EXPERT OPINION ON THERAPEUTIC TARGETS
https://doi.org/10.1080/14728222.2021.1918103

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REVIEW

TLR4 as a therapeutic target for respiratory and neurological complications of SARS-CoV-2

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ABSTRACT

Introduction: The COVID-19 pandemic remains aglobal challenge. While there are mRNA agents on the horizon as apotential prevention, adefinitive drug therapy is an unmet medical need. The hyperinflammatory response, known as the 'cytokine storm', is chiefly responsible for complications and deaths. The binding of spike-glycoprotein of SARS-CoV-2 to TLR4 receptors has been documented in several studies and has been found to play arole in hyperinflammation; hence, there is an interest in TLR4 as apotential drug target.

Areas covered: This review discusses the neurological and respiratory complications of SARS-CoV-2 infection and progresses to examine the role of the 'cytokine storm' and the involvement of TLR4 receptors in these complications. The possibility of using TLR4 modulators to curb the complications are considered and finally, ashort perspective on future potential drug treatments is offered. Various databases were searched including Pub-Med, Google Scholar, and Medline. The search mainly included research articles, meta-analysis, retrospective studies, reports, and systematic reviews.

Expert opinion: TLR4 modulators are being investigated in clinical trials for COVID-19. Challenges in terms of structural diversity of the agents, their natural origin, and efficacy demand extensive research.

ARTICLE HISTORY
Received 19 December 2020
Accepted 13 April 2021

KEYWORDS
Sars-cov-2; covid-19; coronavirus; acute respiratory distress syndrome; toll-like receptors; tlr-4; cytokine storm; neurological complications

1. Introduction

It has been over a year since the first case report of Coronavirus in Wuhan, China. Since then, the search for a drug therapy for COVID-19 is on the cards. The clinical infection of COVID-19 shows a varied picture that ranges from being asymptomatic to being critically ill with acute respiratory distress syndrome (ARDS) so much so that the patient requires multi-organ support in the ICU. Deterioration to the stage of critical disease happens at 7–10 days of clinical infection [1,2]. Multiple organ failure and ARDS lead to death in a short period. Hyper-inflammatory cytokine storm is attributed to be one of the principal causes of this disease aggravation, in both young and old, even if any co-morbidity preexists or not [1,3].

Apart from the detrimental effects on the respiratory system, COVID-19 patients also exhibit neurological symptoms like dizziness, headache, impaired consciousness, and paresthesia. The percentage of such patients is estimated to be around 36.4%. This is more seen in patients that are in the severe stages of the infection than in the mild or moderate ones. Autopsy reports have also come up that show edema of the brain tissue and point toward partial degeneration of neurons in the patients who could not survive the disease. All these findings make it all the more pressing to aware the clinicians of the complications SARS-CoV-2 brings along with it and to devise the necessary solutions [4–6].

With several therapies under exploration, there is still no definitive cure for this disease. In an open-label trial that involved adult hospitalized patients that were given lopinavir-ritonavir, no significant clinical improvement or mortality was observed in patients that were seriously ill [7]. Hydroxychloroquine and Azithromycin have also been tested in a non-randomized open-label clinical trial [8]. The solidarity trial launched by WHO to evaluate four treatment options – Remdesivir, Lopinavir/Ritonavir, Hydroxychloroquine, and Interferon, had its interim results published on 15 October 2020, which stated that these had no or little effect on overall mortality, duration of hospitalization, and initiation of ventilation of COVID-19 patients. Till now, corticosteroids are the only class of drugs proven effective against critical and severe stages of COVID-19 [9]. Drug repurposing includes trying out agents like Doxycycline, Tocilizumab, Auranofin, and Dexamethasone among others that act on the cytokine storm. Agents like Ivermectin, Statins, Nitazoxanide are also under study [10]. Vaccine development is also under process. Currently, more than 100 vaccine candidates for COVID-19 are under development with many of them under human trials [11]. According to the recent data by WHO, 52 candidate vaccines are under clinical evaluation while 162 are under pre-clinical evaluation [12]. The Pfizer-

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Article highlights

- Vaccines potentially offer prevention of SARS-COV2, but not an effective curative treatment; hence finding new drug targets is a necessary starting block.
- The chief culprit behind SARS-COV2 complications is the hyperinflammatory cascade, known as the 'cytokine storm'.
- TLR4 receptors are involved in immune response and further lead to signaling of inflammatory molecules.
- Interaction of spike glycoprotein with TLR4 and elevation of genes associated with TLR4 signaling in COVID-19 point to the possible involvement of these receptors and their inflammatory cascade.
- Modulating TLR4 activity appears to offer promise in the search for new drugs for SARS-COV2

This box summarizes key points contained in the article.

BioNTech COVID-19 vaccine manufactured by Pfizer was recently given the Emergency Use Authorization (EUA) status by FDA [13] while other vaccines like Moderna's COVID-19 vaccine and Oxford-AstraZeneca's COVID-19 vaccine have also completed Phase 3 trials and have applied for EUA status. But, definitive therapy is still at large. So, the need to search for alternative drug targets is quite evident.

One of the potential targets could be eyeing the hyper inflammation induced by SARS-CoV-2 that is the primary cause behind a range of respiratory and neurological complications. A publication has documented the interaction of viral spike glycoprotein with the Toll-like receptors (TLRs), especially strongly to TLR4 [14]. A Korean study also revealed the involvement of TLR4-mediated NF-κB-signaling activation in the hyperinflammatory response in the patients that further suggests strongly the involvement of these receptors in the pathogenesis [15]. Going on these lines, targeting the interaction of TLR4-SARS-CoV-2 appears promising. This review further divulges details on the involvement of TLR4 receptors in the hyperinflammatory cytokine storm and suggests various TLR4 modulators that could be studied upon in the light of COVID-19.

2. Search strategy

Literature was searched from several databases like Pub-Med, Google Scholar, Medline, Embase, and Scopus. Research articles, retrospective studies, meta-analysis, reports, systematic reviews were included in the search. About 200 articles were searched and 117 articles were included in this review. As this review mainly focuses on the respiratory and neurological complications of SARS-CoV-2 and the role of TLR4 modulators, other articles were excluded based on this. SARS-CoV-2, COVID-19, respiratory complications, neurological complications, pathogenesis, cytokine storm, TLR4, TLR4 modulators were some of the keywords used.

3. SARS-CoV-2

3.1. Structure

SARS-CoV-2, a β-coronavirus, is basically an enveloped, single-stranded, positive sense RNA virus that belongs to the family *Coronaviridae*. Its diameter ranges from 80 to 220 nm. On the envelope are 20 nm in length crown-like spikes that bear a resemblance to the corona of the sun as viewed under electron microscopy, thus, giving it the name of coronavirus [16]. There are mainly four structural proteins present that include-small envelope(E) glycoprotein, nucleocapsid(N) protein, spike(S) glycoprotein, membrane(M) glycoprotein, and other accessory proteins [17]. Hemagglutinin esterase (HE) protein is also present. The most abundant is the M glycoprotein spanning the membrane bilayer three times [18]. The spike glycoprotein, weighing about 150 kDa, is a transmembrane protein on the outer surface of the virus. This is responsible for the binding of the virus to host cell receptors (ACE2). The nucleocapsid protein is the structural component bound to nucleic acid material and hence is involved in the viral replication cycle and cellular response to the infection by the host cells [17]. The accessory proteins like the HE protein, 4a/b protein, and 3a/b protein are responsible for virus replication and genome maintenance [18]. Figure 1 represents the structure of SARS-CoV-2.

3.2. Symptoms, clinical features, and transmission

COVID-19 spreads primarily through respiratory droplets, secretions from the respiratory tract, or by direct contact. The incubation period is about 1–14 days and in many cases the symptoms are emerging in 1–7 days. Transmission from human-to-human mainly occurs among family members, friends, and relatives who were in close contact with the infected or with the carriers still in the incubation period [19]. It is highly contagious in the course of its latency period, transmitting rapidly in humans, especially in the case of the elderly or in the people who are already present with an underlying disease or are immune-compromised or have a co-morbidity-like diabetes, hypertension, obesity, cardiac diseases, or respiratory diseases like chronic obstructive lung disease [19–23].

In a study on 138 patients in Wuhan, China, 56 years were the median age of patients. 54.3% of patients were men. The common symptoms were fever (98.6%), fatigue (69.6%), and dry cough (59.4%). Lymphopenia was observed in 70.3% of patients. 26.1% of patients showed complications like ARDS (61.1%), arrhythmia (44.4%), shock (30.6%) and were transferred to ICU. Patients in the ICU were older and had preexisting co-morbidities and showed more likeliness of anorexia (66.7%) and dyspnea (63.9%) [22]. Given the neurological complications, a systematic review analyzed the most common neurological manifestations to be a headache, myalgia, hypogeusia, hyposmia, and altered sensorium. The manifestations of central nervous reported were mainly ischemic stroke, encephalo-myelitis, acute myelitis, and intracerebral hemorrhage. Bell's palsy and Guillain-Barre Syndrome were the peripheral nervous system complications reported while the skeletal muscle manifestation was mainly found to be rhabdomylosis [24].

