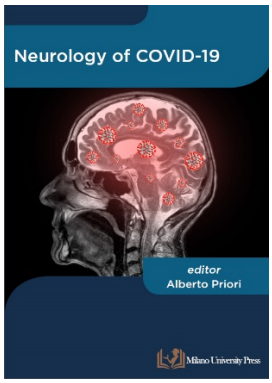

Neurology of COVID-19
Editor Alberto Priori
DOI: https://doi.org/10.54103/milanoup.57

Published by: Milano University Press
Via Festa del Perdono 7 - 20122 Milano
URL: https://milanoup.unimi.it/
E-mail: redazione.milanoup@unimi.it

Chapter 1. SARS-CoV-2 and the nervous system: review on pathogenesis of nervous system SARS-CoV-2 damage

DOI: https://doi.org/10.54103/milanoup.57.1

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Chapter 1. SARS-CoV-2 and the nervous system: review on pathogenesis of nervous system SARS-CoV-2 damage

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The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a newly emerged enveloped virus, positive-sense single-stranded RNA, of the *Coronaviridae* family, belonging to the genus of all highly pathogenic coronaviruses, i.e., Betacoronavirus[1]. After SARS-CoV and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV), SARS-CoV-2 is the third coronavirus to have caused a large outbreak in humans. SARS-CoV emerged in South China in 2002[2], MERS-CoV in Saudi Arabi in 2012[3], and SARS-CoV-2 in the Hubei province of China in 2019 most likely due to species barrier spillover, making the One Health approach a worldwide priority.

SARS-CoV-2 genome is more closely related to the genome of SARS-CoV than to MERS-CoV (80% and 50% identity, respectively)[4-6]. Overall, SARS-CoV-2 appears to be less lethal than SARS-CoV and MERS-CoV but is more highly transmissible. The main cellular target of SARS-CoV-2 is the angiotensin converting enzyme 2 (ACE2), a cell surface carboxypeptidase that is part of the renin-angiotensin system (RAS)[7]. SARS-CoV-2 has a strong affinity for ACE2[7,8]. Two-thirds of the SARS-CoV-2 genome encodes for non-structural proteins necessary for the replicase complex, whereas the remaining genome encodes for accessory and structural proteins[4,5,9]; the latter include spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The S protein is composed of two domains, one containing the receptor binding domain (RBD) and the other the membrane fusion domain, i.e., S1 and S2, respectively. The RBD of the S protein mediates viral entry by binding to the human ACE2. The binding is followed by proteolytic activation between S1/S2 at the plasma membrane by the transmembrane protease serine 2 (TMPRSS2) or at the endosomal membrane by cathepsin L[10]. The genome is then released in the cytosol where it is translated in viral proteins that form the RNA-dependent RNA polymerase. The genomic and sub-genomic RNAs are replicated, with the latter translated in accessory and structural proteins used for virion assembly. Finally, the viral RNA genomes are incorporated into the virions which are released from the

plasma membrane[9,11,12]. ACE2 is expressed by the respiratory tract, and, after having entered the host via epithelial cells, the vascular endothelial cells and macrophages may be the first targets[13-15].

SARS-CoV-2 is the etiologic agent of coronavirus disease 2019 (Covid-19) which is characterized by severe 'flu'-like symptoms that can progress to life-threatening systemic inflammation and multiorgan dysfunction[16]. Severe COVID-19 is observed in about 20% of patients infected with SARS-CoV-2, and the factors that dictate whether or not a patient develops the severe form are not yet known, although older age is one of the main known risk factors associated with severity together with obesity, cardiovascular diseases and male gender. One of the hallmarks of COVID-19 severity is the 'cytokine storm'[17-19], i.e., an uncontrolled increase in pro-inflammatory mediators following innate immune activation. The increased cytokine and chemokine concentration further amplifies the tissue damage by means of endothelial dysfunction and vasodilatation, eventually creating a hypoxic environment and organ failure[20]. Clinical aggravation occurs approximately one week after the onset of symptoms[17,21,22], which roughly corresponds to the temporal bridging of the innate and adaptive immune response. Once the disease becomes systemic, the disease will involve other organs and systems.

SARS-CoV-2 and the nervous system: pathogenetic aspects

Central nervous system and peripheral nervous system manifestations in COVID-19 disease

Central nervous system (CNS) and peripheral nervous system (PNS) involvement were described following SARS and MERS, even if these involved only a small proportion of cases: in fact, the prevalence of CNS and PNS complications ranged from 0.04% for SARS to 0.20% for MERS, and from 0.05% for SARS to 0.16% for MERS, respectively[23]. As the COVID-19 pandemic progressed, neurological manifestations increased. These included neurological symptoms that are present at the time of COVID-19 diagnosis in a considerable number of patients, and neurological complications that could appear later[24-26].

