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REVIEW

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What can cerebrospinal fluid testing and brain autopsies tell us about viral neuroinvasion of SARS-CoV-2

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Abstract

To provide instructive clues for clinical practice and further research of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, we analyzed the existing literature on viral neuroinvasion of SARS-CoV-2 in coronavirus disease 2019 (COVID-19) patients. To date, SARS-CoV-2 has been detected in the cerebrospinal fluid (CSF) or brain parenchyma in quite a few patients, which provide undeniable evidence for the neuroinvasive potential of this novel coronavirus. In contrast with the cerebrum and cerebellum, the detection rate of SARS-CoV-2 was higher in the olfactory system and the brainstem, both of which also showed severe microgliosis and lymphocytic infiltrations. As compared with the number of patients who underwent viral testing in the central nervous system (CNS), the number of patients showing positive results seems very small. However, it seems too early to conclude that the neuroinvasion of SARS-CoV-2 is rare in COVID-19 patients because the detection methods or sampling procedures in some studies may not be suitable or sufficient to reveal the CNS infection induced by neurotropic viruses. Moreover, the primary symptoms and/or causes of death were distinctly different among examined patients, which probably caused more conspicuous pathological changes than those due to the direct infection that usually localized to specific brain areas. Unfortunately, most autopsy studies did not provide sufficient details about neurological symptoms or suspected diagnoses of the examined patients, and the documentation of neuropathological changes was often incomplete. Given the complex pathophysiology of COVID-19 and the characteristics of neurotropic viruses, it is understandable that any study of the CNS infection may inevitably have limitations.

KEYWORDS

autopsy, brain, cerebrospinal fluid, neuroinvasion, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), shows peculiar clinical manifestations, which are challenging current biology. Many COVID-19 patients, whether asymptomatic, mild or severe, had obvious pulmonary pathological changes, but most of them presented only mild flu-like symptoms.[1–4] Large numbers of COVID-19 patients arrived at hospitals with blood-oxygen levels so low that they should have lost consciousness or be on the verge of organ failure, but they were comfortable and denied any difficulty with breathing.[5] Moreover, many severe patients had normal chest imaging findings,[2] which suggests that the respiratory manifestations of some patients with COVID-19 could not be explained only by pulmonary changes.[6,7]

It is possible that the potential neuroinvasion of SARS-CoV-2 plays a role in the peculiar respiratory manifestations.[8,9]

The first-hand clinical report on neurological involvement associated with SARS-CoV-2 infection became available online shortly after the appearance of this novel coronavirus.[10] Thereafter, Moriguchi et al.[11] and Xiang et al.[12] provided the first evidence of SARS-CoV-2 in the cerebrospinal fluid (CSF) of COVID-19 patients. Since then, the neuroinvasive potential of SARS-CoV-2 has attracted more and more attention.[13] However, to date, only a few patients with COVID-19 were tested positive for SARS-CoV-2 in the CSF.[13] Similarly, many decreased patents with COVID-19 who underwent brain autopsies were reported to show negative results for SARS-CoV-2 detection in the brain parenchyma.[7,13] Given these observations, the direct invasion of SARS-CoV-2 in the central nervous system (CNS) is considered to be rare, and therefore may not be responsible for the neurological manifestations of patients with COVID-19.

However, it should be noted that the examined patients in many studies exhibited different neurological features, and therefore probably had different underlying diseases.[13] In many cases, the neuropathological changes caused by hypoxia-ischemia, strokes, toxic metabolic changes, multiorgan failure, or cytokine storming appeared so striking that it was difficult to conclude whether the neuroinvasion of SARS-CoV-2 played a pathogenic role or not.[14]

Since the beginning of 2020, SARS-CoV-2 has spread rapidly all over the world and caused a profound impact on human health, lifestyles, economy, politics, and even the world pattern. As the pandemic of COVID-19 has aroused great public concerns, related scientific papers are increasing in number in an explosive manner.[7] Therefore, it is necessary to make a systematic analysis of the existing literature so as to reveal instructive clues for clinical practice and further research of SARS-CoV-2 infection.

2 | RETRIEVAL STRATEGIES

An exhaustive search of case reports, cohort studies, series of cases, postmortem studies, and clinical trials related to the possible neuroinvasion of SARS-CoV-2 was performed through PubMed/MEDLINE and COVID-19-related preprints from medRxiv and bioRxiv from December 1, 2019, to October 31, 2020. In addition, the references of relevant articles were also scanned for additional studies related to SARS-CoV-2 and CNS infection.

