THE UPCOMING COMPLICATIONS OF COVID-19 ON RECOVERED PATIENTS: MOLECULAR MECHANISMS AND THERAPEUTIC OPPORTUNITIES

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THE UPCOMING COMPLICATIONS OF COVID-19 ON RECOVERED PATIENTS: MOLECULAR MECHANISMS AND THERAPEUTIC OPPORTUNITIES

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EDITORIAL
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Editorial: The Upcoming Complications of COVID-19 on Recovered Patients: Molecular Mechanisms and Therapeutic Opportunities

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Keywords: COVID-19, molecular mechanisms, long-term effects, recovered patients, therapeutic opportunities

Editorial on the Research Topic

The Upcoming Complications of COVID-19 on Recovered Patients: Molecular Mechanisms and Therapeutic Opportunities

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It has been more than 2 years since the world was struck by the COVID-19 pandemic and its emerging variants as Delta, Omicron, and mixed variants. Although global vaccination efforts are effective in controlling COVID-19, recovered patients experienced numerous short- and long-term complications of COVID-19.

Our Research Topic aims to identify the underlying molecular mechanisms and pathways involved of the COVID-19 pathogenesis in the development/reactivation of underlying diseases/disorders. This probably get some more insights for the development of therapeutics, prediction and prevention of the occurrence of life-long morbidities in recovered patients.

In our Research Topic, two studies focused on the pathogenesis, molecular mechanisms, and therapeutic targets of COVID-19. Rahbar et al. highlighted the role of host serine protease 2 (TMPRSS2) in viral infection. Given endosomal and non-endosomal entry of virus in the host cell. In the non-endosomal pathway, the virus entry through S protein is facilitated through its cleavage by furin, which is further activated by TMPRSS2. Clinical trial results showed Bromhexine hydrochloride as a promising intervention for the treatment of early COVID-19 infection by inhibiting the activity of these enzymes. Transcriptional inhibition of TMPRSS2 has no side effects on healthy organs or normal development and homeostasis in the host.

According to the WHO reports, for COVID-19, the current variants of concern (include B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and B.1.1.529 (Omicron) arise due to a high rate of the genetic recombination of S1-RBD/S2 mutations/deletions in the spike protein that have an impact on virus activity. In this respect, Hosseini et al. reviewed the origin and evolution, structure, genetic diversity, route of transmission, pathogenesis, new diagnostic and treatment strategies, as well as the psychological and economic impacts of the COVID-19 pandemic on individuals and their lives around the world.

Other two papers focused on the diagnostic tools/biomarkers for COVID-19 detection. Such that, genome editing targets for site-specific insertions, the CRISPR-Cas system was selected as the 2015 Breakthrough of the Year by Science (Doudna and Charpentier, 2014) and the pioneers won the Nobel Prize of 2020 in Chemistry (Westermann et al., 2021). Shademan et al. reviewed the application of CRISPR technology in the detection and treatment of SARS-CoV-2 infection. For example, a CRISPR/Cas9-

mediated lateral flow nucleic acid assay has been developed to identify infection using the CRISPR/Cas system (Wang et al., 2020), enabling low-cost point-of-care detection methods to identify SARS-CoV-2 infection in the clinical setting (Azhar et al., 2014). Due to the excellent sensitivity, specificity, and reliability of RNA-guided nucleic acid detection, CRISPR/Cas nuclease has recently shown great potential for developing next-generation molecular diagnostics. However, there are challenges in transferring CRISPR/Cas9 into virus-infected cells, the possibility of off-target activity and mutant viruses should also be addressed. Given the impact of cardiovascular disease in SARS-COV-2 infection and the severity of symptoms, cardiac tropin, a known biomarker of cardiovascular disease, has prognostic value in cardiac diseases and COVID-19. Rasmi et al. evaluated the diagnostic value, pathophysiological mechanisms, and novel assessment methods of troponin, including a novel biosensor for troponin in patients with COVID-19.

The rest of the papers in our Research Topic discussed COVID-19 complications such as respiratory, metabolic, oral, cancer, autoimmune disease, and other factors (age and gender). The lung is the primary organ that is affected by the coronavirus and three papers were related to the respiratory complications of COVID-19. As in a study by Matusali et al., autopsies of confirmed COVID-19 patients showed positivity in spindle-like cells infiltrating the sub-mesothelial stroma and the staining confirmed the mesothelial origin. This finding suggests that SARS-COV-2 disrupts the epithelium and after invading the sub mesothelia promotes pleural fibrosis. This disruption is facilitated by neuropilin-1 (NRP1) expression, a co-receptor of vascular endothelial growth factor (VEGF) with a profibrotic activity. SARS-COV-2 infected cells produce massive levels of cytokines with pro-inflammatory abilities (IFNs) and anti-inflammatory, e.g., interleukin 10 (IL-10) at the same time. Among these cytokines, IL-10 creates a double-edged sword as it can dampen the immune system and simultaneously enhance the production of interferon gamma (IFNγ). The cytokine regulation elevates metalloproteases (MMPs) that, in turn, orchestrate fibrosis in the lungs, a significant cause of mortality observed in COVID-19 patients. Calabrese et al. assessed the pulmonary function and exercise capacity 3 months after recovery from pneumonia in patients with COVID-19. They found that COVID-19 patients with a positive clinical history of pulmonary embolism (PE) had more impaired lung function tests compared to COVID-19 patients with a negative clinical history of PE, including a higher percentage of patients who experienced dyspnoeic with exercise and showed a peripheral capillary oxygen saturation (SpO_2)< 90% on the 6-min walk test. Importantly, as severe cases of COVID-19 require hospitalizations, and the significant long-term effect is the reduction in gas transfer, in a meta-analysis Guo et al. stressed that the routine respiratory follow-up of COVID-19 patients is necessary.

Given that lung is not the only organ disturbed during COVID-19 infection, Kaviani et al. summarized the pathogenesis of COVID-19 in the heart, kidney, and liver with a focus on metabolic disease. Moreover, Zhou et al. studied the oral complications (such as ageusia and macroglossia) in patients following COVID-19 infection. Finally, the practical strategies for preventing oral complications are summarized, and a rehabilitation plan for patients with oral complications is constructed. In long run, COVID-19 may result in the development of cancers due to the integration of the virus and the host genome. This possibility may arise due to alteration in the immune system and induction of immunoregulatory pathways that are crucial in the surveillance and eradication of deformed host cells, as reviewed by Rahimmanesh et al.

Hosseini et al. discussed the causes and consequences of the multisystem inflammatory syndrome and autoimmune diseases following SARS-CoV-2 infection which may manifest as Guillain-Barré syndrome and systemic lupus erythematosus to provide a clear view of health care providers and researchers. Finally, age and gender significantly influence the COVID-19 outcomes. Here, Hachim et al. have shown differentially expressed genes in lung tissue of male versus female patients and correlated their findings with signaling pathways such as nuclear factor-kappa B as a regulator of inflammation.

Overall, this Research Topic would serve as a resource to expound the underlying molecular mechanisms of long-term complications in COVID-19 recovered patients and pave the way to consider strategies to reduce these morbidities.

AUTHOR CONTRIBUTIONS

RJE, ZA, and WCC drafted and wrote the editorial. All the authors endorsed the final draft.

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REVIEW
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Host Serine Proteases: A Potential Targeted Therapy for COVID-19 and Influenza

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The ongoing pandemic illustrates limited therapeutic options for controlling SARS-CoV-2 infections, calling a need for additional therapeutic targets. The viral spike S glycoprotein binds to the human receptor angiotensin-converting enzyme 2 (ACE2) and then is activated by the host proteases. Based on the accessibility of the cellular proteases needed for SARS-S activation, SARS-CoV-2 entrance and activation can be mediated by endosomal (such as cathepsin L) and non-endosomal pathways. Evidence indicates that in the non-endosomal pathway, the viral S protein is cleaved by the furin enzyme in infected host cells. To help the virus enter efficiently, the S protein is further activated by the serine protease 2 (TMPRSS2), provided that the S has been cleaved by furin previously. In this review, important roles for host proteases within host cells will be outlined in SARS-CoV-2 infection and antiviral therapeutic strategies will be highlighted. Although there are at least five highly effective vaccines at this time, the appearance of the new viral mutations demands the development of therapeutic agents. Targeted inhibition of host proteases can be used as a therapeutic approach for viral infection.

Keywords: coronavirus, COVID-19, human proteases, TMPRSS2, furin, influenza

HIGHLIGHTS

- Furin and TMPRSS2 mediate SARS-corona virus infection, SARS-CoV-2, and influenza entry into the human cells.
- As a causative factor, TMPRSS2 exerts more severe outcomes for COVID-19.
- Targeted inhibition of TMPRSS2 and furin may be used as a therapeutic approach for COVID-19.
- Bromhexine hydrochloride may be an effective therapeutic drug for COVID-19.

INTRODUCTION

Over the last 2 decades, several outbreaks of coronaviruses (CoVs) have received worldwide attention since they were responsible for the SARS (severe acute respiratory syndrome coronavirus) in China (2002–2003) and the MERS-CoV (Middle East respiratory syndrome) in Saudi Arabia (2012). Currently, the world is struggling with the novel CoV (SARS-CoV-2), initially recognized in China in late 2019. Since its discovery, SARS-CoV-2 has spread globally and its epidemic disease (COVID-19) has claimed thousands of lives. To date, the high number of infected cases with severe respiratory

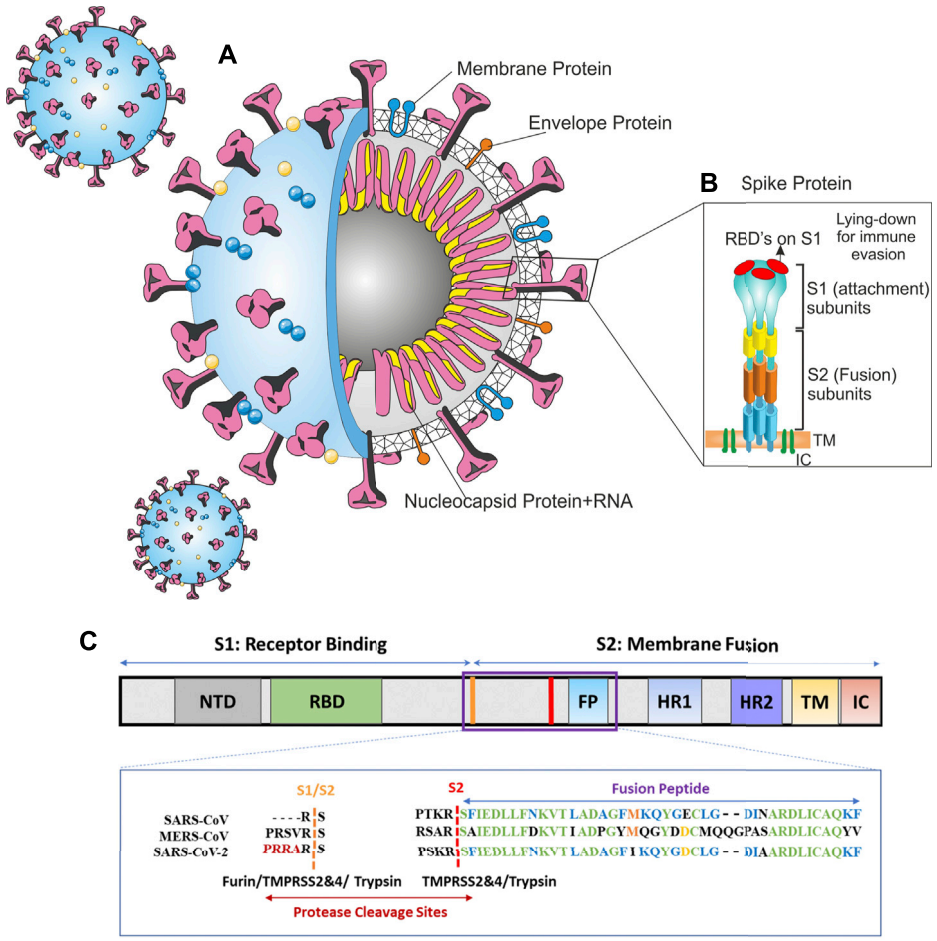

FIGURE 1 | Architecture of SARS-CoV-2 virus **(A)** SARS-CoV-2 schematic **(B)** spike protein, **(C)** alignment of SARS-CoV-2, MERS-CoV, and SARS-CoV sequences in protease cleavage sites S1/S2 and S2. A furin cleavage motif (RRAR) only exists in the SARS-CoV-2 spike. Trypsin, furin, and TMPRSS2 and 4 can cleave the S1/S2 site within the receptor binding domain (RBD) of S protein generating the optimal conformation for viral binding to the host ACE2 receptor. The viral membrane fusion with the host membrane S2 domain can be cleaved by TMPRSS 2 and 4. Panel C is adapted from Luan et al. (2020).

illnesses and viral pneumonia is observed as a risky and rapid human-to-human transmission occurance (Ashour et al., 2020).

