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LEADING TOPIC

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Blood-brain barrier function in response to SARS-CoV-2 and its spike protein

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ABSTRACT

The typical manifestation of coronavirus 2 (CoV-2) infection is a severe acute respiratory syndrome (SARS) accompanied by pneumonia (COVID-19). However, SARS-CoV-2 can also affect the brain, causing chronic neurological symptoms, variously known as long, post, post-acute, or persistent COVID-19 condition, and affecting up to 40% of patients. The symptoms (fatigue, dizziness, headache, sleep disorders, malaise, disturbances of memory and mood) usually are mild and resolve spontaneously. However, some patients develop acute and fatal complications, including stroke or encephalopathy. Damage to the brain vessels mediated by the coronavirus spike protein (S-protein) and overactive immune responses have been identified as leading causes of this condition. However, the molecular mechanism by which the virus affects the brain still needs to be fully delineated. In this review article, we focus on interactions between host molecules and S-protein as the mechanism allowing the transit of SARS-CoV-2 through the blood-brain barrier to reach the brain structures. In addition, we discuss the impact of S-protein mutations and the involvement of other cellular factors conditioning the pathophysiology of SARS-CoV-2 infection. Finally, we review current and future COVID-19 treatment options.

Key words: SARS-CoV-2, spike protein, blood-brain barrier, encephalopathy, stroke, cytokine storm, neuroinflammation

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Introduction

SARS-CoV-2 mainly causes inflammatory lung damage associated with thrombosis and increased pulmonary vascular permeability leading to haemorrhage and oedema [1]. In addition, the cytokine storm during the inflammatory cascade and the direct action of the SARS-CoV-2-derived spike protein affect other organs, including the central nervous system [2–4]. Compelling reports indicate that COVID-19 patients develop neurological symptoms during acute infection. They can also develop a chronic condition, long-COVID or PACS (Post-Acute COVID Syndrome), characterised by fatigue and neuropsychiatric symptoms [5–8]. More than 40% of COVID-19 patients exhibit neurological, potentially lethal, symptoms during SARS-CoV-2, increasing the need to understand the underlying molecular mechanisms and to develop effective countermeasures [9–11].

Neuroinflammation, which typically accompanies central nervous system (CNS) damage, can be mediated directly by the viral invasion of CNS cells or indirectly by mediators that govern the development of systemic inflammation [3, 12]. Briefly, direct action of the SARS-CoV-2 spike protein

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(S1-protein), which circulates in the blood after being cleaved by the proteases during the viral invasion, and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) released by host immunocompetent cells in response to it, impede the blood--brain barrier (BBB) integrity, resulting in viral entry through the endothelium followed by infection of astrocytes [2, 3–15]. Symptoms can range from mild headache, nausea, impaired consciousness or reduced cognition to severe, potentially lethal conditions including encephalopathy, delirium, and acute disseminated encephalomyelitis [9–11]. Multicentre analyses have revealed that stroke (60%+) and encephalopathy (up to 42%) account for most neurological complications associated with SARS-CoV-2 infection. Encephalitis and Guillain-Barré syndrome are considerably less frequent, with incidence rates of 13% and 9%, respectively [11, 16]. Younger patients are more susceptible to ischaemic stroke associated with recurrent vascular occlusion and greater morbidity for COVID-19 patients than influenza patients. Among all neurological symptoms of SARS-CoV-2, patients with stroke and inflammatory syndromes had the worst prognosis [17, 18]. In the majority of cases, neuroimaging reveals ischaemia with large vessel occlusion, perfusion abnormalities, and haemorrhage in locations not typically associated with hypertension. In addition, post mortem analyses of COVID-19 patients have identified a link between microvascular disease and microhaemorrhages [22, 23].

In conclusion, all of these neurological complications may result directly or indirectly from an impairment of the blood-brain barrier (BBB) caused by SARS-CoV-2 infection.

Role of blood-brain barrier in maintaining central nervous system homeostasis

The blood-brain barrier is a specialised system of brain microvascular endothelial cells that guards the brain from circulating toxins, nourishes brain tissues, and filters harmful compounds from the brain to the bloodstream [19]. The proper functioning of the central nervous system is ensured by close interaction between the brain endothelium and other neurovascular/glial components such as astrocytes, pericytes, neurons, and basement membranes [20, 21]. Passage through the BBB is strictly regulated by physical (intercellular tight and adherens junctions) and metabolic (enzymes, transporters etc.) barriers [22, 23]. Notably, there is a functional difference between the brain endothelial cells' abluminal and luminal membrane surfaces due to the differential expression of specific transporters, secretory bodies, and the release of enzymes locally [24]. Due to its restricted permeability, the BBB is a major obstacle for delivering therapeutic agents into the CNS [25]. Leakage or disruption of the BBB plays a crucial role in the pathogenesis of numerous brain diseases such as Alzheimer's Disease, multiple sclerosis, ischaemia, Parkinson's Disease, and PACS/neuro-COVID-19 [26–30].

Interaction of SARS-CoV-2 with host cells

The crown-like (corona) appearance of the virion is attributable to the spike (S) protein, which is assembled in homotrimer form and inserted in multiple copies into the host membrane. In SARS-CoV-2, the S-protein is cleaved by furin into the S1 and S2 subunits during their biosynthesis within the infected cells. In many other coronaviruses, the S-protein is only cleaved when they reach the target cells. Thus, the S-protein on the mature virion consists of non-covalently linked S1 and S2 subunits. The former subunit binds the cell receptor — angiotensin-converting enzyme 2 (ACE2), while the S2 subunit anchors the S-protein to the membrane. ACE2 engagement by the S1-protein triggers conformational changes in both subunits (S1 and S2) that lead to the formation of a fusion pore by bringing the viral and cellular membranes together [31]. As shown in Figure 1, for SARS-CoV-2, this process exposes the S2 site, followed by its subsequent cleavage by a transmembrane protease, serine 2 (TMPRSS2), resulting in bringing the virus closer to the cell surface and ultimately fusion of the S2 subunit to the membrane. Importantly, the S1 subunit is released from the ACE2 receptor to the bloodstream, where it has been detected in unvaccinated and infected patients, leading to inflammation triggered by direct and indirect action of the S-protein throughout the body [15].

Other cell attachment factors/co-receptors for SARS-CoV-2

ACE2 is the primary receptor responsible for the entry and expansion of the SARS-CoV-2 virus in human cells. Nevertheless, several reports have indicated that ACE2 alone cannot ensure SARS-CoV-2's entry into cells, and additional co-receptors or attachment factors have been proposed for this process [32, 33]. Blood-brain barrier endothelial cells express several of these factors, linking SARS-CoV-2 with neurological symptoms. Due to the substantial genetic relatedness between SARS-CoV-2 and other coronaviruses, particularly SARS-CoV-1, specific targets by which the virus interacts with human cells have been described (Fig. 2) [34].

Extracellular vimentin

A link between extracellular vimentin and SARS-CoV-2 infection has been established by several studies [33, 35–38]. Vimentin (Vim) is a type III intermediate filament-forming protein and an essential cytoskeleton element. In addition, Vim exists in the extracellular environment and on the surface of various cells regardless of its expression due to its secretion by immune cells triggered by inflammation [44] and by endothelial cells in tumours [39]. Recent studies have shown that Vim directly interacts with S-protein, and that blocking Vim on the cell surface significantly reduces the entry