The papers on COVID-19 were retrieved by using "novel coronavirus disease 2019 or COVID 19 or severe acute respiratory syndrome coronavirus 2 or SARS CoV 2 or 2019 novel coronavirus or 2019 nCoV" in Title/Abstract (Strategy 1). To reveal the involvement of the nervous system in COVID-19, the following keywords in title/abstract were combined with Strategy 1: "neurological or nervous system or CNS or PNS or brain or cerebrum or cerebral or cerebellum or cerebellar or thalamus or thalamic or hippocampus or hippocampal or pons or pontes or pontine or brainstem or oblongata or medulla oblongata or spinal cord or cerebrospinal or neural or neuron or nerve."

Reviews, meta-analyses, opinion, correspondence, perspective, and letters to the editor containing no original data of interest were excluded from quantitative analysis. Only case reports or series studies that reported patients diagnosed with COVID-19 based on positive SARS-CoV-2 polymerase chain reaction (PCR) or serologic testing were included in this study. The titles and abstracts were first screened, and the full texts and supplementary files were then obtained from the library of Jilin University. The papers were selected based on their relevance as to whether the CSF or the brain was tested for SARS-CoV-2. Due to our limited capacity, the cut-off time of this review was set as October 31, 2020.

3 | RESULTS

3.1 | CSF analysis results for SARS-CoV-2 neuroinvasion

The purpose of this study was to search for evidence of the neuroinvasion of SARS-CoV-2. Therefore, our analysis was focused on the relationship between the primary symptoms of examined patients with COVID-19 and their CSF testing results for SARS-CoV-2, white blood cells (WBCs), and intrathecal antibodies against SARS-CoV-2. By contrast, the results for the measurement of CSF biomarkers of inflammation and neuronal injury were excluded from this study.

In total, we identified 97 relevant papers which reported 468 COVID-19 patients who underwent CSF PCR testing for SARS-CoV-2 (Tables 1–3, S1 and S2). Among these patients, only 30 patients (30/468, 6.4%) from 25 papers were reported to show positive results (Tables 1 and S1).

Among the 30 patients with positive CSF testing, the primary symptoms or possible diagnoses were provided for 24 patients in the papers. Among the 24 patients, 21 (87.5%) presented symptoms that localized to the central nervous system (CNS), including 14 (14/21, 66.7%) with COVID-19-associated encephalitis, 3 (3/21, 14.3%) with encephalopathy, 2 (2/21, 9.5%) with cerebrovascular accidents, and 2 (2/21, 9.5%) with demyelinating disease. Only three patients (3/24, 12.5%) presented symptoms that localized to the peripheral nervous system (PNS), including one with Guillain–Barré syndrome and two with unknown PNS symptoms.

Seven of 26 patients (26.9%) with the virus in the CSF did not present any respiratory symptoms or chest imaging abnormalities, and 6 of 27 (22.2%) showed negative routine tests for SARS-CoV-2 in the nasopharynx, throat, or lower respiratory tract swabs. Among patients with encephalitis who showed positive testing for SARS-CoV-2, 4 of 11 patients (36.4%) did not present respiratory symptoms, and 3 of 10 patients (30.0%) showed negative results for routine tests for SARS-CoV-2. Two patients with the virus in the CSF, including one with meningitis and one with acute disseminated

TABLE 1 The primary symptoms and CSF analysis results in COVID-19 patients with SARS-CoV-2 in the CSF (x/number, percentage)

Neurological diagnosis	Positive for SARS-CoV-2 in CSF	Presence of respiratory symptoms	RTS negative	PRS + RTS negative	Increase of CSF WBCs	Increase of CSF IgGs	Anti-SARS-CoV-2 antibodies in CSF
COVID-19-associated encephalitis	14/30 (46.7%)	4/13 (30.8%)	3/11 (27.3%)	1/11 (9.1%)	6/12 (50.0%)	1/4 (25.0%)	n.d.
COVID-19-associated encephalopathy	3/30 (10.0%)	0/3 (0)	0/3 (0)	0/3 (0)	0/3 (0)	1/3 (33.3%)	n.d.
Cerebrovascular accidents	2/30 (6.7%)	1/2 (50.0%)	0/2 (0)	0/2 (0)	0/2 (0)	½ (50%)	n.d.
Demyelinating disease	2/30 (6.7%)	1/2 (50.0%)	2/2 (100%)	1/2 (50.0%)	1/1 (100%)	1/1 (100%)	n.d.
Guillain-Barré syndrome	1/30 (3.3%)	0/1 (0)	1/1 (100%)	0/1 (0)	0/1 (0)	1/1 (100%)	1
Other PNS involvement	2/30 (6.7%)	0/2 (0)	0/2 (0)	0/2 (0)	n.d.	n.d.	n.d.
Uncharacterized	6/30 (20.0%)	1/3 (33.3%)	0/6 (0)	0/3 (0)	0/3 (0)	1/3 (33.3%)	n.d.
Total	30	7/26 (26.9%)	6/27 (22.2%)	2/24 (8.3%)	7/22 (31.8%)	6/14 (42.9%)	1

Abbreviations: n.d., not data or no detection; PRS, presence of respiratory symptoms; RTS, Routine tests for SARS-CoV-2 in nasopharyngeal, throat or lower respiratory tract swabs; WBC, white blood cell.

encephalomyelitis, did not show either respiratory symptoms or positive routine tests.