Receptor recognition is a significant element of SARS-CoV-2 infection, pathogenesis, determining host range, and a therapeutic target (Wang et al., 2020a; Shang et al., 2020). SARS-COV-2-spike S protein (SARS-COV-2-S), similar to SARS-CoV, exploits human angiotensin-converting enzyme 2 (ACE2) for its entry. Using the same entrance receptor as SARS-CoV, the same set of cells can be targeted and infected by SARS-CoV-2 (Rabi et al., 2020). *In situ* analysis of different tissues has revealed top primary vulnerable cells to SARS-CoV-2, including the AT2 lung cells and macrophages, adrenal gland stromal cells, cardiomyocytes, thyroid, ovary, and stromal testis cells. Some other cells are less likely to be the main targets of SARS-CoV-2 (including enterocytes, cholangiocytes, and the kidney proximal tubule cells) (Zhou et al., 2020).

Although binding to host cells is the initial step of infection, virus entrance necessities the cleavage of S protein via host proteases including cell surface transmembrane protease/serine (TMPRSS) proteases, cathepsins, furin, elastase, factor Xa, and trypsin (Lambertz et al., 2019; Ji et al., 2020; Liu et al., 2020; Luan et al., 2020). Evidence suggests that numerous respiratory viruses hijack host proteases in order to enhance their spread in the host body. Based on the accessibility of cellular proteases needed for SARS-S activation, SARS-CoV-2 entrance and activation can be mediated by two distinct ways; 1) by endocytosis and the cutting of the SARS-COV-2-S by cathepsin L in endosomes (Glowacka et al., 2011; Shirato et al., 2013) and 2) by TMPRSS2 provided that it is co-expressed with ACE2 on the target cells' surface (Heurich et al., 2014). This binding process entails several conformational alternations in the viral envelope glycoproteins (Shen et al., 2017), resulting in virion internalization. Since SARS-CoV-2 employs host proteases as its entrance activators, their inhibitors may exert therapeutic benefits against COVID-19 (Bestle et al., 2020a) and SARS-CoV infections (Shrimp et al., 2020). In the present review, we will highlight the recent updates on the functional role of host proteases, specially TMPRSS2 and furin in viral infection and discuss the possible interventions for inhibiting these enzymes.

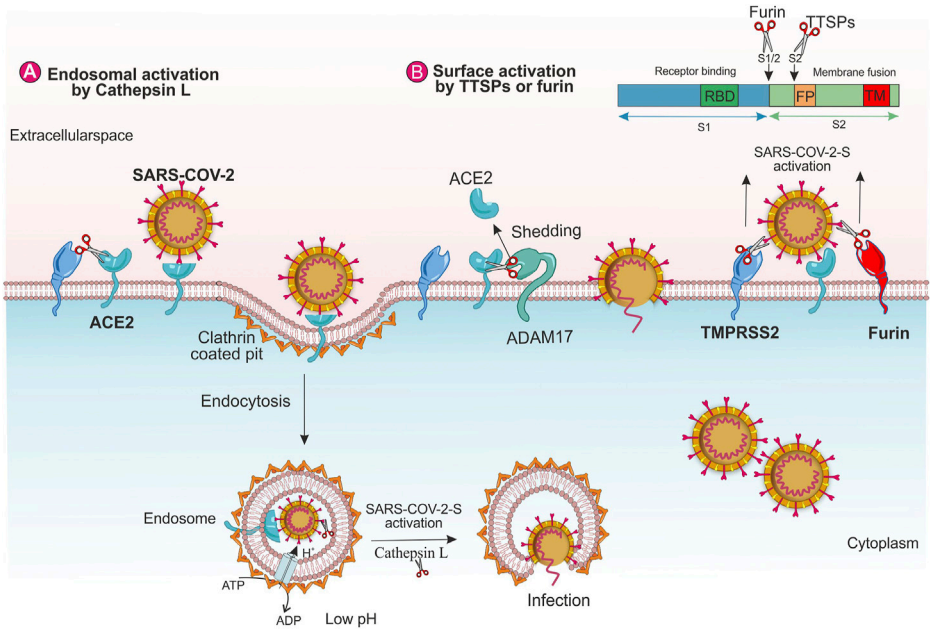

FIGURE 2 | The role of human host proteases on SARS-CoV-2 entry. Virus entry through **(A)** endosomal pathway and **(B)** TMPRSS2 and furin.

SARS-COV-2 VIRION

The SARS-CoV-2 virion is a non-segmented RNA-positive virus (Zumla et al., 2016). Its genome encodes nucleocapsid (N), membrane (M), envelope (E), and spike (S) structural proteins. The M and E proteins are located among the spike proteins in the viral envelope (**Figure 1A**). The virion has a nucleocapsid composed of a single-stranded and positive-sense RNA in a size of 29.9 kb and highly immunogenic phosphorylated N-protein that is covered by the S and the hemagglutinin-esterase (HE) spike proteins, buried inside the phospholipid bilayers (Wu et al., 2020a; Jin et al., 2020).

The SARS-CoV-2 genome has a highly similar identity to the human SARS-CoV (80%) (Matsuyama et al., 2010), hence, they have analogous pathogenesis and biochemical interactions. The S protein eases receptor-binding and viral entrance into the target cells by the fusion of the viral and host cell membranes; hence, it can determine the host range (Heurich et al., 2014; Hoffmann et al., 2020a). Moreover, the spike S is the common target for vaccines and neutralizing antibodies. Spike protein has two functional subunits; the surface unit (S1) and S2 (**Figure 1B**). The S1 recognizes a cellular receptor by a receptor-binding domain (RBD), mediating viral attachment to the host ACE2. The S2 subunit has other basic features needed for the fusion of the viral and cell membranes. The high cleavable properties of the S1/S2 cleavage site of SARS-2-S are attributed to its multi-basic structure (several arginine residues).

In the CoVs, the RBD can be in a standing-up or a lying-down state. The first state permits receptor binding; however, the second state does not (Yuan et al., 2017). SARS-CoV-2-S is frequently in the lying-down state in comparison to SARS-CoV; consequently, despite having high affinity, it is less available to the ACE2. This difference results in a lower or comparable receptor binding affinity for the SARS-CoV-2 whole spike (Shang et al., 2020). The SARS-CoV-2' RBD has a greater affinity to bind to the ACE2 than SARS-CoV; conversely, the whole spike glycoprotein of SARS-CoV-2 cannot bind to human ACE2 stronger than SARS-CoV' spike (Shang et al., 2020). To preserve its RBD less accessible while retaining its high infectivity, SARS-CoV-2 depends on a second strategy; the activation of host proteases (**Figure 1C**).

ENTRY AND PRIMING OF SARS-COV-2 BY THE HOST PROTEASES

The high binding affinity of SARS-CoV-2 to the human receptor is one reason why it is more hostile than SARS-CoV (Wu et al., 2020a; Lu et al., 2020). The viral infectivity highly relies on the cleavage of the S protein by the cell proteases that happens during different steps in the viral life cycle (Heurich et al., 2014). Two cellular proteolytic systems were utilized by SARS-CoV in order to guarantee the adequate processing of viral S protein (**Figure 2**). The SARS-CoV-2 interaction with the human cells is similar to SARS-CoV (Ragia and Manolopoulos, 2020). The proteolysis of the two peptide bonds (i.e., Arg685-Ser686) results in the so-called S1 and S2 subunits separation and the subsequent activation/priming of the S protein of SARS-CoV-2 (Fuentes-Prior, 2021). The presence of receptor-binding domain (RBD) in the N-terminal domain (NTD) of the S1 subunit causes direct binding to the peptidase domain of ACE2, whereas, the C-terminal of the S2 subunit is responsible for its attachment viral envelope after proteolysis at the S1/S2 site and eventually leads to its fusion with the host cell membrane (Benton et al.,

2020; Ragia and Manolopoulos, 2020). Furin-mediated proteolysis results in the pre-activation of the S proteins in the SARS-CoV-2 virions besides entailing the cleavage of a single bond, Arg815-Ser816, for fusion machinery activation. The abovementioned exclusive properties of SARS-CoV-2 are indispensable for S protein-mediated cell–cell fusion and human cell entry (Fuentes-Prior, 2021). Additionally, early priming of the S protein depends on human TMPRSS2, which is necessary for SARS-CoV-2 entry (Ragia and Manolopoulos, 2020).

The binding of the SARS-S to the ACE2 can promote the virions endocytosis. It is reported that Cathepsin L cleaves the S protein of SARS-CoV-2 functionally and promotes viral entry (Zhao et al., 2021). SARS-S/ACE2 complex generates conformational changes in SARS-S, which in turn, may raise the sensitivity of the spike to proteolytic enzymes. Inside the endosome, Cathepsin L, a pH-dependent endosomal or lysosomal protease, facilitates the cleavage of SARS-S and activates the S protein for fusion within the endosomal membrane. During endosome maturation, the low pH of the endosomal environment activates the re-arrangement of HA that exposes the viral fusion peptide into the endosomal membrane (Shen et al., 2017; Simmons et al., 2013), (**Figure 2A**). Moreover, SARS-CoV-2 induces the transcription and enzyme activity of Cathepsin L, which, in turn, elevates viral infection (Chandran et al., 2005; Zhao et al., 2021).

HOST CELL PROTEASES; GENERAL DEFINITION

The SARS-CoV-2's spike protein has cleavage sites for the host cell proteases guaranteeing the exposure of the fusion sequences and viral entry. It is observed the that lysosomal and cell surface proteases can both activate SARS-CoV-2 entrance. Furthermore, other proteases such as furin have accumulative impacts on the entrance of SARS-CoV-2 (Shang et al., 2020).

Furin

Furin and furin-like proteases are members of the proprotein convertases family. Furin, a kind of proprotein convertase, is a type I transmembrane protein expressed in all eukaryotic cells. It is activated by acid pH in the trans-Golgi network (Feliciangeli et al., 2006). Furin plays a key role in the cleavage of a wide range of critical cell surface proteins including adhesion molecules, surface receptors, growth factors, and hormones to produce mature proteins. Additionally, furin cleaves envelope glycoproteins of different viruses, thus, improving the fusion of viral membrane with the host cell (Garten, 2018). Data indicate that the existence of a redundant furin cleavage site at S protein of SARS-CoV-2 is responsible for its infectious nature than other CoVs, leading to its higher efficiency to fuse to the host membrane (Wu et al., 2020b).