The reproduction number, R0, estimates that an infectious case could generate how many possible secondary infections in the early phases in a population that is fully susceptible. The R0 for SARS-CoV-2 was estimated to be 1.4–6.49 by different

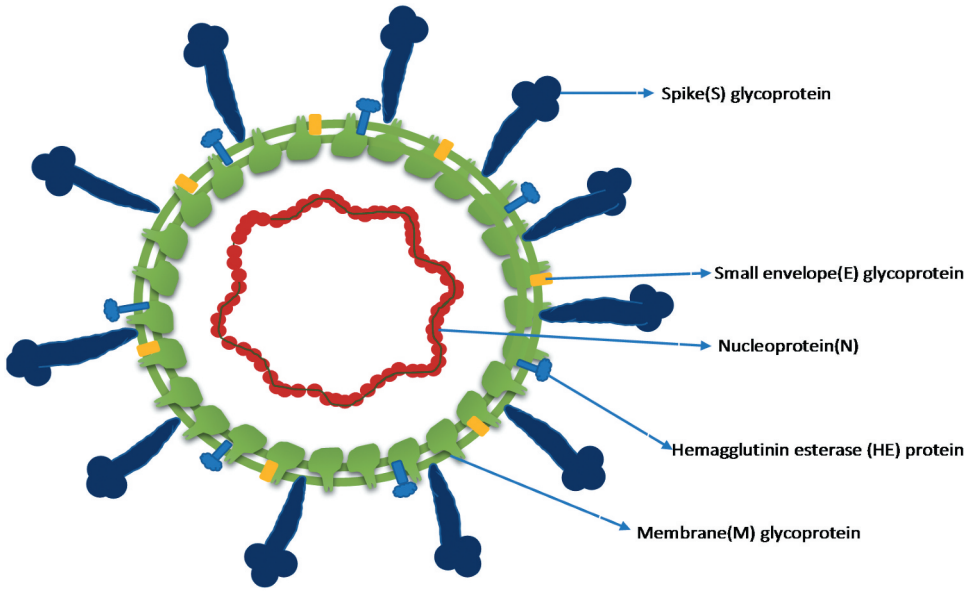

Figure 1. Structure of SARS-CoV-2.

methods. So, an average of 3.28 and a 2.79 median value was calculated by analysis spanning 12 studies. Hence, a range of 2–3 is considered to be reliable. Super spreaders also have to be taken into account that has the potential to infect more than 100 people [25].

3.3. Epidemiology

It all started from the Huanan seafood wholesale market from where the first spread of COVID-19 was reported in December 2019 in Wuhan, although the infection route of the reported first case was unclear. The transmission from human-to-human became evident when cases outside of China came up. Since then, the number of WHO reported COVID-19 cases has been on a continuous rise [16,26]. Till 10 March 2020, about 100 countries recorded COVID-19 cases. In the course of the first 3 weeks after the outbreak, cases were reported in Japan and Thailand that promulgated to the Americas, European and Eastern Mediterranean Region in the upcoming weeks. In weeks 9–11, African Region reported the first of its cases and the South-east Asian countries also saw a rise during this time. Till the above-mentioned date, about 75 countries had their first cases reported of those persons that had a travel history to affected countries (China-22%, Italy-27%, Iran-11%, and Others-15%) [27]. The mortality rate of SARS-CoV-2 is about 3.8% that is lower than SARS-CoV (10%) [28] and MERS-CoV (37.1%) [29], but it has around 10 times higher count of relative infection cases [26].

Till October 11, over 37 million cases and 1 million deaths have globally been reported. Of these, around 48% cases and 55% of deaths have been reported from the Americas region, with America, Argentina, and Brazil topping the list. According to the Weekly Epidemiological Update issued by WHO on October 5, there were about 2.2 million new cases and around 39,000 deaths reported in all the six regions of WHO. According to this, Europe saw the greatest hike in new cases (34%) while a substantial increase (27%) in deaths was reported in the African region. The South-East Asia region saw a 6% decline in new cases and an 8% decline in deaths compared to the week before [30].

According to the Weekly Epidemiological update released by WHO on 24 November 2020, there is a slowdown in the acceleration in global case incidences but death rates are continuously rising. Around 4 million new cases and over 67,000 new deaths have been reported, in which the European region is the largest contributor. Till 22 November 2020, in total over 57.8 million cases and 1.3 million deaths were reported globally. USA, India, Italy, Brazil and France were the five countries that reported the highest number of cases in this weekly update [31]. As of 14 December 2020, the confirmed cases of COVID-19 were 71,051,805 while 1,608,648 were the deaths reported globally. WHO region-wise, America has the highest confirmed cases, followed by Europe, South-East Asia, Eastern Mediterranean, Africa and Western Pacific regions. Country-wise, USA has the maximum number of confirmed cases which are equal to 15,860,675 while the second spot is occupied by India with 9,884,100 confirmed cases [32].

4. Pathogenesis of COVID-19

SARS-CoV-2 is believed to have transmembrane spike glycoprotein(S) that mediates its entry into the host cells. The spike glycoprotein forms homotrimers that protrude from the surface of the virus. The S-glycoprotein has two subunits-S1 and S2. The S1 subunit includes the receptor-binding domain (RBD) for binding to the receptor of the host cell. The S2 subunit is responsible for the fusion of the viral membrane with the cellular membrane. Studies have claimed that the ACE2 receptor (Angiotensin Converting Enzyme 2) in humans is the entry receptor for SARS-CoV-2 [33–35]. A study that analyzed where ACE2 is expressed in the body tissues revealed that the small intestine, kidneys, thyroid, testis, adipose tissue, and heart had the highest level of ACE2

expression. Blood, bone marrow, blood vessels, brain, muscle, and spleen had the lowest ACE2 expression. ACE2 showed a medium expression in the lungs, liver, colon, adrenal gland, and bladder [36].

In a molecular docking study conducted on S-glycoprotein of SARS-CoV-2 to showcase its binding efficiency with ACE2 receptor of pangolin, bat, and human, it was found that the strongest binding was between spike protein and bat ACE2 followed by spike protein and human ACE2. The kind of binding in humans was found to be similar as in bats that consisted majorly of hydrophobic interactions and hydrogen bonding. The efficient binding of SARS-CoV-2 is also believed to be due to the presence of more antigenic peptides as compared to SARS-CoV and MERS-CoV that imparts more antigenicity [14]. In a study using single-molecule force spectroscopy, it was revealed that both the domains of S-glycoprotein interact with ACE2 receptor with similar thermodynamic and kinetic properties that highlights the fact that SARS-CoV-2 binding to ACE2 is through RBD/ACE2 interface, and it binds with quite a high affinity [33].

The S-glycoprotein of SARS-CoV-2 binds to ACE2 receptors after which it undergoes proteolytic activation by a two-step cleavage by TMPRSS2 protease. The first step is priming at the S1/S2 cleavage site that leads to stabilization of the S2 subunit at the site of attachment, while the second cleavage activates the S-glycoprotein that causes conformational changes that lead to the fusion of viral and host cell membranes [37]. Figure 2 depicts the entry of SARS-CoV-2 into the host cell. After the membrane fusion, the viral contents release inside and the viral replication starts. The N protein of the virus binds to the new genomic RNA while the M protein integrates it into the cellular endoplasmic reticulum. These are the new nucleocapsids that are now enclosed in the membrane of the endoplasmic reticulum and are transported to the cell membrane via Golgi vesicles, from where they are exocytosed into the extracellular space. Now, these new viral particles infect the adjacent epithelial cells as well as serve as the infective material for community spread [37].

Alveolar macrophages, dendritic cells, and epithelial cells are three major innate immunity components in the airways that fight against the virus till the involvement of adaptive immunity [38]. In the first stage, the virus binds to the ACE2 receptors on the nasal epithelium. The viral contents release and the viral replication occurs, there is a limited immune response but the individuals can account for the community spread as they are highly infectious. This is the asymptomatic phase that paves way for the infection to reach the upper respiratory tract, where the actual clinical symptoms manifest. The immune response is greater that involves the release of CXCL-10(C-X-C motif chemokine ligand 10) and interferons – IFN-λ and IFN-β, from the virally infected cells. The majority of the patients progress to this stage while about one-fifth of the patients go onto the severe stage that involves lower respiratory tract infection and manifests as ARDS. Here, binding of the viral particles to alveolar epithelium cells takes place and the virus invades Type II alveolar cells which leads to the release of a large amounts of cytokines and inflammatory markers like interleukins, Tumor-necrosis factor – α(TNF-α), CXCL-10, IFN-λ and IFN-β, MCP-1(Monocyte chemoattractant protein-1), etc. This is called as the cytokine storm that attracts neutrophils, CD4 and CD8 cells that sequester in the lung tissue and lead to lung injury while fighting off the viral load [37].