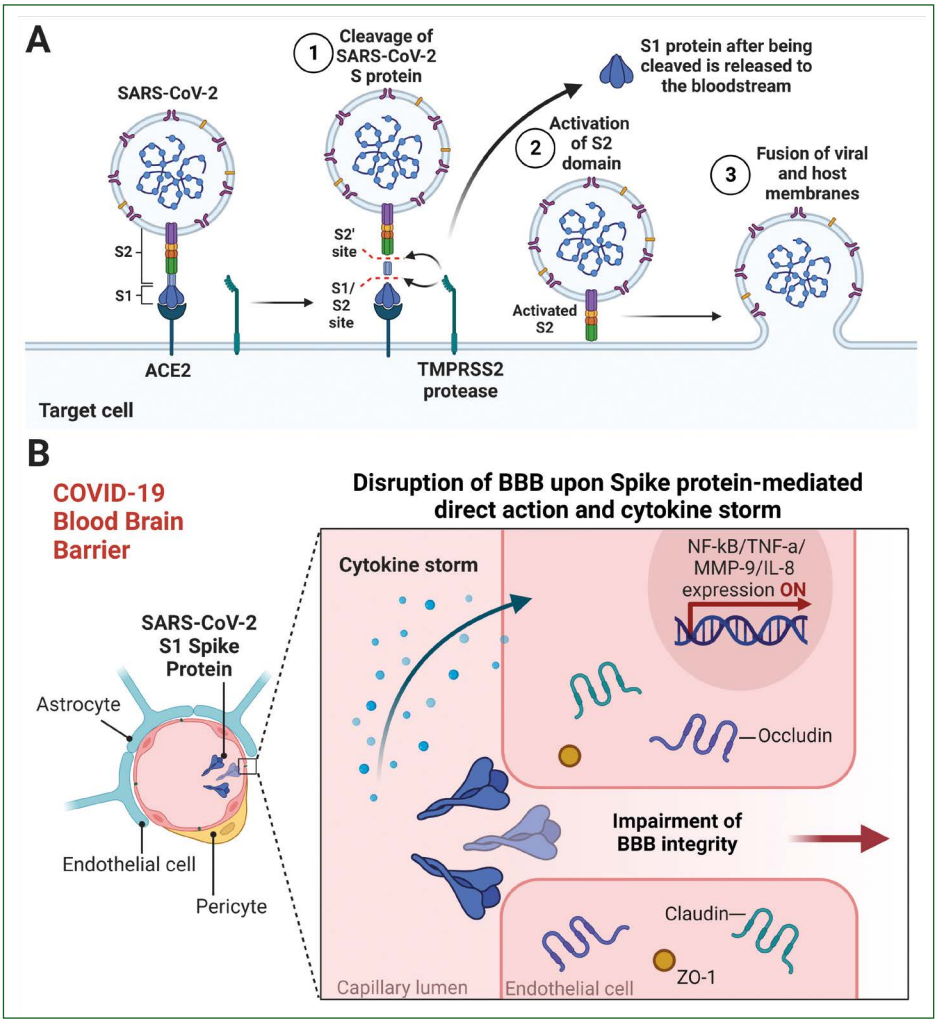

Figure 1. ACE2- and TMPRSS2-mediated SARS-CoV-2 cell entry indicates a possible explanation for presence of S1 protein in free form in circulation **(A)**. Scheme shows SARS-CoV-2 Spike S1 protein-driven disruption of blood-brain barrier. Direct action of S-protein and cytokine storm causes tight junctional complex rupture and enhances autocrine downstream proinflammatory signalling in endothelial cells **(B)**. Illustration created in Biorender

of SARS-CoV-2 into host cells [33]. Specifically, antibodies targeting the vimentin C-terminal domain block entry of the pseudovirus containing S-protein by up to 80% in cells expressing extracellular vimentin and ACE2 [33] *in vitro*, and anti-vimentin antibodies show potential therapeutic value *in vivo* [40]. Similarly, an inhibitory effect on viral uptake has been recorded for other vimentin-blocking compounds [36]. Moreover, limiting the concentration of vimentin in the blood suppresses SARS-CoV-2 replication and, in turn, virus-triggered inflammation [33, 38].

Neuropilin-1

Neuropilin-1 (NRP1) is another putative attachment protein or co-receptor for SARS-CoV-2 since its selective inhibitors or anti-NRP1 antibodies reduce the virus entry to host cells [41]. NRP1 physiologically has the ability to bind all of the furin-cleaved proteins and acts as an inhibitor for neuronal demyelination and a suppressor of inflammatory signalling that preserves BBB integrity [41].

Heparan sulfate

Heparan sulfate (HS), a polysaccharide attached to various cell surfaces or extracellular matrix proteins, is another compound associated with SARS-CoV-2 uptake by host cells. HS participates in several biological processes, including cell adhesion, signalling, and angiogenesis [42]. Interestingly, the S-protein interacts with cellular HS through a domain adjacent to its receptor-binding domain (RBD) [43]. Furthermore, HS inhibitors, such as heparin derivatives and mitoxantrone, may directly block the interaction between HS and S1 protein, inhibiting the uptake of both pseudotyped and authentic SARS--CoV-2 [44]. Similar effects have been observed for sunitinib and BNTX, drugs that indirectly inhibit HS-mediated viral entry by rearrangement of the actin network [49].

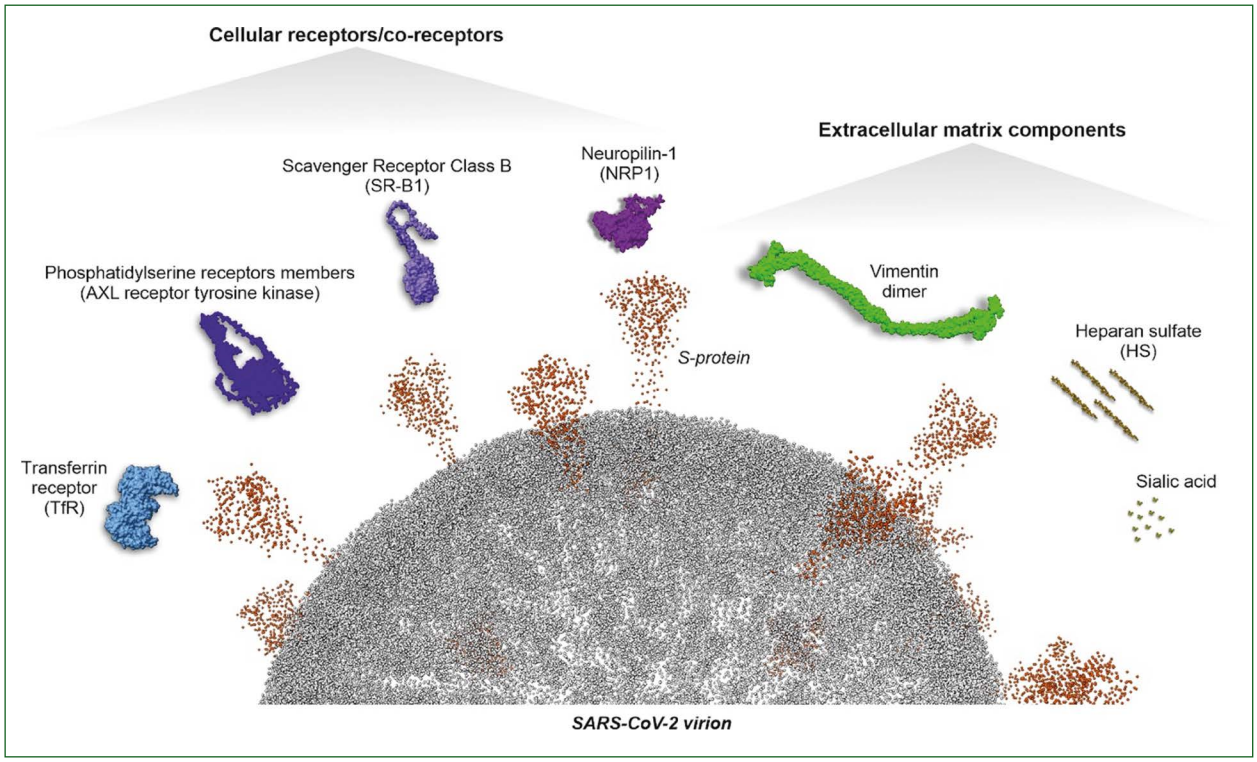

Figure 2. Host molecules interacting with S-protein of SARS-CoV-2. Visualisation performed using ChimeraX v.1.4 software [98] with proportional dimensions, based on 3D model of SARS-CoV-2 virion [99] and 3D models of proteins predicted using AlphaFold v2.2.2 tool [100] as well as heparan sulfate (octamer) and sialic acid molecules

Sialic acid

Sialic acids on the surface of BBB-forming endothelial cells play a vital role in the transport kinetics between the blood and the brain, hence appearing to be naturally associated with the invasion of CNS by SARS-CoV-2. Indeed, the direct binding of S-protein to sialic acid has been observed with electron microscopy and *in silico* studies [45]. However, due to conflicting conclusions, the role of sialic acid in the process of SARS-CoV-2 penetration is not clearly defined [46, 47]. For instance, ACE2 glycosylation inhibition studies with neuraminidase indicate that sialic acids on the ACE2 receptor prevent ACE2/spike protein interaction [46]. On the other hand, it has been reported that gangliosides could serve as ligands for the receptor-binding domain of the SARS-CoV-2 spike protein, thus improving viral uptake [47].