TMPRSS2

TTSPs, a group of membrane serine proteases, occupy a vital role in numerous physiological procedures (Antalis et al., 2010; Antalis et al., 2011; Hoffmann et al., 2018). These enzymes are characterized by an extracellular C-terminal domain with the serine protease activity, a single transmembrane domain, and a short cytoplasmic N-terminal domain. Böttcher et al. considered the TTSPs as the activators of viral infection. They indicated that the TMPRSS2 and the HAT (a trypsin-like protease) result in the activation of the FLUAV-HA and the spread of the FLUAV in the infected host (Hoffmann et al., 2018). TMPRSS2 is a single-pass cell membrane-anchored TTSP protein with 492 amino acids expressed on epithelial cells of some tissues and found to regulate cell-matrix and cell-cell interactions. TMPRSS2 expression seriously stimulates the replication and syncytium formation of coronaviruses *in vitro* and *in vivo* (Matsuyama et al., 2010; Shulla et al., 2011; Shirato et al., 2013; Simmons et al., 2013; Iwata-Yoshikawa et al., 2019a), exerting an important role in their spread (Iwata-Yoshikawa et al., 2019b). Furthermore, the TMPRSS2 might stimulate viral pathogenesis and spread *via* activating spike S for virus-cell and cell-cell fusion and neutralizing antibodies that decrease viral recognition (Glowacka et al., 2011).

Cathepsins

Human cathepsins are endosomal proteases with broad proteolytic activity in acidic pH. Cathepsins activation in lysosomes may exert an important role in SARS-CoV and MERS-CoV entry via endocytosis (Sahebnasagh et al., 2020). Lysosomal cathepsin L cleaves peptide bonds with hydrophobic residues in the P3 position and aromatic residues in the P2 position (Vargas-Alarcón et al., 2020). Cathepsin L incorporates in glycoprotein processing of SARS-CoV and Ebola (Pišlar et al., 2020; Vargas-Alarcón et al., 2020). Recent investigations assessed cathepsins' role in SARS-CoV-2 entry. Further, the role of cathepsin L is highlighted in virion entry into human embryonic kidney 293 cells expressing ACE2; however, CA-074 did not significantly affect SARS-CoV-2 entry (Pišlar et al., 2020).

Neutrophil Elastase

Neutrophils as a part of the host defense system, release a granular serine protease (the so-called elastase) in response to aviral infection (Thierry, 2020; Vargas-Alarcón et al., 2020). Increased elastase activity mediates acute lung injury through increasing inflammatory reactions (e.g., increasing vascular permeability, induction of pro-inflammatory cytokines such as IL-8 and IL-6 secretion by neutrophil vesicles, and conversion of pro-IL-1β to IL-1β) (Bai et al., 2020). Under normal conditions, the NE's function is regulated by the inhibitors of endogenous protease. Nevertheless, under pathophysiological conditions, neutrophil oxidants inactivate the aforementioned inhibitors which result in hydrolyzing the host extracellular matrix proteins such as collagen-IV as well as elastin and subsequent damage to the endothelial barrier and infiltration into the bronchoalveolar space (Korkmaz et al., 2020; Thierry, 2020).

Plasmin

Pathogens, especially viruses, convert plasminogen to plasmin in order to cleave surface proteins and subsequently can evade the

immune system or infect host cells (Medcalf et al., 2020). Plasmin can cleave new furin sites in SARS-CoV S protein which in turn results in increased viral infectivity (Ji et al., 2020). In addition to the contribution of early stages of a viral infection, plasmin can provoke cytokine production and stimulate inflammation *via* factor XII/bradykinin which subsequently can increase edema (Medcalf et al., 2020). The elevated levels of plasmin (ogen) were observed in COVID-19 patients (Ji et al., 2020). Plasmin can cleave hyaline membranes (consist of a fibrin network combined with serum proteins and cellular debris, acts as barriers to gas exchange in the alveoli), which is considered asa histopathological hallmark of acute respiratory distress syndrome (ARDS) induced by SARS-CoV-2. In patients affected by the ARDS, bronchoalveolar lavage samples showed elevated levels of active plasmin and plasminogen (Henry et al., 2020).

THE FUNCTIONAL ROLE OF HOST PROTEASES IN SARS-COV-2 INFECTION

The Role of Furin in SARS-CoV-2 Infection

Bioinformatics analysis on the SARS-CoV-2-S sequences has anticipated a novel polybasic furin cleavage site containing an insertion of amino acid residues (–PRRA–) between S1 and S2 subunits (**Figure 1C**). Based on the genomic characteristics of SARS-CoV-2, furin can cleave the viral spike protein at that site and activate it (Mallapaty, 2020). Coutard et al. identified a furin-like cutting site in the SARS-CoV-2-S which is not available in other SARS-like CoVs such as Pangolin, Bat-CoV, and SARS-CoV-1. MERS has a pseudo-furin- binding site (Coutard et al., 2020; Vankadari, 2020). Likewise, Vankadari found that structurally, furin interacts with the SARS-CoV-2-S that highlighted the mechanism of viral host cell entry (Vankadari, 2020).

In lung cells that highly express furin and fail to express strong cathepsin L levels, pre-cleavage of the S proteins by furin is needed for consequent activation of spike protein by TMPRSS2 in both MERS-CoV and SARS-CoV-2 (Hoffmann et al., 2020b). In order to initiate the membrane fusion of viral and human cells along with the passage of viral genome into the cytoplasm of the host cell, viral spike S glycoprotein needs to be sequentially cleaved at S1/S2 and S2' "sites. Furin cleaves the S1/S2 site, while the TMPRSS2 processes at the S2" site, and these enzymes cannot compensate for each other. Finally, at the Golgi or endoplasmic reticulum compartment, nascent viruses are assembled and released from the infected cells by exocytosis (Hoffmann et al., 2018). Preactivation of furin permits SARS-CoV-2 to be less reliant on host cells, improving its entry into some target cells that relatively express low levels of lysosomal cathepsins and/or TMPRSS2 (Shang et al., 2020).

It is revealed that the furin enzyme is expressed in other potential target organs of the coronavirus such as the intestine, colon, ileum, rectum, heart, and oral mucosa tissues (Dittmann et al., 2015; Mei et al., 2020). The furin universal expression in some tissues and organs may be an explanation for the high pathogenicity and transmissibility of SARS-CoV-2 (Wang et al., 2020a). Therefore, in the course of SARS-CoV-2 infection, the presence of furin may result in some clinical symptoms (Dittmann et al., 2015), for example, the furin-mediated entrance of SARS-CoV-2 into the cardiomyocyte may clarify the cardiac injury in patients with COVID-19 (Dittmann et al., 2015). Moreover, the furin protease activity in the oral mucosa tissues makes them susceptible to SARS-CoV-2 and may be associated with oral symptoms in COVID-19 like taste blindness and dry mouth (Mei et al., 2020).

The Functional Role of TMPRSS2 in Viral Infection

Accumulating evidence reveals that SARS-CoV-2 and relevant viruses such as MERS-CoV, influenza A virus (FLUAV), and SARS-CoV require TMPRSS2 activity as a host cell factor for their spread (Hoffmann et al., 2020a; Kim J et al., 2020). Moreover, it is reported that the VeroE6 cell line, expressing TMPRSS2, is very vulnerable to SARS-CoV-2 infection; indicating that similar to MERS-CoV and SARS-CoV, SARS-CoV-2 infection is boosted by TMPRSS2 (Matsuyama et al., 2020). Furthermore, both lysosomal cathepsins and TMPRSS2 have accumulative effects with a calcium-dependent proprotein/prohormone convertase (furin) on activating the entrance of SARS-CoV-2 in some cells such as lungs. (Shang et al., 2020).

TMPRSS2, by cleaving the SARS-CoV-2-S facilitates virus entrance and activation (Hoffmann et al., 2020a). The co-expression of TMPRSS2 in ACE2-positive lung cells suggests that it exerts a critical role in the spread of the virus in the human respiratory tract (Heurich et al., 2014).

TMPRSS2 eases the infection of SARS-CoV by two independent mechanisms; a) by ACE2 cleavage that enhances the viral entry and stimulates viral uptake by cathepsin L-dependent entry, not activating SARS-S for entry (**Figure 2A**), b) by SARS-S cleavage at the host cell surface that activates the spike protein for membrane fusion (**Figure 2B**) (Heurich et al., 2014). Two amino acid residues (arginine and lysine) within the ACE2 (697–716 and 652–659) are critical for TMPRSS2 and the metalloprotease ADAM17, respectively. These enzymes compete together for the ACE2 cleavage; however, only TMPRSS2-mediated-ACE2 cleavage stimulates SARS S-driven entry (Shulla et al., 2011; Heurich et al., 2014). The ADAM17 eases the ACE2 shedding into the extracellular space and stimulates SARS-CoV uptake into the host cells (**Figure 2B**). Additionally, ADAM17 facilitates the release of the TNF-α (tumor necrosis factor-α) and IL-6 receptors. The TNF-α exerts autocrine and paracrine function and TNF-α/its receptor signaling elevates ADAM17 activity. Viral infection and endocytosed SARS-CoV-2 spike proteins also elevate ADAM17 activity. An increased ACE2 shedding by ADAM17 leads to the down-regulation of ACE2 that increases the angiotensin II levels, resulting in further rises in ADAM17 activity (Gheblawi et al., 2020).

Different residues (R667 and R797) in SARS-S control the TMPRSS2-mediated S activation indicating that these procedures are more complex than initially appreciated (Reinke et al., 2017). Glowacka et al. concluded that based on the location of the TMPRSS2, the processing of the SARS-S cleavage by host cell

TMPRSS2 can have different results. In the secretory pathway of infected cells, when the TMPRSS2 is co-expressed with SARS-S in the same cell, spike cleavage leads to the shedding of SARS-S into the supernatants, and as antibody decoys, the S protein fragments inhibit antibody-mediated neutralization (Glowacka et al., 2011).

Evidence proposes that the TMPRSS2 may control the function of mitochondria through the estrogen-related receptor-α (ERR-α) (Xu et al., 2018). ERR-α, a nuclear receptor, along with its coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) regulate mitochondrial functions and energy homeostasis at the transcriptional level (Xu et al., 2018). Mitochondrial hijacking by SARS-2 may be one of the underlying mechanisms leading to COVID-19. Few human genes including a subunit of ubiquitin-protein ligase complex (FBXO21) and mitochondrial ubiquitin specific peptidase 30 (USP30) appear to be targeted by viral RNA (Pasquier and Robichon, 2020). During infection, viral RNAs can be translocated into the mitochondria to hijack and utilize host mitochondria. By modifying ubiquitination and impacting mitochondrial function, SARS-CoV-2 can repress host immunity in COVID-19 cases *via* different mechanisms. One possible viral mechanism can be mediated by regulating the host USP30. USP30 regulates the mitochondrial homeostasis and dynamics (fusion and fission). The presence of 20 nucleotides in open-reading frame 3a (ORF3a) of SARS-CoV-2 can target a sequence in mitochondrial USP30 transcripts (Pasquier and Robichon, 2020). Utilizing the host mitochondria by viral ORFs can result in mitochondrial DNA (mtDNA) release in the cytoplasm that activates the mtDNA-induced inflammation and represses both innate and adaptive immunity. Finally, the mitochondrial collapsing by virus results in the death of the infected cells (Singh et al., 2020).

