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OPINION

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Neuroinfection may contribute to pathophysiology and clinical manifestations of COVID-19

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The new coronavirus, classified as severe acute respiratory syndrome (SARS)-CoV-2 that emerged in Hubei province in China, causes a new coronavirus disease, which was termed COVID-19 by WHO on 11 February 2020. COVID-19 claimed more than 65 000 lives around the world by 5th of April 2020. It is not the first coronavirus, which infects humans; the pathogenic viruses that cause human diseases (human coronaviruses, HCoV) include 6 other members designated as SARS-CoV, middle east respiratory syndrome (MERS)-CoV, HCoV-HKU1, HCoV-NL63, HCoV-OC43 and HCoV-229E. The clinical presentation is mainly manifested as malignant pneumonia; although many patients present neurological symptoms, such as vomiting, dizziness, headache and delirium.[1] Human coronaviruses were first identified in the mid-1960s; they were named for the crown-like spikes on their surface. The SARS-CoV-2 virus belongs to β-coronavirus, which also include MERS-CoV, SARS-CoV-1, NCoV-OC43 and HCoV-HKU1. The primary target cells for SARS-CoV-2 are the epithelial cells of the respiratory and gastrointestinal tract, which contain angiotensin converting enzyme 2 (ACE2), that is utilized by the virus to enter the cell; it is, however, hard to believe that the penetration of the viral agent into the organism is limited only to these tissues.[2]

Clinical and pre-clinical data from studies with other coronaviruses suggest wider tissue invasiveness and an evident neurotropism, which may result in more complex clinical scenarios. Can the SARS-CoV-2 enter the central nervous system (CNS) and infect neural cells? And if yes, how the CNS damage contributes to pathophysiology of the COVID-19, to its signs, symptoms and progression as well as to its sequelae. In other words, if the SARS-CoV-2 virus had a significant neurotropism, could its presence in the CNS be pathophysiologically relevant?

It has been demonstrated that coronaviruses, and especially β-coronaviruses to which the SARS-CoV-2 belongs, do not limit their presence to the respiratory tract and frequently invade the CNS. This propensity has been convincingly documented for the SARS-CoV, MERS-CoV and the coronavirus responsible for porcine haemagglutinating encephalomyelitis (HEV 67N).[3-5] Previous findings demonstrate that ACE2 represents the key, but not the exclusive, site of entry of the virus into the cell. The ACE2 is expressed in the brain, being in particularly present in the brain stem and in the regions responsible for regulation of cardiovascular function including subfornical organ, paraventricular nucleus, nucleus of the tractus solitarius,

and rostral ventrolateral medulla; expression of ACE2 was found in both neurones and glia.[6,7] Non-ACE2 pathways for virus infection of neural cells also cannot be excluded; the marked penetration of coronavirus into the liver, an organ with lower levels of ACE 2 compared to the CNS, strongly supports the assumption that the cell entry routes can vary.[8] Be this all as it may, the CNS infection with both SARS-CoV-1, MERS-CoV have been reported[2] and SARS-CoV-1 has been identified in neurones from tissues obtained from infected patients.[9]

The intranasal administration of SARS-CoV-1[10] or MERS-COV[11] resulted in the rapid invasion of viral particles into the brain, possibly through the olfactory bulb via trans-synaptic route. This pathway when virus enters peripheral nerves and spreads to the CNS through synaptic contacts has been well-documented for several viruses including CoVs.[12] The brainstem, which hosts the respiratory neuronal circuit in the medulla, was severely infected with both types of viruses, which may contribute to degradation and failure of respiratory centres. When the nasal infecting charges were delivered in extremely low doses, only the CNS was colonized, with virus being absent in other tissues including lungs,[11] corroborating the potent neurotropism of these coronarovirus strains. This testifies a viral property which cannot be ignored for a complete understanding of the impact of the β-coronaviruses on the human organism. Although direct evidence is currently lacking, the high identity between SARS-CoV-1 and SARS-CoV-2 suggests, that the latter viral strain could also infect the CNS, an ability clearly demonstrated by other members of the family to which they belong. The β-coronavirus NCoV-OC43, which causes upper respiratory tract disorder, has been found to infect neural cell lines as well as primary neurones in culture; it was also found to cause encephalitis associated with neuronal apoptosis and necrosis in mice.[13] At least two cases of human encephalitis/encepahlomyelitis caused by NCoV-OC43 were also reported.[14,15] About 12% of children with clinical presentation of acute encephalitis hospitalised at the Children's Hospital of Chenzhou, China between May 2014 and April 2015 had anti-CoV antibodies in serum and in cerebrospinal fluid.[16]

It is of considerable interest that organ distribution studies have shown that the presence of SARS-CoV-1 in the cerebrum, but not in cerebellum.[17] These two parts of the brain exhibit distinct ratios between neurones and neuroglia; in the neocortex the number of non-neuronal cells (most of which are represented by neuroglia) is almost four times larger than the number of neurones, whereas in the cerebellum neurones account for ~90% of all cells.[18] Upon infection and because of other forms of damage neuroglial cells become reactive, representing the most classic neuropathological scenario of the ongoing neuroinflammation. Therefore, it is possible that the SARS-CoV-2 infected brain regions triggers reactive astrogliosis and activation of microglia.