The neurological manifestations in COVID-19 disease have been categorized in three groups: (i) CNS involvement, characterized by dizziness, headache, impaired consciousness, acute cerebrovascular disease and epilepsy; (ii) PNS involvement, including anosmia, hypogeusia, visual impairment and neuralgia; and (iii) skeletal muscle impairment[27]. Neurological symptoms, such as loss of the sense of smell and taste, headache, fatigue, nausea and vomiting, dizziness, are reported in more than one-third of COVID-19 patients[17,28]; but more severe manifestations, as well as encephalopathy, stroke, Guillain-Barré syndrome, acute hemorrhagic necrotizing encephalitis, and acute disseminated

encephalomyelitis seem to be less common[23,29]. However, the prevalence of neurological manifestations associated with COVID-19 varies widely among the different studies, ranging from 7% to over 84%[27,30].

Given the high prevalence of COVID-19 disease worldwide, and the non-specificity of neurological symptoms associated with SARS-CoV-2 infection, that are, in fact, described also in the course of other viral infections, experts advise caution in attributing any specific causal links between SARS-CoV-2 and neurological symptoms[27]. The pathogenesis of CNS infection by SARS-CoV-2 and the neurological complications are still poorly understood and more pathogenetic studies are needed to shed light on this topic.

ACE2 receptors are expressed in different brain regions

ACE2 receptors are widely expressed in human organs, including in multiple CNS structures, such as brainstem, cortex, striatum and hypothalamus, and in several cell types, such as neurons and glia[26,31,32]; recently, ACE2 has also been found in the human cerebral vasculature in postmortem brain samples and appears to be upregulated in brain tissues by oxidative stress, apoptosis and neuroinflammation that characterize several neurological diseases or hypertension[31,32]. ACE2 in brain was also found in the neurons of the subfornical organ, where the virus could more easily find a means of entering the CNS, thanks to the lack of a blood brain barrier (BBB)[7]. Additional receptors may play a role in SARS-CoV-2 invasion of the brain: CD147 (or basigin, BSG) and Neuropilin1 (NRP1), to which the spike protein is capable of binding. Recently, a furin-like cleavage site on the spike protein of SARS-CoV-2 was demonstrated to be specific for this virus; the association between the furin-like cleavage site and its protease in host has been demonstrated to be critical for the neurotropism of the *Coronaviridae* family and thus the presence of this site could explain the ability of SARS-CoV-2 to invade the CNS[33].

Possible mechanisms of neurotropism and neurovirulence of SARS-CoV-2

Neurological manifestations could be caused by SARS-CoV-2 through a direct or indirect mechanism. The possible mechanisms of neurological impairment that have been identified so far are: (i) a direct effect of the virus entry into the CNS; (ii) para-infectious or post-infectious immune-mediated disease; (iii) a secondary involvement of the CNS following the systemic effects of COVID-19, such as systemic inflammatory response syndrome, sepsis and multiorgan failure[23,24]. The different mechanisms are not mutually exclusive and might co-exist. Similarly, Neuro-COVID has also been described as a process in three phases: (i) neuroinvasion; (ii) CNS clearance; and (iii) immune response. In the first phase, the virus reaches the CNS through the systemic circulation and/or through the trans-cribriform route along the olfactory nerve; it seems that the viral load in the CSF gradually increases until the second phase. In the

second phase, the interaction between the subunit S1 of the spike protein and the ACE2 receptor allows viral entry into the neuronal cells with subsequent neuronal damage; early after the infection, neuronal damage leads to the onset of anosmia and/or dysgeusia, while later the involvement of the nucleus of the solitary tract may cause severe respiratory impairment. During the second phase, the viral load starts to decrease until SARS-CoV-2 indirectly affects the CNS. In the third and last phases, the viral infection causes immune-mediated CNS impairment. The virus could stimulate the production of antibodies against glial cells, as a para- or post-infective mechanism, similar to that observed after other viral infections. In this phase, the respiratory symptoms may become even more severe, leading to neurotoxic hypoxia and brain damage[34].

CNS impairment as a consequence of a direct viral effect

Different experimental models were used to study the presence and the consequences of SARS-CoV-2 in the CNS: neural cell lines, animal models and brain organoids[35,36]. Published studies have shown that SARS-CoV-2 can infect and replicate in induced pluripotent stem cell (iPSCs)-derived human neural progenitor cells (hNPCs) and in neurospheres or brain organoids produced from these cells[25,32]. Animal experiments have provided information as to the neuroinvasive potential of SARS-CoV-2: the presence of the virus was found in neurons of different brain areas, and this was later compared with the pulmonary involvement. Interestingly, not all infected animals showed neurological symptoms or signs of CNS infection[24]. The neurotropism and neurovirulence of SARS-CoV-2 have also been demonstrated by the finding of viral acid nucleic in the cerebrospinal fluid (CSF) and in brain tissue samples[26], even if with a low frequency.