Scavenger receptor class B, type I

Scavenger Receptor Class B, Type I (SR-B1) on immune cells is involved in processes of the innate immune response. On the BBB, SR-B1 acts as a major high-density lipoprotein (HDL) receptor that modulates reverse sterol transport. Since the S1-subunit attaches to cholesterol and possibly HDL components, improving viral uptake *in vitro*, SR-B1 may be implicated in this process. For example, the SARS-CoV-2 infection of the cells overexpressing SR-B1 has been shown to be enhanced, while the viral uptake in SR-B1 knockout cells is reduced [48]. Similarly, in a study with cholesterol-poor synthetic biological HDL targeting SR-B1, 80% inhibition of SARS-CoV-2 pseudovirus uptake was observed [49].

Phosphatidylserine receptors

Phosphatidylserine (PS) receptors (PSR) namely TIM (TIM-1 and TIM-4), and TAM (AXL) have been reported as factors that enhance SARS-CoV-2's attachment to cells, promote uptake of virions, and boost ACE2-dependent infection of SARS-CoV-2. Wang et al. indicated that AXL interacts directly with the N-terminal domain of the SARS-CoV-2 S-protein [50]. But contradicting that, Bohan et al. stated that AXL does not bind to S-protein but rather to PS, which is abundant on the surface of the SARS-CoV-2 virion [51]. Furthermore, bemcentinib, an AXL inhibitor, significantly inhibited SARS-CoV-2 viral uptake into Vero 6 cells.

Transferrin receptor

Transferrin receptor (TfR) is highly expressed in brain capillary endothelial cells, but not the peripheral endothelium. TfR is responsible for the transport of iron into the brain parenchyma to maintain iron homeostasis, which is crucial for proper brain function, e.g. metabolism and neural conductivity. TfR is another protein involved in viral uptake by direct interaction with Spike S1 RBD. An anti-TfR antibody shows

a potent inhibitory effect on SARS-CoV-2 infection in mice [52]. *In vitro* studies have indicated that low doses of ferristatin II reduce the uptake of different variants of concern (VOCs) of SARS-CoV-2 [53].

Blood-brain barrier and SARS-CoV-2

Given the unique properties of the BBB, viruses such as SARS-CoV-2 are unable to enter the CNS easily. However, numerous case reports of patients with neurological complications indicate that nearly 60% of all COVID-19 patients have had disrupted BBB, resulting in increased permeability [54]. Post-mortem histopathological examinations indicate the presence of SARS-CoV-2 in different types of glial and endothelial cells within the brain [55]. Due to the large number of co-receptors/attachment factors other than ACE2 that may play a role in viral invasion, the mechanisms utilised by SARS-CoV-2 to enter the brain and cause neurological disorders still need to be fully understood.

So far, two major routes for virus entry into the CNS have been identified: retrograde neuronal transport, and hematogenous transmission including immune cells as vectors in a mechanism known as the 'Trojan horse'. The neuroinvasive route involves direct or indirect contact with the oral mucosa and eyes, and also implicates the olfactory, vagus, and trigeminal nerves as the primary cranial nerves involved in viral entry into the CNS [61]. Other mechanisms involve the indirect action of S-protein and the cascade of events associated with cytotoxicity and systemic inflammatory responses (the cytokine storm) that lead to the deterioration of the BBB. However, this review focuses on the hematogenous route, which involves the brain endothelium more profoundly.

Direct interaction of SARS-Cov-2 with BBB

Although initial *in vitro* studies have shown that SARS-CoV-2 is incapable of infection and replication in endothelial cells, numerous post mortem reports of patients who died from COVID-19 have demonstrated the presence of virus particles within the brain vasculature, pericytes, and neurons [55, 56]. These inconsistencies indicate that BBB infection is more complex and requires further examination. As mentioned previously, SARS-CoV-2 can infect endothelial cells by interacting with ACE2 on their surface [14]. However, the degree of infection is substantially less pronounced than in the primary target lung cells of SARS-CoV-2 — type II pneumocytes. On the other hand, overexpression of ACE2 in the endothelium of the cerebral circulation occurs in patients with hypertension and dementia and positively correlates with the severity of neurological symptoms [2, 57]. In addition, the S-protein may interact directly with the BBB, and S1, S1RBD, and S2 subunits exhibit pro-inflammatory effects, resulting in increased BBB permeability via damage to tight junctions (TJs) but not adherens junctions (AJs) [2, 58].

Trojan horse

One of the hallmarks of virus-induced neuropathogenesis is the breakdown of the BBB, which leads to uncontrolled infiltration of the virus-infected immune cells into the CNS via the 'Trojan horse' mechanism [59]. Using monocytes and macrophages as vectors, the viruses exploit the physiological process of CNS infiltration by immune response cells passing through the BBB. This process of permeation through endothelial cells, known as extravasation, consists of a series of consecutive steps, i.e. tethering, rolling, adhesion, and transmigration/diapedesis [60]. During this process, immune cells interact with adhesion molecules (ICAM, VCAM, E-Selectin) on the surface of the endothelium. Expression of these adhesion molecules is increased during SARS-CoV-2 infection, further contributing to the severity of neurological symptoms [58, 61]. Once the infected cells enter the CNS, the released virions can further infect glial cells, especially astrocytes and pericytes, whose impaired function further disrupts BBB homeostasis, leading to the loss of its function.

Adsorptive transcytosis

Direct passage of the virus across the BBB via interaction with receptors and co-receptors that facilitate infection, as well as the use of immune response cells as transporters, is not the only mechanism exploited by SARS-CoV-2 in order to penetrate endothelial barriers. Rhea et al. showed that iodine-labelled S-protein from SARS-CoV-2 can cross endothelial barriers through adsorptive transcytosis [3]. During this process, glycopeptides, i.e. spike protein with a positive charge circulating in the bloodstream, interact with endothelial cells' negatively charged surface [62]. Subsequently, the interaction causes invagination of the cell membrane and vesicle formation. Vesicles containing S-protein can now enter the intercellular space and may interact with the parenchyma side of the BBB.

Cytokine storm

In severe cases, COVID-19 is accompanied by a systemic inflammatory response, referred to as a cytokine storm, contributing to the disease's lethality. Patients with a poor prognosis frequently have elevated serum levels of cytokines and chemokines, mainly IL-1, IL-6, IL-8, IL-12, and TNF-α [63].

These pro-inflammatory mediators exhibit a positive feedback-like effect that stimulates neighbouring cells to produce even more cytokines, thereby promoting the infiltration of other immune response cells into the site of infection. In addition, S-protein can interact with Toll-like receptors 2 and 4 (TLR2, TLR4) and, in turn, activate signalling pathways involving PI3K, AKT, MAPK, and NF-κB [64, 65]. Subsequently, translocation of NF-κB into the cell nucleus results in increased production of pro-inflammatory cytokines, as well as adhesion factors such as ICAM, VCAM, and E-Selectin. As the aforementioned cytokines exert either direct or indirect effects that increase vascular permeability, the severity of inflammation

correlates closely with BBB damage. For example, IL-1β indirectly increases the expression of matrix metalloproteinase 9 (MMP-9), which breaks down TJS-proteins (ZO-1, occludin, and claudin-5) [66]. Similarly, IL-6, IL-8, IL-12, and TNF-α lead to BBB breakdown [67]. Moreover, S-protein stimulates the production of VEGF, one of the most potent inducers of vascular permeability [68, 69]. The increased vascular permeability significantly contributes to virus entry into the CNS and glial cell infection, resulting in neurological disorders.

TJ disruption through cytoskeleton and glycocalyx involvement

Tight junctions, i.e. close cell-cell connections formed by complexes of proteins, i.e. cytoplasmic scaffolding: ZO-1, -2, -3, and transmembrane proteins: occludins, and claudins, play a critical role in maintaining BBB homeostasis by restricting paracellular permeability [19]. These junctional complexes require the involvement of actin cytoskeleton rearrangement in processes associated with the formation and maturation of cell-cell contacts. Recently, RhoA activation has been associated with SARS-CoV-2-mediated barrier breakdown, indicating that the signalling mechanism involves cytoskeletal components. RhoA is a key regulator of endothelial cytoskeleton and TJ complex dynamics; its activation has been shown to disrupt vascular integrity. SARS-CoV-2 S1 protein triggered activation of RhoA in a 3D microfluidic model of the BBB. The use of C3 transferase, a RhoA inhibitor, mitigated the negative effect of S1 on BBB permeability and TJ degradation [4].