The mechanisms of neurological invasion of SARS-CoV-2 are still not completely clear. After the invasion of nasal epithelium cells, the virus can travel through the olfactory bulb to the brain areas like the thalamus and brainstem that can cause neuroinflammation and demyelination. The viral particles can also travel to the brain via blood circulation. These are the direct invasion routes. Evidence also suggests that SARS-CoV-2 can invade the peripheral nerve terminals

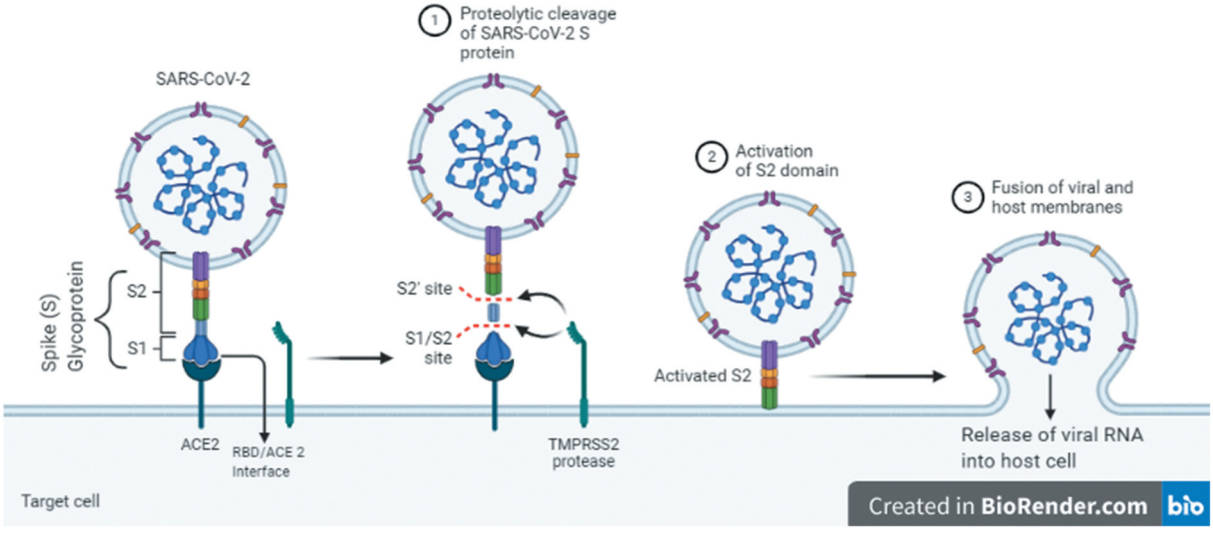

Figure 2. Entry of SARS-CoV-2 through ACE-2 receptor that functions as the entry receptor. S1 and S2 represent subunits of the spike glycoprotein that binds to ACE2 through RBD (Receptor Binding Domain). This is followed by proteolytic cleavage of the spike glycoprotein by TMPRSS2 (Transmembrane Protease Serine 2) protease which leads to the activation of S2 domain spike glycoprotein. Next follows is the fusion of viral and host membranes that releases the viral RNA into the host cell. Created in Biorender.com.

and gain access to the CNS via the synaptic route. Also, the viral particles could infect the gastrointestinal tract and reach CNS through the afferent neurons in the enteric nervous system. The inflammatory cells can also infect the endothelial cells in the blood-brain barrier, thereby disrupting the barrier and gaining entry into the CNS [39]. Figure 3 depicts the pathway of the subsequent events that take place after the entry of the virus into the host cell and the pathogenesis that follows.

5. Complications and the possible mechanisms

5.1. Respiratory complications of COVID-19

The alveolar epithelium and capillary endothelium cells in the lungs have ACE2 receptors that is confirmed by immunolabeling studies [40]. S-glycoprotein binds to ACE2 with a high affinity that allows the entry of SARS-CoV-2 into the cell. This is followed by proteolytic processing of TMPRSS2, that is, Transmembrane Serine Protease 2, on the surface of the host cell that further causes priming of spike protein [40,41]. At this point, the virus propagates locally eliciting a limited response from the innate immunity. Such individuals are carriers of infection, although they have a low viral load. The virus detection is through nasal swabs. Propagation of the virus further down the conducting airways elicits a more vigorous response by innate immune system that points to the clinical manifestation of COVID-19. At this point, testing is done by nasal swabs or sputum samples or we can also rely on the early immune response markers [42]. The disease progresses to the severe stage in around 20% of patients which is characterized by the development of pulmonary infiltrates. Around 5% of them progress to the critical stages [42,43]. The alveolar type II cells are now infected with the virus [42]. A large number of inflammatory mediators and cytokines are released that further attract the neutrophils, CD4, and CD8 cells, known as the cytokine storm, that lead to lung injury through sequestration in the lungs. Eventually, both Type I and Type II alveolar cells are destroyed which manifests as ARDS [37]. Figure 4 depicts the various stages of respiratory infection by SARS-CoV-2.

ARDS (Acute Respiratory Distress Syndrome), which is the most common form of respiratory complication, is a type of lung injury that is characterized by aerated lung tissue loss, increase in the permeability of pulmonary vasculature, and inflammation. Three categories of ARDS are classified according to the degree of hypoxia as mild, moderate, and severe [44]. In a study on 41 patients in Wuhan, China, pneumonia was shown by all. The respiratory complications mainly comprised of ARDS, which was shown by 12 out of 41 patients that constituted about 29%. Around 4% required mechanical ventilation while 5% suffered from refractory hypoxemia. The progression from admission to the hospital to ARDS was just in 2 days and the mortality rate was high, around 15% [23].

In a similar study on 99 patients in Wuhan, China, around 51% of the cases progressed to chronic disease that included respiratory system and nervous system disease among others. ARDS was reported in 17% of the cases out of which 11% worsened and died due to multiple organ failure, while 8% presented acute respiratory injury. Bilateral pneumonia was presented by 75% of the patients [45]. Similar findings were reported in a 138 patient study at the same place in which 26.1% were admitted to the ICU due to organ dysfunction. ARDS was reported in 27% of the patients of which 22% were ICU patients while 5% were non-ICU [22].

5.2. Neurological complications of COVID-19

Spinal neurons and glial cells in the brain also house ACE2 receptors. Although SARS-CoV-2 is not a neurotropic virus primarily with their prime target being the respiratory epithelium, attachment to the ACE2 receptors in the brain can trigger neuronal damage [46–48]. Table 1 enlists the neurological findings of various studies that shed light on the neurological complications involved.

Possible mechanisms:

(1) *Direct infection*: Direct invasion of viruses can be by binding to the receptors present on the axon terminals, causing nerve damage [4,53].

(1) Through blood circulation: The virus affects the circulating leukocytes that may infiltrate the brain parenchyma. Since the leucocytes can naturally cross the blood-brain barrier, the virus is hidden in them also traverses the blood-brain barrier [4,53]. But, the tendency of coronaviruses to cross the epithelium barrier, thus reaching lymph or bloodstream and propagating to the CNS is very bleak and poorly understood [54].
(2) Through the neuronal pathway: Neuronal dissemination occurs through infection of peripheral neurons by the virus and using the active transport mechanics of the cell to access the CNS. An example can be the olfactory nerves-to-neuron transport. It is an effective channel between the epithelium of the nasal cavity and the CNS due to its anatomical organization, taking around 7 days to reach the brain and CSF and cause demyelination and inflammation [4,53,54].

(1) *Hypoxia*: Viral proliferation in lung tissue causes inflammatory exudation, severe pneumonia, edema and transparent membrane formation. All of this leads to the disorder of gas exchange through alveoli that manifests as hypoxia. Due to this, anaerobic metabolism takes place that causes accumulation of acidic compounds. Peripheral vasodilation and hypercarbia can be the other contributing factors. The accumulation of such acidic compounds causes brain swelling, cerebral vasodilation, edema, cerebral blood flow obstruction or headache because of ischemia [4,46].
(2) *Injury through immune system*: The production of cytokines locally in the CNS owing to the viral infection can disrupt the tight junctions of blood rain barrier, for example, expression of MCP-1 or CCL-2 [53]. Systemic Inflammatory Response Syndrome (SIRS) initiates abnormally in the case of severe pneumonia that is