CSF WBC counts were provided for 26 (86.7%) of 30 patients with positive CSF testing, and 7/26 (26.9%) showed > 5 cells/μl in their CSF. The increase of CSF WBC counts was most commonly observed in patients with COVID-19 encephalitis (6/12, 50%).

Among the 30 patients with positive CSF testing for SARS-CoV-2, 6 (42.9%) of 14 who had testing to evaluate for CSF antibodies showed increased immunogloblulin Gs (IgGs) in their CSF. However, only one underwent further testing for the presence of anti-SARS-CoV-2 antibodies in the CSF. Among these patients, no one was evaluated whether there was intrathecal antibody synthesis.

Among 438 patients with negative CSF testing (Tables 2 and S2), the primary symptoms and/or possible diagnoses were provided for 297 patients (297/438, 67.8%) in the papers. Among the 297 patients, 234 (234/297, 78.8%) showed symptoms that localized to the CNS. The most common diagnosis was COVID-19-associated encephalopathy (65/234, 27.8%), followed by encephalitis (51/234, 21.8%), cerebrovascular accidents (27/234, 11.5%), and COVID-19-related autoimmune diseases (21/234, 9.0%). Among the 297 patients, 46 (46/297, 15.5%) presented neurological symptoms that localized to the PNS, and 19 (19/297, 6.4%) who did not present any neurological symptoms were all tested negative for SARS-CoV-2 in the CSF.

CSF WBC counts were provided for 342 of 438 patients (342/438, 78.1%), and 104 (104/342, 30.4%) showed >5 cells/μl in their CSF. The increase of CSF WBC counts was most frequently found in patients with COVID-19-associated encephalitis (30/56, 53.6%), followed by those with encephalopathy (18/56, 32.1%), and with cerebrovascular accidents (5/17, 29.4%).

CSF antibodies were tested in 133 (30.4%) of 438 patients who did not have a positive CSF SARS-CoV-2 PCR, and 62 (62/133, 46.6%) showed increased IgGs in the CSF. Among the 62 patients, the primary symptoms and/or possible diagnoses were provided for 45 patients (45/62, 72.6%) in the papers, and the increase of CSF IgGs was most commonly found in patients with COVID-19-associated encephalopathy (27/45, 60%), followed by those with encephalitis (6/45, 13.3%).

CSF antibodies specific for SARS-CoV-2 were tested in 80 (18.3%) of 438 patients who did not have a positive CSF SARS-CoV-2 PCR, and 37 (37/80, 46.2%) showed the presence of anti-SARS-CoV-2 antibodies in the CSF. Among the 37 patients, the primary symptoms and/or possible diagnoses were provided for 36 patients (36/37, 97.3%) in the papers. The presence of anti-SARS-CoV-2 antibodies in the CSF was most commonly found in patients with COVID-19-associated encephalopathy (23/37, 62.2%), followed by those with encephalitis (6/17, 35.3%).

Additional testing was performed for intrathecal antibody synthesis in 30 (81.1%) of 37 patients with anti-SARS-CoV-2 antibodies in the CSF. Among the 30 patients, intrathecal antibody synthesis was present in 7 patients (7/30, 23.3%), including 2 with COVID-19-associated encephalopathy, 2 with headache, and 1 with seizures.

TABLE 2 The primary symptoms and CSF analysis results in COVID-19 patients without SARS-CoV-2 in the CSF (x/number, percentage)

Neurological diagnosis	Negative for SARS-CoV-2 in CSF	Increase of CSF WBCs	Increase of CSF IgGs	Anti-SARS-CoV-2 antibodies in CSF	Intrathecal antibody synthesis
COVID-19-associated encephalitis	51/438 (11.6%)	30/56 (53.6%)	6/17 (35.3%)	2/7 (28.6%)	0/1 (0)
COVID-19-related autoimmune encephalitis	21/438 (4.8%)	0/13 (0)	n.d.	n.d.	n.d.
COVID-19-associated encephalopathy	65/438 (14.8%)	18/56 (32.1%)	27/37 (71.4%)	23/30 (76.7%)	2/23 (8.7%)
Cerebrovascular accidents	27/438 (6.2%)	5/17 (29.4%)	1/7 (14.3%)	n.d.	n.d.
Seizures	16/438 (3.6%)	7/10 (70.0%)	2/3 (66.7%)	2/2 (100%)	1/1 (100%)
Headache	7/438 (1.6%)	1/7 (14.3%)	2/2 (100%)	2/5 (40.0%)	2/2 (100%)
Myelitis	3/438 (0.7%)	0/3 (0)	2/2 (100%)	2/2 (100%)	n.d.
Miscellaneous CNS symptoms	44/438 (10.0%)	4/23 (17.4%)	3/11 (27.3%)	2/19 (10.5%)	2/2 (100%)
Guillain–Barré syndrome	20/438 (4.6%)	4/19 (21.1%)	2/4 (50%)	2/2 (100%)	n.d.
Miscellaneous PNS involvement	26/438 (5.9%)	5/17 (29.4%)	1/2 (50.0%)	1/12 (8.3%)	0/1 (0)
No neurological symptoms	19/438 (4.3%)	0/7 (0)	0/7 (0)	n.d.	n.d.
Uncharacterized	141/438 (32.2%)	27/114 (23.7%)	16/41 (39.0%)	1/1 (100%)	n.d.
Total	438[a]	104/342[a](30.4%)	62/133 (46.6%)	37/80 (46.2%)	7/30 (23.3%)

