it serves the same mechanisms as typical GBS, consisting of demyelination of peripheral nerve roots. (2) Peripheral nerve damage can be caused by the immune response to SARS-CoV-2, driven by the production of autoreactive antibodies (anti-ganglioside).	(1) Hematological and biochemical examinations have shown leukocytosis, leukopenia, thrombocytosis, thrombocytopenia, and elevated levels of CRP. (2) CSF tests have shown cytological dissociation of albumin. (3) EMG has been associated with a demyelinating process. (4) MRI has revealed an enhancement in the caudal nerve roots and the facial nerve.	The therapeutic protocol to GBS associated with COVID-19 has been typically used for this pathology: IV immunoglobulin or plasma exchange, supportive care, and antiviral drugs.	(144–146)

ACE2, angiotensin-converting enzyme 2; ALT, alanine transaminase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; CNS, central nervous system; CRP, C-reactive protein; CSF, cerebrospinal fluid; DIC, disseminated intravascular coagulation; EEG, electroencephalography; EMG, electromyography; GBS, Guillain-Barre syndrome; IV, intravenous; LDH, lactate dehydrogenase; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; NSAIDs, non-steroidal anti-inflammatory drugs; ONE, olfactory neuroepithelium; ORs, olfactory receptors; ROS, reactive oxygen species.

As discussed previously, the cranial nerves might also be susceptible to a direct or indirect injury caused by SARS-CoV-2. According to a recent study, about 86 and 88% of patients with COVID-19 develop olfactory and gustatory alterations, respectively (138). These findings might be specific for SARS-CoV-2 infection and be useful to distinguish them from other causes. Interestingly, the presence of these dysfunctions can precede the onset of respiratory symptoms (160, 161) and may predict a mild clinical course of the disease (162) (**Table 2**). Besides, it has been proposed that pericytes of the olfactory bulb, which express high levels of the ACE2 receptor, may be responsible for triggering the cytokine storm and thus causing olfactory disorders in COVID-19 patients (163).

On the other hand, neuroimaging data could help us understand the pathological effects of SARS-CoV-2 in the CNS and PNS. Unfortunately, published brain imaging findings from confirmed COVID-19 patients are currently scarce and limited to small case series. However, it has been possible to correlate them with the potential pathophysiological mechanisms involved. For example, in a recent study, it was shown that patients with COVID-19 presented multifocal petechial hemorrhages associated with BBB rupture (164). In another study, microhemorrhages and macrohemorrhages were associated with posterior reversible encephalopathy syndrome (165) (**Table 2**). Although the underlying mechanism of brain abnormalities detected through neuroimaging remains to be understood, these findings provide further evidence that CNS damage can occur in COVID-19 patients. The correct understanding of pathophysiological mechanisms of neurological manifestations may reveal potential therapeutic targets (**Table 3**). Depending on the neurological complications associated with COVID-19, treatments would need to be adjusted accordingly (**Table 2**).

Neurohistopathological Findings by COVID-19

The histopathological analysis of nervous tissue of patients who presented neurological complications and died due to COVID-19 is undoubtedly precious to our understanding of the pathophysiology and potential therapeutic strategies (**Table 4**).

Solomon et al. analyzed nervous tissue from 18 patients infected with SARS-CoV-2 who had also presented with certain comorbidities such as DM, hypertension, cardiovascular disease, hyperlipidemia, chronic kidney disease, and dementia. The histological examination (**Table 4**) revealed a greater number of copies of SARS-CoV-2, acute hypoxic-ischemic injury, neuronal loss, and perivascular inflammation in several brain areas, and even pathological features of Alzheimer's disease (AD) were observed (175). A different case report described the brain from a 73-year-old man with unspecified neurological manifestations (**Table 4**), hypertension and DM and positive for SARS-CoV-2 with cranial computed tomography. The results showed right cerebellar intra-parenchymal hemorrhage, edema, medulla compression, and tonsillar herniation. After 18 h without improvement, the patient died of palliative extubation. Brain histopathology revealed severe global hypoxic changes with scattered hypereosinophilic shrunken neurons in several brain areas and mild perivascular inflammatory infiltrates (**Table 4**) (177). This study also suggested a preference of the virus to the cerebellar Purkinje cell layer. In addition, astrogliosis was noted in the superior frontal and orbital cortices, while microglial activation in the cortex was not evident (177).

A study focused on describing the SARS-CoV-2 tropism within the olfactory mucosa to the CNS examined autopsy material from 33 patients positive for the virus. The authors showed viral RNA for SARS-CoV-2 within the olfactory mucosa sampled directly beneath the cribiform plate. They also found viral RNA in anatomically distinct regions such as cornea, conjunctiva and oral mucosa. Using immunohistochemistry, *in situ* hybridization, and electron microscopy, they suggested that SARS-CoV-2 neuroinvasion to the CNS occurs *via* axonal transport, thus explaining the well-documented neurological symptoms (**Table 4**) (178). The authors also proposed that SARS-CoV-2 infection in the cerebellar region may occur by the migration of the virus-carrying leukocytes across the BBB, without directly connecting this area to the olfactory mucosa.

TABLE 3 | Current drugs used against COVID-19.

Drug	Mechanism of action	Results of clinical case reports	Adverse effects	Dose	References
Antiviral drugs					
Remdesivir	The prodrug, belonging to the group of nucleotide analogs, generates an active metabolite capable of entering cells and inhibits viral RNA polymerase. Inhibitory capacity against SARS-CoV-2 *in vitro* has been observed*.	Decreased recovery time, disease progression, as well as mortality compared to placebo.	- Infusion-related hypotension - Hepatotoxic - Nephrotoxic - Gastrointestinal symptoms	- First dose of 200 mg - 100 mg/day for 5–9 days.	(166)
TMPRSS2 antagonist					
Camostat	Produces GBPA, that inhibits many of the serine proteases that SARS CoV and SARS-CoV-2 use for virus-to-host cell membrane fusion, like TMPRSS2**.	Reduces the likelihood of serious infection, as well as morbidity and mortality.	- Eruption - Pruritus - Oedema - Urticaria	It has been used at different doses in humans and other pathologies. e.g., : 200 mg every 8 h	(167)
Nafamostat mesylate	Inhibited SARS-CoV-2 S protein-mediated entry into host cells with about 15-fold-higher efficiency than camostat, with a 50% effective concentration.	In combination with Favipiravir has shown a decrease in mortality.	Hyperkalemia	0.2 mg per kg/hour by continuous IV infusion, for 14 days.	(168, 169)
Monoclonal antibodies					
Tocilizumab	IL-6 receptor antagonist	May reduce the hospital stay, the need for ICU admission, and the need for invasive mechanical ventilation.	- Increased risk of secondary infections. - Hypersensitivity reactions - Neutropenia and thrombocytopenia - Hepatotoxicity	>75 kg: 600 mg single dose <75 kg: 400 mg single dose***	(170)
Anakinra	IL-1 receptor antagonist	Reduced both needs for invasive mechanical ventilation and mortality in severe COVID-19 patients.	- Elevation of liver enzymes three times higher than their reference value. - Possible thromboembolic events.	100 mg every 6 h for a maximum of 15 days.	(171, 172)
Mavrilimumab	Binds to GM-CSFRα**** and disrupts downstream signaling.	Fast clinical improvement, decrease both the need for mechanical ventilation and mortality.	No adverse reactions to the infusion were observed.	6 mg/kg single dose.	(173)
Steroids					
Dexamethasone	- Anti-inflammatory action. - Inhibits phospholipase A2 and, consequently, prostaglandin, thromboxane, and leukotriene synthesis - Suppresses leukocyte migration - Recovers the BBB by upregulation of ZO-1 tight junction protein	Decreased mortality in patients requiring oxygen therapy and mechanical ventilatory support when treatment is initiated 7 days after symptom onset.	- Hyperglycemia - Increased risk of bacterial and fungal infections.	6 mg/day for 10 days.	(174)

GBPA, 4-[4-guanidinobenzoyl-oxy] phenylacetic acid; GM-CSF, Granulocyte-macrophage colony-stimulating factor (GM-CSF); TMPRSS2, Transmembrane serine protease 2.
**Inhibitory activity against SARS-CoV-1 and MERS-CoV has been demonstrated.*
***The high expression of TMPRSS2 in different brain areas could be a potential therapeutic target for neurological manifestations and complications.*
****According to safety criteria and clinical trial data.*
*****GM-CSF is a cytokine with a cardinal role in inflammation modulation. Ligand binding to the GM-CSF receptor-α (GM-CSFRα) activates multiple pro-inflammatory pathways and, in macrophages and neutrophils, results in increased secretion of pro-inflammatory cytokines.*

Other cranial nerves have been considered as the route for entrance of the virus to the CNS (**Figure 3**) (181, 182). Regarding ageusia, the pathogenesis may involve an alteration in the glossopharyngeal, facial, vagus nerve, or the nucleus tractus solitarii (NTS), at the brainstem level (183). In an immunohistochemistry analysis of the cranial nerves from two individuals, SARS-CoV-2 and viral proteins were found within the medulla oblongata and in both glossopharyngeal and vagal nerves from the lower brainstem (179), suggesting these areas as a potential route for virus entry into the CNS and peripheral tissue (**Table 4**).

Other authors found hypereosinophilia or nuclear and cytoplasmic condensation of neurons in the cerebrum and cerebellum of severe COVID-19 patients due to hypoxic

TABLE 4 | Neurohistopathological findings in patients infected with SARS-CoV-2 and their association with neurological manifestations.

Characteristics of the patients	Tissue and PMI	Histopathological findings	Neurological manifestations	References
Central nervous system				
n = 18 age range: 53–75 years comorbidities: AF, ALL, BPH, CAD, CKD, COPD, DM, ESRD on HD, EtOH use disorder, HF, HTN, ILD, MGUS, NHL, OCD, OSA, PPV, PVD, RA-SLE.	Inferior-frontal lobe with olfactory tract/bulb, corpus callosum, hippocampus, occipital lobe, anterior basal ganglia, thalamus, cerebellum, midbrain, pons, and medulla. PMI: NS.	Acute hypoxic-ischemic injury with neuronal loss in the cerebral cortex, hippocampus, and cerebellar Purkinje cell layer. Arteriolosclerosis with perivascular rarefaction, a microglial nodule, and perivascular inflammation with scattered microglia were also detected.	It is associated with the confusional state, myalgia, headache or, hypogeusia.	(175)
n = 6 age range: 58–82 years comorbidities: EtOH use disorder, HTN, COPD, CKD, PHT, PVD, CAD, AF.	Hippocampus, neocortex, cerebellum, and brainstem nuclei. PMI: NS.	Lymphocytic panencephalitis and meningitis. Neuronal cell loss and axon degeneration in the dorsal motor nuclei of the CN X and V, NTS, dorsal raphe nuclei, and medial longitudinal fasciculus.	Associated with altered consciousness.	(176)
n = 1 age: 73 years commorbidities: DM and HTN.	Cortex, hippocampus, amygdala, striatum. PMI: NS.	Cerebellar hemorrhage, acute infarcts, global hypoxic changes with scattered hypereosinophilic shrunken neurons in the cerebral cortex, striatum, thalamus, amygdala, hippocampus, and the Purkinje cell layer.	Headache, nausea, vomiting, and loss of consciousness.	(177)
Cranial nerves and peripheric nervous system				
n = 33 age range: 67–79 years commorbidities: DM, HTN, CVD, HLD, CKD, PS and dementia.	Olfactory mucosa, bulb and tuber, oral mucosa, trigeminal ganglion, medulla oblongata, and cerebellum. PMI: NS.	High levels of viral SARS-CoV-2 RNA (RT–qPCR) and protein within the olfactory mucosa. Lower levels were found in the cornea, conjunctiva, and oral mucosa; and in only a few COVID-19 autopsy cases, the cerebellum was positive for SARS-CoV-2.	Alterations of smell and taste perception, impaired consciousness, headache, and behavioral changes	(178)
n = 2 age: 51 and 94 years commorbidities: COPD, IHD and AML	Glossopharyngeal, vagal nerves and other brain areas. PMI: 3.3 days	SARS-CoV-2 viral proteins mapped to isolated cells.	Ageusia	(179)
n = 21 age range: 41–78 years commorbidities: DM, CVD, COPD, asthma, ASM and AHM.	Olfactory bulbs, NTS and other brain areas. PMI: NS.	Extensive inflammation and infiltrating immune cells.	Anosmia and dampening of the respiratory system.	(180)