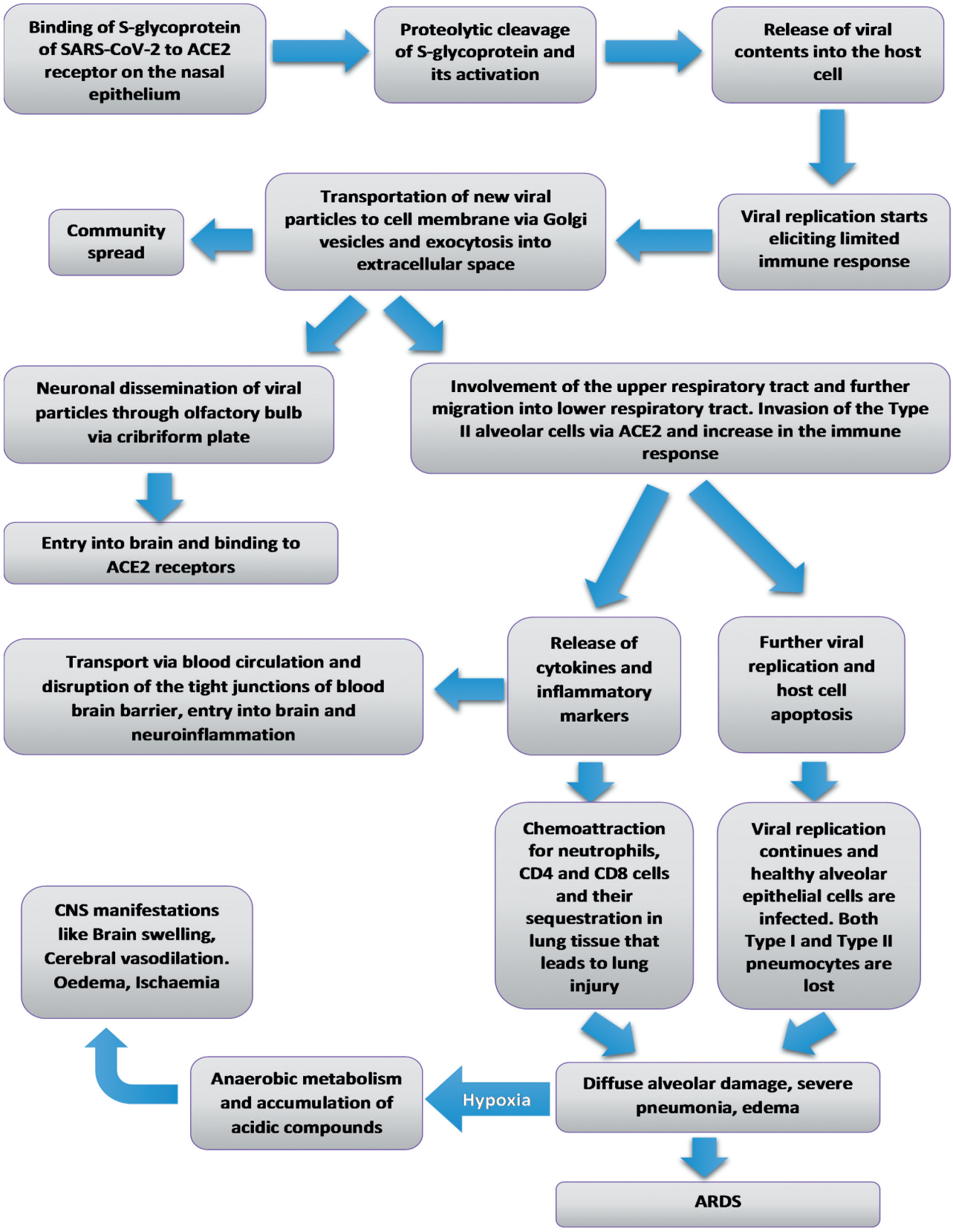

Figure 3. Pathogenesis of COVID-19 and the routes by which infection reaches the brain and lungs. The virus enters in the body and a limited immune response is elicited. Exocytosis of the new viral particles formed after viral replication infect the upper respiratory tract and travel down further to the lower respiratory tract eliciting a higher immune response. Here, the virus can also enter the brain via the olfactory bulb. These events lead to a release in a larger number of inflammatory markers that cause the cytokine storm and further manifest the ARDS (Acute respiratory distress syndrome). The virus can also reach the brain via blood circulation or it can also cause CNS manifestations through hypoxia. These events lead to the neurological complications due to SARS-CoV-2.

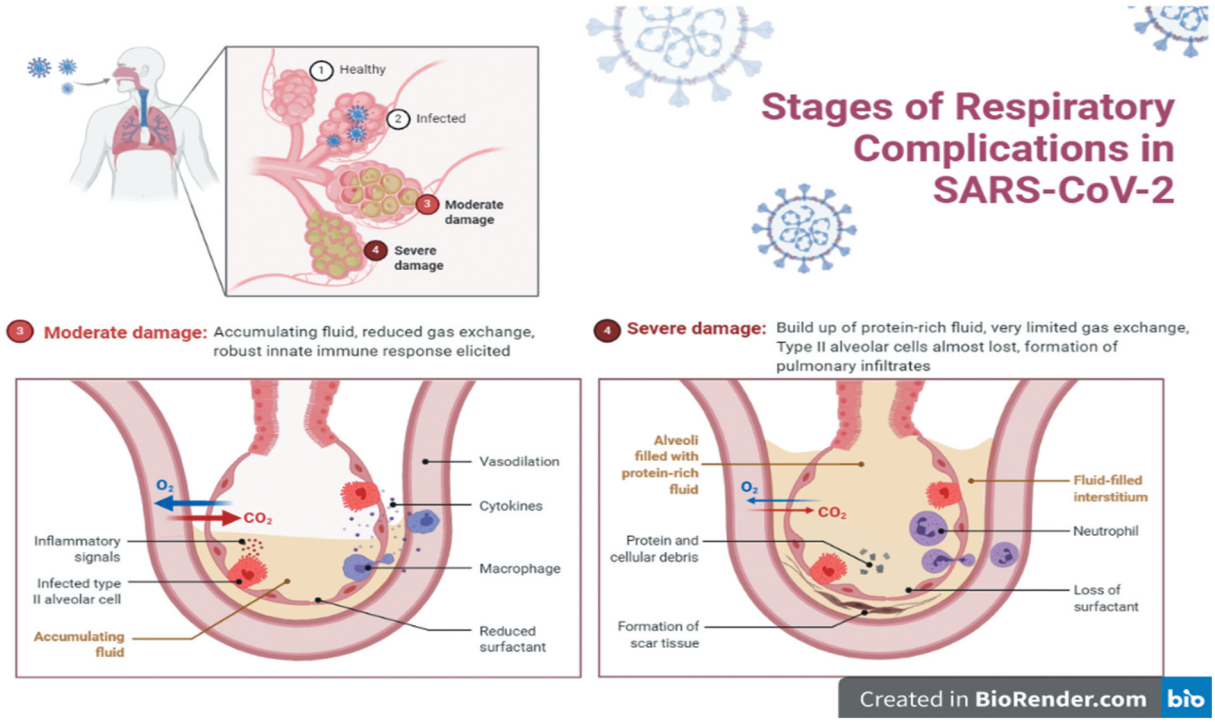

Figure 4. Stages of respiratory complications in SARS-CoV-2. First, the virus infects the alveolar epithelial cells which further progresses to the moderate form of the disease characterized by fluid build up, vasodilation, infection of Type II alveolar cells. In some patients, it progresses to the severe stage where the fluid completely fills up and alveolar type II cells are destroyed. Cytokine storm prevails and ARDS manifests. Created with BioRender.com.

caused by SARS-CoV-2. There is evidence that COVID-19 is associated with a severe cytokine storm [4]. A study of 150 patients in China showed the fatality predictors as elevated ferretin and IL-6 levels that suggest the mortality may be because of viral induced hyperinflammation [55]. A study also showed the involvement of NF-κB pathway in the pathogenesis of COVID-19. The genes involved in this signaling were found to be upregulated that points to the role of NF-κB in the hyper inflammation [15].

Nrf2(Nuclear factor erythroid 2-related factor 2) is a transcription factor responsible for protecting the cells against electrophile or oxidative stress-induced death and tissue damage. It also regulates the inflammatory response as it transcriptionally represses the inflammatory genes. Along with the cytokine storm, the generation of ROS (Reactive Oxygen Species) and oxidative stress is inevitable. Normally, Nrf2 is bonded to its inhibitor Keap1 in the cytoplasm. When electrophiles or ROS are present, it leads to dissociation of Keap1-Nrf2 complex and migration of Nrf2 to the nucleus where it stimulates the transcription of the genes with the anti-oxidant response, redox homeostasis, etc., thus, protecting the cells from inflammation. Nrf2 also stimulates the expression of HO-1 (Hemoxygenase) that degrades pro-inflammatory free heme and forms anti-inflammatory compounds (CO, Bilirubin). It also upregulates the production of NAD(P)H quinone oxidoreductase(NQO1), an anti-oxidant enzyme, and other enzymes in glutathione biosynthesis that is the main cellular anti-oxidant [56]. COVID-19 patient's biopsies revealed that the genes associated with the antioxidant response by Nrf2 were downregulated. Repression of the Nrf2 pathway is also supported by in-vitro experiments where Nrf2 inducible proteins had a reduced expression [57]. In a study on the role of Nrf2 activator on COVID-19, it was concluded that the genes involved in virus infectivity and the immune response were responsive to Nrf2 activation that may attenuate the replication rate of the virus, limit microvascular injuries and allow successful control of the cytokine storm [58].

(1) *ACE2*: On binding to the ACE2 receptors, the virus can also lead to elevated blood pressure that further increases the risk of a cerebral hemorrhage. Also, as the interaction between ACE2 and S-glycoprotein can take place in capillary endothelium as well, as ACE2 is expressed here, it can also cause damage to the blood-brain barrier, entering CNS through vascular system [4,48]. The dysregulation of ACE2 can also give rise to cerebrovascular accidents. A Dutch study on COVID-19 infected patients showed abnormal blood clotting. The clots can break and travel to various body parts, as the lungs where it can cause pulmonary embolism, or it can clog the arteries in the brain leading to stroke [59]. A study on 219 confirmed SARS-CoV-2 patients, 11 developed cerebrovascular diseases, out of which 10 presented with ischemic stroke while 1 had intracerebral hemorrhage. They also showed elevated D-dimer levels in that indicated hypercoagulable state. Also,

Table 1. Various studies that observed neurological findings in COVID-19 patients.