The TMPRSS2 Gene

The *TMPRSS2* gene consists of 14 exons and 13 introns (44 kb in length) and is located on human chromosome 21. It is more expressed in prostate cancer cells (Paoloni-Giacobino et al., 1997). One of the important characteristics of the *TMPRSS2* gene is located at position −148 with several 15-bp androgen response elements (AREs) at the upstream of the transcription start origin (Lin et al., 1999; Afar et al., 2001). In prostate cancer cells, androgenic hormones up-regulate the *TMPRSS2* gene which probably is mediated by the androgen receptor (Antalis et al., 2011; Shen et al., 2017; Ashour et al., 2020). Studies in knock-out murine models revealed that these mice are resistant to the spread and pathogenesis of some subtypes of the FLUAV. As mentioned earlier, TMPRSS2 can be regulated by androgen and its receptor (Chen et al., 2019) and the presence of AREs on the promoter of the *TMPRSS2* gene may be the underlying cause of the severity and higher mortality of COVID-19 in men (Zununi Vahed et al., 2020). Moreover, the promoter of the human *TMPRSS2* gene has a guanine-rich region forming G-quadruplex secondary structures that can block or reduce *TMPRSS2* transcription in the presence of potassium ions (Shen et al., 2020). Furthermore, studies regarding the *TMPRSS2* gene polymorphisms that cause *TMPRSS2* gene overexpression in humans, showed its association with severe influenza (Hoffmann et al., 2018). It is demonstrated that genetic variation in TMPRSS2 including two identified SNP in *TMPRSS2* (rs383510 and rs2070788) had a strong correlation with the A (H7N9) influenza susceptibility (Cheng et al., 2015).

Complete computational analyses indicated that functional single nucleotide polymorphisms of *TMPRSS2* gene and epigenetic mechanisms play important roles in the diverse susceptibility of different populations to SARS-CoV-2 (Paniri et al., 2020). *TMPRSS2* genetic variants including rs383510, rs2070788 37, rs469390, and rs464397 strongly increase the TMPRSS2 expression in lung tissue, where these variants at higher frequencies are present in European and American populations than the Asian populations. This result suggests these populations may be quite more vulnerable to SARS-CoV-2 infection (Irham et al., 2020).

TMPRSS2 Expression in Different Cells

Remarkably, the TMPRSS2 has a highly variable expression in humans and its expression may be positively associated with COVID-19 severity. The TMPRSS2 is mainly expressed in the epithelium cells of the prostate and has a vital role in its carcinogenesis (Lucas et al., 2008). Matsuyama et al. (2010) indicated an interaction of the TMPRSS2-expressing cells with viral tropism and pathogenicity of SARS-CoV infection. The TMPRSS2 is also expressed in the lungs (Bertram et al., 2012), digestive tract, kidney, the cardiac endothelium proposing that these organs may be essential targets for SARS-CoV-2 infection (Bertram et al., 2012). Indeed, the COVID-19 clinical manifestations include complications from gastrointestinal symptoms, higher liver enzymes, acute kidney injury (AKI), and acute myocardial damage. It was approved that the expression of the ACE2 and the TMPRSS2 provides SARS-CoV-2 entrance on the ocular surface cells. Moreover, the co-express of the TMPRSS2 and the ACE2 in the prostate epithelial cells may be involved in more pathogenicity of COVID-19 disease in males than females (Song et al., 2020). The TMPRSS2 and TMPRSS4 promote virus entry into the intestinal cells by facilitating SARS-CoV-2 spike fusogenic activity (Zang et al., 2020).

Based on the different datasets of gene expression, it is found that the expression levels of the TMPRSS2 and the ACE2 are significantly higher in the nasal epithelium in comparison to saliva and blood, where their levels decrease in lower airway tissues. Significantly, the expression levels of these genes in the bronchial and nasal tissues are lower in children than adults. The result of this study indicates that the severity of COVID-19 between adults and children, in part, can be attributed to the different expression levels of the TMPRSS2 and the ACE2 in airways tissues (Saheb Sharif-Askari et al., 2020).

Mechanisms that influence SARS-CoV-2 infectivity and clinical outcomes of COVID-19 are reported by analyzing the nasal airway transcriptome of children. In this study, it is found that the ACE2 expression is upregulated by interferon response to respiratory viruses. Moreover, the action of IL-13a upregulates the *TMPRSS2* as a mucus secretory network gene (Sajuthi et al., 2020).

TABLE 1 | A list of host protease inhibitors against COVID-19

Targets/Inhibitors	Mechanism of action	Studied models	References
Furin protease inhibitors			
MI-1851	-Inhibits virus entry by preventing furin cleavage at S1/S2 site of S protein -Inhibits SARS-CoV-2 replication	*In vitro*	Bestle et al. (2020a)
Diminazene (an anti-parasitic drug)	It occupies the substrate-binding pocket of furin.	*In vitro*	Wu et al. (2020b)
Decanoyl-RVKR-chloromethylketone (CMK)	CMK blocks SARS-CoV-2 entry, suppresses cleavage of spikes and the syncytium. Also, it affects the early stage of the virus replication cycle	*In vitro*	Cheng et al. (2020)
Naphthofluorescein	It suppresses SARS-CoV-2 RNA transcription rather than virus entry.	*In vitro*	Cheng et al. (2020)
TMPRSS2 protease inhibitors			
MI-432	Inhibits virus entry by hindering TMPRSS2 cleavage at S2 site of S protein	*In vitro*	Meyer et al. (2013)
MI-1900			
Aprotinin	-Inhibits virus entry by hindering TMPRSS2 cleavage at S2 site of S protein-Prevents double-stranded RNA formation in SARS-CoV-2 infected cells	*In vitro*	Bojkova (2020)
Excavatolide M, Dictyosphaeric Acid A, Durumolide K, Schisphenin ACytidine (5)-Diphosphocholine (Citicoline), 5-Methoxyhydnocarpin D Polyphenol (-)-Epicatechin 3-O-(30-O-Methyl) Gallate, Curtisian L, Microcarpin, Geniposide, NPC306344, Isogemichalcone B	These compounds interact with the active site residues of TMPRSS2 and inhibit it	In silico	Rahman et al. (2020)
Nafamostat mesylate	Prevents S-glycoprotein activation by inhibiting TMPRSS2	Clinical trial	
Camostat mesylate	-Decreases SARS-S-, MERS-S-, and SARS-2-S- significantly -Decreases authentic SARS-CoV-2 infection in the Calu-3 lung cell line -Inhibits SARS-S- and SARS-2-S entry into primary human lung cells	*In vitro*	Hoffmann et al. (2020a)
	Beyond its antiviral activity, camostat may decrease the uncontrolled cytokine storm observed in severe COVID-19, since the expression of TMPRSS2 is necessary for cytokine release upon exposure of mice to polyIC	*In vivo*	Iwata-Yoshikawa et al. (2019c)
	- when applied with inhibitor E-64d, completely blocks the SARS-2-S-driven entry -Partially blocks SARS-CoV-2-S and SARS-CoV-driven entry by inhibiting the TMPRSS2	Clinical trial	
Gabexate			
Bromhexine		Clinical trial	Ansarin et al. (2020)
Cathepsin B and L inhibitors			
CA-074 (#HY-103350): an inhibitor of Cathepsin B	Had no marked effect on virus entry		Sahebnasagh et al. (2020)
E64D: an endosomal cysteine proteases (CatB/L) inhibitor	Reduced entry of SARS-CoV-2 S pseudovirions	*In vitro*	Sahebnasagh et al. (2020)
	Had no effect on virus replication in Calu-3 (human airway epithelial cells)	*In vitro*	Bestle et al. (2020a)
	E63D interfere efficiently with SARS-2-S-driven entry into the TMPRSS2-cell lines 293 T and Vero	*In vitro*	Hoffmann et al. (2020e)
Dalbavancin (a lipoglycopeptide antibiotic) :an inhibitor of Cathepsin L	By preventing cathepsin L in the late endosome/ lysosome represents an antiviral effect. in a dose-dependent manner it could hinder the entry of SARS-CoV-2	*In vitro*	Zhang (2020)
Teicoplanin (a glycopeptide antibiotic): an inhibitor of Cathepsin L	Prevents HIV-luc/2019-nCoV-S pseudoviruses entry in a dose-dependent manner	*In vitro*	Zhang (2020)
SID 26681509 (#HY-103353): an inhibitor of Cathepsin L	Reduced entry of SARS-CoV-2 S pseudovirions in 293/ hACE2	*In vitro*	Sahebnasagh et al. (2020)
Other protease inhibitors			
BenHCl, an inhibitor of Factor Xa	Factor Xa could cleave the full-length recombinant S protein into S1 and S2 subunits, and this cleavage		Du et al. (2007)
Rivaroxaban, apixaban, edoxaban, andbetrixaban, inhibitors of Factor Xa	They present anti-inflammatory, antiviral, and anticoagulants effects		Al-Horani (2020)

THERAPEUTIC STRATEGIES TO TARGET HOST PROTEASES FOR COVID-19

Unraveling the viral RBD features can open a new horizon to block the spike cleavage sites and develop protease inhibitors. Recent studies suggest that host proteases are vital for the activation of the SARS-CoV-2 in human epithelial cells, hence, they can be hopeful drug targets for the management of COVID-19 (Bestle et al., 2020a). Therapeutic inhibition of the TMPRSS2 and furin may be used as a therapeutic approach for

COVID-19. A list of host proteases inhibitors is listed in **Table 1**. In the following section, we review the potential drugs or compounds that may alter the TMPRSS2 expression and activity.

Modulating the Expression and Activity of TMPRSS2

Besides SARS-COV, the TMPRSS2 is critical for viral spread of the H3N2 influenza A virus (IAV) and mono-basic H1N1 virus (Hatesuer et al., 2013) and also for the replication and pathogenesis of the H10 subtype of IAV in mice (Lambertz et al., 2019), where knock-out Tmprss2 mice were resistant to the virus. This subtype can also infect humans emphasizing the significance of the TMPRSS2 for drug development against multiple IAV subtypes (Lambertz et al., 2019). The TMPRSS2 can also activate HCV infection at the entry and post-binding stages and involve in the persistence, pathogenesis, and sensitivity of HCV infection (Nickols and Dervan, 2007). Since the TMPRSS2 is involved in other viral infections such as coronavirus (MERS-CoV, SARS-CoV, hCoV-EMC, and HCoV-229E) (Glowacka et al., 2011; Bertram et al., 2013; Gierer et al., 2013; Shirato et al., 2013), hepatitis C virus (Esumi et al., 2015), and influenza A virus (Shen et al., 2020), it would be an attractive alternative against a wide spectrum of respiratory viruses, especially SARS-CoV-2. Taken together, the TMPRSS2 is a target for antiviral therapy.

As we mentioned earlier, the AREs are involved in the TMPRSS2 expression and can be striking drug targets. A polyamide compound can bind to the ARE in the TMPRSS2 promoter and moderately repress its expression (Nickols and Dervan, 2007). Wang et al. (2020) by mining publicly available data on gene expression, identified that the estrogen-related compounds including genistein, androgen receptor antagonist enzalutamide and estradiol can down-regulate the TMPRSS2. These reports suggest that the aforementioned drugs can be promising therapeutic candidates for the treatment of COVID-19. (Wang et al., 2020b).

The promoter of the human *TMPRSS2* gene has a guanine-rich region forming G-quadruplex secondary structures that can block or reduce the TMPRSS2 transcription in the presence of potassium ions (Shen et al., 2020). Benzoselenoxanthene analogs could significantly down-regulate the TMPRSS2 expression by stabilizing G-quadruplex structure and could prevent the growth and spread of influenza A virus *in vitro* (Shen et al., 2020). Therefore, the down-regulation of the TMPRSS2 mRNA through G-quadruplex structure stabilizers can be a promising strategy in developing novel small molecule drugs against SARS-CoV-2.