Another study has implicated the interaction between hyaluronic acid (HA) and CD44 in the endothelial barrier breakdown during COVID-19 [70]. This work analysed circulating glycosaminoglycans levels and their degradative enzymes' activities among SARS-CoV-2-infected patients [70], and found that the glycocalyx was severely damaged, which corresponded with disease outcome. Moreover, the circulating HA fragments and hyaluronidase level strongly correlated with organ failure assessment scores and hyperinflammation. In conclusion, the authors suggested that HA fragments are released into blood circulation due to COVID-19-mediated dysregulation of HA biosynthesis and degradation, which leads to direct endothelial barrier disruption in a ROCK- and CD44--dependent manner, indicating a role for HA in the vascular pathology of COVID-19.

Coagulopathy

Since the beginning of the COVID-19 pandemic, numerous reports have indicated an increased incidence of thrombotic events in patients with SARS-CoV-2. The syndrome of life-threatening clotting complications has been named COVID-19--associated coagulopathy (CAC) [71]. Cohort studies have shown that the incidence of ischaemic stroke as a complication of thromboembolic events oscillated around 2% [72, 73]. In most cases, elevated levels of fibrinogen, D-dimers, C-reactive protein, and P-selectin are recorded in the plasma of patients with CAC [74].

Endothelial cells have an antithrombotic system that determines platelet aggregation (NO, prostacyclin) and coagulation (TPFI, EPCR, thrombomodulin). In addition, the heparan sulfate in the glycocalyx layer exhibits anticoagulant activity [75]. During SARS-CoV-2 infection, there is a state of hyperactivation of the immune response, which results in activation of the complement system, increased release of neutrophil extracellular traps, and overproduction of cytokines, chemokines, and reactive oxygen species [76].

This cascade of events results in an imbalance between fibrinolysis and coagulation. Furthermore, due to damage to the glycocalyx and a reduction in the production of thrombomodulin, nitric oxide, and prostacyclins, the anti-thrombogenic fibrinolytic activity of the endothelium decreases. Additionally, an increase in free fibrin, tissue factors, and P-selectin is noted, which is associated with an increase in the prothrombogenic activity of the endothelium. Thrombin, fibrinogen, and plasminogen levels increase in CAC and directly or indirectly damage the endothelial barrier. Thrombin increases BBB permeability by indirectly damaging TJ-forming proteins through phosphorylation of MMPs and TJs and upregulation of VEGF by Src kinase [77]. Fibrinogen at pathologically high levels destroys the junctions between actin filaments and TJ, which widens intercellular junctions, impairing endothelial integrity [78]. Ventricular administration of tissue plasminogen activator results in dose-dependent increased BBB permeability, suggesting plasminogen's direct involvement in endothelial injury [79].

ACE2, which is the master receptor for SARS-CoV-2, has a dual function, i.e. it regulates the renin-angiotensin-aldosterone system as well as the kallikrein-kinin system. Kinins are peptides with the ability to increase vascular permeability. An occupied ACE2 receptor during SARS-CoV-2 infection can cause dysregulation of kinin degradation, resulting in what has been called a 'kinin storm', leading to increased vascular permeability, inflammation, and ultimately organ damage, including to the brain [80].

Lung hypoxia increases BBB permeability

COVID-19 is a predominantly pulmonary disease that causes infection of the alveoli, impairing their function, and patients with SARS-CoV-2 are chronically hypoxic due to pathological changes in the lungs. Many studies have described so-called 'happy hypoxia' in SARS-CoV-2 infected patients, i.e. hypoxemia without signs of shortness of breath, which leads to chronic mild hypoxia (CMH) [81].

Hypoxia, encephalitis, and stroke are the leading long-term causes of neurocognitive symptoms in COVID-19 patients [88]. Multiple mechanisms of chronic hypoxia can result in BBB impairment. Hypoxia can induce cell death in endothelial and glial cells via the apoptosis pathway by activating hypoxia--inducible factor 1 (HIF-1) [89]. In addition, CMH has been shown to cause a temporary vascular leak in brain blood vessels, along with microglial activation and infiltration around

damaged vessels. CMH-induced increased endothelial permeability exerts a regional preference, with considerably higher prevalence within the brainstem and olfactory bulb compared to the cerebellum and cerebral cortex [82]. These regions have also been reported to have the most significant levels of angiogenic development, with a peak in VEGF production that may additionally influence hypoxic-induced BBB disruption. Furthermore, i*n vitro* coculture models of endothelial cells and astrocytes have shown that hypoxia causes a decrease in ZO-1 expression, which correlates with the rearrangement of activated filaments resulting in increased permeability to molecular tracers [83].

Mutations and their impact on severity of neurological complications

The high transmissibility and genetic diversity of SARS-CoV-2 have led to the emergence of mutated virus strains, some of which have been termed 'variants of concern' (VOCs) due to their increased infectiousness. VOC strains have critical point mutations within spike proteins that affect the virus's ability to survive in harsh conditions and evade the host's immune response. The most dangerous VOCs hold several common mutations.

For example, the D614G and P681R/H mutations in the S-protein increase the transmission and infectivity of the virus due to the increased affinity of the S1 subunit to the ACE receptor [84]. Remarkably, certain VOCs with a higher virulence can eliminate from circulation less pathogenic variants. Such displaced variants are then referred to as de-escalated variants if they meet at least one of the following criteria: (i) the variant is no longer circulating; (ii) although the strain has circulated for a long time in the population, it has no impact on the epidemiological situation; or (iii) the variant does not have specific, more virulent, properties based on scientific evidence.

SARS-CoV-2 is still evolving, and the emergence of more pathogenic mutants in the future is possible. For this reason, freshly discovered variants are closely monitored and listed as Variants of Interest (VOI) or Variants under Monitoring (VUM). A summary of the most common mutations, along with their impact on transmissibility and induction of the immune response, is set out in Table 1.

Recently, Taquet et al., in a massive two-year cohort study including almost 1.3 million patients, analysed risk trajectories of neurological and psychiatric outcomes after SARS-CoV-2 infection [85]. They compared the risk for adverse effects within CNS in cohorts (a minimum of 39,845 patients per group) of patients diagnosed with different VOCs of SARS-CoV-2, e.g. alpha (B.1.1.7), delta (B.1.617.2), and omicron (B.1.1.529) comparing them to control cohorts, before the emergence of those VOCs. Only minor changes were observed for the alpha variant in the six-month hazard risk of neurological and psychiatric events. In contrast, patients infected with the delta variant were at a significantly higher risk of anxiety, insomnia, cognitive disorders, seizures, and ischaemic stroke incidents, but at a lower risk of dementia. Mortality among patients diagnosed with the delta strain was also higher. The omicron variant caused an increased risk of dementia, as well as mood, nerve, and plexus disorders. However, the death rate was significantly lower than among patients before the emergence of omicron.

Unfortunately, despite extensive research into the pathogenicity and molecular mechanisms of SARS-CoV-2 infection, little is known about the direct impact of mutations in the S-protein on the integrity of the BBB. Most *in vitro* BBB studies are concerned with the effects of spike protein subunits derived from the Wild Wuhan strain of SARS-CoV-2.

SARS-CoV-2 treatment

Since the beginning of the COVID-19 pandemic, concerted efforts by researchers worldwide have led to the development of therapies, some using previously developed broad-spectrum drugs, and some that have been newly developed and are specifically placed to treat SARS-CoV-2. Implementing vaccination against COVID-19, using, among others, innovative mRNA vaccine technology, has been a great success, which significantly reduced severe adverse events (SAE), the frequency of hospitalisations, and consequently, mortality [86, 87].

Vaccination — current status

As of January 2023, nearly 5.6 billion people, 69.1% of the world's population, have received at least one dose of a COVID-19 vaccine. 13.19 billion doses have been administered globally, and 2.2 million are administered daily. Almost 26% of people in low-income countries have received at least one dose. In Poland, over 57.8 million doses have been administered so far, and 22.6 million people are considered fully vaccinated, with at least two doses administered [88]. There are several types of vaccines against SARS-CoV-2 [89]. mRNA-based vaccines use mRNAs developed in the laboratory that are encapsulated in liposome lipid envelopes that enable entry into human cells, where they stimulate ribosomes to produce viral S-protein or its fragment, which further triggers antibody production by plasmocytes conditioning a specific immune response. Other vaccines trigger an immune response using weakened, modified, or inactivated viruses. Viral vector vaccines contain a modified version of a vector virus that includes fragments of SARS-CoV-2 but cannot cause infection. In contrast, protein subunit vaccines contain the spike protein fragment but without vector virus. Additionally, protein subunit vaccines contain an adjuvant that modulates immune system responses in the future when the human body meets viral particles during infection again. Due to the high ability of SARS-CoV-2 to mutate, vaccine manufacturers regularly update their formulations to maintain a high level of effectiveness in preventing COVID-19, which requires repeated booster doses.