AF, atrial fibrillation; AHM, active hematological malignancy; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ASM, active solid malignancy; BPH, benign prostatic hyperplasia; CAD, coronary artery disease; CKD, chronic kidney disease; CN, cranial nerves; COPD, chronic obstructive lung disease; CVD, cardiovascular disease; DM, diabetes mellitus; ESRD on HD, end stage renal disease on dialysis; EtOH use disorder, alcohol use disorder; HF, heart failure; HLD, hyperlipidemia; HTN, hypertension; IHD, ischaemic heart disease; ILD, interstitial lung disease; MGUS, monoclonal gammopathy of undetermined significance; n, number of patients; NHL, non-Hodgkin lymphoma; NS, not specified; NTS, nucleus tractus solitarius; OCD, obsessive compulsive disorder; OSA, obstructive sleep apnea; PHT, pulmonary hypertension; PMI, postmortem interval; PS, prior stroke; PVD, peripheral vascular disease; RA-SLE, rheumatoid arthritis - systemic lupus erythematosus; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

brain changes (**Table 4**). The olfactory bulb histopathological analysis showed many activated microglia with enlarged bodies and T-cell extravasation into the parenchyma and elevated levels of reactive astrocytes (180). Interestingly, the NTS showed astrogliosis and a massive microglial activation with the formation of a microglia nodule and T cells in the leptomeninges of the medulla oblongata (180). This implies extensive inflammation in this area, which results in a dysregulation of the respiratory system (184). One of the main limitations of these studies is that it is not clear whether the histopathological findings are the result of patients' comorbidities/aging, or due to SARS-CoV2 neuro-infection. Further investigations are necessary to make the corresponding comparison with healthy subjects with appropriate age ranges and to understand the neuropathological mechanism of SARS-CoV-2 and its relationship with neurological manifestations and patient comorbidities.

The Potential Role of SARS-CoV-2 in the Pathogenesis of Neurodegenerative Diseases

The SARS-CoV-2 neurotropism has already been documented in several reports (**Table 2**) (185–187). However, it remains unknown whether SARS-CoV-2 contributes to neurodegenerative pathogenesis. It has been hypothesized that viruses can cause neurological problems by affecting neurotransmitter release, lysing the cells, inducing apoptosis, commanding neuronal transcriptional pathways or indirectly activating the immune response (188).

Neuroinvasive animal CoVs, such as the porcine hemagglutinating encephalitis virus (PHEV) or MHV have been shown to induce different types of neuropathology. Similarly, human CoVs, such as HCoV-229E and HCoV-OC43, have been implicated in establishing or exacerbating neurodegenerative diseases (189–192).

AD is the most common cause of dementia among the elderly. Neuropathological hallmarks of AD include the neurofibrillary tangles, consisting of intraneuronal and hyperphosphorylated tau, and the extracellular accumulation of amyloid β-peptide (Aβ) in the brain parenchyma in the form of neuritic plaques (193, 194). The neurovascular unit (NVU) and BBB dysregulation are also critical pathophysiological events in neurodegenerative diseases, including AD. Previous studies have suggested a relationship between AD and infectious agents. *Chlamydia pneumoniae, Helicobacter pylori, Borrelia burgdorferi*, and herpes simplex virus have been reported in post-mortem AD brain (195) and a relationship between virus infection and Aβ has been suggested. Soscia et al. noticed that Aβ exerted antimicrobial activity against relevant microorganisms and was modulated in response to some environmental stressors. Thus, transient viral infection could initiate or accelerate Aβ accumulation in the brain and neuronal damage (**Figure 2B** steps 5 and 6) (196). ACE2 can induce an increase of nitric oxide (NO) in the brain, which becomes neurotoxic. NO and other reactive species, which could be produced as a consequence of viral internalization and impairment of cell organelles (mitochondria, lysosomes), could, in turn, increase misfolding and aggregation of cellular proteins (197).

It has been proposed that SARS-CoV-2 could increase the hyperphosphorylation of tau in the axonal region (52, 198–200), promoting disassembly of microtubules and, subsequently, neuronal degeneration (**Figure 2B** steps 5 and 6) (201). Likewise, it has been suggested that persistent CoV infections can induce a neuroimmune response and a pro-inflammatory state, and activate glial cells (202). Microglial cells may be chronically activated by a single stimulus, such as pathogen infection, resulting in slow and progressive neuronal loss through multiple neurotoxic factors (203). Interferon (IFN), which has a role in mediating AD pathology, directly activates microglia and stimulates a pro-inflammatory response derived from SARS-CoV-2 infection (204). Therefore, it seems that the neurotropism of SARS-CoV-2 can lead to the activation of microglial cells, trigger chronic neuroinflammation, and finally, neurodegeneration (**Figure 2B**).

Finally, it has been found that ACE2 is upregulated in the cerebral vasculature of dementia cases. ACE2 increases the intracellular level of angiotensin 2, causing vasoconstriction and promoting brain degeneration (205). Buzhdygan et al. demonstrated that S1 could promote BBB alteration in an advanced 3D microfluidic model of the BBB, being able to induce different types of neuropathology (98).

Like AD, viral agents have been associated with parkinsonism disorders. These include the post-encephalitic parkinsonism linked to the 1918 influenza A H1N1 pandemic (206) and the parkinsonism associated with Epstein Barr, Coxsackie, West Nile, herpes and the human immunodeficiency (HIV) viruses (207–209). Parkinson's disease (PD) is the second most common and fastest-growing neurodegenerative disorder (210). It is characterized by dopaminergic neuronal loss in the *substantia nigra pars compacta* and the accumulation of misfolded α-synuclein (α-syn), which is found as intracytoplasmic inclusions called "Lewy bodies" (211). SARS-CoV-2 could play a role in the epidemiology of PD, since pro-inflammatory events triggered by viral infections could act as predisposing factors to the development of PD (**Figure 2B** steps 5 and 6) (**Table 2**) (212–214). This inflammatory environment can trigger the long-term neuronal loss, misfolding, aggregation, and spread of α-syn through the CNS (215, 216). Interestingly, it has been proposed that α-syn plays an essential role in response to infection, promoting a higher expression of α-syn, as occurs in the West Nile virus encephalitis (217, 218). Furthermore, α-syn aggregation can activate microglia, favoring the pro-inflammatory response and cellular damage signals, leading to slow and progressive neuronal death (219). However, it remains unknown whether SARS-CoV-2 could contribute to neurodegenerative pathogenesis, or whether it only uses the CNS as a reservoir, making it difficult for the virus to replicate, due to the low level of ACE2 receptors expressed in CNS (220).

CONCLUSION

COVID-19 pandemic has become a real challenge for the scientific community around the world. Although SARS-CoV-2 mainly affects the respiratory tract, more evidence suggests that this virus can also invade the CNS causing neurological manifestations. The possible routes of SARS-CoV-2 neuroinvasion include: (1) the hematopoietic pathway *via* the BBB, (2) *via* the B-CSF, (3) *via* retrograde axonal transport through the cranial nerves, and (4) *via* the circumventricular organs. Once the virus enters the CNS, it binds to cell receptors, including ACE2. This receptor is expressed in several brain areas and in both neuronal and non-neuronal cell types. The binding of SARS-CoV-2 with ACE2 can promote neuroinflammation, hypercoagulation, microhemorrhages, BBB dysfunction, generation of reactive species, phosphorylation of tau, protein misfolding and aggregation, and neuronal death, features that are closely related to the appearance or progression of neurodegenerative diseases. Further studies on the molecular changes in the brain triggered by SARS-CoV-2 infection would facilitate timely diagnosis and therapeutic approaches.

AUTHOR CONTRIBUTIONS

MP-H, JL-M, and LS-R contributed to the idea formulation, reviewing of the literature, and writing and revision of the manuscript. YF-M, CH, MV-R, AL-A, PM-G, BC-C, VB-A, RA-P, CC-T, and JD-G contributed to reviewing of the literature and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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† Deceased.

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Acute Transverse Myelitis (ATM): Clinical Review of 43 Patients With COVID-19-Associated ATM and 3 Post-Vaccination ATM Serious Adverse Events With the ChAdOx1 nCoV-19 Vaccine (AZD1222)

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Introduction: Although acute transverse myelitis (ATM) is a rare neurological condition (1.34-4.6 cases per million/year) COVID-19-associated ATM cases have occurred during the pandemic.

Case-finding methods: We report a patient from Panama with SARS-CoV-2 infection complicated by ATM and present a comprehensive clinical review of 43 patients with COVID-19-associated ATM from 21 countries published from March 2020 to January 2021. In addition, 3 cases of ATM were reported as serious adverse events during the clinical trials of the COVID-19 vaccine ChAdOx1 nCoV-19 (AZD1222).

Results: All patients had typical features of ATM with acute onset of paralysis, sensory level and sphincter deficits due to spinal cord lesions demonstrated by imaging. There were 23 males (53%) and 20 females (47%) ranging from ages 21- to 73- years-old (mean age, 49 years), with two peaks at 29 and 58 years, excluding 3 pediatric cases. The main clinical manifestations were quadriplegia (58%) and paraplegia (42%). MRI reports were available in 40 patients; localized ATM lesions affected ≤3 cord segments (12 cases, 30%) at cervical (5 cases) and thoracic cord levels (7 cases); 28 cases (70%) had longitudinally-extensive ATM (LEATM) involving ≥4 spinal cord segments (cervicothoracic in 18 cases and thoracolumbar-sacral in 10 patients). Acute disseminated encephalomyelitis (ADEM) occurred in 8 patients, mainly women (67%) ranging from 27- to 64-years-old. Three ATM patients also had blindness from myeloneuritis optica (MNO) and two more also had acute motor axonal neuropathy (AMAN).

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Conclusions: We found ATM to be an unexpectedly frequent neurological complication of COVID-19. Most cases (68%) had a latency of 10 days to 6 weeks that may indicate post-infectious neurological complications mediated by the host's response to the virus. In 32% a brief latency (15 hours to 5 days) suggested a direct neurotropic effect of SARS-CoV-2. The occurrence of 3 reported ATM adverse effects among 11,636 participants in the AZD1222 vaccine trials is extremely high considering a worldwide incidence of 0.5/million COVID-19-associated ATM cases found in this report. The pathogenesis of ATM remains unknown, but it is conceivable that SARS-CoV-2 antigens –perhaps also present in the AZD1222 COVID-19 vaccine or its chimpanzee adenovirus adjuvant– may induce immune mechanisms leading to the myelitis.