Antioxidants serve as regulators of the protease/antiprotease balance that can prevent viral infection. Antioxidants—the so-called free radical scavengers- are natural or man-made substances that can prevent or neutralize free radicals' damage to cells. It has been elucidated that the master antioxidant transcriptional factor (Nrf2) could down-regulate the expression of the TMPRSS2 in prostate cancer cell lines, thus causing alternations in the protease/antiprotease components balance and subsequently, and result in protection against the respiratory infections (Meyer and Jaspers, 2015). The Nrf2 has a critical role in the reduction of oxidative stress; besides, it exerts beneficial effects in respiratory epithelial responses to respiratory viral infection. Various types of antioxidants are available nowadays. Sulforaphane (SFN) is a potent antioxidant belonging to the class of isothiocyanates, a sulfur-containing organic compound naturally found in cruciferous vegetables (e.g., cauliflower and broccoli). It may decrease oxidative stress and inflammation besides exerting antimicrobial effects. It has been shown that the beneficial effects of the SFN supplementation depend on promoting the Nrf2, cellular antioxidants such as heme oxygenase-1 (HO-1) and NADPH quinone oxidoreductase 1 (NQO1) activities, thus, in turn, prevents the secretion of pro-inflammatory mediators. The SFN down-regulates TMPRSS2 levels and results in the protection against infection. Available reports elucidated that the Nrf2 negatively regulates the TMPRSS2 (Meyer and Jaspers, 2015). Kesic and colleagues demonstrated that the SFN exerts protective effects against respiratory viruses and reduces the IAV entry into respiratory epithelial cells probably as a result of a reduction in TMPRSS2 expression (Kesic et al., 2011).

In addition to the possible androgen receptor-targeted treatments to modify the expression of the TMPRSS2, impairing its protease activity would be an alternative approach. Camostat mesylate, known as FOY 305, is clinically used to treat chronic pancreatitis. It could protect cultured lung epithelia and mice from infection with the H1N1 influenza virus (Bahgat et al., 2011). Shirato et al. report that the treatment with camostat (a single dose) could sufficiently hamper the MERS-CoV entry into Calu-3 cells (a lung-derived cell line) and possibly into the lung (Shirato et al., 2013). Moreover, the viral multistep growth was significantly repressed and virus-induced host cell death was hindered. They concluded that the camostat-inhibited TMPRSS2 or other serine proteases may determine virus pathogenesis and tropism in the lung (Shirato et al., 2013). It is also reported that the SARS-CoV pathogenesis and spread can be efficiently prohibited by camostat (Zhou et al., 2015). This drug can target the TMPRSS2 protease and reduce the replication rate of SARS-CoV-2 (Hoffmann et al., 2020c).

Bromhexine hydrochloride (BRH) is a TMPRSS2 inhibitor that can attenuate metastasis in prostate cancer mice models (Lucas et al., 2014). Being an FDA-approved drug in mucolytic cough suppressants with no significant adverse effects, it can effectively be used against coronavirus infections (Barzegar et al., 2020; Wang et al., 2020c; Fu et al., 2020; Tolouian et al., 2020). In this regard, Li et al. conducted a clinical pilot study on Chinese patients to evaluate the beneficial effects of BRH tablets in moderate COVID-19 treatment. Their findings illustrated that BRH enhanced chest computed tomography (CT), the need for oxygen therapy, and the discharge rate within 20 days (Irham et al., 2020). Nafamostat –an anticoagulant- is another drug that can inhibit the activity of the TMPRSS2, so, it can reduce the viral entrance and block MERS-CoV infection *in vitro* (Yamamoto et al., 2016). Recently, evidence from the latest studies showed the advantages of nafamostat in COVID-19 patients (Asakura and Ogawa, 2020; Hoffmann et al., 2020d). A case study performed on three elderly COVID-19 patients in South Korea demonstrated

that nafamostat administration leads to disease prevention through regulating the complement cascade and blocking DIC. Furthermore, it may inhibit virus invasion by impeding virus fusion on the cell membrane (Jang and Rhee, 2020). Altogether, the TMPRSS2 has potential therapeutic benefits against respiratory coronavirus infections. Plasminogen activator inhibitor-1 (PAI-1) is an effective membrane-anchored serine protease inhibitor. It can inhibit TMPRSS2-mediated hemagglutinin cleavage and repress the influenza virus in animals.

Mc Cord et al. (2020) discovered that the PB125 could be used as a therapeutic agent in COVID-19 patients, evidenced by upregulated LIF, suppressed inflammatory responses, inhibited *TMPRSS*2 gene expression directly or by prevention of PAI-1, encoded by the SERPINE1 (Dittmann et al., 2015) and increased Nrf2 activity by HDAC5 downregulation (Hu et al., 2019). In the human airway epithelial cell line, aprotinin and synthetic inhibitors of the TMPRSS2 could inhibit the replication of SARS-CoV-2. Combining several inhibitors of the TMPRSS2 could result in a more effective antiviral activity against the virus than a single serine protease inhibitor. The top 12 natural compounds that significantly can interact with the active sites of the TMPRSS2 are reviewed by Rahman et al. (202).

Furin Inhibitors

Unlike the TMPRSS2, furin and furin-like enzymes are essential for several pathways and normal development, therefore, its prolonged blockade may lead to some adverse and toxic effects (Hasan et al., 2020). However, a brief furin inhibition may exert a therapeutic benefit and be tolerated (Sarac et al., 2002).

To achieve satisfactory outcomes, a mixture of protease inhibitors would be required. The combination of furin inhibitors that target different proteases of SARS-CoV-2 might be an interesting therapeutic strategy (Wu et al., 2020b). Moreover, a combined administration of furin and the TMPRSS2 inhibitors can be used to target both of these proteases. Evidence shows that the combination of furin inhibitor MI-1851 with several TMPRSS2 inhibitors (MI-1900 and MI-432) could produce more effective antiviral activity against the newly emerged virus than any single serine protease inhibitor (Bestle et al., 2020a).

Cathepsin Inhibitors

The inhibitors of cathepsin B (CA-074), cathepsin L (SID 26681509), and calpain (E64D) were tested in HEK 293/hACE2 cells. The findings illustrated that 293/hACE2 cells treated with E64D, had a decreased entry of the SARS-CoV2 S pseudovirions (about 92.5%), which in turn highlight the role of at least one of the calpain or cathepsins for SARS-CoV-2 entry. Moreover, treatment with cathepsin L inhibitor by 76% reduced the entry of SARS-CoV-2 S pseudovirions, indicating the probable role of lysosomal cathepsin L in priming SARS-CoV-2 S protein in 293/hACE2 cells. However, cathepsin B inhibitor showed no significant effects on virus entry (Korkmaz et al., 2020; Sahebnasagh et al., 2020). Applying both the E-64D and camostat mesylate results in a complete inhibition of SARS-CoV-2 S protein-driven entry into Vero-TMPRSS2 cells and Caco-2 cells which proposed that both cathepsins B, L and TMPRSS2 is required for priming cells (Hoffmann et al., 2020a). The inhibition of cathepsin L offers two possible steps for the coronavirus infection, blocking virus entry on the host cell surface and viral material release and replication inside the host cell endosomes (Liu et al., 2020).

Other Protease Inhibitors

Utilizing sivelestat- a neutrophil elastase inhibitor- leads to acute lung injury (ALI) alleviation *via* enhancing alveolar epithelium, vascular endothelium injuries and reducing vascular permeability induced by neutrophils. Though it can be considered as a novel treatment approach in controlling ALI/ARDS or coagulopathy in COVID-19 patients (Sahebnasagh et al., 2020). The suppression of plasmin activity by antiproteases may prevent SARS-CoV-2 entry into respiratory cells and alleviate the clinical outcomes of COVID-19 patients (Ji et al., 2020).

CLINICAL TRIALS

Ansarin et al. for the first time performed an open-label randomized clinical trial, to investigate the efficacy of early administration of bromhexine in patients with COVID-19 pneumonia in Tabriz, Iran. The oral administration of bromhexine results in a significant reduction in ICU admissions, intubation and mortality in the treated group in comparison to the control group. Furthermore, bromhexine treatment exhibited an improvement in C-reactive protein (CRP), lactate dehydrogenase (LDH), and neutrophil/lymphocyte ratio (NLR) levels within two weeks when compared to the control group. However, their results demonstrated that there was no significant difference in the length of hospital stay among treated and control groups (Ansarin et al., 2020).

Li and colleagues conducted an open-label randomized controlled pilot study to examine the efficacy and safety of bromhexine hydrochloride in the treatment of moderate COVID-19. The oral administration of bromhexine hydrochloride (for 14 consecutive days) improved chest computed tomography, a need for oxygen therapy, and discharge rate in 20 days. Nevertheless, their data were not statistically significant (Shang et al., 2020).

Hofmann-Winkler et al. evaluated the efficacy of camostat mesylate in critically ill COVID19 patients with organ failure admitted in ICU of University Hospital Göttingen, Germany. Their findings revealed that the Sepsis-related Organ Failure Assessment score declined in the camostat mesylate-treated group; however, in the hydroxychloroquine group it remained high. Besides, camostat mesylate administration results in a reduction of disease severity, inflammatory markers and amelioration of oxygenation within 8 days in comparison to patients receiving hydroxychloroquine (Hofmann-Winkler et al., 2020). Doi et al. reported the efficacy of nafamostat

mesylate in combination with favipiravir in critically ill COVID19 patients admitted to the ICU at The University of Tokyo Hospital. Nafamostat therapy targets the virus entry in host epithelial cells and impedes intravascular coagulopathy (Doi et al., 2020).

CONCLUSION

Unique landscapes of SARS-CoV-2 entry are low frequency of RBD standing up that is implicated for immune evasion; moreover, its RBD has a high binding affinity to hACE2 that provides an efficient entry. Finally, the pre-activation of the spike by furin enhances viral entry into some cells. All these features compensate for SARS-CoV-2' hidden RBD and possibly permit the virus to preserve an effective cell entry yet evading immune surveillance. All these characteristics may be responsible for the viral widespread.

Among different host factors that are involved in the entrance of SARS-CoV-2, transcriptional inhibition of the TMPRSS2 seems to be a hopeful strategy. As with scientific coincidences, our TMPRSS2 insights are obtained from cancer research. TMPRSS2 inhibitor could decrease prostate cancer severity in a mouse model. Additional evidence for effective inhibition of the TMPRSS2 comes from a report indicated that aprotinin could successfully inhibit influenza virus infections (Zhirnov, 1987), although the HA activating protease TMPRSS2 was unknown at that time—thereafter identified by Böttcher et al. (2006). Since the TMPRSS2 is vital for SARS-CoV-2 entry into the host cells, we propose that the same TMPRSS2 inhibitors may decrease or prevent SARS-CoV-2 infection and can be an affordable medicine in blocking the TMPRSS2. The results of our clinical trial supported this idea and indicated that the administration of bromhexine is promising in the early stage of the COVID-19. It should be noted that transcriptional inhibition of the TMPRSS2 may not be destructive since it seems the TMPRSS2 has no important role in any organ and its blockade does not compromise normal development and homeostasis in the host. However, there are conflicting data on the role of TMPRSS2 blocking in the prevention and/or treatment of COVID-19. Well-designed and large-scale clinical trials are required to shed light on this issue in clinical practice.