Table 1. Summary of key, emerging mutated variants of SARS-CoV-2 and its impact on immune response and severity of infection compared to Wild Wuhan strain of SARS-CoV-2. Its current status, according to World Health Organisation, is denoted as either VOC (Variant of Concern), VUM (Variant under Monitoring), VOM (Variant of Interest), or DEV (De-escalated Variants). Table created according to current (as of January 2023) reports of WHO (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports)

WHO label and lineage	WHO status	Spike mutations of interest	First detection date and place	Transmissibility	Impact on immunity	Severity
Alpha B.1.1.7	DEV	N501Y, D614G, P681H	September 2020, United Kingdom	↑	Comparable	↑
Beta B.1.351	DEV	K417N, E484K, N501Y, D614G, A701V	September 2020, South Africa	↑	↑	↑
Gamma P.1	DEV	K417T, E484K, N501Y, D614G, H655Y	December 2020, Brazil	↑	↑	↑
Delta B.1.617.2	DEV	L452R, T478K, D614G, P681R	December 2020, India	↑	↑	↑
Omicron BA.1	DEV	A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212, ins215EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N6 79K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	November 2021, South Africa and Botswana	↑	↑	↓
Omicron BA.2	VOC	142D, N211I, Δ212, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K	November 2021, South Africa	↑	↑	↓
Omicron BA.3	DEV	A67V, Δ69-70, Δ143-145, N211I, Δ212, G339D, S371F, S373P, S375F, D405N, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, D796Y, Q954H, N969K	November 2021, South Africa	No evidence	No evidence	No evidence
Omicron BA.4	VOC	As BA.2 + L452R, F486V, R493Q	January 2022, South Africa	No evidence	↑	No evidence
Omicron BA.5	VOC	As BA.2 + L452R, F486V, R493Q	February 2022, South Africa	No evidence	↑	Unclear
Omicron BA.2.75	VOI	W152R, F157L, I210V, G257S, D339H, G446S, N460K, Q493	May 2022, India	No evidence	↑	No evidence
Omicron XBB	VOI	As in BA.2.75 + N460K, F490S	Unclear	No evidence	↑	No evidence
Omicron BA.2.3.20	VUM	K444R, L452M, N460K	Unclear	No evidence	No evidence	No evidence
Omicron XBC	VUM	N440K, F486P	Unclear	No evidence	No evidence	No evidence

Antiviral drugs

Despite the high vaccination prevalence in all age groups, many people have yet to receive a full dose. In addition, among people with impaired immunity, such as the elderly or those undergoing hematological treatment, the response to vaccination has been insufficient. Therefore, it became necessary to look for antiviral therapies. Several drugs have been found useful and approved by global organisations that control the approval of medicines for COVID-19 treatment. Examples of such antiviral drugs used in treating SARS-CoV-2 are remdesivir, molnupiravir, nirmatrelvir, and ritonavir.

Remdesivir is approved for COVID-19 treatment in adults and pediatric patients who are at least 28 days of age and weigh at least 3 kilograms. Remdesivir is used both in clinical practice and at home by people diagnosed with mild to moderate SARS-CoV-2 at high risk for severe disease progression. Remdesivir is a nucleoside analogue that inhibits RNA-dependent RNA polymerase (RdRp) of different coronaviruses. Incorporation by RdRp results in highly limited RNA synthesis [90].

Molnupinavir, like remdesivir, is a nucleoside analogue with a broad antiviral spectrum. It also inhibits the replication of other coronaviruses, i.e. SARS-CoV-1, SARS-CoV-2, and MERS, as well as viruses from different families, RSB, HCV, and Ebola [91].

Nirmatrelvir and ritonavir are used in concurrent therapy because of their synergistic effect [92]. Nirmatrelvir is an oral protease inhibitor active against m^{PRO}, a viral protease involved in the replication process.

Ritonavir is a cytochrome p450 (CYP) 3A4 inhibitor that enhances the pharmacokinetics of protease inhibitors. Coadministration is required to increase nirmatrelvir concentration to the therapeutic range. Molnupinavir, nirmatrelvir, and ritonavir, unlike remdesivir, have yet to receive authorisation from the US Food and Drug Administration (FDA). However, the FDA granted Emergency Use Authorisation (EUA) status for these formulations in late 2021.

Monoclonal antibodies

SARS-CoV-2 targeting monoclonal antibodies (mAbs) are artificial antibodies that target specific epitopes within S-protein that act as neutralising agents limiting viral entry. Bebtelovimab and tixagevimab co-packaged with cilgavimab are SARS-COV-2-targeting mAbs authorised for use through a EUA [93, 94]. However, the COVID-19 Treatment Guidelines Panel recommends against using anti-SARS-CoV-2 mAbs to treat SARS-CoV-2 because the currently dominant Omicron variant is unlikely to be susceptible to mAbs, given its high variability within the S-protein sequence.

Immunomodulatory agents

Unlike previously mentioned compounds, immune modulators are therapeutic agents that do not target virus-infected cells but instead activate, suppress or boost immune responses. During SARS-CoV-2, immune modulators are especially useful during the cytokine storm due to their ability to suppress hyperinflammation. Several immunomodulatory drugs have been authorised to treat COVID-19, including anakinra, baricitinib, and tocilizumab.

Anakinra is an IL-1 receptor agonist used to treat hospitalised adults with SARS-CoV-2 requiring supplemental oxygen [95]. Baricitinib is a Janus kinase (JAK) inhibitor, preventing downstream activation of signalling pathways leading to the secretion of various interleukins, interferons, and growth factors [96]. Baricitinib treats COVID-19 in pediatric patients requiring oxygen supplementation, mechanical ventilation, or extracorporeal membrane oxygen (ECMO). Tocilizumab inhibits IL-6 action by competitive blockage of its receptor IL6-R, which inhibits downstream signal transduction that leads to infiltration of immune cells, e.g. B and T cells [97]. Tocilizumab has found use among pediatric patients requiring mechanical ventilation and ECMO, with concomitant administration of corticosteroids.

Conclusions

SARS-CoV-2 remains a substantial threat to global health and the global economy. Neurological and psychiatric disorders involve disruption of blood-brain barrier integrity, and their longitudinal effects are unknown. Despite the introduction of effective vaccines, continued research toward a deeper understanding of the intricate and complex processes involved in the pathophysiology of SARS-CoV-2 infections is still needed due to its high genetic variability and evolutionary dynamics. In particular, understanding the effects of S-protein from different VOCs on the integrity of the BBB is essential if we are to recognise the neurological consequences of COVID-19. Equally important is the education of medical professionals and patients about the aetiopathology, prevention, treatment, and mechanism of action of SARS-CoV-2.

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References

1. Cascella M, Rajnik M, Aleem A, et al. Features, evaluation, and treatment of coronavirus (COVID-19). Statpearls [internet]; 2022.
2. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, et al. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in vitro models of the human blood-brain barrier. bioRxiv. 2020, doi: 10.1101/2020.06.15.150912, indexed in Pubmed: 32587958.
3. Rhea EM, Logsdon AF, Hansen KM, et al. The S1 protein of SARS-CoV-2 crosses the blood-brain barrier in mice. Nat Neurosci. 2021; 24(3): 368–378, doi: 10.1038/s41593-020-00771-8, indexed in Pubmed: 33328624.