Keywords: COVID-19, neurological complications, SARS-CoV-2 neurotropism, myelitis, transverse myelitis, COVID-19 ChAdOx1 nCoV-19 vaccine

INTRODUCTION

Neurological complications of coronavirus disease 2019 (COVID-19) are well recognized (1–3) and affect both the central nervous system (CNS) and the peripheral nervous system (PNS). Neurological injury results from the affinity of the COVID-19 etiological agent, the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), for the angiotensin-converting enzyme 2 (ACE2) receptor present in neurons and glial cells endowing high neuroinvasive potential to SARS-CoV-2 compared to previous coronaviruses. The high frequency of anosmia during the acute infection probably reflects viral invasion of the olfactory bulbs. Cells with abundant ACE2 receptors are infected first by this coronavirus including nasal epithelium cells, ciliated bronchial epithelial cells and type II pneumocytes, explaining the severity of the pulmonary involvement. Also, the presence of ACE2 receptors for the viral S protein in endothelial cells correlates with the frequent vascular complications of COVID-19 resulting from endotheliitis and microvascular brain injury (4) that induces the host's immune response with cytokine storm, hyperinflammation, coagulopathy, thrombosis and embolism resulting in ischemic and hemorrhagic strokes and multisystemic complications affecting lungs, heart, kidneys and liver.

According to Borchers and Gershwin (5), ATM is a rare neurological condition in adults with an estimated incidence ranging between 1.34 and 4.6 cases per million annually with a mean age of 35-40 years. We report a patient with SARS-CoV-2 infection in Panama who developed acute transverse myelitis (ATM) and we present the results of a comprehensive review of COVID-19-associated myelitis that yielded 42 additional cases reported in 21 countries worldwide (6–44) published from March 2020 to January 2021 during year 1 of the pandemic (**Table 1** and **Supplementary Table 1A**). Furthermore, 3 ATM serious adverse events were reported with the ChAdOx1 nCoV-19 (AZD1222) vaccine trials (45, 46).

CASE DESCRIPTION

A previously-healthy 72-year-old man presented to the emergency department at a hospital in Panama City, Panama, complaining of sudden difficulty to urinate. The urologist diagnosed neurogenic bladder and placed a Foley catheter. Three days later the patient developed dysesthesias in arms and legs and weakness of all four limbs. Neurologic examination showed 3+/5 strength in the upper extremities and 1+/5 in the lower limbs with spastic paraplegia, generalized hyperreflexia, bilateral Babinski, and spontaneous pyramidal jerking of both legs; sensory examination disclosed decreased proprioception in the legs and a tactile sensory level below Th_9. The patient was alert and oriented; higher cortical functions, cranial nerves and cerebellar examination were all intact.

He denied fever, headache, ageusia, anosmia, fatigue, diarrhea or upper respiratory symptoms during the past 3 weeks. Past medical history was negative except for hypertension controlled with enalapril. The SARS-CoV-2 RNA PCR nasal swab test was negative on 2 occasions. His wife was asymptomatic, but her nasal swab test was positive, and she had SARS-CoV-2 antibodies. The patient's serology demonstrated recent infection with SARS-CoV-2 IgG index = 3.53 (normal <1.6) and IgM index = 5.1 (normal <0.6). Chest X-rays showed mild cardiomegaly but no evidence of consolidation or pleural effusion. Chest computerized tomography (CT) scan was normal. Electrocardiogram showed mild left ventricular hypertrophy. The patient was afebrile and his general physical evaluation was normal. Respiratory rate 16 breaths per minute, oxygen saturation 98% on room air, blood pressure 130/80 mmHg, heart rate 78 beats per minute and temperature 36.8°C.

Laboratory results showed normal white blood cell count (8,100/μL) with normal hemoglobin (14.7g/dl). Inflammatory markers showed elevated C-reactive protein at 1.7mg/dl and high erythrocyte sedimentation rate at 51mm/hr (normal range 0-10mm/hr). Coagulation profile was normal. Protein C, Protein S, Antithrombin III, and activated Protein C resistance were within normal limits. Hematology consult found no associated pathology. Hepatic and thyroid function tests were normal. VDRL and HIV tests were negative. Autoimmune immunological screening was negative for lupus anticoagulant, anti-nuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies, rheumatoid factor, anti-cardiolipin, and

TABLE 1 | Summary of SARS-CoV-2-Associated Myelitis Published Cased from March-2020 until January-2021.

Case	Country	Sex/Age years	Myelitis Type		Lesion Level				Other Clinical Features	Ref.
			ATM	LEATM	C	CT	Th	Conus		
1	CN	M/66	ATM				Th10			Zhao (6)
2	IR	M/60		LEATM	C1-4					Saberi (7)
3	GB	M/40	ATM		C1-2				ADEM Brainstem rhombencephalitis	Wong (8)
4	IT	W/54		LEATM		C2-Th6			Bulbomedullary lesions	Zanin (9)
5	DK	W/28		LEATM	X	X	X	X	Medulla oblongata to conus medullaris	Sarma (10)
6	ES	W/69		LEATM		C7 - Th1			Medulla to C7- Th1	Sotoca (11)
7	DE	M/60	ATM				Th9		Late lesions Th3-5 Th9-10	Munz (12)
8	IT	W/64	ATM				Th8		ADEM – NMO Monoclonal gammopathy CSF SARS-CoV-2 (+)	Novi (13)
9	IT	W/22	ATM		X					Giorgianni (14)
10	US	W/61		LEATM		C1-Th1			AMAN	Valiuddin (15) Maideniuc (16)
11	AE	M/32		LEATM		C2-Th-L				Al Ketbi (17)
12	BR	W/42	ATM		C5				Trigeminal nucleus	Barros- Domingues (18)
13	IR	M/21		LEATM		C1-Th				Zoghi (19)
14	US	M/24		LEATM			Th7-12			Durrani (20)
15	CH	M/63	ATM				Th10			Zachariadis (21)
16	TR	M/48	ATM		C2-3				ADEM CSF: SARS- CoV-2 (+)	Otluoglu (22)
17	QA	M/52		LEATM			Th3-10			Abdelhady (23)
18	US	M/44		LEATM		C5-7 Th3-6		conus medullaris	ADEM	Utukuri (24)
19	US	W/40	ATM		C1				ADEM Pons, medulla	McCuddy (25)
20	AU	M/60		LEATM			Th7-10			Chow (26)
21	US	G/3		LEATM		C1 to Th6			Lower medulla to Th6	Kaur (27)
22	MD	M/27		LEATM		C4-Th5			HIV (+)	Lisnic (28)
23	IN	W/59	ATM				Th6-7			Chakraborty (29)
24	IR	M/63		LEATM		C7-Th12				Hazrati (30)
25	BR	W/51		LETM			Th6-10		Lumbar radiculitis	Corrêa (31)
26	IT	W/70	ATM			C7-Th1			AMAN Anti-GD1b IgM	Masuccio (32)
27	IR	W/53	ATM				Th8-10			Baghbanian (33)
28	IT	M/64	ATM							Rifino (34)
29	IT	M/64	ATM							Rifino (34)
30	ES	M/50	ATM		C5-C6					Águila- Gordo (35)
31	TR	G/14		LEATM	C2-5					Güler (36)
32	MX	M/73		LEATM	C1 - C3-6				Atlas to C3-C6 cervical spondylotic myelopathy	Guadarrama - Ortiz (37)
33	US	M/26		LEATM		C4-7 Th5-8			Edema optic nerves MOG-IgG-mediated NMOSD	Zhou (38)
34	ID	W/45	ATM				Th3-4			Munir (39)
35	GB	W/33		LEATM		C1-7 Th2			ADEM Brain & pontomedullary	Paterson (40)
36	GB	W/27	ATM					Conus medullaris	ADEM diffuse T2 white matter and corticospinal lesions	Paterson (40)

(Continued)

TABLE 1 | Continued

Case	Country	Sex/Age years	Myelitis Type		Lesion Level				Other Clinical Features	Ref.
			ATM	LEATM	C	CT	Th	Conus		
37	GB	M/48		LEATM			Th5-6 Th10-11	Conus medullaris		Paterson (40)
38	IR	M/47		LEATM		C2-Th2				Advani (41)
39	IR	W/67		LEATM	C3-C6					Advani (41)
40	PK	M/56		LEATM			Th4-Th8			Ali (42)
41	IR	G/11		LEATM			Th3-Th6			Nejad- Biglari (43)
42	BE	W/38		LETM		C3-Th4				Fumery (44)
43	PA	M/72		LEATM		C2-Th9				Román-Gracia (this report)

Names of Countries: Australia = AU, Belgium = BE, Brazil = BR, China = CN, Denmark = DK, Germany = DE, India = IN, Indonesia = ID, Iran = IR, Italy = IT, Mexico = MX, Moldova = MD, Panama = PA, Pakistan = PK, Qatar = QA, Spain = ES, Switzerland = CH, Turkey = TR, United Arab Emirates = AE, United Kingdom = GB, United States of America = US.
ADEM, acute disseminated encephalomyelitis; AMAN, acute motor axonal neuropathy; ANA, antinuclear antibodies; AQP4, aquaporin-4; ATM, acute transverse myelitis; (C, cervical; CSF, cerebrospinal fluid; G, girl; HIV, human immunodeficiency virus; LEATM, longitudinally-extensive acute transverse myelitis; M, man; MOG-IgG, myelin oligodendrocyte glycoprotein antibody-immune globulin G; NMOSD, neuromyelitis optica spectrum disorder; SARS-CoV-2, severe acute respiratory syndrome-coronavirus-2, Th, thoracic; W, woman.

complement C3, C4. Rheumatology consult found no underlying disease. Aquaporin-4 antibody (anti-AQP4) and myelin oligodendrocyte glycoprotein antibody IgG (anti-MOG-IgG) in serum were both negative (Quest diagnostics Nichols Institute, CA).

Gadolinium-enhanced magnetic resonance imaging (MRI) of the brain was normal. MRI of the spinal cord revealed mild cervical and thoracic cord enlargement and swelling with diffuse hyperintensities. On the axial projections, cord hyperintensities at C_4-C_5 and Th_3-Th_4 were observed with irregular patchy imaging but without contrast enhancement consistent with ATM (**Figure 1**). No apparent hemorrhagic components were present, and the conus medullaris had normal appearance.

Cerebrospinal fluid (CSF) showed no cells, hyperproteinorraquia of 76mg/dl and normal glucose. Meningitis CSF panel was negative for bacteria, yeast and viruses. Gram, acid-fast bacilli and fungus stains were negative. CSF oligoclonal bands (IgG) demonstrated 3 well-defined gamma restriction bands that were not present in serum (Quest diagnostics Nichols Institute, CA).

The patient was treated with a pulse dose of IV methylprednisolone 1g/d for 5 days, enoxaparin 40 mg daily, followed by IV gamma-globulin (IVIG) 30g/day for five days. Oral prednisone was prescribed for the next 30 days. He recovered partial strength in his upper limbs (4+/5) but the severe spastic paraplegia (1+/5) and the neurogenic bladder remained unchanged. He is undergoing physical therapy and rehabilitation treatment.