AUTHOR'S CONTRIBUTION

MA and SZV developed the concept and designed the study. SMHK and YRS did systematic search and prepared the first draft. All authors participated in the revising of the manuscript before submission.

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The Molecular Basis of Gender Variations in Mortality Rates Associated With the Novel Coronavirus (COVID-19) Outbreak

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Since the outbreak of the novel coronavirus disease (COVID-19) at the end of 2019, the clinical presentation of the disease showed a great heterogeneity with a diverse impact among different subpopulations. Emerging evidence from different parts of the world showed that male patients usually had a longer disease course as well as worse outcome compared to female patients. A better understanding of the molecular mechanisms behind this difference might be a fundamental step for more effective and personalized response to this disease outbreak. For that reason, here we investigate the molecular basis of gender variations in mortality rates related to COVID-19 infection. To achieve this, we used publicly available lung transcriptomic data from 141 females and compare it to 286 male lung tissues. After excluding Y specific genes, our results showed a shortlist of 73 genes that are differentially expressed between the two groups. Further analysis using pathway enrichment analysis revealed downregulation of a group of genes that are involved in the regulation of hydrolase activity including (CHM, DDX3X, FGFR3, SFRP2, and NLRP2) in males lungs compared to females. This pathway is believed to be essential for immune response and antimicrobial activity in the lung tissues. In contrast, our results showed an increased upregulation of angiotensin II receptor type 1 (AGTR1), a member of the renin-angiotensin system (RAS) that plays a role in angiotensin-converting enzyme 2 (ACE2) activity modulation in male lungs compared to females. Finally, our results showed a differential expression of genes involved in the immune response including the NLRP2 and PTGDR2 in lung tissues of both genders, further supporting the notion of the sex-based immunological differences. Taken together, our results provide an initial evidence of the molecular mechanisms that might be involved in the differential outcomes observed in both genders during the COVID-19 outbreak. This maybe essential for the discovery of new targets and more precise therapeutic options to treat COVID-19 patients from different clinical and epidemiological characteristics with the aim of improving their outcome.

Keywords: gender, transcriptomics, COVID-19, ras, hydrolase activity, sex-based immunological differences

INTRODUCTION

Since the outbreak of the novel coronavirus disease (COVID-19) in the end of 2019, this disease has become a public health emergency with a global impact that attracts international interest (Wenham et al., 2020). While most of the COVID-19 patients were found to suffer from only mild to moderate symptoms, the other 19% of patients suffer from a more severe disease which in some cases progress to a critical condition (Wu and McGoogan, 2020). Interestingly, the fatality rate showed great variability between various populations as well as risk groups. This was attributed to several factors including age, presence or absence of co-morbidities like obesity, diabetes, cardiovascular diseases as well as chronic respiratory diseases (Caramelo et al., 2020). For that reason, better understanding and early identification of risk factors that might predispose to more aggressive clinical course may be essential for the adoption of more effective management strategies including early intensive care and more personalized therapeutic options.

Interestingly, while several reports from different parts of the world revealed an equal distribution of cases between men and women, the mortality rate showed a significant difference between both genders with men forming around two-thirds of the deceased patients compared to only one third of women (Huang et al., 2020; Epidemiology Working Group for NCIP, 2020; Zhou et al., 2020).

This difference in mortality rates can also be attributed to gender-related factors including hormonal variations. This was supported by *in vivo* studies which showed that ovariectomized females in addition to male animals had higher levels of ACE2 activity compared to non-ovariectomized females. Indeed this indicates the possible role of sex hormones in regulation of ACE2 activity Walter and McGregor (2020), which was found to be essential for the COVID-19 virus binding and entry to the host cells in both upper and lower respiratory tracts (Markus, 2020).

Social and behavioural differences such as smoking and alcohol consumption, which are closely associated with comorbidities including cardiovascular and lung diseases The Lancet (2020), were also proposed as essential factors that might contribute in the variable mortality rates between both genders. Sex-based immunological differences were also suggested to play a role in this variation (Chen et al., 2020). For example, while the IgG antibody levels in both genders were found to be similar in the mild cases of COVID-19, female patients with severe cases showed a significantly higher levels of SARS-CoV-2 IgG antibodies compared to male patients (Zeng, 2020).Other mechanisms that were proposed to play a role in the higher vulnerability rate of males in COVID-19 pandemic is the gender-defined genetic polymorphisms and molecular variations (Zeng, 2020).

While these observations showed a possible role of gender-related genetic and molecular variations in determining the clinical behaviour of the disease including the higher mortality rate in male patients compared to females, the full mechanisms underlying such differences still need to be more clarified.

Indeed, a better understanding of the molecular mechanisms that differentially affect males and females leading to variable infection vulnerability as well as mortality is essential for the discovery of novel pathways and targets. This might help in the implementation of new therapeutic options aiding in a more effective, personalized, and comprehensive approach to treat the COVID-19 outbreak.

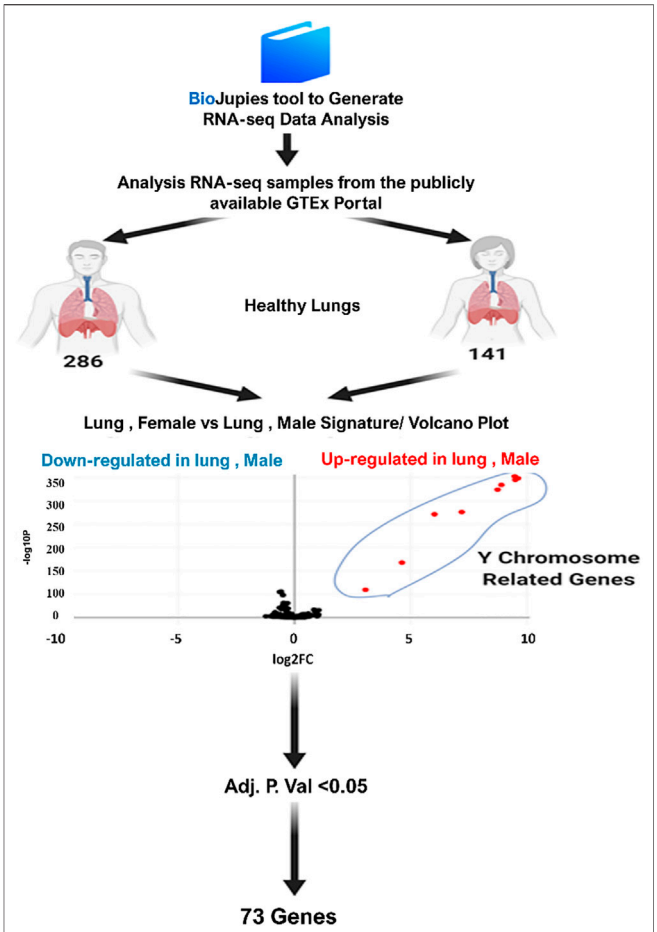


FIGURE 1 | Schematic representation to bioinformatics analysis of differentially expressed genes in males compared to females' lung tissues using BioJupies tools (Caramelo et al., 2020).

METHODS

Differential Expressed Genes (DEGs) Between Males' and Females' Lung Tissues

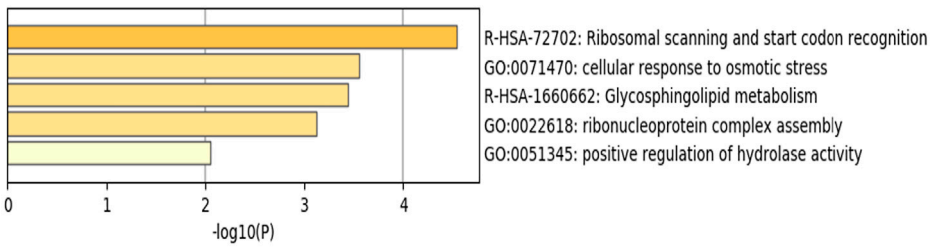
To have a better understanding of the molecular basis of the variable gender-related response to COVID-19 infection and due to the fact that the lung tissue damage is an essential event in the disease pathogenesis, we investigated the differentially expressed genes (DEGs) from lung tissues obtained from males and females using publicly available database (https://www.gtexportal.org/home/) BioJupies tool (Torre et al., 2018). A schematic representation of the bioinformatics analysis is shown in **Figure 1**.

Enriched Ontology Clustering of the Identified DEGs

Enriched Ontology Clustering for the identified genes was performed to explore if the identified genes are sharing common pathways using the Metascape (a web-based tool used for comprehensive gene list annotation and analysis resource) Zhou et al. (2019), as shown in **Figure 1**.

TABLE 1 | Top differential genes between males' and females' lung tissues.

DDX43	KCNIP3	GEMIN8	SLC2A1	ITGAD	EFHC2	SYTL5
SFRP2	SLC4A3	CA5B	LANCL3	STS	KDM6A	
OOEP	AGTR1	RHOH	ZRSR2	RPS4X	PNPLA4	
GRM8	KRBOX1	NAP1L2	KDM5C	RNF183	ERCC6L	
NOX5	MRC2	PLIN4	FAM3B	UGT8	RIBC1	
SPESP1	MAN2C1	SMC1A	CEACAM6	SRRM4	LYPD6B	
AJAP1	CHM	EIF2S3	DDX3X	ARSD	AQP5	
FAM228A	TRAPPC2	SYAP1	GYG2	TNFRSF13B	NLRP2	
PTGDR2	UBA1	FGFR3	ADD2	CP	BEND2	
GPAT2	ZDHHC2	TXLNG	KEL	ZFX	MAP7D2	
MMEL1	EIF5	PRKX	EIF1AX	PCDHA1	SAA4	
PRPH2	OFD1	FBXL16	PLEKHG4B	HS6ST2	SAA2	



Term	Description	LogP	Log(q-value)	InTerm_InList	Symbols
R-HSA-72702	Ribosomal scanning and start codon recognition	-4.54091	-0.222	4/58	EIF1AX, EIF2S3, EIF5, RPS4X, DDX3X, GEMIN8
GO:0071470	cellular response to osmotic stress	-3.55394	0.000	3/42	AQP5, DDX3X, SLC2A1
R-HSA-1660662	Glycosphingolipid metabolism	-3.43638	0.000	3/46	STS, ARSD, UGT8
GO:0022618	ribonucleoprotein complex assembly	-3.12694	0.000	5/236	DDX3X, EIF2S3, EIF5, ZRSR2, GEMIN8
GO:0051345	positive regulation of hydrolase activity	-2.04604	0.000	7/777	AGTR1, CHM, DDX3X, EIF5, FGFR3, SFRP2, NLRP2

FIGURE 2 | Top pathway enrichment for the differentially expressed genes between males' and females' lung tissues. Genes related to the hydrolase activity are enriched in females' lung tissue compared to males'.

Deciphering Organ and Sex-specific Gene Expression Levels Variation

The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) was used to evaluate the variation in the gene expression levels of the selected genes according to sex and organs including the lung and kidney (Consortium, 2017). This platform includes the human transcriptomic data across different individuals and allow researchers to investigate the reference values of the gene expression levels for a range of normal primary tissues and organs. In addition, it allows the stratification of the gene expression levels with some clinical parameters including gender.

The average expression of our candidate genes was evaluated across different cell population of human lung tissue using Lung Gene Expression Analysis Web Portal "LungGENS" (https://research.cchmc.org/pbge/lunggens/), which is a web-based tool used to investigate the expression levels of different genes in single-cell population.

Statistical Analysis

For the shortlisted DEGs, we select 2-fold change as a threshold between different groups and adjusted p value < 0.05 was used as cut-offs.