4. DeOre BJ, Tran KA, Andrews AM, et al. SARS-CoV-2 Spike Protein Disrupts Blood-Brain Barrier Integrity via RhoA Activation. J Neuroimmune Pharmacol. 2021; 16(4): 722–728, doi: 10.1007/s11481-021-10029-0, indexed in Pubmed: 34687399.
5. Raveendran AV, Jayadevan R, Sashidharan S, et al. Long COVID: An overview. Diabetes Metab Syndr. 2021; 15(3): 869–875, doi: 10.1016/j.dsx.2021.04.007, indexed in Pubmed: 33892403.
6. Aiyegbusi OL, Hughes SE, Turner G, et al. TLC Study Group, TLC Study Group. Symptoms, complications and management of long COVID: a review. J R Soc Med. 2021; 114(9): 428–442, doi: 10.1177/01410768211032850, indexed in Pubmed: 34265229.
7. González-Herazo MA, Silva-Muñoz DC, Guevara-Martínez PA, et al. Post-COVID 19 Neurological Syndrome: a fresh challenge in neurological management. Neurol Neurochir Pol. 2021; 55(4): 413–414, doi: 10.5603/PJNNS.a2021.0052, indexed in Pubmed: 34346051.
8. Hirschfeld AS, Hirschfeld AS. Autoimmune mediated hyperkinetic movement disorders in SARS-CoV-2 infection — a systematic review. Neurol Neurochir Pol. 2021; 55(6): 549–558, doi: 10.5603/PJNNS.a2021.0069, indexed in Pubmed: 34637137.
9. Xiong W, Mu J, Guo J, et al. New onset neurologic events in people with COVID-19 in 3 regions in China. Neurology. 2020; 95(11): e1479–e1487, doi: 10.1212/WNL.0000000000010034, indexed in Pubmed: 32554771.
10. Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. Lancet Neurol. 2020; 19(9): 767–783, doi: 10.1016/S1474-4422(20)30221-0, indexed in Pubmed: 32622375.
11. Varatharaj A, Thomas N, Ellul MA, et al. CoroNerve Study Group, CoroNerve Study Group. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. Lancet Psychiatry. 2020; 7(10): 875–882, doi: 10.1016/S2215-0366(20)30287-X, indexed in Pubmed: 32593341.
12. Amruta N, Chastain WH, Paz M, et al. SARS-CoV-2 mediated neuroinflammation and the impact of COVID-19 in neurological disorders. Cytokine Growth Factor Rev. 2021; 58: 1–15, doi: 10.1016/j.cytogfr.2021.02.002, indexed in Pubmed: 33674185.
13. Andrews MG, Mukhtar T, Eze UC, et al. Tropism of SARS-CoV-2 for Developing Human Cortical Astrocytes. bioRxiv. 2021, doi: 10.1101/2021.01.17.427024, indexed in Pubmed: 33469577.
14. Liu F, Han K, Blair R, et al. SARS-CoV-2 Infects Endothelial Cells and . Front Cell Infect Microbiol. 2021; 11: 701278, doi: 10.3389/fcimb.2021.701278, indexed in Pubmed: 34307198.
15. Letarov AV, Babenko VV, Kulikov EE, et al. Free SARS-CoV-2 Spike Protein S1 Particles May Play a Role in the Pathogenesis of COVID-19 Infection. Biochemistry (Mosc). 2021; 86(3): 257–261, doi: 10.1134/S0006297921030032, indexed in Pubmed: 33838638.
16. Meppiel E, Peiffer-Smadja N, Maury A, et al. contributors to the NeuroCOVID registry, contributors to the NeuroCOVID registry. Neurologic manifestations associated with COVID-19: a multicentre registry. Clin Microbiol Infect. 2021; 27(3): 458–466, doi: 10.1016/j.cmi.2020.11.005, indexed in Pubmed: 33189873.
17. Nannoni S, de Groot R, Bell S, et al. Stroke in COVID-19: A systematic review and meta-analysis. Int J Stroke. 2021; 16(2): 137–149, doi: 10.1177/1747493020972922, indexed in Pubmed: 33103610.
18. Merkler AE, Parikh NS, Mir S, et al. Risk of Ischemic Stroke in Patients With Coronavirus Disease 2019 (COVID-19) vs Patients With Influenza. JAMA Neurol. 2020 [Epub ahead of print]; 77(11): 1–7, doi: 10.1001/jamaneurol.2020.2730, indexed in Pubmed: 32614385.
19. Daneman R, Prat A, Daneman R, et al. The blood-brain barrier. Cold Spring Harb Perspect Biol. 2015; 7(1): a020412, doi: 10.1101/cshperspect.a020412, indexed in Pubmed: 25561720.
20. Banerjee S, Bhat MA, Banerjee S, et al. Neuron-glial interactions in blood-brain barrier formation. Annu Rev Neurosci. 2007; 30: 235–258, doi: 10.1146/annurev.neuro.30.051606.094345, indexed in Pubmed: 17506642.
21. Cabezas R, Avila M, Gonzalez J, et al. Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. Front Cell Neurosci. 2014; 8: 211, doi: 10.3389/fncel.2014.00211, indexed in Pubmed: 25136294.
22. Stamatovic SM, Johnson AM, Keep RF, et al. Junctional proteins of the blood-brain barrier: New insights into function and dysfunction. Tissue Barriers. 2016; 4(1): e1154641, doi: 10.1080/21688370.2016.1154641, indexed in Pubmed: 27141427.
23. Ghosh C, Puvenna V, Gonzalez-Martinez J, et al. Blood-brain barrier P450 enzymes and multidrug transporters in drug resistance: a synergistic role in neurological diseases. Curr Drug Metab. 2011; 12(8): 742–749, doi: 10.2174/138920011798357051, indexed in Pubmed: 21568937.
24. Cornford EM, Hyman S, Cornford EM, et al. Localization of brain endothelial luminal and abluminal transporters with immunogold electron microscopy. NeuroRx. 2005; 2(1): 27–43, doi: 10.1602/neurorx.2.1.27, indexed in Pubmed: 15717055.
25. Dong X, Dong X. Current Strategies for Brain Drug Delivery. Theranostics. 2018; 8(6): 1481–1493, doi: 10.7150/thno.21254, indexed in Pubmed: 29556336.
26. Al-Bachari S, Naish JH, Parker GJM, et al. Blood-Brain Barrier Leakage Is Increased in Parkinson's Disease. Front Physiol. 2020; 11: 593026, doi: 10.3389/fphys.2020.593026, indexed in Pubmed: 33414722.
27. Sweeney MD, Sagare AP, Zlokovic BV, et al. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018; 14(3): 133–150, doi: 10.1038/nrneurol.2017.188, indexed in Pubmed: 29377008.
28. Balasa R, Barcutean L, Mosora O, et al. Reviewing the Significance of Blood-Brain Barrier Disruption in Multiple Sclerosis Pathology and Treatment. Int J Mol Sci. 2021; 22(16), doi: 10.3390/ijms22168370, indexed in Pubmed: 34445097.
29. Finsterer J, Scorza FA, Scorza CA, et al. Neuro-COVID due to response against the virus. Neurol Neurochir Pol. 2022; 56(1): 103–104, doi: 10.5603/PJNNS.a2021.0089, indexed in Pubmed: 34939661.
30. Bratosiewicz-Wąsik J, Bratosiewicz-Wąsik J. Neuro-COVID-19: an insidious virus in action. Neurol Neurochir Pol. 2022; 56(1): 48–60, doi: 10.5603/PJNNS.a2021.0072, indexed in Pubmed: 34642927.
31. Jackson CB, Farzan M, Chen B, et al. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022; 23(1): 3–20, doi: 10.1038/s41580-021-00418-x, indexed in Pubmed: 34611326.
32. van Eijk LE, Binkhorst M, Bourgonje AR, et al. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). J Pathol. 2020; 251(3): 228–248, doi: 10.1002/path.5471, indexed in Pubmed: 32418199.
33. Suprewicz Ł, Swoger M, Gupta S, et al. Extracellular vimentin as a target against SARS-CoV-2 host cell invasion. bioRxiv. 2021; 18(6): e2105640, doi: 10.1101/2021.01.08.425793, indexed in Pubmed: 33442680.
34. Wang H, Li X, Li T, et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis. 2020; 39(9): 1629–1635, doi: 10.1007/s10096-020-03899-4, indexed in Pubmed: 32333222.
35. Lalioti V, González-Sanz S, Lois-Bermejo I, et al. Cell surface detection of vimentin, ACE2 and SARS-CoV-2 Spike proteins reveals selective colocalization at primary cilia. Sci Rep. 2022; 12(1): 7063, doi: 10.1038/s41598-022-11248-y, indexed in Pubmed: 35487944.