CASE-FINDING REVIEW

We performed a comprehensive search of the literature using PubMed, Medline, Scopus, Web of Science, EMBASE, and Google Scholar up to January 5, 2021. For PubMed we used the following key search terms: ("Myelitis, Transverse" [MeSH] OR "Myelitis" [All Fields] OR "Myelitis, Acute" [All Fields] OR "Encephalomyelitis" [All Fields] OR "Neuromyelitis Optica" [MeSH] OR "Myeloneuropathy" [All Fields] OR "Encephalomyelitis, Acute Disseminated" [MeSH] OR "Acute Disseminated Encephalomyelitis" [All Fields]) AND ("COVID-19" [MeSH Term] OR "SARS-CoV-2" [MeSH Term] OR "coronavirus" [All Fields]). **Table 1** and **Supplementary Table 1A** list the total 43 patients reported in 21 countries worldwide, as follows: 7 cases from Iran (IR), 6 each from Italy (IT) and the United States of America (US), 4 from the United Kingdom (GB), 2 cases each from Brazil (BR), Spain (ES), and Turkey (TR), plus single case reports from Australia (AU), Belgium (BE), China (CN), Denmark (DK), Germany (DE), India (IN), Indonesia (ID), Mexico (MX), Moldova (MD), Panama (PA), Pakistan (PK), Qatar (QA), Switzerland (CH), and the United Arab Emirates (AE). Patient 10 from the US was published twice (15, 16).

RESULTS

Early reports of neurological complications of COVID-19 from China (2) and France (3) included no cases of ATM. Therefore, it was unexpected to collect 43 cases of COVID-19-associated myelitis in a period of 10 months around the world. Given a total of 86 million COVID-19 cases as of 5 January 2021 (coronavirus.jhu.edu) the incidence of myelitis is 0.5 per million. Based on a single hospital COVID-19 series with 1760 patients from Italy (34), SARS-CoV-2-associated ATM may represent 1.2% of all neurological complications of COVID-19.

COVID-19-associated ATM was reported in 23 males (53%) and 20 females (47%) ranging in age from 21 to 73 years (mean age 49 years) excluding children. There were three age groups: (i)

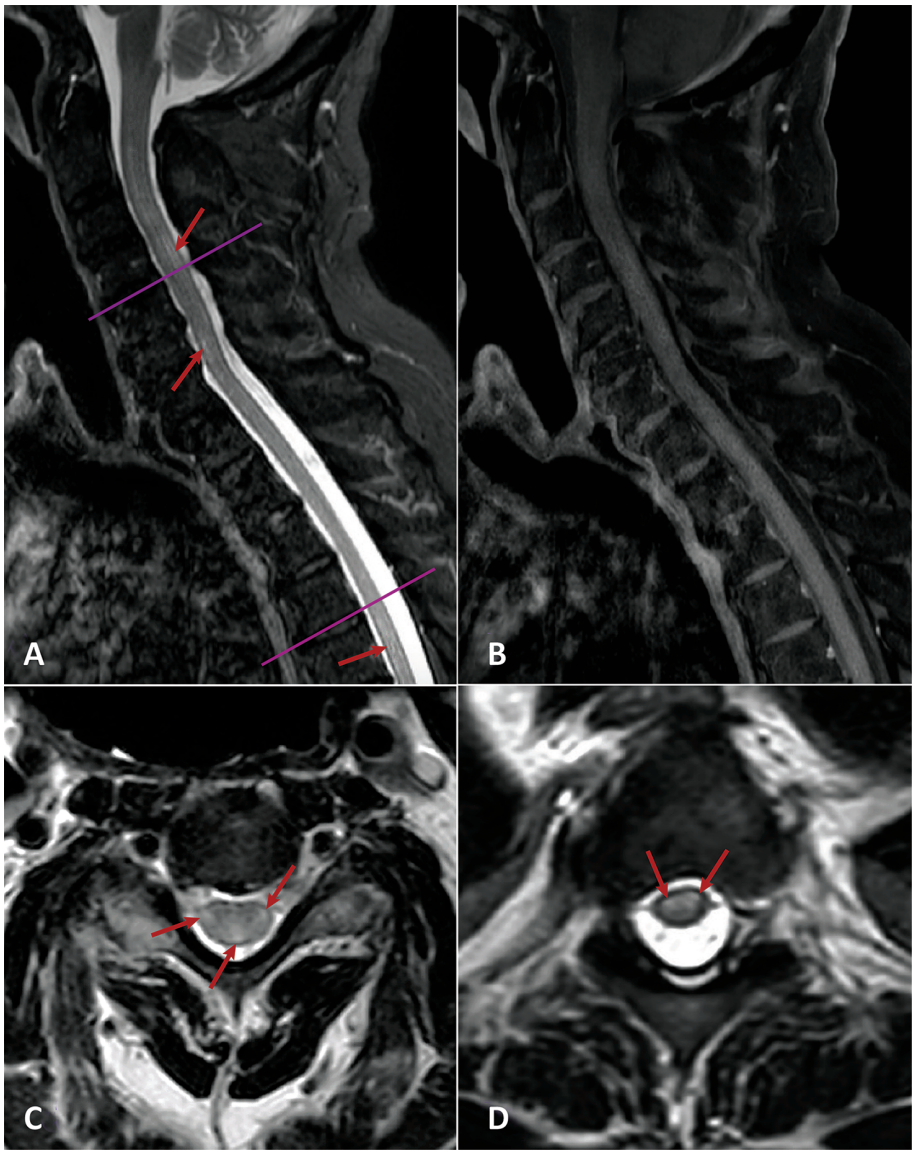

FIGURE 1 | Spinal cord MRI. **(A)** Sagittal Short-T1 Inversion Recovery: Mild cervical cord thickening and diffuse hyperintensities in cervical and dorsal cord (red arrows). **(B)** Sagittal T1 + gadolinium. No contrast enhancement. **(C, D)** Axial T2 (C4-5 and T3-4 levels) showing diffuse cord hyperintensities (red arrows).

Pediatric cases: Patient 21, a 3-year-old Navajo girl in the USA (27), Patient 31, a 14-year-old girl in Turkey (36), and Patient 41, an 11-year-old girl from Iran (43). (ii) *Young adult cases:* 13 patients, 7 men and 6 women, ages 21-42 years with mean age of 29 years. (iii): *Older adults:* 27 patients, 18 men and 9 women, ranging from 44-73 years (mean age 58 years).

The main manifestations of the spinal cord lesions based on clinical examination included two major groups, quadriplegia and paraplegia. There were 27/40 patients (58%) with tetraparesis/quadriplegia resulting from cervical cord-upper thoracic cord lesions compared with 15/40 (42%) with acute paraparesis/paraplegia from thoracic cord lesions. The anatomical distribution of the spinal cord lesions by MRI imaging was reported in 40 cases (**Table 1** and **Supplementary Table 1A**). Localized ATM lesions affected ≤3 cord segments in 12 cases (30%) at cervical (5 cases) and thoracic cord (7 cases) levels, and 28 cases (70%) had longitudinally-extensive ATM (LEATM) involving ≥4 spinal cord segments in cervicothoracic (18 cases) and thoracolumbar-sacral regions (10 cases). In 3 case reports the lesions are described as 'myelitis' without further clinical information. Cervical cord lesions extended in some cases to the brainstem causing rhombencephalitis (8), as well as involvement of pons and medulla oblongata. Patient 4 from Denmark (10) had the most extensive lesions reported affecting the entire spinal cord from the medulla oblongata to the conus medullaris. Patients 18, 36 and 37 had lesions affecting the conus medullaris.

The latency period from the onset of COVID-19 symptoms to the first neurological manifestations followed a dual distribution: (i) *Short latency:* 15 hours to 5 days in 11/34 patients (32%) and (ii) *Long latency:* 10 days to 6 weeks in 68% (**Table 1** and **Supplementary Table 1A**). The shorter latency period may indicate a direct neurotropic effect of SARS-CoV-2 during the initial infection causing para-infectious myelitis. Longer latency periods may indicate a post-infectious neurological complication resulting from the host response to the virus. No particular geographic origin, distribution by sex or age group, nor clinical picture were associated with shorter or longer latency periods. Treatments included steroids in most patients, along with IVIg in a few instances; some patients also received respiratory support. There were 2 deaths reported (Patient 17 from Qatar and Patient 23 from India). SARS-CoV-2 RNA PCR in the CSF was positive in 2 cases (18, 24).

ATM AND OTHER NEUROINFLAMMATORY SYNDROMES

According to Hartung and Aktas (47) a number of neuroimmunological disorders affecting CNS and PNS are expected to occur during COVID-19. Although the immune mechanisms causing ATM remain unknown other neurological disorders of neuroimmune nature were reported concurrently with the myelitis (**Table 1** and **Supplementary Table 1A**). These included acute disseminated encephalomyelitis (ADEM), neuromyelitis optica (NMO) and acute motor axonal neuropathy (AMAN).

ACUTE DISSEMINATED ENCEPHALOMYELITIS (ADEM)

Reichard et al. (48) reported the neuropathological findings, extensive vascular lesions and perivenous demyelination of ADEM in association with COVID-19. Myelitis as part of ADEM was diagnosed in 8/40 patients (20%) summarized in **Table 1** and **Supplementary Table 1A**. In contrast with the overall male preponderance in this series, ADEM with ATM affected predominantly women (67%) ranging in age from 27-64 years (mean age 43 years). Lesions revealed by spinal cord MRI included LEATM from medulla oblongata and cervicothoracic cord (Th_6) in Patient 3 (8); cervicothoracic spinal cord lesions down to the conus medullaris in Patient 18 (24); $C_{1\text{-}7}$-Th_2 in Patient 35 (40); and, $Th_{5\text{-}6}$ and $Th_{10\text{-}11}$ down to the conus medullaris in Patient 37 (40). ATM at Th_8 level occurred in Patient 8 (13); at $C_{2\text{-}3}$ level in Patient 16 (22); pons and medulla-cord junction in Patient 19 (25); and, intramedullary lesion of the conus medullaris in Patient 36 (40). Brain MRI lesions consistent with ADEM included among others, multiple T1 post-Gd enhancing white matter lesions plus bilateral edema of the optic nerves; hyperintense FLAIR lesions in the medial temporal lobe; bilateral lesions involving cerebral white matter, corpus callosum and brainstem including pons and medulla-cord junction.

NEUROMYELITIS OPTICA (NMO) AND NMO SPECTRUM DISORDERS (NMOSD)

NMO and NMOSD are relatively common conditions in neuroimmunology (49–51) previously reported after SARS-CoV-2 infection (52). We found 3 patients with myelitis and visual loss due to optic nerve edema diagnosed with NMO. Patient 8 (13), is a 64-year-old woman with ATM and visual loss. Patient 33 (38), is a 26-year-old Hispanic man from the USA with positive MOG-IgG antibodies who developed papilledema, blindness and dysesthesias of the upper extremities due to bilateral optic neuritis and LEATM cord lesions from C_4-Th_2. Patient 25, from Rio de Janeiro, Brazil (31) is a 51-year-old Caucasian woman, with a 2-week history of COVID-19 who developed band-like dysesthesias at the $Th_{6\text{-}10}$ dermatomes, urinary retention, leg numbness and paraparesis. Brain MRI showed enhancing T2/FLAIR lesions in anterior fornix and subfornical organ. Spinal cord MRI demonstrated LEATM at Th6-10 with lumbar radiculitis. Serum ANA was positive (1:320). Anti-AQP4 antibodies were positive in serum and CSF. This encephalomyeloradiculitis is probably a form of COVID-19-associated NMOSD. According to Jarius et al. (53) NMO and NMOSD are caused in >80% of cases by pathogenetic IgG autoantibodies to AQP4 but only 1 case was positive in this cohort. A 15-year-old Caucasian boy with SARS-CoV-2-associated NMO reported by de Ruijter at al (52). had blindness without myelitis but with positive anti-MOG-IgG antibodies.