Detection of SARS-CoV-2 in cerebrospinal fluid

Neurotropism is a common characteristic of human coronaviruses (HCoVs) and several studies have demonstrated that SARS-CoV-2 is able to invade the CNS[33,37]. The detection of the viral RNA in the cerebrospinal fluid (CSF) by Real-Time Reverse Transcription PCR (RT-PCR) has been described by different authors in some cases of encephalopathy with a prevalence of 6.4%[38-43]. However, in the majority of patients diagnosed with encephalopathy, SARS-CoV-2 RNA was not detected in the CSF[41,42,44,45]. A possible explanation for this could be that the virus is cell-bound and spreads from cell to cell and does not transit freely in the CSF. Alternatively, the viral concentrations in the CSF could be below the level of detection of the test or the PCR reaction is inhibited by the presence of hemoglobin products for the breakdown of erythrocytes. The detection rate is also highly dependent on the type of neurological disease and the time of sample collection[41]. Finally, the absence of the virus in the CSF despite inflammation (confirmed by high levels of CSF white blood cells and

protein levels described in some patients with encephalitis) suggests that the encephalitis could be the result of systemic immune-mediated inflammation and is not driven only by a direct neuroinvasion of SARS-CoV-2[27,42,46].

The detection rate of SARS-CoV-2 RNA in the CSF was generally higher in patients diagnosed with encephalitis and much lower in patients with encephalopathy, cerebrovascular accidents or Guillain-Barré syndrome. Interestingly, patients without neurological manifestations were all negative for CSF SARS-CoV-2 RNA[41]. Moreover, almost half the patients with a negative test for SARS-CoV-2 in the CSF showed the presence in the CSF of antibodies specific for SARS-CoV-2, and, according to available data, 23.3% of tested patients present intrathecal antibody synthesis[41] that could suggest the invasion of the virus into the CNS[47].

Detection of SARS-CoV-2 in brain tissue samples

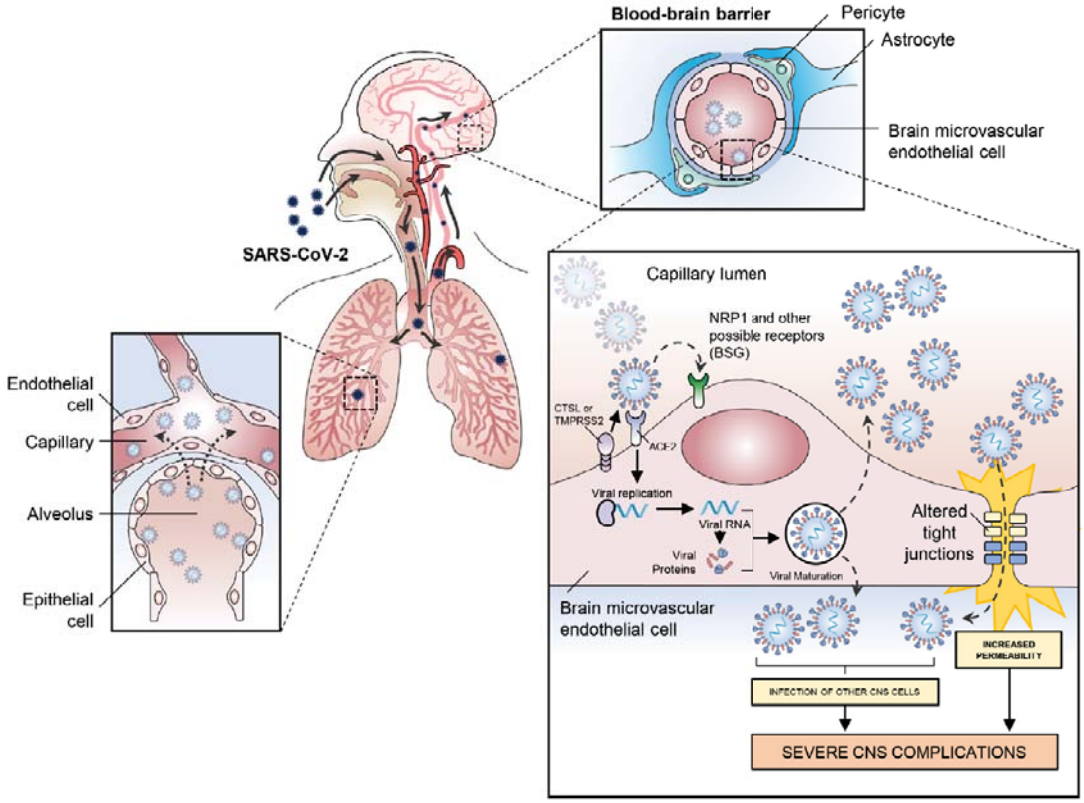
Postmortem examination is the definitive means of assessing viral neuroinvasion, in addition to that of the CSF, and previous studies have investigated the detection of SARS-CoV-2 in postmortem human brain samples, but so far with contrasting results. Some authors, in fact, reported the detection of the virus in brain autopsies (even though the viral load was low), by PCR and quantitative PCR (qPCR), or of viral nucleocapsid and/or spike proteins by immunohistochemistry[1,46,48]. Conversely, in other studies, the virus was not detected in brain cells. Thus, the hypothesis that the virus is intrinsically neuroinvasive and is able to create a persistent infection in the CNS requires clarification[27,49,50]. SARS-CoV-2 was detected in different cell types as well as frontal lobe neurons, glial cells, endothelial cells, pericytes of brain capillaries and vagus nerve fibers[45,51-53], and viral proteins were found in a smaller proportion of patients in different brain regions, as well as brainstem, cerebellum, cerebrum and the olfactory system[41]. The highest detection rate of SARS-CoV-2 RNA was found in brainstem, as well as the most severe microgliosis and lymphocytic infiltration, suggesting that the brainstem could be a major target of SARS-CoV-2 in the CNS[41].