For the LungGENS, we used the "Query by single gene" tool. The search engine in that database use t test to compare the expression levels of the selected genes among the different cell types. Further analysis was done using pie chart to show the percentage of each gene and its expression in each cell type using the p value < 0.05 as a cut-off.

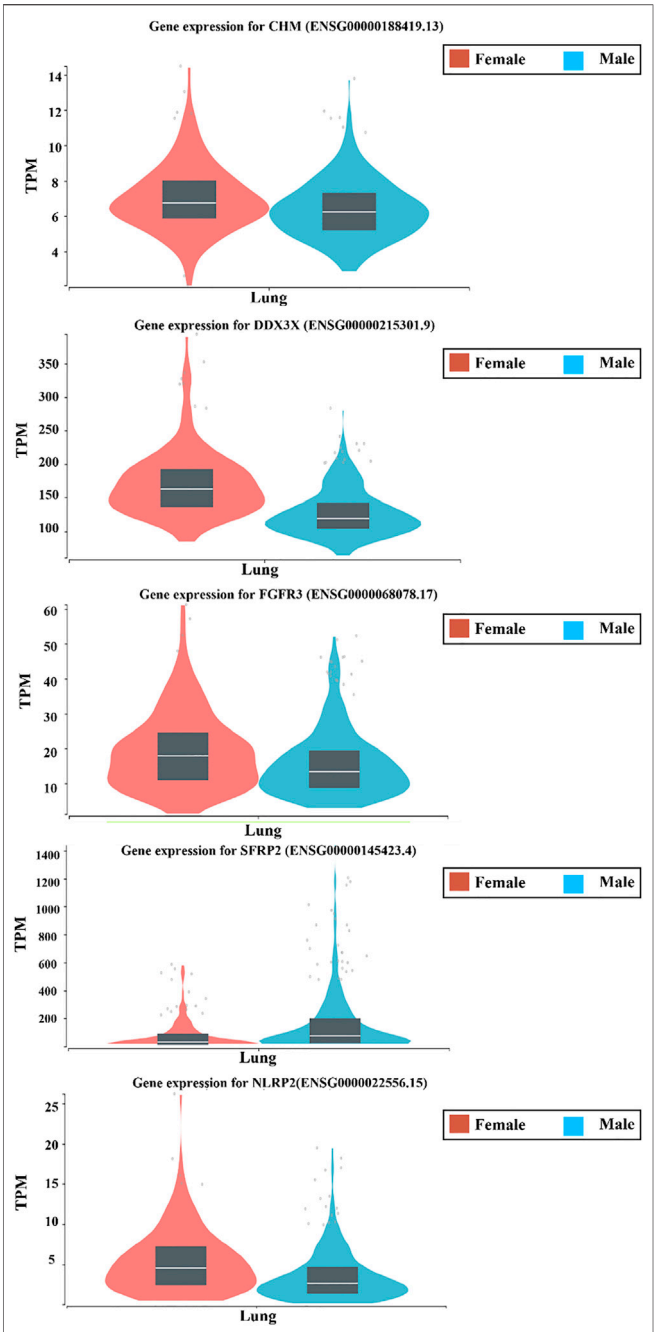


FIGURE 3 | The expression level of different genes related to the lung hydrolase activity between males' and females' lung tissues using The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) portal.

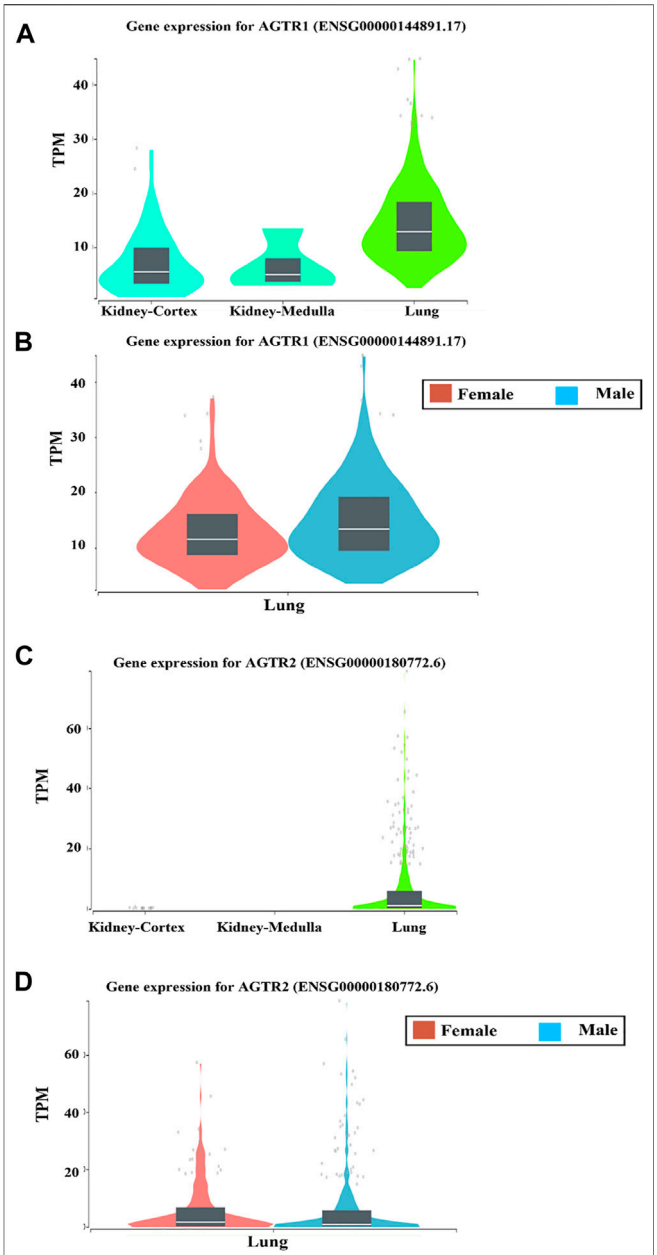


FIGURE 4 | The mRNA expression levels of AGTR1 and AGTR2 in lung and kidney tissues using The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) portal.The prostaglandin D2 receptor 2 (PTGDR2), involved in type 2 innate immune response and well known for its role in airway inflammation is another gender differential gene in the lung tissue.

RESULTS

Lung tissues from both genders showed differential expression of 73 genes in male and female lung tissues.

Initially, we compared the lung transcriptomic data of 141 females with 286 males. Our results showed 85 genes with significant variation in the expression levels between the two groups (**Table 1**). After excluding Y specific genes, 73 genes were selected.

Significant pathways in which the identified DEGs are differentially expressed between males' lung tissues compared to females'

Further analysis of the DEGs that differentiated between males' and females' lung tissues revealed that our top differential genes are enriched with several pathways. These include a pathway related to positive regulation of hydrolase activity (AGTR1, CHM, DDX3X, FGFR3, SFRP2, and NLRP2), which is believed to be important in the lung physiology as well as

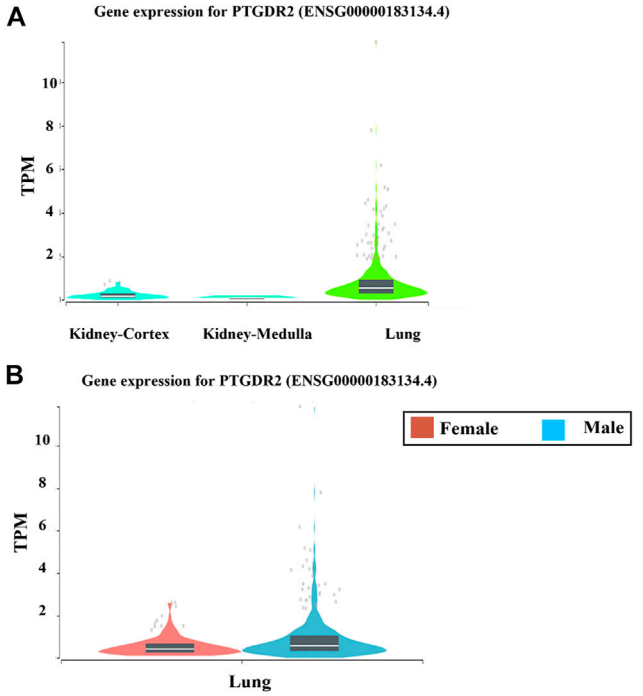


FIGURE 5 | The mRNA expression levels of PTGDR2 in lung and kidney tissues using The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) portal Cellular localization of the differentially identified gene candidates.

inflammation. Pulmonary surfactant contains homeostatic and antimicrobial hydrolases that are found to play a significant role in the terminal bronchioles and alveoli microbes control (Arcos et al., 1950) (**Figure 2**). Moreover, some of our top differentially expressed genes belong to the glycosphingolipid metabolism pathway. Reports highlighted that this pathway is essential in the virus–host interactions through regulating the ability of viruses to bind to gangliosides and determining the internalization pathway into cells.

Previous reports showed a possible role of several hydrolases in human lung tissues and alveolar lining fluid, in the modulation of microorganism envelope suggesting their possible role in infection control (Arcos et al., 1950). For that reason, we try to further validate the expression levels of our top differentiated genes involved in the lung hydrolase activity using The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) portal, which is another independent publicly available database. Interestingly, our results showed that CHM, DDX3X, FGFR3, and NLRP2 are more expressed in lung tissues obtained from female's lungs compared to males. This further highlights a possible role of these genes in controlling the COVID-19 infection through regulating the lung hydrolases activity (**Figure 3**).

Angiotensin II type 1 receptor (AGTR1) is among the differentially expressed genes in both genders and its expression is significantly higher in the lung tissue compared to the kidney tissue.

Our short-listed genes also revealed the presence of AGTR1 among the gender-related differentially expressed genes. Interestingly, AGTR1 is one of the 2 G protein-coupled receptors essential for the physiological effects of angiotensin II (ANG II). Since many reports highlighted that the role of human receptor ACE2 belongs to the renin-angiotensin system (RAS) and facilitates the binding of the SARS-CoV-2 to host cells, we further analysed the mRNA expression levels of AGTR1 and AGTR2 in lung tissue compared to kidney tissue as an example of other tissues, using The Genotype-Tissue Expression (GTEx) project (www.gtexportal.org) portal. Our results showed that both receptors were shown to be upregulated in the lung tissue compared to the kidney tissue (**Figures 4A, C**). Next, we analysed their expression in the lung tissues from both genders. Interestingly, the results revealed that while AGTR2 showed no significant difference in its expression in both genders, AGTR1 expression levels were higher in tissues obtained from male individuals compared to females (**Figures 4B,D**). This further supports our initial finding that AGTR1 is among the top differential genes in both genders.

Another interesting gene that we also found in our short list is the prostaglandin D2 receptor 2 (PTGDR2). This gene is essential for cells involved in type 2 immune responses through its interaction with prostaglandin D2 (PGD2). Also, it is known to play an essential role in the pathogenesis of asthma, through its role in induction of the pro-inflammatory cytokines and cationic proteases. Our analysis also revealed that PTGDR2 expression is higher in the lung tissue compared to the kidney tissue (**Figure 5A**). Moreover, its expression was found to be upregulated in male lungs compared to females' (**Figure 5B**). This further confirms our initial findings.

To have a better idea of the cellular localization of our short listed genes in the lung tissue, we investigated the gene expression levels of those genes using single-cell profiling of human lung tissues (the LGEA portal: https://research.cchmc.org/pbge/lunggens/mainportal.html) (**Figure 6**). Our results showed a variable expression of the candidate genes in different cell populations. Interestingly, all genes were expressed in the alveolar type I (AT1) and alveolar type II (AT2) cells in the respiratory system. FGFR3, PTGDR2 and NLRP2 were predominately expressed in the epithelial cells of the respiratory system including the airway epithelial cells, alveolar type I (AT1) and alveolar type II (AT2). In contrast, AGTR1 showed a predominant expression in the matrix fibroblasts and pericytes compared to other cells.