ACUTE MOTOR AXONAL NEUROPATHY (AMAN)

AMAN may be found in patients with clinical Guillain-Barré syndrome (GBS), an acute immune-mediated polyradiculoneuropathy reported as the most common form of peripheral nerve lesion in patients with COVID-19 (54, 55). Based on electrophysiological features GBS can be classified into several subtypes including acute inflammatory demyelinating polyneuropathy (AIDP), acute motor and sensory axonal neuropathy (AMSAN), and AMAN (54, 56).

We found reports of 2 patients that presented concurrently AMAN with ATM indicating simultaneous involvement of CNS and PNS. Patient 10, a 61-year-old woman from the US (15, 16) developed quadriparesis due to LEATM affecting C_1-Th_1 and concurrent AMAN with negative anti-MOG-IgG and anti-AQP4-IgG antibodies. Patient 26, a 70-year-old woman from Italy (32) developed quadriparesis from ATM at C_7-Th_1 and AMAN with positive anti-GD1b IgM antibodies.

OTHER IMMUNE/INFLAMMATORY FACTORS

In addition to the above patients other immune or inflammatory mechanisms may have contributed to COVID-19-associated ATM. Patient 22 is a 27-year-old HIV-positive man from Moldova (28) who developed paraplegia due to LEATM involving C_4-Th_5. Patient 32 is a 72-year-old man from Mexico (37) with preexisting cervical spondylotic myelopathy that evolved to tetraparesis after SARS-CoV-2 infection due to ATM at the C_1-C_3-C_6 levels.

DISCUSSION

The neurotropism of the coronaviruses in general and SARS-CoV-2 in particular has been well demonstrated (57–60). Moreover, the numerous neurological complications of COVID-19 are well recognized (1–4, 34, 40, 47, 48, 54, 55, 57, 61). Symptoms reflecting central nervous system involvement include headache, anosmia and dysgeusia, agitation, delirium, and impaired consciousness (1, 61). Stroke is common, probably reflecting the endoteliitis (62) and small-vessel brain lesions (4) causing brain hemorrhages, arterial and venous thromboses, and subarachnoid hemorrhage, as well as rare cases of acute hemorrhagic necrotizing encephalopathy (48, 61). Neuropathological examination of fatal adult cases of COVID-19 (63–65) showed in addition to vascular lesions (4) low-grade localized encephalitis affecting brainstem respiratory and cardiovascular centers (63).

According to Paterson et al. (40) the postulated mechanisms causing ATM and the various neurological syndromes associated with SARS-CoV-2 include, either individually or in combination, direct viral neuronal injury (57–60) and the host's secondary hyperinflammation syndrome (61, 66, 67). SARS-CoV-2 enables interleukin (IL)-1 synthesis and release (68) leading to inflammasome activation. Also, IL-6, a proinflammatory mediator, is elevated in COVID-19 and induces CNS immune responses (68). Type I interferon (IFN) is dysregulated in COVID-19 and can affect innate and acquired immunity (69). COVID-19 patients exhibit increased circulating levels of IL-2, IL-8, IL-17, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon gamma-induced protein 10, and monocyte chemoattractant protein 1 (69, 70). IFN release can result in inflammation and immune system suppression (70). These immune factors may lead to the so-called "cytokine storm" syndrome that triggers coagulopathy and thrombosis (71). Also, of critical importance during COVID-19 are the para- and post-infectious inflammatory or immune-mediated neurological disorders (72–75), also observed after vaccination (76, 77), that affect both the CNS and the PNS causing GBS, ADEM, NMOSD, and ATM, among others.

ACUTE TRANSVERSE MYELITIS (ATM)

Definition and Differential Diagnosis

The term ATM is used here to identify patients with myelitis described during COVID-19. Most COVID-19-associated ATM cases reported here fulfill the strict definition of the Transverse Myelitis Consortium Working Group (78) requiring clinical evidence of bilateral sensory, motor, or autonomic dysfunction referable to the spinal cord, and confirmed by MRI images.

ATM is different from acute flaccid myelitis or AFM (79) the predominantly pediatric form of acute flaccid paralysis with anterior myelitis or "polio-like syndrome" with spinal cord gray matter lesions. Epidemiological data for pediatric cases of AFM from a national surveillance program in the US (80) reported as of July 2020 a total of 633 cases of AFM with a median age of 5.3 years with peaks in 2014, 2016 and 2018. Non-polio enteroviruses, including EV-D68 and EV-A71, are the most frequent etiological agents (81). Children are relatively unaffected by SARS-CoV-2, probably because of low ACE2 receptors in the olfactory mucosa (82) and there has been no increase in cases of AFM up to July 2020. Patient 21 (27), a 3-year-old Navajo girl, is the youngest pediatric case of SARS-CoV-2-associated ATM reported in this series. Three weeks after an asymptomatic COVID-19 infection she developed a flaccid quadriparesis as a result of LEATM extending from the lower medulla and C_1 to the Th_6 spinal cord segments. These lesions are clearly different from those of pediatric AFM (83).

According to West and colleagues (84), ATM remains a rare immune-mediated neurological condition with an estimated incidence of up to 3 per 100,000 patient years (0.003%). ATM can be caused by autoimmune, inflammatory, and infectious agents but the main differential diagnosis is with multiple sclerosis. Clinical features and imaging usually eliminate from the differential diagnosis of ATM other noninflammatory conditions such as traumatic, compressive, neoplastic or vascular lesions (84).

Pathogenesis of COVID-19-Associated ATM

The latency period between SARS-CoV-2 infection and onset of the neurological symptoms was unknown in many instances because of asymptomatic COVID-19. In most cases (68%) the latency period ranged from 10 days to 6 weeks; in the remaining 32% (11/34 cases) the latency period ranged from hours to 5 days; the shortest period was 15 hours for Patient 22, the HIV-positive man from Moldova (28). Very short latency periods of respectively 2 and 3 days occurred for Patient 11 from UAE (17) and Patient 17 from Qatar (23). It is unknown if these two patients had been previously infected with the Middle East Respiratory Syndrome coronavirus (MERS-CoV).

The neurological complications of viral infections can be either para-infectious, i.e., due to direct viral neurotropism, or post-infectious, i.e., resulting from immune-mediated reactions against the virus (48, 57–59, 61–68). Except for the three para-infectious cases mentioned above, most cases of SARS-CoV-2-associated myelitis had longer latency periods suggesting a post-infectious origin.

According to Blackburn and Wang (72), the proposed mechanisms of post-infectious neurological disorders include molecular mimicry, epitope spreading, bystander activation and polyclonal B-cell activation. Molecular mimicry is due to the presence in microorganisms of epitopes that share marked similarity in peptide sequence or three-dimensional structure

to host's antigens. Therefore, lymphocytes activated by the infection may cross-react with self-antigens. In epitope spreading the specific initial response to an antigen is broadened to include other different epitopes. Also, during the immune response to a highly virulent pathogen autoreactive lymphocytes may be activated during the inflammatory cascade resulting in autoimmunity by "bystander activation." Finally, polyclonal B-cell activation may occur with chronic viral infections that persist in the host such as herpesviruses. Molecular mimicry and bystander activation appear to be the most likely mechanisms explaining SARS-CoV-2-associated ATM. The antibodies reported in patients with COVID-19-associated ATM included 3 cases of positive anti-MOG-IgG antibodies and single cases of positive anti-GD1b IgM antibodies, ANA, and anti-AQP4 antibodies in serum and CSF.

POST-VACCINATION ATM

Neurological complications of vaccination were first recognized in 1885 with Pasteur's rabies vaccine obtained from rabbits' spinal cords. More recently, in 1977, we reported 21 cases of GBS and brain demyelination (77) resulting from the use in Colombia of the suckling mouse brain (SMB) rabies vaccine containing neural tissue antigens causing neurological complications that included GBS, ADEM, chronic leukoencephalitis, and myelitis (77). Concurrent involvement of CNS and PNS occurs in post-viral infections such as Zika (75). These lesions resemble those of experimental autoimmune encephalomyelitis induced in animals with the use of myelin antigens and Freund's adjuvant (74, 76, 84). Current rabies vaccine obtained from tissue culture of human diploid cells eliminated this problem.

The US national vaccination campaign in 1976 against the A New Jersey "swine flu" influenza using the A/NJ/1976/H1N1 vaccine was associated with increased incidence of GBS (85). Nachamkin et al. (86) postulated that *Campylobacter jejuni* antigens that mimic human gangliosides capable of inducing an anti-GM_1 antibody response could have caused GBS. *Campylobacter* antigens were not present in any of the vaccines examined. However, these authors demonstrated that the 1976, 1991-1992, 2004-2005 influenza vaccines induced IgG and IgM anti-GM_1 antibodies in mice. Recent cases of ATM following H1N1 vaccination have been reported (87–90) indicating that influenza vaccines may induce immune mechanisms targeting the spinal cord. It may be important to notice that the COVID-19 ChAdOx1 nCoV-1 vaccine (AZD1222) contains chimpanzee adenovirus antigens as adjuvant.

In 2003, an experimental vaccine (AN1792) containing synthetic aggregated Aβ42 fragments with QS-21 as adjuvant targeting the amyloid precursor protein with the aim of preventing the development of Alzheimer's disease resulted in meningoencephalitis in 6% of the vaccinated patients (91). The trial was discontinued and neuropathology studies (92) confirmed the presence of meningoencephalitis with strong MCH class I immunoreactivity in collapsed amyloid plaques and multinucleated giant cells suggesting that a fragment of β-amyloid was the possible origin of the post-vaccination reaction.

Numerous other vaccines have caused neurological post-vaccination complications including diphtheria-tetanus-polio, measles, mumps, rubella, Japanese B encephalitis, and pertussis. According to Karussis and Petrou (76), the most recent cases of CNS demyelination after vaccination include vaccines against influenza, human papilloma virus, hepatitis A or B, measles, rubella, yellow fever, anthrax, meningococcus and tetanus. Other than GBS, ATM, and ADEM, other post-vaccination reactions include neuromyelitis optica (NMOSD), isolated ophthalmoplegia, brachial neuritis and other mononeuropathies. Post-vaccination reactions have declined with the use of recombinant proteins, rather than *in vivo* infected animal tissue (73). Recent research on the immunopathogenesis of ATM (93) has emphasized the role of interleukins IL-6 and IL-17. In myelitis, IL-6 is elevated in the CSF and predicts disability (94). Production of both IL-6 and IL-17 by peripheral blood mononuclear cells is increased in ATM (94). IL-17 regulates cytokines (TNFα, IL-1β and IL-6) to stimulate IL-6 production by astrocytes. The role of adjuvants as contributing factors to the immune and inflammatory reactions to vaccines has also been emphasized (95).

ChAdOx1 nCoV-19 Vaccine (AZD1222) Trials

The ChAdOx1 nCoV-19 vaccine (AZD1222) consists of a replication-deficient chimpanzee adenoviral ChAdOx1 containing the SARS-CoV-2 structural surface vector glycoprotein antigen (spike protein; nCoV-19) gene (45). The safety and efficacy report of four randomized controlled trials conducted in Brazil, South Africa and Great Britain for the AZD1222 COVID-19 vaccine informed the occurrence of three cases of ATM as serious adverse events (45, 46).

One participant developed ATM 14 days after ChAdOx1 nCoV-19 booster vaccination and was diagnosed as idiopathic, short segment, spinal cord demyelination possibly related to vaccination (45, 46). The second participant developed ATM 10 days after a first vaccination with ChAdOx1 nCoV-19. It was initially assessed as possibly vaccine-related but later considered unlikely when further investigation revealed pre-existing, but previously unrecognised, multiple sclerosis (45, 46). The third patient with ATM occurred in a control subject 68 days after receiving the meningococcal conjugate (MenACWY) vaccine. Initially considered possibly related, it was finally considered unlikely to be vaccine-related by neurological experts (45, 46). However, no information was provided regarding COVID-19 infection in this unvaccinated subject. These three ATM serious adverse events resulted in temporarily halting of the vaccine trial until the affected participants began to show signs of recovery.