The most common findings in the CNS of patients who died from COVID-19 are hypoxic injury and vascular accidents[41] and microglial activation was found in the compromised brain areas in more than half the patients. The detection rate of SARS-CoV-2 was higher in regions with microgliosis and lymphocytic infiltration than in areas with hypoxic injury and vascular impairment[41].

Routes of neuroinvasion

Coronaviruses may reach the CNS through any of three different pathways: (i) hematogenous dissemination; (ii) the "Trojan horse" mechanism; and (iii) neuronal retrograde propagation[27,28,54,55]. In the first case, after a phase of viremia, the virus can cross the BBB and enter the CNS.

Figure 1.1: Possible SARS-CoV-2 entry to the central nervous system (CNS) via blood circulation



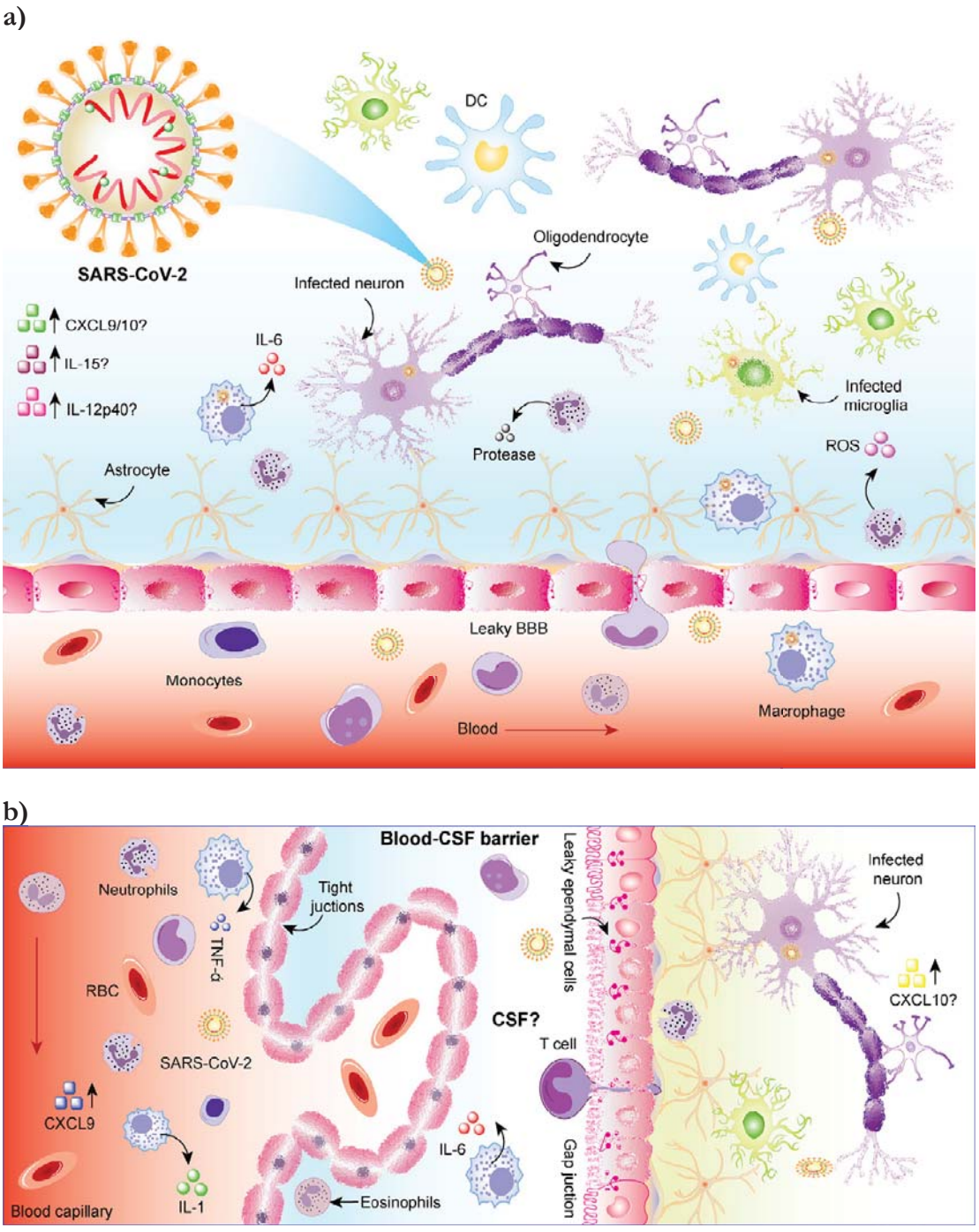
Passage of SARS-CoV-2 from the upper respiratory tract and the alveolar epithelial cells to the blood circulation; crossing of the Blood Brain Barrier (BBB) and invasion of the CNS. Reproduced from [25] with permission.