STUDY	NUMBER OF SUBJECTS	MAJOR FINDINGS
Neurological manifestations in hospitalized patients [5]	214	36.4% patients showed neurological findings CNS-24.8% [Dizziness(16.8%,headache(13.1%,impairedconsciousness(7.5%), acute cerebrovascular disease(2.5%),ataxia(0.5%),epilepsy(0.5%) PNS-8.9% [Hypogeusia(5.6%),hyposmia(5.1%),hypopsia(1.4%),neuralgia(2.3%)
Observational study of neurological features in France [49]	58	84% patients showed neurological findings Agitation(69%), Corticospinal tract signs(67%), Dysexecutive syndrome(14/39 patients), Leptomeningeal enhancement(8/13 patients), Perfusion abnormalities(11/11 patients), Cerebral ischemic stroke(3/13 patients)
MRI Brain Findings in COVID-19 [50]	126	Acute or subacute infarcts(32 cases), Leukoencephalopathy(17 cases), Cortical FLAIR Signal abnormality(15 cases), Microhaemorrhages(14 cases), Leptomeningeal enhancement(14 cases), Demyelinating lesions(3 cases), Acute hemorrhagic necrotizing encephalopathy(1 case), Rhombencephalitis(1 case), Miller-Fisher Guillain Barre Syndrome(1 case), Hypoxic-ischemic encephalopathy(1 case)
Retrospective Study on Deceased patients in Wuhan [51]	113 deceased and 161 recovered (Total 274)	Disorders of consciousness: 22% of deceased subjects, 1% of recovered subjects Hypoxic encephalopathy: 20% of deceased subjects, 1% of recovered subjects
Neuropsychiatric and Neurological complications-UK wide surveillance study [68]	153	62% cerebrovascular events including – 74% ischemic stroke, 12% Intracerebral hemorrhage, 1% cerebral vasculitis, 13% other cerebrovascular events 31% altered mental status including – 18% encephalitis, 23% unspecified encephalopathy, 59% neuropsychiatric disorders (43% psychosis, 26% neurocognitive disorder, 30% other psychiatric disorders) 5% peripheral disorders including – 67% Guillain Barre Syndrome and variants, 23% other peripheral disorders 2% other neurological disorders
Self-reported taste and olfactory disorders [52]	59 interviewed out of 88 hospitalized patients	33.9% reported either taste or olfactory disorder: Taste disorders only: Dysgeusia (8.5%), Ageusia (1.7%) Olfactory Disorders only: Hyposmia (5.1%), Anosmia (0) 18.6% reported both taste and olfactory disorders: Dysgeusia and Hyposmia (3.4%), Ageusia and Hyposmia (3.4%), Dysgeusia and anosmia (3.4%), Ageusia and Anosmia (8.5%)

the patients who developed the cerebrovascular disease were more likely to be already presenting comorbidities like diabetes mellitus and hypertension [60]. Another study on ICU patients with COVID-19 reported the prevalence of thrombotic complications to be 31% [61].

6. Cytokine storm-the real culprit

DAMPs or Damage Associated Molecular Patterns released into the cytosol during viral replication. The circulating macrophages sense these DAMPs by receptors known as PRRs (Pattern Recognition Receptors) and RLRs (RIG-I-like receptors). This leads to the release of cytokines involved in acute phase response, namely TNF – α (Tumor Necrosis Factor α), IL-1 β, IL-6, and Type-1 interferons, which causes initiation of chemotaxis and further recruiting of immune cells. Besides these, the macrophage bound receptors include TLRs (Toll-like Receptors), STING/cGas (Interferon related), and NLRs (NOD-like receptors) [1]. Reports suggest elevated cytokine levels in COVID-19, which include IL-1 β, IL-6, IFN – γ, and TNF – α among others. The outcome of this manifests as cytokine release syndrome (CRS) or Cytokine Storm, which is considered to be the major culprit of tissue damage seen in COVID-19 [40]. The basic definition of CRS states that it is a state of overactive response by the immune system that causes an excessive increase in the pro-inflammatory cytokines systemically that occurs as a response to an auto-immune disease, tumor generation or to an external stimuli [40].

The first step to CRS is the release of cytokines from innate immune, epithelial and endothelial cells to combat the viral replication and simultaneous recruitment of effector cells to get rid of infected cells. This is innate immunity response. This is followed by a second step in which there is downstream induction of cytokine cascade due to the release of initial cytokines or by signalling due to immune cells [40]. Figure 5 represents the steps leading to the cytokine storm.

IL-6 and TNF – α, the cytokines concerned with acute phase response, are considered to have the major hand in hyper inflammation seen in COVID-19 [1]. The cytokine storm activates a range of signaling pathways that take place in the infected cells or tissues. Cytokines like IL-6, IFN – γ, and IL-1 β activate the JAK/STAT pathway and induce signaling of NF – κB. IL-6 also can induce Angiotensin II expression which further promotes IL-6 expression via JAK/STAT. This creates a feedback loop [40]. The NF – κB family causes an inflammatory signaling overlay which leads to the increased gene expression of many molecules. This is also elicited by infection of SARS-CoV-2. This pathway is triggered by antigen or ligand binding to TLRs. Another pathway involves MAPKs (Mitogen-Activated Protein Kinases) that have their role in differentiation and proliferation of cells followed by cell death as a response to environmental stimuli. Out of the three families of MAPKs, p38 MAPKs have been suggested to have a part in the infection [40].

IL-6 leads to macrophage activation syndrome (MAS) that triggers pro-inflammatory cytokines mass production and the fibroblasts and neutrophils migration into the pulmonary epithelium. This increases collagen and fibrin deposition that damages the underlying lung tissue (Image 3). Increased levels

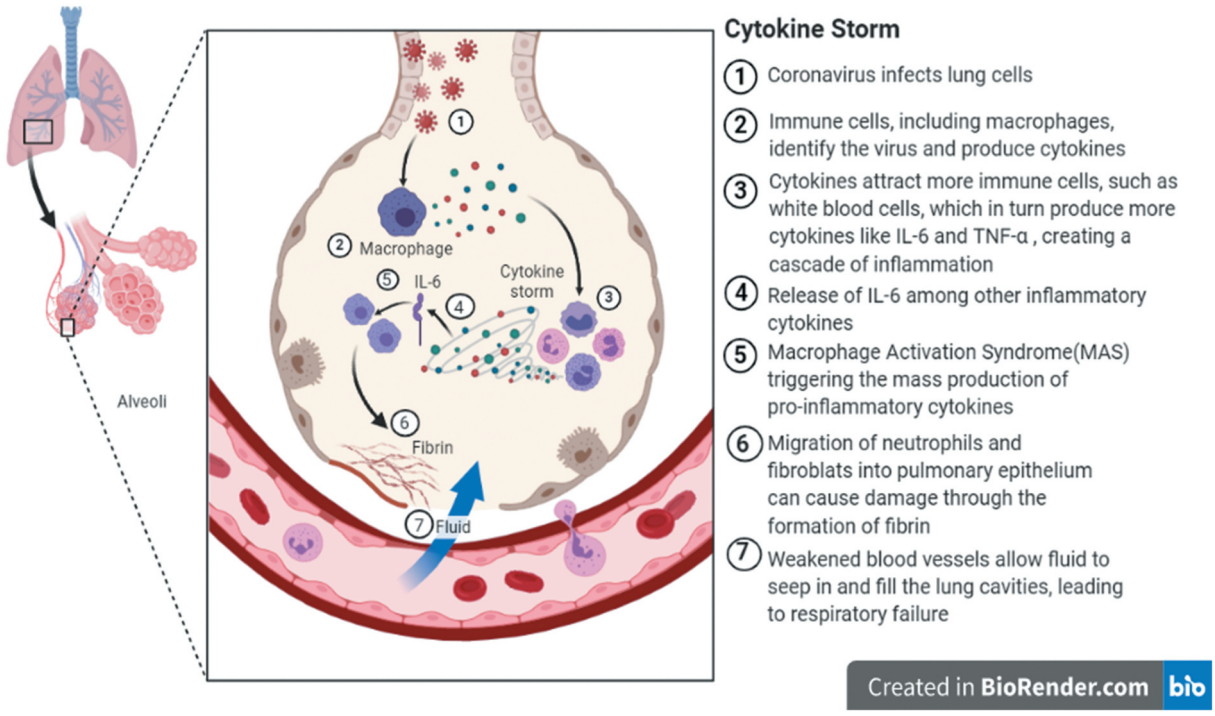

Figure 5. Cytokine storm in COVID-19-the steps involved finally leading to lung tissue injury. Coronavirus infects the lung cells in response to which macrophages produce cytokines. These attract more immune cells and the production of cytokines increases like IL-6 (Interleukin-6) and TNF-α (Tumor necrosis factor-α). This causes a series of steps as depicted that finally lead to deposition of fibrin that manifests as lung injury. Created with BioRender.com.

of TNF – α increase the severity of CRS and also induces lymphocyte apoptosis [1]. This leads to a reduced count of CD4/8 known as lymphopenia that is a common clinical finding when the COVID-19 takes a severe turn. IL-6 and IL-23 together lead to activation of JAK/STAT pathway that differentiates Th_{17} cells leading to production of IL-17 that drives the COVID-19 CRS [1].

The mast cells express TLR and are present in the submucosa of respiratory tract and in nasal cavity. These work like a protective barrier against microorganisms. SARS-CoV-2 can also activate mast cells that causes early release of inflammatory compounds like protease and histamine. Late activation leads to the production of pro-inflammatory members of IL-1 family that include IL-1 and IL-33, in addition to the production of IL-6 and TNF-α, that further activate the macrophages leading to airway inflammation [62]. IL-1 and TNF-α are responsible for causing thrombosis, pulmonary edema, and bleeding. IL-1 can also cause thrombocytopenia and leukopenia. Formation of thrombi is an important factor leading to morbidity and mortality and is one of the major complications of circulatory system. IL-1 is also responsible for causing platelet vascular thrombogenicity by formation of Thromboxane A2 that is released at the inflammation site. It also induces fever as it is involved in arachidonic acid metabolism. Thromboxane increase leads to systemic inflammation. Hence, inhibition of its inducer, IL-1, can also be a strategy for reducing inflammation [63]. Drugs that suppress IL-1 or TNF-α are used for rheumatoid arthritis treatment. These agents can be considered to be explored for Covid-19 treatment as well to reduce the patient mortality [64]. On the other hand, cytokine IL-37 shows suppression of innate and acquired immune response and can also inhibit inflammation by its action on IL-18Rα receptor. It also inhibits class II histocompatibility complex (MHC) by suppressing MyD88 pathway and subsequently the production of interleukins and chemokines. IL-38 can also suppress IL-1β and other IL-family members. Using these cytokines can also provide other pathway for curbing the inflammation [65].