The occurrence of three reported ATM cases among 11,636 participating subjects is extremely high considering the worldwide incidence of 0.5/million COVID-19-associated ATM cases found in this report during year 1 of the pandemic. Moreover, Agmon-Levin et al. (96) in a systematic review (1970–2009) fund in 39 years only 37 reported cases of ATM in association with several different vaccines.

CONCLUSION

This review confirms that ATM is not uncommon as a neurological complication associated with COVID-19 infection around the world, responsible perhaps for 1.2% of all neurological complications caused by this coronavirus. It occurs acutely in a small number of patients as a para-infectious manifestation but most cases of SARS-CoV-2-associated ATM have longer latency periods suggesting a post-infectious origin. These facts suggest probable viral antigen(s) in SARS-CoV-2 target the spinal cord –perhaps also present in the COVID-19 vaccine AZD1222 or its chimpanzee adenovirus adjuvant– and may induce immune mechanisms leading to ATM. Research to identify the responsible antigen(s) and the immunopathogenesis of COVID-19-associated ATM must be encouraged.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available. Requests to access the datasets should be directed to gcroman@houstonmethodist.org.

AUTHOR CONTRIBUTIONS

GR and FG drafted the manuscript. FG, AT, AP, KG, and DH provided clinical data. GR, FG, and KG participated in the search of the literature. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.653786/full#supplementary-material

Supplementary Table 1A | Clinical and MRI Data of Published Cases of SARS-CoV-2-Associated Myelitis (March-2020 January-2021).

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frontiers in Neurology

CASE REPORT
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Case Report: Bilateral Palsy of the Vocal Cords After COVID-19 Infection

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During the COVID-19 pandemic, adverse neurological effects have been described. In addition to unspecific neurological symptoms, cranial nerve deficits have appeared as part of SARS-CoV-2 infection. In this case report, we describe a 74-year-old patient who developed bilateral paralysis of the vocal cords some weeks following his dismissal in stable condition after COVID-19 pneumonia. After ruling out central lesions, peripheral tumors, and other possible causes, therapy was initiated with methylprednisolone, inhalations, and oxygen. The patient showed no improvement, so laterofixation after Lichtenberger was performed. The dyspnea worsened after several weeks, so a laser posterior cordectomy was performed with satisfactory outcome.

Keywords: COVID-19, SARS-CoV-2, vocal cords, palsy, cranial nerve, recurrens

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INTRODUCTION

Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is a novel corona virus that was first detected in the Chinese city of Wuhan in 2019 (1). The resulting disease, Corona Virus Disease 2019 (COVID-19), has spread pandemically and is now a critical threat to the global healthcare system.

The pandemically significant pathomechanism leads to pneumonia with, in the most dramatic case, serious organ failure and respiratory decompensation, which leads to the need for ventilation. In addition, the majority of infections are a- or oligo-symptomatic, with the symptoms of a mild-to-moderate flu-like infection of the respiratory tract. In addition to cough and fever, sensory impairments, such as a reduction in smell and taste, are also observed (2). Besides these relatively harmless limitations, however, critical neurologic complications such as encephalitis and encephalomyelitis have been reported, especially in clinically severe cases (3).

In addition, affection of peripheral and cranial nerves in the context of a COVID-19 infection were also reported in several case reports and series, with paralysis of the eye muscles described most commonly (3).

Nevertheless, neuropathies of the facial nerve (4) and more caudally located nerves such as the phrenic nerve (5) have also been observed in COVID-19. The exact pathomechanisms are still unclear and in the interest of current research, a direct neurotoxic effect of the virus or a virally triggered autoimmune vasculitis of the nerves have been hypothesized (6).

In this context, the tenth cranial nerve, the vagus nerve, plays a special role. In addition to its sensitive, sensory and vegetative parts, it and its branches innervate various muscles in the head and neck region. The fibers innervating the larynx and the vocal cords also run over the vagus nerve and regulate the glottis, the narrowest and thus most critical point in the human airway. While unilateral vocal cord palsy mainly causes hoarseness, bilateral vocal cord palsy, especially

in the acute situation, represents a dangerous respiratory emergency requiring immediate action. Neuropathy of the vagus nerve in the context of COVID-19 has already been described, at least partially, but with deficits in the more cranial pharyngeal region and simultaneous impairment of the ninth cranial nerve, the glossopharyngeal nerve (7).

This report describes the case of a patient with bilateral vocal cord palsy after undergoing COVID-19 infection, which could be a rare neuronal manifestation of SARS-CoV-2.

CASE REPORT

A 74-year-old female was admitted to our hospital after syncope, most likely of orthostatic genesis, during a bronchopulmonary infection and suspicion of COVID-19 pneumonia. Pre-existing conditions included high blood pressure, as well as implantation of knee endoprosthesis on both sides and hip endoprosthesis on the right side.

A computed tomography (CT) scan of the thorax showed frequently reported CT features of COVID-19 pneumonia and the nasopharyngeal smear for SARS-CoV-2 was PCR positive.

With the patient's consent, therapy with off-label use hydroxychloroquine was initiated. Due to persistent recurrent fevers, additional empirical antibiotic therapy with clarithromycin was initiated.

Six days after admission, the patient developed increasing respiratory insufficiency with deterioration of the peripheral SpO2, despite oxygen administration, and was urgently transferred to the intensive care unit. She was intubated and ventilated for a total of 17 days and was extubated successfully after that. The patient was then transferred to a peripheral hospital for early rehabilitation where she stayed for 9 days. At that time, she did not show any significant dyspnea.

Two weeks after discharge from the rehabilitation center, the patient was again urgently transferred to our hospital due to dyspnea. After acute cardiopulmonary reasons for the dyspnea were ruled out, an inspiratory stridor was noticed, and the patient was presented to the department of otorhinolaryngology, head and neck surgery as well as in our phoniatrics section.

Using the "RBH-scale," the Roughness, Breathiness and Hoarseness of the speaking voice are each subjectively rated by the examiner as 0 (no disorder), 1 (low-grade disorder), 2 (moderate-grade disorder) or 3 (high-grade disorder). With the Voice Handicap Index (VHI), 3 domains (functional, physical, and emotional aspects of the voice disorder) are self-assessed by the patient with 10 questions each. Patients indicate whether the described stresses caused by the voice disorder occur in their everyday life never (0 points), almost never (1 points), sometimes (2 points), almost always (3 points), and always (4 points) (8). The patient showed a high graded dysphonia with a R3B1H3 in the RBH-*scale* and 67 points in the VHI. The voice sounded highly rough with pathological phonatoric effort. In the laryngostroboscopic examination, we found a bilateral vocal cord palsy with hyperplastic vestibular folds (**Figure 1**). During phonation we detected a small glottal gap with missing mucosal waves and amplitudes. Only some passive vibrations could be seen. During respiration the vocal folds were also fixed in medial to mediolateral position without recognizable lateral movements. During inspiration, the vocal folds were driven medially because of the Bernoulli effect. During pauses, it could be heard how the patient breathed heavily. The voice range profile was narrow with little dynamic range and no projection of voice.

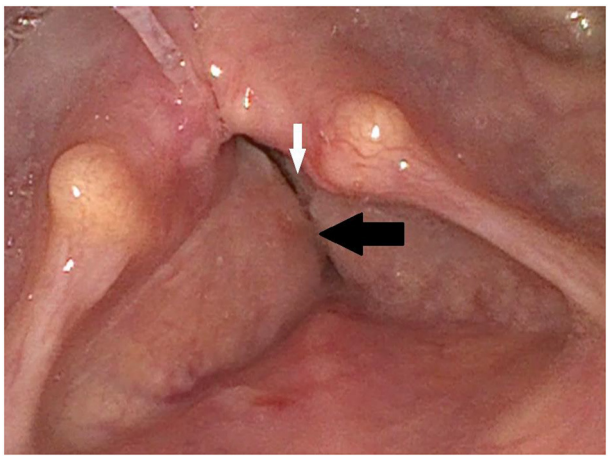

FIGURE 1 | Initial finding via laryngoscopy: The paralyzed vocal cords (white arrow) are partially covered by the hyperplastic vestibular folds (black arrow).

Imaging via MRI and CT scan found no morphological cause for vocal cord palsy, no cerebral ischemia, bleeding, or cerebral or pulmonary tumor or other cervical or thoracic mass that could compress or affect recurrent laryngeal nerve function. Serological laboratory testing ruled out increased autoimmune parameters or an ongoing or past neurotropic viral infection. Furthermore, a neurological consultation showed no evidence of critical illness neuropathy or other signs and symptoms of neurological disease that could affect vocal cord function, e.g., progressive bulbar palsy. Consequently, it was assumed that the SARS-CoV-2 virus resulted in a neurological affection of the recurrent nerves.

We started a therapy with intravenous methylprednisolone, inhalations, and oxygen via nasal mask. While there was no improvement, we performed a laterofixation according to Lichtenberger (9) under intubation anesthesia, using a special endo-extralaryngeal needle carrier instrument to lateralize the paralyzed vocal fold in the sense of an arytenoidopexy. Apart from hyperplastic vestibular folds, which occur compensatory after vocal cord palsy, no morphologic features such as tumors or scars on the vocal cords were found intraoperatively.

Thereafter, there was a marked improvement in dyspnea (**Figure 2**) and the patient could be discharged to outpatient follow-up care The patient still showed a high graded dysphonia that changed to a R1B3H3 in the "RBH"-*scale* and improved to 34 points in the VHI. The voice quality changed from a rough to a breathy voice due to the unmodulated air passing the post-therapeutic significant glottal gap. In the laryngostroboscopic examination, the fixed vocal folds hardly vibrated and there is a large glottal gap during attempted phonation. Still, there were no recognizable mucosal waves and amplitudes. The voice

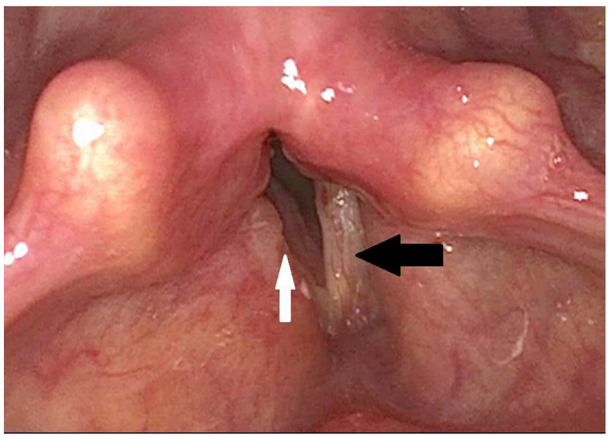

FIGURE 2 | Post-operative finding via laryngoscopy after laterofixation on the right side: The right vocal fold is fixed laterally (white arrow) and thus widens the glottic gap, while the position of the left vocal fold is unchanged (black arrow). The vestibular folds are less swollen.