The passage of the virus to the blood circulation may follow the infection of type II alveolar epithelial cells, which highly express ACE2, and the epithelial cells of the gastrointestinal tract, which also express ACE2 receptors and can be infected by SARS-CoV-2[53]. The most plausible scenario is that of access through the respiratory tract: the damage to lung blood vessels following SARS-CoV-2 infection, including endothelial necrosis and capillary injury, has been demonstrated in postmortem analyses, and suggests that the virus can translocate from the lungs to the pulmonary microcirculation and then spread to other organs[25,56].

The "Trojan horse" mechanism is the process by which the virus infects lymphocytes and monocytes, and the latter, activated by the infection, can disseminate and cross the BBB; infected macrophages have been described in COVID-19, suggesting that latent SARS-CoV-2 infection can establish in immune cells[25,56]. Finally, the trans-synaptic dissemination can be retrograde or antegrade and use an exocytosis/endocytosis mechanism or the rapid axonal transport; it could be facilitated by proteins called dinein and kinesin, both possible targets of the virus[57,58].

The virus can either infect endothelial cells of the BBB or epithelial cells of the blood-CSF barrier in the choroid plexus to enter the CNS.

Figure 1.2: Passage of SARS-CoV-2 through the Blood Brain Barrier triggering a neuroinflammation process



SARS-CoV-2 infection of microglia and neurons triggers an inflammatory cascade with release of pro-inflammatory cytokines and chemokines in the Central Nervous System (CNS). b) These pro-inflammatory cytokines and chemokines, in turn, reduce the integrity of the Blood Brain Barrier (BBB) and allow the entry of other viruses and mediators of inflammation into the CNS. This neuroinflammation process eventually causes neurotoxicity and neuronal death. Reproduced from [65] with permission.

During transcellular migration, the virus invades host endothelial cells to cross the BBB, while during paracellular migration, the virus invades the tight junctions formed by the endothelial cells[59]. ACE2 and NRP1 have been found in human brain microvascular endothelial cells (BMVECs) and may allow viral entry to the CNS; the examination of postmortem brain samples by transmission electron microscopy has found the presence of viral-like proteins inside BMVECs in the frontal lobe[45]. Human choroid plexus expresses ACE2 and, thanks to its greater permeability compared to BBB, it could provide a second entry route to the CNS[25,60]; on the other hand, the low viral load in the blood makes this an unlikely means of entry to the CSN[25].

BBB is negatively affected by viral infections, not only thanks to the productive or non-productive infection of endothelial cells, but also by the host immune response that stimulate the release of pro-inflammatory cytokines, chemokines and cell adhesion molecules, finally leading to changes in the structural and functional integrity of the BBB[31]. The inflammatory mediators could break down the BBB by reducing the integrity of the tight junction proteins[61]. In turn, BBB dysfunction creates a vicious circle, allowing the passage of other free viral particles and infected immune cells to the CNS.

The increase in BBB permeability has been described in COVID-19 patients with neurological symptoms[62] and, recently, a possible direct role for SARS-CoV-2 in modifying BBB properties has been reported. In a BBB-on-a-chip *in vitro* model of the human BBB, the subunits S1, S2 and RBD of the spike protein are able to promote a loss of barrier integrity, triggering a proinflammatory response on brain endothelial cells that includes upregulation of Matrix Metalloproteinases (MMP), cell adhesion molecules (ICAM-1 and VCAM-1), leukocyte chemotaxis factors (CXCL10 and RANTES) and cytokines (IL-1β and IL-6), and may finally contribute to a destabilization of BBB function[31].

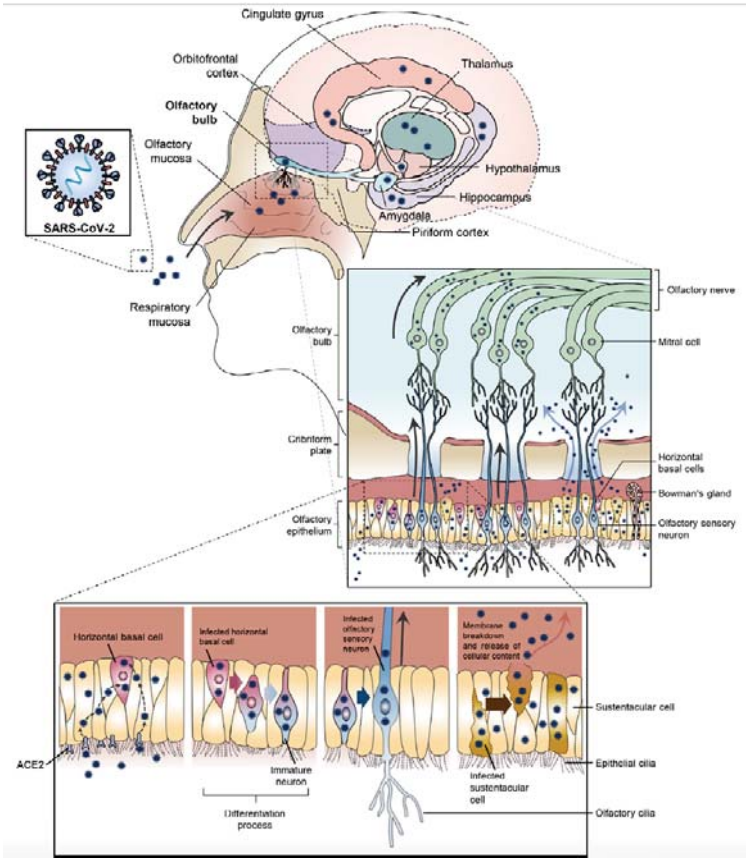
On entry into the CNS, SARS-CoV-2 may cause active infection of resident cells, thanks to the presence of its receptors, such as ACE2, NRP1 and BSG)[45]. Furthermore, the virus or infected lymphocytes could stimulate the production of pro-inflammatory cytokines, such as TNF-α and IL-6, and chemokines, such as CCL5, CXCL10 and CXCL11, that can induce chemoattraction of other activated T cells in the CNS. Activated astrocytes can, in turn, produce chemokines and participate in the recruitment of leukocytes. This process, for which the viral infection is the first trigger, finally causes neuroinflammation and neurotoxicity, damaging oligodendrocytes and neurons[63].