The cytokine storm can also disturb the homeostasis of nervous system that manifests as neurological complications [40]. A study also established correlation of IL-6 with olfactory and gustatory dysfunction. The dysfunctions were high in patients that showed higher IL-6 levels. The patients who showed both the dysfunctions together had even raised IL-6 levels [66]. In a study on cerebrovascular disease in COVID-19 infected patients, higher inflammatory response was reported in the patients who presented with cerebrovascular disease, which included high white blood cell count, neutrophil count, and C-Reactive protein, while the lymphocyte count was lower that pointed to immunosuppression. This further supports the fact that acute inflammation due to COVID-19 is followed by states of high coagulation due to an increase in the levels of pro-inflammatory cytokines. This eventually led to higher incidences of stroke [60]. The first reported case of encephalitis was a 24-year male in Japan [67]. Since then, encephalopathy has been reported further also [68]. Acute Haemorrhagic Necrotizing Encephalopathy (ANE) also develops due to the cytokine storm and disrupts

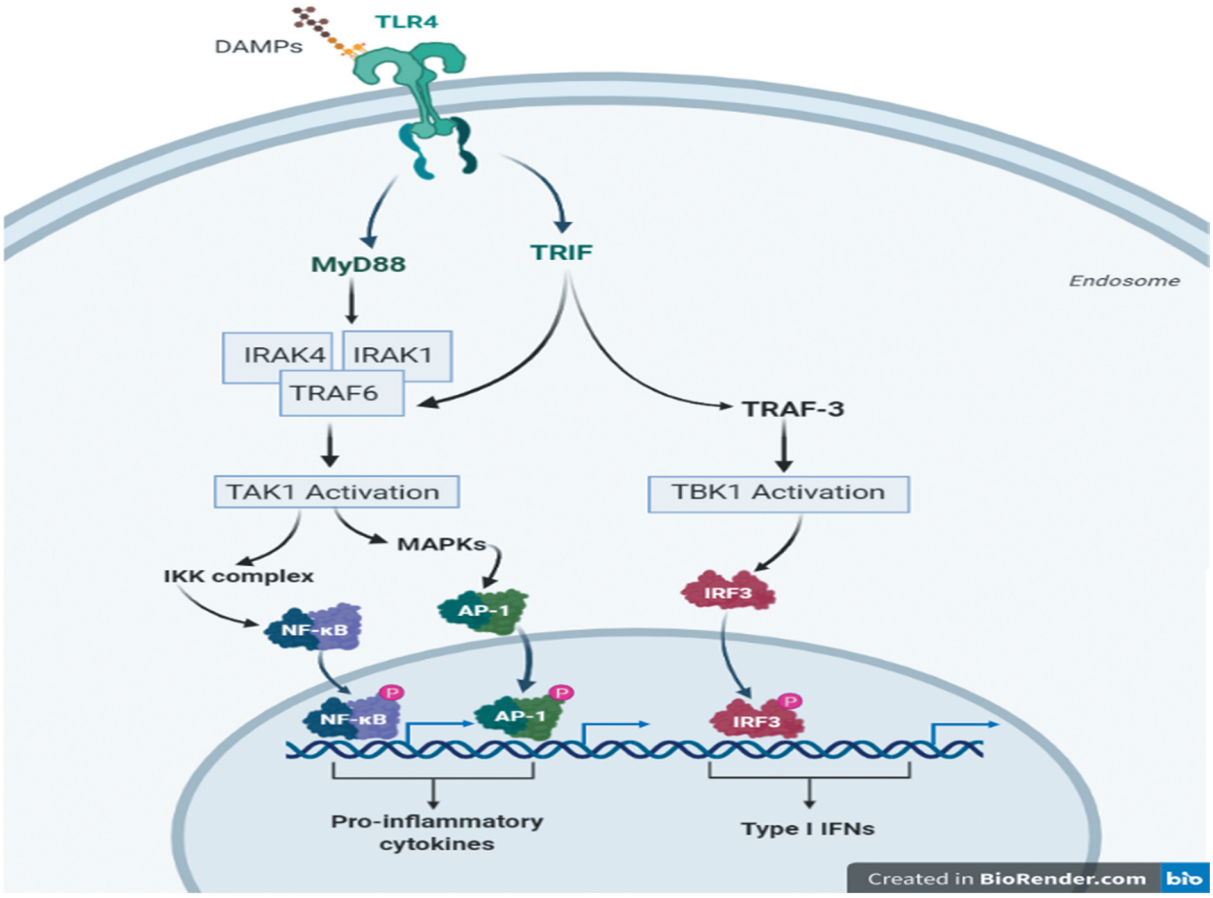

Figure 6. Signaling through TLR4 and the release of pro-inflammatory cytokines and interferons. TLR4 receptors recognize the DAMPs released in the host that bind to these receptors and lead to the activation of MyD88 dependent pathway. Recruitment of IRAK4 and IRAK1 takes place that further associate temporarily with TRAF6, thus inducing TAK1 activation. This further couples to IKK complex and phosphorylates IκB that causes localization of NF-κB in the nucleus. MAPKs are also activated by TAK1 that releases transcription factor AP-1 which induces transcription of inflammatory mediators. In MyD88 independent pathway, TRIF attaches to TIR domain and can bind to both TRAF-6 and TRAF-3. Binding to TRAF-6 leads to MyD88 dependent pathway while binding to TRAF-3 leads to TBK1 activation which further activates transcription factor IRF3 that leads to transcription of IFN-α and IFN-β.

DAMPs-Damage associated molecular patterns; MyD88-Myeloid differentiation primary response 88; TRIF – TIR domain-containing adapter inducing interferon-β; IRAK-Interleukin-1 receptor-associated kinase; TRAF-Tumor necrosis factor receptor-associated factor 6; TBK1-Tank binding kinase 1; TAK1-Transforming growth factor-β activated kinase 1; IKK – I-kappa B kinase complex; MAPKs – Mitogen-activated protein kinases; NF-κB – Nuclear factor-kappa B; AP-1 – Activator protein 1; IRF3 – Interferon regulatory factor 3; IFNs – Interferons. Created with Biorender.com

the blood-brain barrier leading to neurological dysfunction [39].

7. What are TLRs?

The innate immune system has a set of receptors known as PRRs (Pattern Recognition Receptors) [69] which recognize exogenous PAMPs (Pathogen Associated Molecular Patterns) as well as endogenous DAMPs (Danger Associated Molecular Patterns) [70]. TLRs that identify fungal and bacterial components are usually on the cell surface while those that identify microbial or viral nucleic acids are present in intracellular membranes. Small molecules and antibodies can target cell surface TLRs while modified oligonucleotides target the TLRs localized in intracellular membranes [71]. Out of all the TLRs present, the ones on the cell surface include TLR1, TLR2, TLR4, TLR5, TLR6, TLR10 while the ones present intracellularly include TLR3, TLR7, TLR8, TLR9 [72]. All these are part of the innate immune system. Cell surface TLRs generally recognize the membrane components of microbes such as lipoproteins, proteins, and lipids. The intracellular TLRs generally recognize bacterial and viral nucleic acids as well as self-nucleic acids in case of auto-immune disorders. All the TLRs recruit their adaptor molecules leading to the activation of various transcription factors that govern the innate immune response [73].

8. TLR4 and its location

TLR4 is a subtype of TLR and is expressed on immune system cells that include macrophages, monocytes, and dendritic cells. TLR4 senses a PAMP known as Lipopolysaccharide (LPS) which is a component of the outer cell wall in gram-negative bacteria. Series of steps follow after LPS activates TLR4 which eventually results in the release of pro-inflammatory mediators [69]. TLR4 has also been shown to respond to DAMPs which are endogenous molecules released by injured or dying cells of the host [74,75]. Apart from these, PAMPs are also recognized from fungi, viruses, and parasites by TLR4 [69,76].

TLR4 is demonstrated to express in the epithelial cells of alveoli and bronchi and the endothelial cells of vascular

Table 2. TLR4 modulators that are being explored for COVID-19 and those that can be explored for COVID-19.