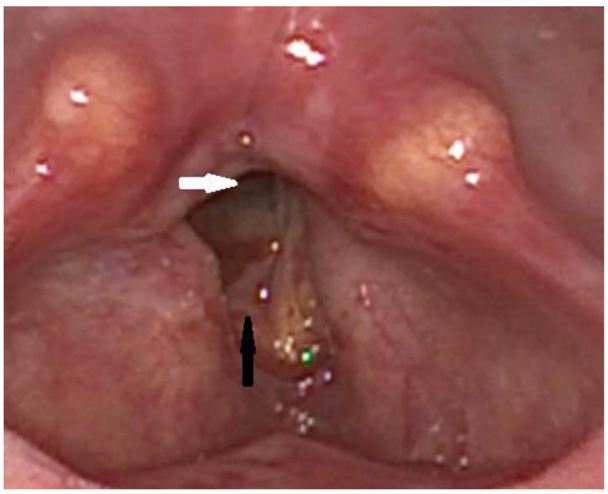

FIGURE 3 | Post-operative finding via laryngoscopy after laser posterior cordectomy: The posterior portion of the glottic gap is open (white arrow), while the anterior portion of the right vocal fold is still swollen postoperatively (black arrow).

range profile was slightly improved, but there was still a high graded hoarseness as hearable in the additional material. After several weeks, the patient returned with worsening of dyspnea, and a laser posterior cordectomy was performed, which resolved the dyspnea, nevertheless it did not improve the patient's voice quality (**Figure 3**). The patient is currently stable and continues to be seen in regular intervals.

DISCUSSION

Different neurological symptoms have been described in the context of a SARS-CoV-2 infection.

In a systematic review, an overall average of 59.45% of patients experienced olfactory disturbance (3). Ageusia and anosmia are specific symptoms of COVID-19 and, in contrast to other flu-like infections of the upper airway, do not seem to be caused by nasal congestion or rhinorrhea (2). Rather, a neurotoxic effect of the virus on the sensory cells of the nasal and oral mucosa may occur (10). To determine whether the presented patient also showed sensory deficits, we performed olfactory testing. Hyposmia, (specifically identification, discrimination, and threshold) was found. Besides presbyacusis, other testing of the vestibular, gustatory, and hearing functions showed no signs of impairment. In light of the currently hypothesized pathomechanism of SARS-CoV-2 by binding to the angiotensin-converting enzyme 2 (ACE2) as his receptor and entering the nervous system (11), the evidence of anosmia suggests that the virus had infiltrated the patient's nervous system. This makes a neurotoxic effect of the virus on other nervous structures, such as the brain nerves, seem plausible, at least in theory. However, due to the fact that olfactory impairment seems to occur at a very high frequency in the context of COVID-19 infections, whereas motor paralysis is reported as a very rare phenomenon, no definite causal relationship can be deduced here either.

Besides a differential diagnosis of COVID-19 associated vocal cord palsy with the infection, we discussed intubation damage. However, there was no stridor during the inpatient stay in the rehabilitation center, and dyspnea was not pronounced at that time. In addition, intraoperatively there were no morphologic changes of the vocal folds (such as scarring strands) that would go beyond compensatory hyperplasia of the vestibular folds. Therefore, it seems unlikely that any bilateral intubation trauma would have become symptomatic and clinically noticeable only weeks later. However, no clear causal association between focal paralysis and infection with SARS-CoV-2 can be demonstrated at this stage of research.

The time from the classic symptoms of COVID-19, such as fever and cough, to the onset of neurologic failure was longer in our case, 46 days, than in most published cases of cranial nerve palsy. Specifically, it appears that the palsy of the eye muscles (controlled by the fourth, fifth and sixth cranial nerves) occurs earliest within the scope of the infection, namely within a few days (12–14). However, it should also be noted that there were still failures of the cranial nerves that control the eye muscles in individual cases with a significantly longer latency; e.g., bilateral trochlear palsy was only described after 11 days (15).

Palsy of the cranial nerves that lie further caudally, such as the seventh cranial nerve, has also been described after about 1 week, thus leading to COVID-associated Bells paralysis (4). Also branches of the cervical plexus like the phrenic nerve, which innervates the diaphragm, have also been described as the sites of COVID-associated paralysis. In these patients, specific symptoms appeared ∼1 week after general symptoms of the disease began (5).

An affection of the ninth cranial nerve (the glossopharyngeal nerve) was described in a patient who developed oropharyngeal dysphagia 29 days after the first symptoms of COVID-19 (7).

To the best of our knowledge, a report on paralysis of the 11th or 12th cranial nerves (accessory and hypoglossal nerves) has not yet been published.

Syndromes in which multiple cranial nerves fail, similar to Miller Fisher syndrome, are described with an onset latency of 3–5 days (12).

In summary, it can be speculated that cranial nerves, which are further cranial, are affected more often and show earlier failures, whereas caudal cranial nerves are affected less frequently, and if they are affected, they are affected later in the course of the disease. This observation is congruent with the fact that an affection of the brain stem was detected in a mouse model used in a previous study on the SARS-CoV virus and that this virus enters the brain primarily via the olfactory bulb (16).

The temporal differences in the involvement of the different can be determined by the anatomical location of the different nerves and their core areas; however, different theories exist on the pathophysiological mechanism of nerve damage. The virus appears to have a direct effect on the affected nervous structures, such as the olfactory bulb, when early-onset failures, such as odor reduction occur. For late-onset failures, the time course contradicts a direct neurotoxic effect of the virus because the late-onset palsy described above developed at times when the virus no longer caused systemic symptoms. Rather, it is currently assumed that molecular mimicry leads to a virally triggered autoimmune response of the infected person, which is directed against the nerves and their supplying vessels. This corresponds to the suspected pathomechanism in the post-viral olfactory dysfunction (17). Fotuhi et al. suggest a classification system called Neurocovid Stage I–III (6). In Neurocovid Stage I, the extent of SARS-CoV-2 binding to the angiotensin-converting-encyme-2 (ACE2) receptor is limited to the nasal and oral mucosa, resulting in a limitation in smell and taste. Persistence and progression of the infection can lead to Neurocovid Stage II where focal palsy occurs due to the above-mentioned molecular mimicry, possibly due to vasculitis in the nerves and muscles triggered by the hyperactivated immune system. In stage III, a cytokine storm damages the blood-brain barrier, including symptoms, such as delirium, encephalopathy and/or seizures.

Previous electron microscopic studies indicate that the virus has a high affinity for the ACE2 receptor and that the receptor plays an important role in penetrating the nervous system (18). Reports show that the incidence of hyposmia as a result of COVID-19 infection is different by region. While relatively few patients (5%) experienced hyposmia (19) in the initial infection area of Wuhan, China, European studies show significantly higher incidences of hyposmia (up to 88% of those infected) in the context of COVID-19 (20). Initial studies also show that a higher allele frequency of an intron variant of ACE2 (rs4646127) can be found in Asia (21) and that the different variants of the ACE2 receptor have a different affinity for SARS-CoV-2 (22). However, a clear risk gene for the neuronal manifestation of COVID-19 infection has yet to be identified. Therefore, it is speculated that therapy with ACE inhibitors might increase the risk of a nerve attack due to SARS-CoV-2. In our case, the patient was treated with an ACE inhibitor (ramipril) due to arterial hypertension. However, retrospective studies (23) do not seem to prove this and advocate for the continuation of drug therapy because of the organo-protective benefits of ACE inhibitors.

A retrospective study found neurological manifestations especially in patients with lymphopenia, thrombocytopenia, and higher blood-urea concentrations (19). Our case showed lymphopenia with 1.05*10E9/L (reference range 1.4–3.2*10E9/L) and thrombocytopenia with 117 * 10E9/L (reference range 165–387*10E9/L), but no increased urea values during the phase of nerve paralysis. However, during the initial COVID-19 pneumonia, there was an increased urea level with 108.3 mg/dl (reference range 21–43 mg/dl) and lymphocytopenia (1.04 * 10E9/L) with normal thrombocytes at the same time.

In most of the published case reports, therapy with i.v. corticoids and i.v. immunoglobulins were initiated, resulting in a clear improvement in symptoms overall. Recurrence palsy plays a special role in the case described in this report because its occurrence (especially bilateral) can lead to an acute life-threatening risk to the airway and spontaneous healing cannot occur.

CONCLUSION

In addition to the typical symptoms and forms of manifestation, COVID-19 can cause complications in individual cases that do not correspond to the average appearance. Sensory and motor deficiency symptoms (a neuronal affection of SARS-CoV-2) should be considered, especially for high-risk patients, and further diagnostics and specific therapies should be initiated. Vocal cord paralysis can also occur as a rare complication in the setting of or following COVID-19 infection and may be due to the neurotoxic effects of SARS-CoV-2. It is important for clinicians to consider this as a possible differential diagnosis in COVID-19 patients with dyspnea. Close interdisciplinary coordination is also highly recommended.

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

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

FJ did the literature research and wrote the manuscprit. AS initiated the writing of the manuscript, coordinated the different disciplines involved in the case and reviewed the manuscript with overall expertise and in terms of language. RH and FL performed the clinical examination, documentation, creation of the additional material, and reviewed the manuscript with regard

to phoniatric expertise. NR and SL reviewed the manuscript with regard to expertise in otorhinolaryngology, head- and neck-surgery. VH reviewed the manuscript with regard to neurologic expertise. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2021.619545/full#supplementary-material

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Mitigating Long-Term COVID-19 Consequences on Brain Health

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COVID-19 is increasingly being linked to brain health impacts. The emerging situation is consistent with evidence of immunological injury to the brain, which has been described as a resulting "brain fog." The situation need not be medicalized but rather clinically managed in terms of improving resilience for an over-stressed nervous system. Pre-existing comparisons include managing post-concussion syndromes and/or brain fog. The objective evaluation of changes in cognitive functioning will be an important clinical starting point, which is being accelerated through pandemic digital health innovations. Pre-morbid brain health can significantly optimize risk factors and existing clinical frameworks provide useful guidance in managing over-stressed COVID-19 nervous systems.

Keywords: SARS-CoV-2, coronavirus, brain inflammation, cognition, neuromodulation, neuroplasticity, mental health, moral injury

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INTRODUCTION

The Coronavirus 2019 (COVID-19) pandemic has manifested in many clinical presentations. Central nervous system (CNS) involvement is not a rare complication of this virus. In the current analysis, we postulate that: (1) pre-morbid brain health may be a significant modifiable risk factor when considering clinical sequelae; and (2) a framework similar to concussion management may provide helpful guidance in next-steps for COVID-19 clinical management. In this way, CNS related concerns can be represented as treating an "over-stressed" nervous system. It is not desirable, from a public health perspective, to create a unifying diagnosis that potentially "medicalizes" COVID-19 related CNS symptomatology and dysfunction to the detriment of empowering environmental and lifestyle choices that are within a patient's control.

This article first summarizes current state of knowledge pertaining to neuroinflammation and the neurological consequences related to COVID-19, then suggests "brain health" risk factors amenable to modification, and proposes means to measure cognitive brain function in a cost-effective and efficient manner, with simple strategies to potentially mitigate long-term consequences of post COVID-19 "brain fog." Similar concepts around brain fog have been identified in association with concussion, anoxic insult, and chemotherapy. While any one of these may serve as a potential model, given its prevalence the concussion model is adopted in this brief analysis.

IMMUNOLOGICAL INJURY TO THE BRAIN

CNS involvement with COVID-19 is real, measurable, and potentially modifiable. Increasing evidence has demonstrated that COVID-19 is associated with CNS dysfunction, as demonstrated by a range of neurological and mental health symptoms spanning from acute to potentially chronic conditions (see literature review below). We propose an approach to prevent, mitigate and rapidly identify the small but significant minority of those with residual symptoms realizing that the reasons for residual symptoms might be complex and multiple. The underlying pathophysiology of this involvement appears to revolve around neuroinflammation, a common factor amongst many conditions affecting neurological function. The propensity toward inflammation is dependent on many variables, of which some are modifiable. Consequently, there is a constellation of physical, psychological, and cognitive factors that contribute to whether or not a specific injury leads to functional disability or not. This model is not dissimilar to those currently in place for concussion care (1).