The presence of SARS-CoV-2 in the brain was not always associated with the severity of neuroinflammation and immune-activation, probably underlining the fact that the virus can hide in neurons and elude the surveillance of the immune system, or that the immune response is not effectively activated in the infected areas unless the neurons that were infected first have been significantly impaired[41].

As regards peripheral nerve dissemination, coronaviruses are known to invade peripheral nerve terminals and spread retrogradely across nerve synapses, reaching the CNS. Several viruses may spread to the peripheral nerves by binding to specific receptors on the axons or dendrites of the neurons[64]. Once the neurons are infected, the viruses reside in endosomal vesicles, resulting from the cytomembrane during viral entry, and use dinein to transport the vesicles along the microtubule to the centrosome beside the nucleus. Gradually, the viral capsid disassembles, according to the change in pH in the endosomal vesicle, and the viral nucleic acids are then released to the cytoplasm, allowing viral replication. Finally, viral nucleic acids and viral proteins are transported to synaptic membrane for further assembly and transmission to the next neuron and to the CNS[7].

Recently, a rapid accumulation of SARS-CoV-2 in the brain was reported after intranasal injection using a new humanized ACE knock-in-mouse model[56]. The most likely way of entering the CNS following intranasal infection is through the olfactory receptor neurons, also known as olfactory sensory neurons[27,29].

Figure 1.3: Possible SARS-CoV-2 entry to the central nervous system (CNS) via the olfactory nerve



SARS-CoV-2 entry through the olfactory nerve. SARS-CoV-2 can infect the olfactory epithelium thanks to the ACE2 receptor expressed by the horizontal basal cells. Horizontal basal cells can mature in infected olfactory neurons that are connected with neurons in the olfactory bulb; these neurons allow the viral spread to other areas in the CNS. Furthermore, the infected olfactory epithelial cells can release the virus at the cribriform plate. Reproduced from [25] with permission.

The virus can pass the neuroepithelium of the olfactory mucosa and reach the olfactory bulb, the olfactory nerve, and, from there, eventually spread to the hippocampus or other brain structures[27,32]. The ability to enter the olfactory bulb was reported for SARS and another coronavirus, OC43, using murine models of human coronavirus infection[65], and several studies have recently proposed this process in the context of SARS-CoV-2[25,33,56,59,66,67]. Figure 1.3 shows the proposed mechanism for SARS-CoV-2 entry into the CNS through the olfactory receptor neurons[25]. Given that SARS-CoV-2 spreads through the respiratory tracts, the olfactory nerve may serve as a major retrograde route for the spread of the virus to the CNS[7,68]. Furthermore, the proximity of the cribriform plate to the infected nasal epithelium, possible traumas due to sneezing, and the detection of the highest SARS-CoV-2 viral load in nasal swabs compared to bronchoalveolar lavage or pharyngeal swabs, all seem to confirm the different means of entry into the CNS through this route[25,32].

SARS-CoV-2 could infect the olfactory neurons thanks to its binding to NRP1 and BSG. In fact, both these proteins are expressed in the olfactory bulb at higher levels than ACE2 or TMPRSS2; NRP1 is also expressed in the olfactory epithelium[69-73]. In contrast, in another model, the first target of the virus could be the sustentacular cells (SUSs) thanks to their expression of ACE2 and TMPRSS2; the infection of these cells triggers a cascade of events leading to anosmia and eventually allows access of the virus to the CNS.

The possible entry through olfactory receptor neurons has also been hypothesized after the detection of SARS-CoV-2 RNA and viral proteins, with associated microgliosis and/or lymphocytic infiltrations, in the olfactory mucosa of most of the autopsies that have been carried out[41]. In animal models, immunostaining for SARS-CoV-2 revealed extensive staining in secondary and tertiary brain regions connected with the olfactory bulb, and the possibility of invasion of the brain in a retrograde manner along gustatory and trigeminal pathways at the early stage of infection was shown[24].