Name	Mechanism of action	Category	Stage of Drug Development
EB05	TLR4 Antagonist		Under Phase 2 Clinical trials for COVID 19(NCT04401475) [92]
SHINGRIX(Zoster Vaccine Recombinant, Adjuvanted)	TLR4 Agonist		Under the early phase 1 trial for COVID-19(NCT04523246) [93]
Eritoran	TLR4 Antagonist		Under clinical trials for COVID-19, Community-acquired Pneumonia, Influenza(NCT02735707) [94]
Naltrexone	TLR4 Antagonist Competitive inhibitor Inhibits TRIF/TRAM pathway [95]	Opioids	-Under phase 2 trials for COVID-19 along with dietary supplement NAD +(NCT04604704) [96] -Under phase 2 trials for COVID-19 along with Metformin(NCT04604678) [97] -Under phase 2 trials for COVID-19, ARDS, Coronavirus infections along with Ketamine(NCT04365985) [98]
Curcumin	TLR4 Antagonist Non-covalent binding to MD-2 Competes with LPS for binding to TLR4/MD-2 complex which inhibits both the TRIF-dependent pathway and MyD88-dependent pathway [99,100]	Flavonoid	Under phase 2 trials for COVID-19 as a medical spray ArtemiC as add-on therapy(Comination of Artemisinin, Curcumin, Frankincense, Vitamin C) (NCT04382040) [101]
Quercetin	TLR4 Antagonist	Flavonoid	-Under phase 2 trials for COVID-19 as Quercetin phytosome (NCT04578158) [102] -Under clinical study for prophylaxis and treatment of COVID-19 (NCT04377789) [103] -Under phase 4 for COVID-19 as Quadruple therapy(Zinc, Quercetin, Bromelain, Vitamin C) (NCT04468139) [104] -Under phase 1 for COVID-19 as a dietary supplement with Hydroxychloroquine(NCT04590274) [105]
Isoquercetin	TLR4 Antagonist	Flavonoid	-Under phase 2 trials for COVID-19 along with Masitinib(NCT04622865) [106] -Under phase 2 trials for COVID-19(NCT04536090) [107]
Resveratrol	TLR4 Antagonist	Stilbene phytoalexin phenolic compound	-Under phase 2 trials for COVID-19 along with Zinc therapy(NCT04542993) [108] -Under clinical study as one of the ingredients in an Immuno-formulation for COVID-19(NCT04666753) [109] -Under clinical study as one of the ingredients of Nutritional Support System(NSS) for COVID-19(NCT04507867) [110]
Berberine	TLR4 Antagonist Binds to TLR4/MD2 receptor [111] Blocks the transduction of TLR4/NF-κB by binding to cysteine 179 residue of IKK (IκB Kinase) so suppresses NF – κB activation [112]	Isoquinoline alkaloid (extracted from *Rhizoma coptidis)*	Under clinical trials for COVID-19(NCT04479202) [113]
Ferulic acid	TLR4 Antagonist Suppresses NF-κB and MAPK signaling [114]	Phenolic compound (found abundantly in herbs, vegetables, fruits, extracted from *Ligusticum wallichii)*	Under clinical study as one of the ingredients of an Immuno-formulation for COVID-19(NCT04666753) [115]
Glucopyranosyl Lipid Adjuvant	TLR4 Agonist	Lipid A mimetic	-Under phase 2 trials as an adjuvant with H5N1 vaccine for influenza (NCT01147068) [116] -Under phase 1 trials as an adjuvant with HIV Vaccine(NCT01966900) [117]
TAK-242	TLR4 Antagonist Noncompetitive inhibitor Disrupts the conformation of TIR domain and interacts with TIRAP and TRIF, thus inhibits both MyD88 dependent and independent pathways [118,119]	Cyclohexene carboxylic ester derivative (Non-glycolipid based)	-Under phase 3 trials for sepsis(NCT00143611) [120] -Under phase 2 for Acute-On-Chronic Liver Failure(NCT04620148) [121]

(*Continued*)

Table 2. (Continued).

Name	Mechanism of action	Category	Stage of Drug Development
Parthenolide	TLR4 Antagonist Binds to TRAF6 [122] and blocks both pathways (MyD88 dependent and independent) [123,124]	Sesquiterpene lactone	Under phase 3 trials for Contact dermatitis(NCT00640614) [125]
Naringenin	TLR4 Antagonist	Flavonoid	-Under phase 1 trials for Hepatitis C virus(NCT01091077) [126] -Under clinical trials for Cardiovascular risk factor, Type 2 Diabetes Mellitus, Insulin sensitivity and Metabolic Syndrome(NCT02527277) [127]
FP7 Like	TLR4 Antagonist Competitive inhibition	Lipid X mimetic Anionic Monosaccharide glycolipid based	'In-vivo' stage of drug development (Binding studies for anti-inflammatory conditions) [85,128]
Calixarene	TLR4 Antagonist Competitive inhibition Direct action on TLR4/MD-2 complex [129]	Non-glycolipid based	'In-vitro' stage of drug development (Molecular docking studies that showed calixarenes inhibited TLR4 induced cytokine storm) [128,129]
PIP2	TLR4 Antagonist Competitive inhibitor Inhibits LPS-induced production of TNF-α and IL-6 [130]	Non-glycolipid based	'In-vivo' stage of drug development (Inhibits induction of inflammatory markers in rheumatoid arthritis) [128,130]
Unsaturated cardiolipins	TLR4 Antagonist Competitive inhibition	Non-glycolipid based	'In-vitro' stage of drug development (Cardiolipins inhibit LPS-induced cell activation) [128,131]
Alpinetin	TLR4 Antagonist Agonist of PPAR-γ that downregulates the expression of TLR4 [132]	Natural flavonoid Non-glycolipid based	'In-vitro' stage of drug development (Inhibits inflammatory and oxidative responses in LPS/D-Galactosamine induced liver injury) [128,133]

endothelium [77]. In an RT-PCR study, the expression of TLR4 was demonstrated on epithelial cells of the respiratory system. It states that alveolar and tracheobronchial epithelial cells show TLR4 at mRNA level [78]. This suggests that pulmonary epithelium may participate in local innate immune response by cytokines or antimicrobial peptide secretion [77].

In a study on mice, it was revealed that all kinds of TLRs have mRNA expression in the brain. TLRs 2,3,4 have a higher degree of gene expression while TLRs 1,5,7,8,9 have a lower degree of gene expression. TLR genes 1-9 were upregulated substantially on infection. TLR4 was detected in microglia and neurons upon infection while it was undetected on astrocytes and oligodendrocytes. They were also expressed on CD11b+ myeloid cells as the infiltrating leucocytes [79].

In a study on 48 Korean patients, it was revealed that TLR4 mediated activation of NF – κB signaling pathway was involved in the hyperinflammatory responses in COVID-19 patients which suggests that TLR4 signaling is very much involved in the pathogenesis. IL-1 β and all its downstream signaling molecules were also elevated that results in uncontrolled pathological inflammation [15]. Molecular docking studies have also suggested substantial binding of SARS-CoV-2 spike protein to TLRs 1,6,4, out of which the binding between TLR4 and spike protein was found to be the strongest. The binding is mainly constituted of hydrophobic interactions or hydrogen bonds [14].

9. TLR4 signaling

TLR4 has an extracellular domain and an intracellular domain that is made of 608 residues and 187 residues respectively. Two distinct pathways form the signaling cascade by the intracellular domain-MyD88 dependent pathway which causes pro-inflammatory cytokines production, and MyD88 independent pathway that stimulates Type-1 IFNs [74]. TLR4 is present intracellularly and transfers to the cell surface for recognizing the pathogen [78]. MyD88 has three domains – Carboxy terminal TIR domain (Toll/IL-1R), Amino-terminal Death Domain (DD), and Short linker sequence. IRAK4 (IL-1R Associated Kinases) is connected to an intermediate domain (ID) of MyD88 that is involved in signaling. MyD88 independent pathway is through TRIF, a protein that binds indirectly to TLR4 through TRAM (TRIF Related Adaptor Molecule) [72,80]. The DAMPs by the host in response to viral replication have been detected in the lungs of SARS-CoV infected patients in a study. These DAMPs are recognized by TLR4, and they bind to the receptor [81]. Studies have also showcased the binding of S-glycoprotein of SARS-CoV-2 to TLR4 which can also activate the signaling pathway [14]. MyD88 pathway gets activated and recruits IRAK4 and IRAK1 (activated by phosphorylation by IRAK4). These now temporarily associate with TRAF6. This further induces TAK1 activation. TAK1 now further couples to the IKK complex which further phosphorylates IκB and causes NF-κB to localize in the nucleus [74]. TAK1 also activates MAPKs that release the transcription factor AP1 which bind to their respective DNA segments and induce transcription of a range of inflammatory mediators, namely cytokines, interferons, chemokines, etc. In the MyD88 independent pathway, TRIF attaches to the TIR domain through TRAM. Now from here, TRIF can bind to TRAF3 and TRAF6. Binding to TRAF6 activates MyD88 dependent pathway while binding to TRAF3 activates TBK1 which further results in activation of IRF3 (IFN Regulatory Factor 3) which is a transcription factor. This will bind to its respective DNA segment and leads to transcription of IFN – α and IFN – β [72]. Figure 6 represents the TLR4 signaling pathway.

In the neuronal cells, different cells have different signalling pathways. In astrocytes, MyD88 dependent pathway is activated that leads to transcription of TNF-α, VCAM-1(Vascular cell adhesion molecule 1), IL-27 and other inflammatory mediators. In microglia, dendritic cells and NPCs(Neural Progenitor cells), the signalling occurs through Myd88 – and TRIF-dependent pathways [82].