Abbreviations: CSF, cerebrospinal fluid; n.d., not data or no detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cells.

[a]A case reported by Neumann et al.[15] showed both encephalopathy and Guillain–Barré Syndrome[77]; a case reported by Parsons et al.[16] showed both acute disseminated encephalomyelitis and cerebral hemorrhage[78].

Among all 86 patients with encephalitis who underwent CSF testing for SARS-CoV-2, 14 (14/86, 16.3%) were found to have SARS-CoV-2 in the CSF (Table 3). By contrast, the detection rate of SARS-CoV-2 was much lower in patients with encephalopathy (3/68, 4.4%), cerebrovascular accidents (2/30, 6.7%), or Guillain–Barré syndrome (1/21, 4.8%). Importantly, patients without neurological manifestations were all negative for CSF SARS-CoV-2 testing.

3.2 | Autopsy findings for SARS-CoV-2 neuroinvasion

In total, we identified 28 autopsy studies that reported structural abnormalities in 134 (66.4%) of 202 patients who died from COVID-19 (Tables 4 and S3). Among 202 patients, 108 (108/202, 53.5%) were further tested for SARS-CoV-2 in the neural tissues (Tables 5 and S3). Among 134 patients with brain abnormalities, SARS-CoV-2 RNA and viral proteins were detected in 31 (33.3%) of 93 and 18 (24.7%) of 73 tested patients, respectively, and 9 (20.9%) of 43 tested patients showed positive results for both SARS-CoV-2 RNA and viral proteins (Table 4). Among all patients who underwent viral detection, SARS-CoV-2 RNA was detected in the brain in 56 (51.9%) of 108 tested patients, while viral proteins were detected in the brain in 25 (29.4%) of 85 tested patients (Table 5). Among 48 patients who underwent testing with different methods, 9 (9/48, 18.7%) were positive for both SARS-CoV-2 RNA and viral proteins (nucleocapsid and/or spike proteins).

Structural abnormalities in the olfactory bulb/nerve were reported in 52 (38.8%) of 134 examined patients. Among the 52 patients, 50 (50/52, 96.2%) showed microglial activation and/or lymphocytic infiltrations, but no patient was further tested for SARS-CoV-2 in the olfactory bulb/nerve (Table 4). On the other hand, some other patients, whose pathological changes in the olfactory system were not documented in the papers, have been tested for SARS-CoV-2 in the olfactory system. Among them, SARS-CoV-2 RNA and viral proteins were detectable in the olfactory mucosa in 14 (58.3%) of 24 and 5 (83.3%) of 6 tested patients, respectively, and were detectable in the olfactory bulb/nerve in 11 (25.6%) of 43 and 5 (26.3%) of 19 tested patients, respectively.

Brainstem abnormalities were reported in 78 (58.2%) of 134 examined patients, including 15 (15/78, 19.2%) with brainstem hypoxic injury, 18 (18/78, 23.1%) with brainstem vascular accidents, and 65 (65/78, 83.3%) with microgliosis/lymphocytic infiltrations in the brainstem (Table 4). Among the 78 patients with brainstem abnormalities, SARS-CoV-2 RNA and viral proteins were detected in the brainstem in 5 (26.3%) of 19 and 17 (32.7%) of 52 tested patients, respectively, and 3 (37.5%) of 8 tested patients showed positive results for both viral RNA and proteins. Relative to the patients

TABLE 3 The relationship between neurological diagnosis and CSF testing for SARS-CoV-2 in COVID-19 patients (x/number, percentage)