SARS-CoV-2 was initially thought to infect primarily the lower respiratory tract and cause lung damage. However, a growing body of evidence suggests that under certain circumstances SARS-CoV-2 can infect the CNS and cause neurological complications (2). It was clear even in the early months of the COVID-19 pandemic that neurological manifestations, such as headache, dizziness, confusion, ageusia and anosmia were common in more than 50% of the hospitalized COVID-19 patients (3, 4). Surprisingly, acute cerebrovascular disease with increased risk of stroke is also emerging as an important neurological complication in hospitalized patients with severe COVID-19 disease (5, 6). The long-term consequences of these symptoms on brain health may not be realized for years or decades. While our knowledge on the neurological impacts of SARS-CoV-2 continues to evolve, the lessons learnt from recent studies can provide an important roadmap to advance our understanding of SARS-CoV-2 pathogenesis (7–9). Neuropathological examination of post-mortem brain specimens obtained from COVID-19 patients have shown acute hypoxic-ischemic changes in the cerebellum and loss of neurons in cerebral cortex, hippocampus and cerebellar Purkinje cell layer. Further testing of brain tissue using molecular and immunohistochemical analysis revealed the presence of low levels of viral ribonucleic acid (RNA) or nucleocapsid proteins (9). In a case series study, early post-mortem brain magnetic resonance imaging (MRI) scans of COVID-19 patients demonstrated hemorrhage, posterior reversible encephalopathy syndrome and non-specific deep white matter changes, possibly due to the blood-brain barrier (BBB) breakdown (8). Another study showed elevated plasma levels of neurofilament light chain protein (NfL), a marker of neuronal injury and glial fibrillary acidic protein (GFAP), a marker of astroglial injury in COVID-19 patients, suggestive of direct CNS damage (7). Although these studies provide evidence of CNS damage in COVID-19 patients, whether these lesions are due to direct viral infection of the brain could not be established and further mechanistic studies are warranted.

The blood-brain barrier (BBB) represents a formidable barrier that prevents harmful substances, including viruses from entering the CNS. SARS-CoV-2 possibly deploys several strategies to evade the innate immune responses, traverse the BBB and gain entry into the CNS (10). The spike protein of SARS-CoV-2 binds the angiotensin converting enzyme-2 (ACE-2) receptor to infect brain endothelial cells and activate inflammatory and thrombotic pathways (11, 12). Given the importance of the BBB for the maintenance of cerebral blood flow, cerebrovascular endothelial cell dysfunction could lead to alterations in BBB function. SARS-CoV-2 infection has been associated with meningitis and pan-encephalitis and viral RNA was detected in the cerebrospinal fluid (CSF) of a COVID-19 patient (13, 14). A case of acute hemorrhagic necrotizing encephalitis on MRI scans has been reported in COVID-19 patients, suggestive of hyperinflammation (15). In addition, increased serum and CSF levels of proinflammatory cytokines, IL-1β, and IL-6 have been reported in SARS-CoV-2-associated encephalitis (16). Systemic exposure to pathogenic levels of proinflammatory mediators could result in immunopathogenic and neuronal injury (17), eventually leading to cognitive dysfunction. Similar to the 1918 Spanish Flu pandemic, with reports of encephalitis lethargica and post-encephalitis parkinsonism (18), case study evidence of parkinsonism has emerged after SARS-CoV2 infection (19). Therefore, it is important to diagnose and treat the neurological manifestations in COVID-19 patients at an early stage in the disease process to limit the long-term sequelae.

FACTORS AFFECTING RESILIENCE AND RECOVERY

Public health measures to limit the transmission of SARS-CoV-2 have also consequently impacted conventional evaluation and treatment options and practices. Like any complex condition, a bio-psycho-social model is needed that integrates the inflammation effects with those related to psychosocial and other stressors. CNS care in a COVID-19 era involves treating an already over-stressed nervous system. Care can involve addressing multiple factors spanning from direct neurological injury, mental health, post-traumatic stress disorder (PTSD), and potentially extending to the emerging concept of "moral injury" when the impact to front-line responders and many others is also considered.

In this respect, COVID-19 can expose vulnerabilities in brain resiliency and challenge a host of underlying factors that support brain health. Cognitive and mental health complaints appear to be emerging as early reported symptoms (20). In essence, COVID-19 has stressed the nervous system, but a system that in many cases was already stressed to begin with. As any biological homeostatic system, resilience can be seen as a common thread that brings together CNS injury, cognitive functioning, general mental health and resiliency, PTSD, and moral injury. Prevention and recovery therefore involves searching for links (diet, exercise, sleep, et cetera) to some key factors that can be influenced in

order to manage common symptoms that have previously not been understood to be connected.

CLINICAL MANAGEMENT NEXT STEPS

By comparison, all consensus guidelines for concussion management show that reassurance and expectation for a positive outcome are the single most important factors in management. It is reasonable to assume that for a multi-factorial condition such as COVID-19, barring any evidence to the contrary, a similar approach should be implemented. Given the significant amount of media-coverage and the already escalating toll on mental health, public policies that encourage and promote pro-active approaches to pre-habilitation and "normalization" of non-specific symptoms might avoid potentially inappropriate and costly labeling of non-specific neurological symptoms. As any practicing neurologist will certainly attest, there is no shortage of "functional neurological disorder" in daily practice. A consensus statement has been recently published to guide rehabilitation post-COVID-19 (21).

A recent review has outlined five major categories of modifiable personal factors that contribute to baseline brain health and which may be protective against various CNS conditions: (1) exercise; (2) cognitive stimulation; (3) sleep; (4) dietary considerations; and (5) social connectedness (22). The detailed review of these is beyond the scope of this article, but a few points are worthy of mention. The effects of physical activity on brain function are well-established. The effects of brain-derived neurotrophic factor (BDNF) on neuroplasticity and neuromodulation are well-known. Regrettably, with some exceptions, COVID-19 public health measures have affected the access to exercise facilities or outdoor activity for many. Physical activity needs to increase prominently in public health messaging as lack of exercise is a major cause of chronic diseases (23). In the now famous video "23 ½ h," Dr. Michael Evans makes a very compelling argument that it only takes 30 min of brisk walking per day to achieve most exercise related health benefits (24). Clearly this mode of exercise should be available to all in a COVID-19-safe manner. The role of cognitive stimulation has gained greater awareness, with various applications and technologies available to help facilitate cognitive and mental well-being (25). The importance of sleep and diet for brain health has been validated and amply documented, with dietary considerations particularly focused on anti-inflammatory diets (e.g., omega-3 fatty acids and medium chain triglyceride supplementation, magnesium, Intermittent fasting and Ketosis, etc.) (26). Simple modifications in this domain could have significant impacts in reducing the pre-morbid burden of dietary-related CNS inflammation that may predispose individuals to an "inflammatory storm" that may have otherwise been potentially less severe. Finally, if social connectedness is key to cognitive and brain health, there is an interesting paradox brewing insofar as the precise tools used to contain the spread of disease may be contributing directly or indirectly to long-term disease burden from a CNS perspective.

THE IMPORTANCE OF COGNITIVE FUNCTION

By the same token, measuring cognitive function in an objective manner would allow for serial tracking of CNS involvement and recovery. In contrast to traditional neuropsychological testing, which can be time-consuming and is increasingly challenging to access in a post-COVID-19 era, there have been several recent digital health advances in cognitive evaluation. A number of user-friendly, portable cognitive/behavioral evaluation technologies have been developed (e.g., BrainFX, CBS Health, BrainHQ, etc.). Novel portable neurophysiological options are also emerging. In terms of the most recent advances in neurotechnology capable of deployment to clinical frontlines, portable electroencephalography (EEG) can now provide non-invasive, objective, neurophysiological, monitoring systems (e.g., BrainScope, NeuroCatch® Platform, and eVox System) and may prove beneficial in mitigating the long-term consequences of cognitive impairments from COVID-19. The key clinical management advance here is early and sensitive evaluation, which can lead to earlier and more effective interventions.

As the medical axiom states: You can't treat what you can't measure. In HIV, it took more than a quarter of a century to establish the HIV-associated neurocognitive disorder HAND (27). Accordingly, it will be important to start with evaluating basic cognitive complaints within the framework of an over-stressed nervous system and anticipate the rise of descriptors such as "brain fog" similar to that which occurs in concussion. Historically, rapid, objective, and accurate clinical evaluation of cognitive function has been difficult, particularly now in the pandemic era. However, digital health advances have overcome the inherent challenges of cognitive screening and neuropsychological testing that rely on subjective behavioral responses that can be error prone (28). Through the use of portable EEG, it is possible to obtain objective neurophysiological testing through low-cost, clinically accessible technologies (29). These devices have been used, particularly for point-of-care concussion management, and are actively used in the current pandemic era (30).

Specifically, cognitive evoked potentials are increasingly being shown to be useful within a vital sign framework that overcomes historical clinical limitations of EEG. The timing for such an advance is critical. Recent CNS COVID-19 reports have highlighted the importance of objectively monitoring vital neurocognitive functions over the longer term (30). Modeled from vital sign frameworks, cognitive brain function can be monitored as an objective, sensitive, physiological evaluation of evoked responses that is clinically accessible, particularly in pandemic times (31–35). The basic framework uses a portable EEG for rapid, automated, standardized evaluation of three established cognitive evoked potentials for Auditory sensation (36), Basic attention (37), and Cognitive processing (38) in under 10 min. In a post-pandemic era, the deployable features of this type of digital health technology are becoming increasingly important tools to investigate subjective cognitive complaints. The detection of subtle cognitive changes may provide further

alignment between COVID-19 and concussion in terms of mitigation strategies. Here, emerging cognitive rehabilitation advances will be important to begin evaluating in future prospective studies.

DISCUSSION

COVID-19 has pushed our personal and collective resources to their limits, with evidence-based impacts on cognitive and related brain health issues. The good news remains that there is much that can be done to improve individual resilience to CNS injury/inflammation.

As we have seen with the relative concussion epidemic, in order to fully understand multisystem, complex issues one needs to start at the beginning with simple yet solid foundational evidence-based interventions that are: (1) Cost effective; (2) Clinically effective; and (3) Result in functional improvements or, at the very least, mitigate functional impairment and (4) Can be implemented on a large-scale.

What other epidemics and pandemics have taught us is that, reducing complex problems to simple organ system injury models rarely provides tangible and lasting solutions at least in the domain of human functioning and participation in life [as per the World Health Assembly's International Classification of Functioning, Disability and Health, (39)]. In fact often, well-intentioned medically focussed interventions can inadvertently contribute to the problem for which the solution was intended. Notwithstanding the immense and commendable work that is being done in the realm of these other conditions, it is paramount that over-medicalization or dramatization of CNS symptomatology and dysfunction be kept to the minimum required to recognize and treat serious pathology but not to create a new pandemic of "worried-well" as has been the case in other areas of health care, specifically in the case of concussion. In the current analysis, we conclude that pre-morbid brain health optimization can significantly modify the risk factors and that a framework similar to concussion management provides useful guidance in clinically managing over-stressed nervous systems.

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

RD and JS contributed to initial concept and perspectives. RD, JS, and MB developed the initial version of the manuscript. SM reviewed and edited the manuscript along with all other authors. All authors read and approved the final manuscript.

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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.

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