10. TLR4 modulators being explored for COVID-19 and that can be explored for it

In a mouse model that corresponds to human ARDS in the acute phase, it was found out that when TLR4 was genetically inactivated, the acute lung injury was markedly reduced with improved lung elastance and lesser edema formation, and a healed tissue histology. In the same study, inactivation of TRAF6 also greatly ameliorated acute lung injury. There was also much less nuclear translocation of NF-κB. In short, it can be said that the signaling pathway of acute lung injury was identified to be TLR4—TRIF—TRAF6—NF – κB. IL-6 deficient mice also showed improved lung function which points to the fact that IL-6 has a major role in acute lung injury in combination with other cytokines released by TLR4 signaling [81]. In the study on 48 Korean subjects quoted earlier, TLR4 expression and the related molecules involved in downstream signaling were found to be elevated in COVID-19 patients. The genes involved in NF – κB signaling pathway were also upregulated. The high levels of IL-1β and its related signaling molecules, chemokines and increased S100A9 (a marker of hypoalbuminemia which is a characteristic of COVID-19 patients in severe stages [83]) that acts as a DAMP for TLR4 further strengthens the belief that TLR4 mediated hyper inflammation is the major contributing factor to the molecular pathogenesis of COVID-19 [15].

Cytokines are also known to disrupt the tight junctions in the blood-brain barrier [53] and the higher mRNA expression of TLR4 in the brain, specifically more on microglia and neurons in case of infection, documented in a mice study makes it all the more believable that TLR4 receptors have a hand in neurological complications as well [79].

Thus, modulating TLR4 signaling provides a promising route that can be taken to combat the hyperinflammatory reaction. Modulating the TLR4 signaling could be by both ways, by using agonists and antagonists. Lipid A, a lipid component of endotoxin has an acyl-chain region that is recognized by TLR4 and CD14 in most of the cells. This triggers the innate immune signaling pathway and leads to the translocation of NF-κB and the release of pro-inflammatory cytokines and inflammatory mediators. There could be some potency differences in Lipid A depending upon the microbial species, the humidity and temperature, and the interaction with the

immune system of the host [84]. The backbone of Lipid A is formed by 1,4-diphosphorylated glucosamine to which fatty acid acyl chains of variables lengths can be attached. The development of novel TLR4 agonists is based on the development of simpler structures than Lipid A. Lipid A has a biosynthetic precursor Lipid X that shows antagonistic activity for TLR4 is the simpler monosaccharide that can be used as the basis for TLR4 antagonists, like FP7-like compounds [85–87].

TLR4 agonists are generally developed as immunomodulators and vaccine adjuvants. Generally, vaccine adjuvants are used along with antigen to provide a stronger, faster, and long-lasting immune response as compared to when immunization is done with only antigen [88]. MPLA (Monophosphoryl Lipid A), a TLR4 agonist is a vaccine adjuvant approved for HPV virus and Hepatitis B virus by US-FDA [72]. Lipid A derivatives like Aminoalkyl glucosaminide phosphates are effective as well as safe adjuvants in the vaccine for influenza virus [72,89]. A purified, recombinant anchorless RSV F protein-based vaccine that was formulated along with MPLA showed increased protection against RSV without the induction of a cytokine storm [90]. These previous studies show that TLR4 agonists have been able to provide immunity against specific viruses that opens the doors of their possibilities to be explored against SARS-CoV-2.

TLR4 antagonists should be capable of antagonizing the harmful effects of TLR4 signaling, without any detrimental effects on the normal host defense mechanisms [74]. Many several compounds have had animal testing to demonstrate their capacity to block the cytokine production through TLR4 by acting at various levels in the signaling pathway, with many reaching the clinical phases as well. These compounds, both natural and synthetic, belong to diverse chemistries – mainly being the glycolipids mimicking the precursor of Lipid A, that is, Lipid X, and others belonging to peptides, opioids, heterocycles, steroids, flavonoids, etc [91]. Table 2 depicts the various TLR4 modulators, both agonists and antagonists, being explored for COVID-19 and that can be explored for COVID-19.

11. Conclusion

SARS-CoV-2 surely comes with its own set of complications and injury to multiple organs as implicated by various studies conducted worldwide. The major cause is attributed to be the hyperinflammatory response that occurs due to the viral infection. A large number of cytokines exert deleterious effects on the body organs that takes the infection to a critical turn. The TLR4 receptors are intended to be responsible for this response as has been demonstrated by studies. So, the role of TLR4 modulators to reduce the hyper inflammation becomes all the more evident. Various TLR4 modulators in the various stages of drug development have been suggested, although some of them have not yielded any satisfactory results when tried in other models of inflammation or auto-immune diseases in the preclinical or clinical phases. Their role in suppressing the inflammatory response in SARS-CoV-2 needs to be explored. Also, this is indicative of a pressing need for research in this area to develop novel TLR4 modulators that could certainly work for ameliorating the hyper inflammation-induced complications and stop them before the infection takes a serious turn.

12. Expert opinion

Definitive drug therapy for SARS-CoV-2 has not been achieved so far. Success has come in the form of vaccines which is the only solution in the current scenario. With some vaccines already hitting the immunisation process, many are under clinical trials as well [134]. Around 7 different vaccines have been rolled out in the countries while more than 200 vaccine candidates are under development. The first vaccine to get a nod for the Emergency Use Authorization (EUA) status was the one developed by Pfizer BioNTech. Recently, the vaccine developed by Astra Zeneca/Oxford was given the green light for emergency use. A version of this vaccine is manufactured by The Serum Institute of India with the name Covishield. Covaxin is another whole virus vaccine developed by Bharat Biotech currently in use. Sputnik V vaccine is developed by Russia that uses the spike protein of adenoviruses. Dr. Reddy's has recently completed Phase 3 trials for Sputnik V and the results are awaited. Moderna Covid-19 vaccine is also given EUA status for distribution in the US.

But vaccines would provide a preventive cure only. Once a person contracts the infection, vaccine is not the resort as then therapeutic agents combating the disease progression are required. The impact of the vaccines on the progression of the disease depends on factors like safety and efficacy of the vaccine, time taken for dissemination and speedy delivery. Scientists also anticipate that COVID-19 vaccines may not show 100% efficacy. Also, as large-scale clinical trials for the major vaccines are still going on, there is not a complete information on the safety profile of these and idiosyncratic reactions may occur. Apart from this, a new challenge that has taken shape is the arrival of the variants of the virus in some countries, including Brazil, UK and South Africa. Scientists are still not sure if the vaccine will be equally effective against the new mutants that are rapidly transmissible. A study published in Nature showed slight decrease in the neutralization capacity of the antibodies against the new strains [135]. Also, some vaccines focus on the spike protein-receptor binding and may not work at all entry points. All these pointers do not obviously deny the effectiveness of vaccines but they surely are points of challenge that require proper research to be sure of.

COVID-19 kickstarts as lung infection with the common symptoms being fever, dry cough, fatigue, etc. This is the point where anti-viral agents including nucleoside analogs (Favipiravir, Ribavirin, Remdesivir) and protease inhibitors (Lopinavir, Ritonavir) have been tried but none have been able to ameliorate the disease. Monoclonal antibodies is another therapeutic area that is being explored. Some percentage of the patients suffer from the severe form of the infection. Regardless of the type or strain or mutation of the virus, hyperinflammatory cascade is the major culprit in

deterioration of the patients to critical stages. Hence, developing agents eyeing the cytokine storm is a rational lookout that would provide a treatment rather than a preventive cure. Hence, the advice to fellow researchers is to look out for new therapeutic moieties that could slow down the progression of COVID-19.

Theoretically, as discussed in this review, TLR4 receptors could serve as the ideal therapeutic target for combating the infection as these are the major receptors for cytokine storm. These emerging approaches can be appealing to the researchers as already a number of TLR4 modulators are under trials for COVID-19. The potential is huge as these agents have been on trials for a number of other inflammatory conditions earlier as well. Naltrexone, that is an approved opioid antagonist and is in use already, is also being repurposed for COVID-19 as TLR4 antagonist. The clinical trials for this are going on and a ray of hope could come in the form of its success. The TLR4 modulators discussed in this review have all been evaluated for their efficacy in a range of diseases, particularly in inflammatory and auto-immune disorders. For most of the molecules, studies regarding their molecular docking and their binding to TLR4 receptors are available. Still, there are loads of challenges in their development. These molecules show a great range of structural diversity that inevitably diversifies their effects, potencies, mode of actions, and pharmacodynamics. Also, TLR4 signals through two pathways so some of the molecules work well with one pathway and some with the other. The structural diversity could also lead to pharmacokinetic and targeting differences. Natural products showcasing TLR4 antagonist activity may have multiple targets of action as they may have certain other components too. These knowledge gaps do exist in terms of binding, structural diversity and efficacy and demand a lot of hard-core research to be developed fully and give us potentially beneficial agents. Researchers are doing their job by continuously working on these agents and modifying them that could bring positive results. It is never easy to study the interaction of the virus with the host cells as it tends to operate through a multitude of pathways that may be tough to grasp or point out and whose drug targets would eventually be difficult to develop.

In the coming 5 years, this area can be expected to grow and there are chances that it could unfold useful drug therapies that could be used against the deadly COVID-19. Combination therapies can be tried as well. Also, with the advent of Computer-Aided Drug Designing, new agents can be developed in this area and certain targeted therapies can be devised that could specifically target these receptors. We can also hope that this drug therapy would be effective against the mutant strains as well because hyperinflammation is a characteristic of all strains. An approach of interest can be the drug Naltrexone. If this presents a promising result in the trials, it can surely be explored for COVID-19. An in-depth exploration of this area can give us an actual remedy for the cytokine storm and put a stop to the severe stages of the disease.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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