Neurological diagnosis	CSF testing for SARS-CoV-2	x/number	Increase of CSF WBCs	Increase in CSF IgGs	Anti-SARS-CoV-2 antibodies in CSF
COVID-19-associated encephalitis/autoimmune encephalitis	Negative	72/86 (83.7%)	30/68 (44.1%)	6/17(35.3%)	2/7(28.6%)
	Positive	14/86 (16.3%)	6/12 (50%)	1/4(25%)	n.d.
Total		86	36/80 (45%)	5/21 (23.8%)	2/7 (28.6%)
COVID-19-associated encephalopathy	Negative	65/68 (95.6%)	18/56 (32.1%)	27/37 (71.4%)	23/30 (76.7%)
	Positive	3/68 (4.4%)	0/3 (0)	1/3(33.3%)	n.d.
Total		68	18/59 (30.5%)	28/40 (70%)	23/30 (76.7%)
Cerebrovascular accidents	Negative	28/30 (93.3%)	5/28 (17.8%)	1/7 (14.3%)	n.d.
	Positive	2/30 (6.7%)	0/2(0)	1/2 (50%)	n.d.
Total		30	5/30 (16.7%)	2/9 (22.2%)	n.d.
Miscellaneous CNS symptoms	Negative	70/72 (97.2%)	12/43 (27.9%)	9/18 (50%)	8/28 (28.6%)
	Positive	2/72 (2.8%)	1/1 (100%)	1/1 (100%)	n.d.
Total		72	13/44 (29.5%)	10/19 (52.6%)	
Guillain–Barré syndrome	Negative	20/21 (95.2%)	4/19 (21.1%)	2/4 (50%)	2/2 (100%)
	Positive	1/21 (4.8%)	0/1 (0)	1/1 (100%)	1/1 (100%)
Total		21	4/20 (20%)	3/5 (60%)	3/3 (100%)
Miscellaneous PNS involvement	Negative	26/28 (92.9%)	5/17(29.4%)	1/2(50%)	1/12(8.3%)
	Positive	2/28 (7.1%)	n.d.	n.d.	n.d.
Total		28	5/17 (29.4%)	1/2 (50%)	1/12 (8.3%)
Uncharacterized	Negative	141 (97.2%)	27/114 (23.7%)	16/41 (39.0%)	1/1 (100%)
	Positive	6 (2.8%)	0/3 (0)	1/3 (33.3%)	n.d.
Total		145	27/117(23.1%)	17/44 (38.6%)	1/1 (100%)

Abbreviations: CSF, cerebrospinal fluid; n.d., not data or no detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cells.

TABLE 4 Structural abnormalities in different brain areas and the detection of SARS-CoV-2 in the abnormal regions (x/number)

Abnormalities	Cases	Detection of SARS-CoV-2 in the abnormal region		
		RNA	Protein	RNA + Protein
Abnormalities in the olfactory nerve/bulb/cortex	52/134 (38.8%)	n.d.	n.d.	n.d.
Microgliosis/lymphocytic infiltration in the olfactory system	50/52 (96.2%)	n.d.	n.d.	n.d.
Abnormalities in the brainstem	78/134 (58.2%)	5/19 (26.3%)	17/52 (32.7%)	3/8 (37.5%)
Hypoxic injury in the brainstem	15/78 (19.2%)	1/11 (9.1%)	0/1 (0)	n.d.
Vascular accidents in the brainstem	18/78 (23.1%)	1/10 (10%)	n.d.	n.d.
Microgliosis/lymphocytic infiltration in the brainstem	65/78 (83.3%)	5/14 (35.7%)	16/51 (31.4%)	2/8 (25%)
Abnormalities in the cerebellum	94/134 (70.1%)	2/4 (50%)	0/11 (0)	n.d.
Hypoxic injury in the cerebellum	49/94 (52.1%)	1/3 (33.3%)	0/11 (0)	n.d.
Vascular accidents in the cerebellum	13/94 (13.8%)	1/1 (100%)	n.d.	n.d.
Microgliosis/lymphocytic infiltration in the cerebellum	51/94 (54.2%)	1/1 (100%)	0/11 (0)	n.d.
Abnormalities in the cerebrum	114/134 (85.1%)	20/61 (32.8%)	2/31 (6.5%)	1/9 (11.1%)
Hypoxic injury in the cerebrum	56/114 (49.0%)	4/19(21.0%)	0/29 (0)	n.d.
Vascular accidents in the cerebrum	42/114 (36.8%)	11/27 (40.7%)	0/1 (0)	n.d.
Microgliosis/lymphocytic infiltration in the cerebrum	58/114 (50.9%)	7/29 (24.1%)	0/11 (0)	n.d.
Abnormalities in the brain	134	31/93 (33.3%)	18/73 (24.7%)	9/43 (20.9%)

Abbreviation: n.d., not data or no detection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

with brainstem hypoxic injury (1/11 [9.1%] positive for viral RNA and 0/1 [0%] for viral proteins in the brainstem) or brainstem vascular accidents (1/10 [10%] positive for viral RNA in the brainstem and no data available for viral protein detection), the detection rate of SARS-CoV-2 in the brainstem was higher in the patients who showed microgliosis/lymphocytic infiltrations in the brainstem (5/14 [35.7%] positive for viral RNA and 16/51 [31.4%] positive for viral proteins). Among all patients who underwent viral detection in the brainstem, SARS-CoV-2 RNA and viral proteins were detectable in the brainstem in 16 (32.7%) of 49 and 18 (25.3%) of 71 tested patients, respectively, and 3 (25%) of 12 tested patients showed positive results for both viral RNA and proteins (Table 5).