Other potential routes of brain infection through nerve dissemination could be possible, as well as via vagus, trigeminal and nasopharyngeal nerves; in fact, ACE2 and NRP1 are expressed in the vagus nerve in animal models, and trigeminal and nasopharyngeal nerves are easily exposed to SARS-CoV-2[25,53]. SARS-CoV-2 fragments have been found in a patient's conjunctiva, where the sensory nerve endings of the trigeminal nerve are found. In addition, local peripheral nerves of the gastrointestinal tract may play a role in the retrograde penetration of SARS-CoV-2 to the CNS[53].

However, additional data on humans are needed to understand if these mechanisms are likely to produce CNS infection[25].

Indirect CNS damage in the course of SARS-CoV-2 infection: non-specific complications of systemic disease

The CNS could also be damaged by hyperinflammation syndrome and the "cytokine storm" that is triggered outside the brain by SARS-CoV-2, or by the severe effects of systemic disorders, such as sepsis, hyperpyrexia, hypoxia, hypercoagulability and critical illness with multiorgan failure[25,32,74,75].

The excessive levels of proinflammatory cytokines and chemokines in the systemic circulation, caused by a maladaptive innate immunity, may increase the permeability of the BBB; the passive flow of cytokines/chemokines to the CNS, together with infected immune cells, could damage the brain[58,61]. Furthermore, IL-6, IL-1 and TNF-α are all upregulated in the brain of infected animal models and are produced in the human CNS following brain injuries[37]. In fact, sepsis and the subsequent inflammatory "cytokine storm" has been implicated in cases of an altered state of consciousness[37]. Increased levels of pro-inflammatory cytokines in the CNS could also persist for a lengthy period of time, leading to a post-infectious proinflammatory state that may contribute to possible long-term neuroinflammation[61].

Metabolic imbalances, including disorders of blood calcium, sodium and glucose, and renal and/or liver dysfunction, may have secondary negative effects on CSN function[56].

Systemic factors could also be responsible for the increased risk of cerebrovascular disease in COVID-19 patients. The SARS-CoV-2 binding to the ACE2 receptor on endothelial cells may result in increased blood pressure. Arterial hypertension can also complicate severe or critical COVID-19 as consequence of the viral infection or kidney damage, and can result in ischemic or cerebral bleeding[74]. Together with an increase in blood pressure, thrombocytopenia and thrombus formation due to hypercoagulability in the brain or in peripheral veins could finally lead to stroke. Indeed, the marked systemic inflammation and hypercoagulability that characterize severely affected patients are associated with increased risk of thrombotic events and stroke. Cases of myocarditis associated with SARS-CoV-2 have also been described and can lead to ischemic stroke through heart failure, with a reduction in the cerebral blood supply, or supra- and ventricular arrhythmias causing intra-ventricular thrombus formation. Finally, systemic and CNS inflammatory vasculitis have been reported from autopsies of COVID-19 patients[56].

Severe COVID-19 with acute respiratory failure, severe acute respiratory distress syndrome (ARDS), or even cardiac arrest can be associated with cerebral hypoxia[32,74]. Hypoxia may cause indirect neuronal damage[56]. There is, however, some evidence that the possible hypoxic injury is not an underlining mechanism of CNS impairment in COVID-19. In fact, hypoxia causes specific features on cerebral imaging: (i) the localization of lesions in grey matter; (ii) the edema, loss

of grey/white matter differentiation, reversal sign, white cerebellum sign, linear hyperdensity outlining the cortex and pseudo-subarachnoid bleeding on a CT scan; or (iii) cytotoxic edema within the first 24 hours with T2-hyperintensity of the lesions, subsequent pseudo-normalization of the lesions after 1 week and T1-hyperintensity after 1-2 weeks suggesting cortical laminar necrosis on MRI[76]. The evolution of these lesions has not been described in COVID-19 patients. Moreover, most patients are intubated and on mechanical ventilation before they develop cerebral hypoxia[74].

It has been hypothesized that the gut-brain axis could also be responsible for the CNS impairment during the clinical course of SARS-CoV-2 infection; however, the actual impact of SARS-CoV-2 on gut microbiota has yet to be established. The possible relationships between gut and brain could be due to a direct viral invasion of the CNS through systemic circulation or the vagal nerve after entry from the gut, or the disruption of the homeostasis of mucosal immunity and gut microbiota with repercussions on the CNS. In fact, a gut dysbiosis induced by the virus could make the CNS even more susceptible to harmful agents including pathogens and SARS-CoV-2 itself[77].

One last mechanism of possible CNS damage is the effect of empiric treatments for COVID-19; rhabdomyolisis was sometimes described after treatment with antivirals, such as remdesivir and lopinavir/ritonavir, while toxic myopathy can be caused by chloroquine or some antibiotics[74]. Finally, a myasthenic syndrome was reported after treatment with chloroquine. However, we must consider that most of these therapies are no longer recommended in the guidelines for COVID-19 treatment, and this mechanism could explain only a small part of the neurological complications associated with COVID-19[78,79]. A possible alteration of the adaptive cellular immune response to viruses could be caused by the use of steroids, even if their role in switching off the inflammatory response seems to be more important than any possible negative effects[46].