This framework, as learned from studies of Tick-borne encephalitis virus (TBEV) and Zika virus (ZIKV), predicts a strong role of astrocytes and microglia in orchestrating the nervous tissue response to neuroinfection and spread of the virus in the brain. One of the fundamental events in the neuroinfection is the pathogen crossing of the blood-brain barrier (BBB). Astrocytes form the parenchymal portion of the BBB through their endfeet, which extensively plaster (~98% of coverage) intracranial blood vessels. In the grey mater astrocytes occupy separate territorial domains and integrate neural elements with vasculature forming the neurovascular unit.[19]

Both TBEV and ZIKV belong to the *Flaviviridae* family, and both viruses enter astrocytes by endocytosis[20,21] thus instigating a neuroinfection. Internalization of TBEV into astrocytes is mediated by the clathrin-dependent endocytosis also known for several members of *Flaviviridae* family including West Nile virus, Dengue virus, Hepatitis C virus and Bovine Viral Diarrhoea virus.[21] Whether SARS-Co-V2 infects astroglial cells and enters astrocytes by endocytosis remains to be studied, although the interneuronal transfer of another coronavirus HEV67 utilises the clathrin-dependent endocytotic/exocytotic pathway.[4]

At least in the rodent brain, infection by TBEV has no detrimental effect on astroglial viability and hence astrocytes likely represent a reservoir for TBEV from where further infection and re-infection can occur. Once within a cell, virus can traffic to different compartments. In astroglia the TBEV uses the endosomal system for the spread within the cytoplasm.[22] The spread of virus-loaded vesicles exhibits directional mobility, which is driven by protein motors carrying vesicles along the cytoskeletal elements, including microtubules, actin and intermediate filaments. On the other hand, virus-loaded vesicles may also exhibit non-directional mobility, characterized by randomness of free diffusion. As a function of time, there is a series of events in virus-infected cells, leading to an increased number of TBEV particles per astrocytes, with a pronounced increase in virus particle mobility.[22] Similar to the infiltration of TBEV, endocytosis was recently confirmed to be the mechanism of ZIKV infection of astrocytes and microglia.[23] Among human cells, astrocytes were more susceptible to ZIKV infection than neurones, released more progeny virus and tolerated higher virus loads than neurones.[20]

The occurrence of the virus in the brain stem may affect chemosensing neural cells associated with respiratory and cardiovascular regulation as well as respiratory centre neurones thus damaging ventilatory lung function. Further support for the hypothesis that the nasal route may contribute to the entry of the virus into the organism, including the brain, is provided by clinical observations of an early and profound

marked anosmia in SARS-CoV-2 infected subjects (Ear, Nose and Throat surgery society, ENT UK; https://www.entuk.org/sites/default/files/files/LossofsenseofsmellasmarkerofCOVID.pdf).

Another fundamental aspect of the effect of SARS CoV2 infection and CNS is that this infection triggers a substantial systemic inflammatory storm with a massive release of cytokines, chemokines, and other inflammation signals with a subsequent significant break of BBB, which instigates and amplifies the neuroinflammatory process. Numerous preclinical and clinical studies consistently demonstrate that systemic inflammation, regardless of its nature, be it bacterial, viral or toxic, compromises BBB, injures glia limitans, activates Toll-like receptors residing in microglia and astrocytes and is associated with the innate immunity, ultimately promoting neuroinflammation that may severely disturb brain homeostasis and cause neuronal death.[24] Therefore, the neuroinflammatory process associated with functional brain damage could explain the clinical experience according to which even in patients who overcome pneumonia, the onset or the progression of cognitive impairment associated with behavioural changes is observed. Delirium and cognitive deficits and behavioural abnormalities are clearly caused by a situation in which systemic inflammation associated with conditions of prolonged hypoxia induces a persistent and uncontrolled neuroinflammation—responsible, in turn, for damage to hippocampus and cortical areas associated with cognitive functions and behavioural alterations.[25]

Elderly patients recovering from pneumonia often exhibit delirium or deficits in attention and memory that persist over time and require treatment, which is frequently remarkably demanding. Delirium is commonly provoked by peripheral infection associated with systemic inflammation. Elevated concentrations of serum pro-interleukins and S100B, (recognized as index of BBB disruption), have been observed during delirium in elderly patients.[26] Neuroinflammation appears as an almost obligatory component in neurodegenerative disorders[27] and has been implicated in psychiatric pathologies from acute psychosis to schizophrenia, autism spectrum disorder, affective disorders to name but a few.[28] There is a strong association between systemic inflammation and depressive syndromes with infections rising the risk of depressive episodes by ~60%.[28] In animal models, injections of cytokines instigates sickness behaviour[29]; which is very similar to a human "flu-like syndrome" manifested by anhedonia, anorexia, fever, fatigue, increased pain, sleep disturbances, and confusion. Furthermore, severe respiratory failure accompanying COVID-19 triggers long-lasting hypoxia, which arguably affects the brain and causes neurocognitive alterations.

To conclude: coronaviruses are neurotropic, and SARS-CoV-2 most likely is not an exception; coronaviruses may enter the CNS through several routes, most notably through intranasal inoculation and though peripheral nerves using trans-synaptic pathways. Coronaviruses can infect both neurones and neuroglia; neural cells express the entry protein ACE2, although direct endocytotic infection (similar to those demonstrated for ZIKA and TBEV viruses) cannot be excluded. Coronavirueses predominantly infect neurones in the brain stem in the nuclei associated with cardio-respiratory control; injuries to these areas may exacerbate or even lead to respiratory failure. Direct CNS infection together with systemic inflammation, which accompanies COVID-19, compromises the blood brain barrier and triggers a massive neuroinflammatory response manifested by reactive astrogliosis and activation of microglia. Neuroinflammation together with prolonged hypoxia may promote neuropsychiatric developments and cognitive impairments both acute and chronic. The neurological and psychiatric aspects of the viral attack must therefore be considered in designing the therapeutic strategies and for rehabilitation paradigms aimed at victims of SARS-CoV-2.

CONFLICT OF INTEREST

No conflict of interest to declare.

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