Cerebellar abnormalities were reported in 94 (70.1%) of 134 examined patients, including 49 (49/94, 52.1%) with cerebellar hypoxic injury, 13 (13/94, 13.8%) with cerebellar vascular accidents, and 51 (51/94, 54.2%) with microgliosis/lymphocytic infiltrations in the cerebellum (Table 4). SARS-CoV-2 RNA was detected in the cerebellum in 4 (16.7%) of 24 tested patients, but viral proteins were absent in all 11 tested patients (Table 5).

Cerebral abnormalities were reported in 114 (85.1%) of 134 patients including 56 (56/114, 49%) with cerebral hypoxic injury, 42 (42/114, 36.8%) with cerebrovascular accidents, and 58 (58/114, 50.9%) with microglial activation and/or lymphocytic infiltrations in the cerebrum (Table 4). SARS-CoV-2 RNA was detected in the cerebrum in 4 (21%) of 19 with cerebral hypoxic injury, 11 (40.7%) of 27 with cerebrovascular accidents, and 7 (24.1%) of 29 with microgliosis/lymphocytic infiltrations in the cerebrum, respectively. However, no viral proteins were confirmed in the abnormal cerebral regions in these patients. Among all the patients who underwent testing for SARS-CoV-2 in the cerebrum, SARS-CoV-2 RNA and viral proteins were detected in the cerebrum in 22 (34.4%) of 64 and 3 (8.8%) of 34 tested patients, respectively, and only 1 showed positive results for both (Table 5).

TABLE 5 The detection of SARS-CoV-2 in different brain areas (x/number)

Test of SARS-CoV-2	RNA	Viral protein	RNA + viral protein
In olfactory mucosa	14/24 (58.3%)	5/6 (83.3%)	n.d.
In olfactory bulb/nerve	11/43 (25.6%)	5/19 (26.3%)	0/7 (0)
In the brainstem	16/49 (32.7%)	18/71 (25.3%)	3/12 (25%)
In the cerebellum	4/24 (16.7%)	0/11 (0)	n.d.
In the cerebrum	22//64 (34.4%)	3/34 (8.8%)	1/8 (12.5%)
In the brain	56/108 (51.9%)	25/85 (29.4%)	9/48 (18.7%)

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

4 | DISCUSSION

4.1 | What can CSF testing tell us about SARS-CoV-2 neuroinvasion

Since Moriguchi et al. reported the first case of COVID-19-associated encephalitis who showed a positive reaction for SARS-CoV-2 in the CSF,[11] increasing studies have been conducted to confirm whether the virus invaded the CSF or not.

In this study, we identified 97 relevant papers and found the presence of SARS-CoV-2 in the CSF in 30 (6.4%) of 468 patients who underwent CSF testing.[11,12,17–38] In addition, we found several important clues supporting the possible invasion of SARS-CoV-2 in the CNS.

First, among the patients with SARS-CoV-2 in the CSF, 7 of 26 patients (26.9%) did not present respiratory symptoms.[17,23,28,29,32,34,36] There were 6 (23.1%) of 25 tested patients who showed negative routine tests for SARS-CoV-2 in respiratory samples.[11,20,25,29,31,34] Moreover, 2/24 patients (8.3%) did not show either respiratory symptoms or positive routine tests.[29,34] Interestingly, almost all these patients exhibited symptoms suggestive of encephalitis or demyelinating diseases, suggesting these symptoms may be related to the direct invasion of SARS-CoV-2 in the CSF.

Second, quantitative analysis shows that the positive detection of SARS-CoV-2 is closely correlated with the neurological symptoms of tested patients. The detection rate of SARS-CoV-2 in the CSF is the highest in patients with encephalitis (16.3%), followed by those with cerebrovascular accidents (6.7%), encephalopathy (4.4%), and Guillain–Barré syndrome (4.8%). Importantly, patients without neurological manifestations all tested negative for SARS-CoV-2 in the CSF.[30,39,40] Unfortunately, in many studies, detailed clinical records or possible neurological diagnoses have not been provided for patients who underwent CSF testing.

Third, 46.2% of tested patients with negative detection of SARS-CoV-2 in the CSF showed the presence of antibodies specific to SARS-CoV-2 in the CSF. Unfortunately, in other studies, no further testing was performed to distinguish between autoimmune and antiviral antibodies. Of note, the presence of anti-SARS-CoV-2 antibodies in the CSF is also related to the neurological symptoms of tested patients. Furthermore, intrathecal antibody synthesis has been demonstrated in 23.3% of tested patients with anti-SARS-CoV-2 antibodies in the CSF.