36. Li Z, Wu J, Zhou Ji, et al. A Vimentin-Targeting Oral Compound with Host-Directed Antiviral and Anti-Inflammatory Actions Addresses Multiple Features of COVID-19 and Related Diseases. mBio. 2021; 12(5): e0254221, doi: 10.1128/mBio.02542-21, indexed in Pubmed: 34634931.
37. Amraei R, Xia C, Olejnik J, et al. Extracellular vimentin is an attachment factor that facilitates SARS-CoV-2 entry into human endothelial cells. Proc Natl Acad Sci U S A. 2022; 119(6), doi: 10.1073/pnas.2113874119, indexed in Pubmed: 35078919.
38. Kim H-M, Wang M, Kim S, et al. Hong S-T. Removal of Extracellular Vimentin in Blood Confers Resistance to Viral Infection; 2022.
39. van Beijnum JR, Huijbers EJM, van Loon K, et al. Extracellular vimentin mimics VEGF and is a target for anti-angiogenic immunotherapy. Nat Commun. 2022; 13(1): 2842, doi: 10.1038/s41467-022-30063-7, indexed in Pubmed: 35606362.
40. Prasenohadi P, Burhan E, Dhunny S, et al. Double-Blind, Randomized, Placebo-Controlled Study on hzVSF-v13, a Novel Anti-Vimentin Monoclonal Antibody Drug as Add-on Standard of Care in the Management of Patients with Moderate to Severe COVID-19. J Clin Med. 2022; 11(11), doi: 10.3390/jcm11112961, indexed in Pubmed: 35683351.
41. Cantuti-Castelvetri L, Ojha R, Pedro LD, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020; 370(6518): 856–860, doi: 10.1126/science.abd2985, indexed in Pubmed: 33082293.
42. Zhang Qi, Chen CZ, Swaroop M, et al. Heparan sulfate assists SARS--CoV-2 in cell entry and can be targeted by approved drugs. bioRxiv. 2020; 6(1): 80, doi: 10.1101/2020.07.14.202549, indexed in Pubmed: 32699847.
43. Kearns FL, Sandoval DR, Casalino L, et al. Spike-heparan sulfate interactions in SARS-CoV-2 infection. Curr Opin Struct Biol. 2022; 76: 102439, doi: 10.1016/j.sbi.2022.102439, indexed in Pubmed: 35926454.
44. Zhang Qi, Radvak P, Lee J, et al. Mitoxantrone modulates a heparan sulfate-spike complex to inhibit SARS-CoV-2 infection. Sci Rep. 2022; 12(1): 6294, doi: 10.1038/s41598-022-10293-x, indexed in Pubmed: 35440680.
45. Tortorici MA, Walls AC, Lang Y, et al. Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol. 2019; 26(6): 481–489, doi: 10.1038/s41594-019-0233-y, indexed in Pubmed: 31160783.
46. Gong Y, Qin S, Dai L, et al. The glycosylation in SARS-CoV-2 and its receptor ACE2. Signal Transduct Target Ther. 2021; 6(1): 396, doi: 10.1038/s41392-021-00809-8, indexed in Pubmed: 34782609.
47. Li B, Wang L, Ge H, et al. Identification of Potential Binding Sites of Sialic Acids on the RBD Domain of SARS-CoV-2 Spike Protein. Front Chem. 2021; 9: 659764, doi: 10.3389/fchem.2021.659764, indexed in Pubmed: 34368076.
48. Wei C, Wan L, Yan Q, et al. HDL-scavenger receptor B type 1 facilitates SARS-CoV-2 entry. Nat Metab. 2020; 2(12): 1391–1400, doi: 10.1038/s42255-020-00324-0, indexed in Pubmed: 33244168.
49. Henrich S, McMahon K, Palacio N, et al. Targeting Scavenger Receptor Type B-1 (SR-B1) and Cholesterol Inhibits Entry of SARS-CoV-2 Pseudovirus in Cell Culture. , doi: 10.1101/2020.12.14.420133.
50. Wang S, Qiu Z, Hou Y, et al. AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. Cell Res. 2021; 31(2): 126–140, doi: 10.1038/s41422-020-00460-y, indexed in Pubmed: 33420426.
51. Bohan D, Van Ert H, Ruggio N, et al. Phosphatidylserine receptors enhance SARS-CoV-2 infection. PLoS Pathog. 2021; 17(11): e1009743, doi: 10.1371/journal.ppat.1009743, indexed in Pubmed: 34797899.
52. Tang X, Yang M, Duan Z, et al. Transferrin receptor is another receptor for SARS-CoV-2 entry. , doi: 10.1101/2020.10.23.350348.
53. Sokolov A, Isakova-Sivak I, Grudinina N, et al. Ferristatin II Efficiently Inhibits SARS-CoV-2 Replication in Vero Cells. Viruses. 2022; 14(2), doi: 10.3390/v14020317, indexed in Pubmed: 35215911.
54. Bellon M, Schweblin C, Lambeng N, et al. Cerebrospinal Fluid Features in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Reverse Transcription Polymerase Chain Reaction (RT-PCR) Positive Patients. Clin Infect Dis. 2021; 73(9): e3102–e3105, doi: 10.1093/cid/ciaa1165, indexed in Pubmed: 32770235.
55. Matschke J, Lütgehetmann M, Hagel C, et al. Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. Lancet Neurol. 2020; 19(11): 919–929, doi: 10.1016/S1474-4422(20)30308-2, indexed in Pubmed: 33031735.
56. Constant O, Barthelemy J, Bolloré K, et al. SARS-CoV-2 Poorly Replicates in Cells of the Human Blood-Brain Barrier Without Associated Deleterious Effects. Front Immunol. 2021; 12: 697329, doi: 10.3389/fimmu.2021.697329, indexed in Pubmed: 34386007.
57. Numbers K, Brodaty H. The effects of the COVID-19 pandemic on people with dementia. Nat Rev Neurol. 2021; 17(2): 69–70, doi: 10.1038/s41582-020-00450-z, indexed in Pubmed: 33408384.
58. Suprewicz Ł, Tran KA, Piktel E, et al. Recombinant human plasma gelsolin reverses increased permeability of the blood-brain barrier induced by the spike protein of the SARS-CoV-2 virus. J Neuroinflammation. 2022; 19(1): 282, doi: 10.1186/s12974-022-02642-4, indexed in Pubmed: 36434734.
59. Percivalle E, Sammartino JC, Cassaniti I, et al. Macrophages and Monocytes: „Trojan Horses" in COVID-19. Viruses. 2021; 13(11), doi: 10.3390/v13112178, indexed in Pubmed: 34834984.
60. Filippi MD. Mechanism of Diapedesis: Importance of the Transcellular Route. Adv Immunol. 2016; 129: 25–53, doi: 10.1016/bs.ai.2015.09.001, indexed in Pubmed: 26791857.
61. Tong M, Jiang Yu, Xia Da, et al. Elevated Expression of Serum Endothelial Cell Adhesion Molecules in COVID-19 Patients. J Infect Dis. 2020; 222(6): 894–898, doi: 10.1093/infdis/jiaa349, indexed in Pubmed: 32582936.
62. Hervé F, Ghinea N, Scherrmann JM. CNS delivery via adsorptive transcytosis. AAPS J. 2008; 10(3): 455–472, doi: 10.1208/s12248-008-9055-2, indexed in Pubmed: 18726697.
63. Soy M, Keser G, Atagündüz P, et al. Cytokine storm in COVID-19: pathogenesis and overview of anti-inflammatory agents used in treatment. Clin Rheumatol. 2020; 39(7): 2085–2094, doi: 10.1007/s10067-020-05190-5, indexed in Pubmed: 32474885.
64. Khan S, Shafiei MS, Longoria C, et al. SARS-CoV-2 spike protein induces inflammation via TLR2-dependent activation of the NF-κB pathway. bioRxiv. 2021; 10, doi: 10.1101/2021.03.16.435700, indexed in Pubmed: 33758854.
65. Zhao Y, Kuang M, Li J, et al. SARS-CoV-2 spike protein interacts with and activates TLR41. Cell Res. 2021; 31(7): 818–820, doi: 10.1038/s41422-021-00495-9, indexed in Pubmed: 33742149.
66. Yang F, Zhao K, Zhang X, et al. ATP Induces Disruption of Tight Junction Proteins via IL-1 Beta-Dependent MMP-9 Activation of Human Blood-Brain Barrier . Neural Plast. 2016; 2016: 8928530, doi: 10.1155/2016/8928530, indexed in Pubmed: 27795859.
67. Huang X, Hussain B, Chang J. Peripheral inflammation and blood--brain barrier disruption: effects and mechanisms. CNS Neurosci Ther. 2021; 27(1): 36–47, doi: 10.1111/cns.13569, indexed in Pubmed: 33381913.
68. Miao Z, Dong Y, Fang W, et al. VEGF increases paracellular permeability in brain endothelial cells via upregulation of EphA2. Anat Rec