Indirect CNS damage during SARS-CoV-2 infection: para-infectious and post-infectious immune-mediated disease

SARS-CoV-2 could cause indirect damage to the CNS because of an infection-triggered excessive and detrimental immune activation[47]. Autoimmune responses to the virus have also been proposed as para- or post-infectious mechanism of CNS damage and could explain some neurological manifestations, such as Guillain-Barré syndrome, acute necrotizing encephalopathy, and acute disseminated encephalomyelitis, that have been described in COVID-19 patients[26,37,43].

As regards immune-mediated process, SARS-CoV-2 infection may be associated with a process of neuroinflammation, similar to that described in other

viral infections and in neurological diseases, such as Alzheimer's and Parkinson's disease. SARS-CoV-2 could escape from the immune system and spread to all CNS tissue, causing increased viral replication or over-reactive innate immune responses[61]. The activation of glial cells by SARS-CoV-2 could result in further production of cytokines/chemokines that ultimately damage neurons[61].

The neuroinflammation could also be associated with cellular senescence and a state of cell proliferative arrest, as an adaptive response to the viral infection, eventually resulting in neurodegenerative processes. The possibility that neurodegenerative diseases could follow the viral infection in certain patients as long-term consequences is still under investigation[32,58]. The possible causes could be the establishment of a latent reservoir of SARS-CoV-2 in the CNS and long-term neuroinflammation[26,61]. Following latent viral infection, vascular endothelium dysfunction and oxidative stress could persist, and both these processes have been well described as determinants of neurodegeneration[61,80].

Immune-mediated demyelinating diseases have been described for other coronaviruses, following the virus-induced inflammation and activation of glial cells; the same mechanism could lead do demyelinating diseases also after SARS-CoV-2 infection[67].

Brain regions affected by SARS-CoV-2 infection

Brainstem could be one of the major areas affected by SARS-CoV-2 infection in the CNS; one hypothesis is that the infection of respiratory centers in the medulla oblongata and the pons could contribute partially to the respiratory breakdown of COVID-19 patients. The spread via synapse-connected route into the brainstem cardiorespiratory centers of the medulla oblongata has been demonstrated for other coronaviruses and is thus likely also for SARS-CoV-2[8]. Grey matter could be directly impacted by the infection as demonstrated by edema and partial neuronal degeneration observed in autopsies. Finally, demyelinating lesions have been described in the white matter and the spine[8].

Neuropathological findings of COVID-19 patients

Histological findings described in COVID-19 patients are heterogeneous[81]. The most frequent are: (i) microglial activation, mostly confined to the brainstem, the cerebellum, the frontal lobe and meninges; (ii) lymphoid inflammation including perivascular lymphocytosis, parenchymal lymphocytic infiltration and leptomeningeal lymphocytic inflammation with a prevalence of infiltration of CD8+ cytotoxic T lymphocytes; (iii) hypoxic-ischemic changes; (iv) variable degrees of astrogliosis in all brain regions; (v) myelin loss; (vi) acute/subacute brain infarcts; and (vii) primary hemorrhage and microthrombi[48,81,82].

Conclusions

SARS-CoV-2 may be neurotropic and it has been demonstrated that it can reach the CNS. The exact mechanisms of neuropathogenesis of SARS-CoV-2 infection are still unknown and are likely to be multifactorial. It is likely that the virus could harm the CNS both directly, entering the CNS and triggering a process of neuroinflammation, and indirectly, thanks to systemic inflammation and immune-mediated processes. Viral entry to the CNS is under investigation: similar to other viruses and coronaviruses, SARS-CoV-2 could enter the CNS through the systemic circulation crossing the BBB, through the infection of immune cells, such as lymphocytes and monocytes (the "Trojan horse" mechanism) or through a neuronal retrograde dissemination, via olfactory mucosa or gastrointestinal tract. Future studies will promote a better understanding of the real neurovirulence of SARS-CoV-2.

Take-home message

- SARS-CoV-2 infection can involve the Central Nervous System (CNS) causing neurological symptoms at the time of the diagnosis or neurological complications that could appear later, defined as "Neuro-COVID".
- SARS-CoV-2 could determine neurological impairment directly invading the CNS or indirectly by para/post-infectious immune-mediated disease or by a secondary involvement of the CNS in the course of systemic COVID-19 disease.
- The virus reaches the CNS through the blood circulation crossing the Blood Brain Barrier (BBB) and/or through the olfactory nerve. The exact mechanisms of viral entry into the CNS are still not completely understood.
- SARS-CoV-2 can infect the microglia and neuronal cells and the viral presence in the CNS triggers an inflammatory cascade; pro-inflammatory cytokines and chemokines increase the permeability of the BBB, allowing the arrival of new viral particles, as well as immune system cells and inflammatory mediators, in the CNS. A process of neuroinflammation is then established which ultimately causes neurotoxicity.
- The long-term consequences of "Neuro-COVID" and the possibility of establishing a latent viral reservoir in the CNS or developing neurodegenerative diseases following the acute viral infection is still under investigation.

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