Previous studies on other neurotropic viruses show that the invasion of viruses into the CNS is associated with the increase of intrathecal antibodies.[41,42] Song et al.[43] further found that the CSF antibodies against viruses appeared or increased only when the CNS was infected. Therefore, the presence of anti-SARS-CoV-2 antibodies in the CSF in patients with an intact blood–brain barrier may be associated with the direct invasion of SARS-CoV-2 in the CNS.

At the time of writing, Lewis et al.[44] published an elegant systematic review on CSF testing for SARS-CoV-2 in patients with COVID-19. Different from ours, their study is limited to the patients who had CSF testing due to neurologic symptoms.

CSF testing is currently the only clinically acceptable invasive method to evaluate CNS responses to infection. However, the detection rate of SARS-CoV-2 in the CSF is highly dependent on the types of diseases and the time of sample collection.[45] The titers of viruses in the CSF may change over the course of a patient's illness due to possible CSF clearance. Therefore, CSF testing may fail to give positive results due to delayed sampling.[44] Although some patients showed negative results for SARS-CoV-2 in the CSF, the possibility of CNS infection cannot be completely excluded in these patients, as demonstrated in some autopsy studies.[46,47]

On the other hand, viral detection via PCR testing is not 100% sensitive due to genetic variability in the virus itself or technical factors.[44] It has been pointed out that CSF testing will give false-positive results if a sample has been contaminated by blood. Moreover, a positive SARS-CoV-2 PCR does not definitively indicate that neuroinvasion is responsible for a given constellation of symptoms.[44]

Taken together, from the data of CSF testing, the presence of SARS-CoV-2 or anti-SARS-CoV-2 antibodies with intrathecal antibody synthesis has been found in the CSF in a total of 37 of 468 (7.9%). Although these findings are not conclusive evidence, they strongly indicate the possible invasion of SARS-CoV-2 in the CNS in some COVID-19 patients.

4.2 | What can autopsy studies tell us about SARS-CoV-2 neuroinvasion

Postmortem examination is known as the most definitive mean to assess viral neuroinvasion in patients with COVID-19.[14] Since Paniz-Mondolfi et al.[46] reported the first autopsy evidence of SARS-CoV-2 viral particles in the brain parenchyma of a COVID-19 patient by electron microscopy and PCR assays on April 21, 2020, increasing autopsy studies on patients with COVID-19 have been published.[39,46–72]

Among these studies, structural abnormalities are widely observed in the olfactory bulb/tract, brainstem, cerebellum, and cerebrum. The most common findings are hypoxic injury and vascular accidents, which is well consistent with the known hypoxemia and hypercoagulable state of blood in most decreased COVID-19 patients. However, this is not contradictory to the neuroinvasion of SARS-CoV-2, as SARS-CoV-2 RNA and/or viral proteins have been detected in the brain in some patients with hypoxic brain injury and/or brain vascular accidents.[48,54,59,63,66,71]

Microglial activation is a common pathological feature during neuronal injury induced by a variety of insults. Therefore, it may not be surprising to find microglial activation in the compromised brain regions in more than half of the examined cases. Interestingly, the detection rate of SARS-CoV-2 is much higher in the brain regions with microgliosis and/or lymphocytic infiltrations, relative to those with hypoxic brain injury or vascular accidents. Moreover, severe microglial proliferation is most commonly observed in the medulla oblongata.[48,54,56,59,67,72] These findings indicate that the inflammatory responses in some specific brain areas cannot be

attributed to only the hypoxemia or vascular accidents in critical patients with COVID-19.

Trans-neuronal transfer is known as a unique way for neurotropic viruses to infect the nervous system.[73] The high incidence of olfactory disorder in COVID-19 patients supports the hypothesis that olfactory mucosa/nerve may be one of the portals for SARS-CoV-2 to enter the CNS.[8,9,73,74] Consistently, SARS-CoV-2 RNA and viral proteins have been detected in the olfactory system in 14 (58.3%) of 24 and 5 (83.3%) of 6 tested patients, respectively. Moreover, 50 (37.3%) of 134 tested patients showed severe microgliosis and/or lymphocytic infiltrations in the olfactory nerve, olfactory bulb, or olfactory cortex.[59,67]

The brainstem is comprised of many important structures, and the wide anatomical connections make it an easily accessible CNS target for SARS-CoV-2 from peripheral infection sites.[7,75] Consistently, the brainstem was found to show a high detection rate of SARS-CoV-2,[59,60,62] as well as the most severe microgliosis and/or lymphocytic infiltrations.[48,54,56,58,67,72] These findings support the speculation that the brainstem may be a major target in the CNS for SARS-CoV-2.