(Hoboken). 2014; 297(5): 964–972, doi: 10.1002/ar.22878, indexed in Pubmed: 24458982.
69. Veerabathiran R, Ragunath B, Kaviarasan V, et al. Identification of selected genes associated with the SARS-CoV-2: a therapeutic approach and disease severity. Bull Natl Res Cent. 2021; 45(1): 79, doi: 10.1186/s42269-021-00540-y, indexed in Pubmed: 33907373.
70. Queisser KA, Mellema RA, Middleton EA, et al. COVID-19 generates hyaluronan fragments that directly induce endothelial barrier dysfunction. JCI Insight. 2021; 6(17), doi: 10.1172/jci.insight.147472, indexed in Pubmed: 34314391.
71. Franchini M, Marano G, Cruciani M, et al. COVID-19-associated coagulopathy. Diagnosis. 2020; 7(4): 357–363, doi: 10.1515/dx-2020-0078.
72. Lodigiani C, Iapichino G, Carenzo L, et al. Humanitas COVID-19 Task Force. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020; 191: 9–14, doi: 10.1016/j.thromres.2020.04.024, indexed in Pubmed: 32353746.
73. Klok FA, Kruip M, Meer Nv, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thrombosis Research. 2020; 191: 145–147, doi: 10.1016/j.thromres.2020.04.013.
74. Han H, Yang L, Liu R, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020; 58(7): 1116–1120, doi: 10.1515/cclm-2020-0188, indexed in Pubmed: 32172226.
75. van Hinsbergh VWM. Endothelium--role in regulation of coagulation and inflammation. Semin Immunopathol. 2012; 34(1): 93–106, doi: 10.1007/s00281-011-0285-5, indexed in Pubmed: 21845431.
76. Behzadifard M, Soleimani M. NETosis and SARS-COV-2 infection related thrombosis: a narrative review. Thromb J. 2022; 20(1): 13, doi: 10.1186/s12959-022-00375-1, indexed in Pubmed: 35354492.
77. Liu DZ, Ander BP, Xu H, et al. Blood-brain barrier breakdown and repair by Src after thrombin-induced injury. Ann Neurol. 2010; 67(4): 526–533, doi: 10.1002/ana.21924, indexed in Pubmed: 20437588.
78. Tyagi N, Roberts AM, Dean WL, et al. Fibrinogen induces endothelial cell permeability. Mol Cell Biochem. 2008; 307(1-2): 13–22, doi: 10.1007/s11010-007-9579-2, indexed in Pubmed: 17849175.
79. Yepes M, Sandkvist M, Moore EG, et al. Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. J Clin Invest. 2003; 112(10): 1533–1540, doi: 10.1172/JCI19212, indexed in Pubmed: 14617754.
80. McCarthy CG, Wilczynski S, Wenceslau CF, et al. A new storm on the horizon in COVID-19: Bradykinin-induced vascular complications. Vascul Pharmacol. 2021; 137: 106826, doi: 10.1016/j.vph.2020.106826, indexed in Pubmed: 33358968.
81. Couzin-Frankel J. The mystery of the pandemic's ‚happy hypoxia'. Science. 2020; 368(6490): 455–456, doi: 10.1126/science.368.6490.455, indexed in Pubmed: 32355007.
82. Meinhardt J, Radke J, Dittmayer C, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nat Neurosci. 2021; 24(2): 168–175, doi: 10.1038/s41593-020-00758-5, indexed in Pubmed: 33257876.
83. Mark KS, Davis TP. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. Am J Physiol Heart Circ Physiol. 2002; 282(4): H1485–H1494, doi: 10.1152/ajpheart.00645.2001, indexed in Pubmed: 11893586.
84. Khatri R, Siddqui G, Sadhu S, et al. Intrinsic D614G and P681R/H mutations in SARS-CoV-2 VoCs Alpha, Delta, Omicron and viruses with D614G plus key signature mutations in spike protein alters fusogenicity and infectivity. Med Microbiol Immunol. 2023; 212(1): 103–122, doi: 10.1007/s00430-022-00760-7, indexed in Pubmed: 36583790.
85. Taquet M, Sillett R, Zhu L, et al. Neurological and psychiatric risk trajectories after SARS-CoV-2 infection: an analysis of 2-year retrospective cohort studies including 1 284 437 patients. Lancet Psychiatry. 2022; 9(10): 815–827, doi: 10.1016/S2215-0366(22)00260-7, indexed in Pubmed: 35987197.
86. Graña C, Ghosn L, Evrenoglou T, et al. Efficacy and safety of COVID-19 vaccines. Cochrane Database Syst Rev. 2022; 12(12): CD015477, doi: 10.1002/14651858.CD015477, indexed in Pubmed: 36473651.
87. Nojszewska M, Kalinowska A, Adamczyk-Sowa M, et al. COVID-19 mRNA vaccines (Pfizer-BioNTech and Moderna) in patients with multiple sclerosis: a statement by a working group convened by the Section of Multiple Sclerosis and Neuroimmunology of the Polish Neurological Society. Neurol Neurochir Pol. 2021; 55(1): 8–11, doi: 10.5603/PJNNS.a2021.0016, indexed in Pubmed: 33555604.
88. Ritchie H, Mathieu E, Rodés-Guirao L, et al. Coronavirus pandemic (COVID-19). Our world in data; 2020.
89. Mascellino MT, Di Timoteo F, De Angelis M, et al. Overview of the Main Anti-SARS-CoV-2 Vaccines: Mechanism of Action, Efficacy and Safety. Infect Drug Resist. 2021; 14: 3459–3476, doi: 10.2147/IDR.S315727, indexed in Pubmed: 34511939.
90. Kokic G, Hillen HS, Tegunov D, et al. Mechanism of SARS-CoV-2 polymerase stalling by remdesivir. Nat Commun. 2021; 12(1): 279, doi: 10.1038/s41467-020-20542-0, indexed in Pubmed: 33436624.
91. Jayk Bernal A, Gomes da Silva MM, Musungaie DB, et al. MOVe-OUT Study Group. Molnupiravir for Oral Treatment of Covid-19 in Nonhospitalized Patients. N Engl J Med. 2022; 386(6): 509–520, doi: 10.1056/NEJMoa2116044, indexed in Pubmed: 34914868.
92. Hung YP, Lee JC, Chiu CW, et al. Oral Nirmatrelvir/Ritonavir Therapy for COVID-19: The Dawn in the Dark? Antibiotics (Basel). 2022; 11(2), doi: 10.3390/antibiotics11020220, indexed in Pubmed: 35203821.
93. Westendorf K, Žentelis S, Wang L, et al. LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2 variants. bioRxiv. 2022; 39(7): 110812, doi: 10.1101/2021.04.30.442182, indexed in Pubmed: 33972947.
94. Levin MJ, Ustianowski A, De Wit S, et al. PROVENT Study Group. Intramuscular AZD7442 (Tixagevimab-Cilgavimab) for Prevention of Covid-19. N Engl J Med. 2022; 386(23): 2188–2200, doi: 10.1056/NEJMoa2116620, indexed in Pubmed: 35443106.
95. Pasin L, Cavalli G, Navalesi P, et al. Anakinra for patients with COVID-19: a meta-analysis of non-randomized cohort studies. Eur J Intern Med. 2021; 86: 34–40, doi: 10.1016/j.ejim.2021.01.016, indexed in Pubmed: 33581979.
96. Zhang X, Zhang Y, Qiao W, et al. Baricitinib, a drug with potential effect to prevent SARS-COV-2 from entering target cells and control cytokine storm induced by COVID-19. Int Immunopharmacol. 2020; 86: 106749, doi: 10.1016/j.intimp.2020.106749, indexed in Pubmed: 32645632.
97. Samaee H, Mohsenzadegan M, Ala S, et al. Tocilizumab for treatment patients with COVID-19: Recommended medication for novel disease. Int Immunopharmacol. 2020; 89(Pt A): 107018, doi: 10.1016/j.intimp.2020.107018, indexed in Pubmed: 33045577.
98. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004; 25(13): 1605–1612, doi: 10.1002/jcc.20084, indexed in Pubmed: 15264254.
99. Yu A, Pak AJ, He P, et al. A Multiscale Coarse-grained Model of the SARS-CoV-2 Virion. bioRxiv. 2020; 120(6): 1097–1104, doi: 10.1101/2020.10.02.323915, indexed in Pubmed: 33024966.
100. Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021; 596(7873): 583–589, doi: 10.1038/s41586-021-03819-2, indexed in Pubmed: 34265844.