To date, PCR or quantitative real-time polymerase chain reaction (qRT-PCR) techniques revealed positive results for SARS-CoV-2 in the brain in 56 (51.9%) of 108 tested cases, while immunohistochemistry, using antibodies against viral nucleocapsid and/or spike proteins, revealed positive results in the brain in 25 (29.4%) of 85 tested cases. The detection rate by PCR assays was much higher than that by immunohistochemistry in almost all tested brain regions (Table 5).

It is reported that some COVID-19 patients had a detectable level of SARS-CoV-2 in the blood.[76,77] Therefore, viral PCR detection may give false-positive results due to the blood contained in the neural tissue. On the other hand, because neurotropic viruses usually infect some neurons only in specific brain regions, a sample homogenate containing uninfected neuronal and glial cells may have extremely low viral RNA, leading to false-negative results by PCR assays. Moreover, PCR detection cannot distinguish the types of cells which are infected in the neural tissue. Probably for these reasons, not all the results of PCR assays can be confirmed with in situ hybridization or immunohistochemistry.

Due to antigenic variability and technical factors, immunohistochemistry is also not 100% accurate for the elucidation of viruses in the CNS. However, the in-situ detection of SARS-CoV-2 by this technique seems more reliable and has provided convincing evidence for the presence of the virus in neuronal cells in specific brain areas.

Among the published autopsy studies, Matschke et al.[59] provided the most detailed account of neuropathological alterations in patients who died from COVID-19. In their meticulous study, immunohistochemical staining revealed that the nucleocapsid protein of SARS-CoV-2 was present in neuron-like cells in the medulla oblongata and in the cranial nerves which originated from the lower brainstem. Of note, in 13 patients for whom SARS-CoV-2 was detected in the brain by qRT-PCR, SARS-CoV-2 viral proteins could be confirmed in 8 (61%) using immunohistochemistry with antibodies against nucleocapsid and spike proteins. On the other hand, in 5 patients who were tested negative on qRT-PCR analysis of SARS-CoV-2 RNA in the brain tissues, viral proteins were detectable by immunohistochemistry in the medulla oblongata.[59]

Noteworthily, Matschke et al.[57] found that the presence of SARS-CoV-2 in the brain was not associated with the severity of neuroimmune activation. At first sight, this finding seems surprising, but it may be consistent with the characteristics of neurotropic viruses as they can hide in neurons from the surveillance of the immune system.[78] Therefore, the immune response will not be effectively activated in the infected areas unless the initially infected neurons have been significantly damaged.[7]

Whether technically or ethically, it is a great challenge to carry out brain autopsies on human beings, especially for patients with infectious diseases. It is a pity that most autopsy studies did not provide sufficient details about neurological symptoms or suspected diagnoses of the examined patients. Moreover, documentation of neuropathological changes in COVID-19 patients was often incomplete in many reports. These are not conducive for further analysis of the relationship between the autopsy findings and clinical manifestations. Due to technical or ethical factors, complete brain removal was difficult or even was not permitted in some studies. However, incomplete or random sampling of brain tissue is not suitable for the study of CNS infection caused by neurotropic viruses, because these viruses usually only infect the brain regions with neural connections to the peripheral invasion sites.

5 | CONCLUSION

At present, SARS-CoV-2 has been detected in the CSF or brain parenchyma in quite a few patients, which provide undeniable evidence for the neuroinvasive potential of this virus. The detection of SARS-CoV-2 in the olfactory mucosa/nerve/bulb coincides with the olfactory dysfunction reported in most COVID-19 patients and therefore supports the use of the olfactory pathway by SARS-CoV-2 to enter the CNS. The discovery of brainstem abnormalities and the presence of SARS-CoV-2 in some medullary neurons support the brainstem as a major target of SARS-CoV-2 in the CNS. The possible damage to the brainstem respiratory center is worthy of further study, as it may be responsible for the high incidence of severe respiratory distress syndrome in COVID-19 patients.

As compared with the number of patients who underwent viral testing in the CNS, the number of patients with positive results seems very small. However, it should not be simply concluded that the neuroinvasion of SARS-CoV-2 is rare in COVID-19 patients because the detection methods or sampling procedures in some studies may not be suitable or sufficient to reveal the CNS infection induced by neurotropic viruses. Moreover, the primary symptoms and/or causes of death were significantly different among COVID-19 patients, and probably caused more conspicuous pathological changes than those due to direct infection, which usually localized to specific

brain areas. Given the complex pathophysiology of COVID-19 and the characteristics of neurotropic viruses, it is understandable that any study of the CNS infection may inevitably have limitations.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Yan-Chao Li conceived and designed the study. Yan Zhang and Bai-Hong Tan helped to search and interpret the literature. All authors critically reviewed and approved the final version of the paper.

DATA AVAILABILITY STATEMENT

The data in this study can be obtained upon request from the corresponding author.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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