DISCUSSION

Despite the fact that COVID-19 is characterized by a milder clinical course of disease for the majority of patients compared to other coronavirus infections like SARS-CoV and MERS-CoV Lukassen et al. (2020), patients belonging to specific ethnic and demographic subgroups and those presented with pre-existing comorbidities showed higher rates of serious adverse outcomes including high mortality rates compared to the general

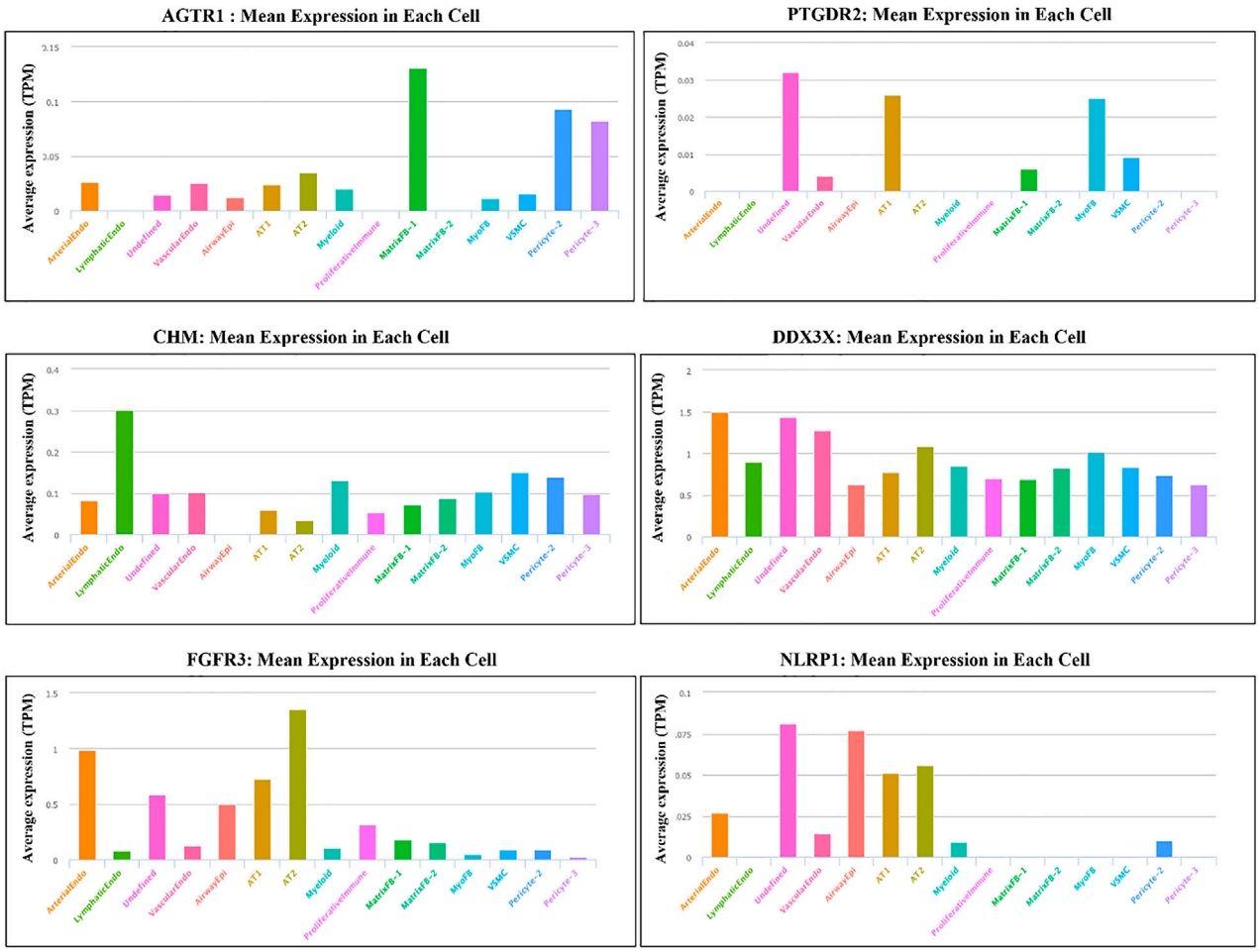


FIGURE 6 | The mRNA expression levels of different candidate genes in different lung cell populations using Single-cell RNA analysis of LungGENS web-based tool.

population (Chen et al., 2020; Guan, 2020; Guan et al., 2020; Huang et al., 2020; Pareek et al., 2020).

For that reason, the identification of different risk factors that predisposes for adverse outcome and the discovery of the molecular pathways that promote such severe course might be an essential step to provide more effective management plans Pareek et al. (2020), in addition to the discovery of new targets and tools that may lead to the adoption of more precise targeted therapies.

One of the striking findings related to COVID-19 outbreak is the significant difference in the mortality rates between male and female patients despite the equal numbers of infection for both genders (Epidemiology Working Group for NCIP, 2020). According to the available reports, elderly male patients with comorbidities are more likely to die from COVID-19 compared to females in a ratio reaching to 3:1 (Di Stadio et al., 2020; Huang et al., 2020; Epidemiology Working Group for NCIP, 2020; Wenham et al., 2020; Zhou et al., 2020).

The difference observed between both genders was suggested to be associated with some social and behavioural habits including smoking and alcohol consumption The Lancet (2020) as well as sex-based immunological differences between both genders (Chen et al., 2020; Zeng, 2020). Despite all these preliminary findings, there is no in-depth analysis to understand the genetic and molecular basis of this difference.

The here presented data sheds the light on some possible molecular pathways that might be responsible for this gender difference. Our results showed a downregulation of genes that are involved in regulating hydrolase activity including (CHM, DDX3X, FGFR3, and NLRP2) in the lung tissues obtained from males compared to that obtained from females. Interestingly, recent reports linked the hydrolase activity with lung immune response and inflammation as well as to antimicrobial effect (Arcos et al., 2017; Arcos et al., 1950). Moreover, alveolar lining fluid (ALF) hydrolases was found to be involved in the regulation of macrophages function as well as the host immune response, essential for infection control (Arcos et al., 1950; Arcos et al., 2017; Scordo et al., 2017). For that reason, the downregulation of genes involved in this pathway in male's lung tissues might explain the higher vulnerability of male patients to more severe clinical course.

Another important finding in this study, is the upregulation of the AGTR1 gene (also known as AT1 receptor), in the lung tissue

obtained from males compared to that obtained from females. This gene is considered as a fundamental component of the renin-angiotensin system Mottl et al. (2008) and discovered to have strong interaction with the ACE2. Indeed, the fact that ACE2 is considered as an essential component mediating COVID-19 virus entry to the human respiratory cells Mottl et al. (2008) may highlight a possible specific role of AGTR1 in the ACE2 mediated binding of the COVID-19 to the host cells in male patients. This suggestion is supported by recent findings that blockage of AGTR1 with losartan in experimental models helped in reducing the pulmonary edema as well as the severe acute lung injury associated with SARS-Coronavirus infection (Kuba et al., 2005; Gurwitz, 2020). For that reason, researchers suggested the use of AGTR1 (AT1R) antagonists like losartan and olmesartan and other angiotensin II receptor blockers (ARBs) as a possible tentative therapeutic option for COVID-19 infection that may protect COVID-19 patients from the severe symptoms and reduce their mortality rate (Gurwitz, 2020; Sun et al., 2020).

Finally, our findings showed a differential expression of some genes that play a role in the immune response. This includes the NLRP2, which is involved in the suppression of the NF-κB signaling pathway leading to the modulation of the inflammatory response (Fontalba et al., 2007). In addition, it is considered as an important component of the inflammasome, which is a multiprotein intracellular complex, essential for the detection of the pathogenic microorganisms and activation of the pro-inflammatory cytokines including interleukin-1β (IL-1β) and IL-18 (de Rivero Vaccari et al., 2014; Rossi et al., 2019). Similarly, PTGDR2, which we found to be upregulated in male lungs compared to females, is believed to be essential for the pro-inflammatory cytokines induction as well as asthma pathogenesis (Huang et al., 2016). Moreover, recent reports highlighted prostaglandin D2 as a mediator of lymphopenia and adverse clinical course, suggesting it as a possible therapeutic target in COVID-19 patients (Gupta and Chander Chiang, 2020).

Indeed, this study is an essential proof of concept, however, further studies are needed to confirm the significance of those genes in determining the distinct clinical course between both genders in response to COVID-19 infection.

All together, our results shed the light on some of the molecular mechanisms and pathways that might predispose to the poor outcome and high mortality rates observed in male patients during the COVID-19 outbreak. Better understanding of such mechanisms might be essential in the discovery of new therapeutic approaches based on targeting those specific pathways, which in turn might improve the outcome of patients with different ethnic and epidemiological characteristics.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

IH and MH, conceptualization, research design, data analysis and wrote the manuscript. IT, V-O, N-A, SH, RH: data analysis and drafting of the manuscript. QH: research design, supervised the study and drafting the manuscript. All authors made substantial contributions to the conception, design, acquisition of data, analysis and interpretation of data, all authors have been involved in revising and critically evaluating the manuscript for important intellectual content. In addition, each author has agreed to be accountable for the accuracy and integrity of this research work.

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Respiratory Outcomes in Patients Following COVID-19-Related Hospitalization: A Meta-Analysis

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Background: To determine the respiratory outcomes in patients following COVID-19-related hospitalization.

Methods: Systematic review and meta-analysis of the literature.

Results: Forced vital capacity (FVC, % of predicted): 0–3 months post discharge: 96.1, 95% CI [82.1–110.0]; 3–6 months post discharge: 99.9, 95% CI [84.8, 115.0]; >6 months post discharge: 97.4, 95% CI [76.8–118.0]. Diffusing capacity of the lungs for carbon monoxide (DLCO, % of predicted): 0–3 months post discharge: 83.9, 95% CI [68.9–98.9]; 3–6 months post discharge: 91.2, 95% CI [74.8–107.7]; >6 months post discharge: 97.3, 95% CI [76.7–117.9]. Percentage of patients with FVC less than 80% of predicted: 0–3 months post discharge: 10%, 95% CI [6–14%]; 3–6 months post discharge: 10%, 95% CI [2–18%]; >6 months post discharge: 13%, 95% CI [8–18%]. Percentage of patients with DLCO less than 80% of predicted: 0–3 months post discharge: 48%, 95% CI [41–56%]; 3–6 months post discharge: 33%, 95% CI [23–44%]; >6 months post discharge: 43%, 95% CI [22–65%].

Conclusion: The meta-analysis confirms a high prevalence of persistent lung diffusion impairment in patients following COVID-19-related hospitalization. Routine respiratory follow-up is thus strongly recommended.

Keywords: COVID-19, follow-up, pulmonary function test, FVC, DLCO, synthesis review, meta-analysis

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INTRODUCTION

To date, over 200 million people worldwide have recovered from COVID-19 (https://www.worldometers.info/coronavirus/) (Worldometers (2020). Worl, 2020), but concern remains that some organs, including the lungs, might suffer long-term impairment following recovery from acute infections. Individual studies have shown that residual abnormalities of pulmonary function were observed in a subgroup of recovered COVID-19 patients, with the most common finding being a reduction in gas transfer as measured by diffusing capacity of the lungs for carbon monoxide (DLCO) (Hull et al., 2020; Dhawan et al., 2021;

Abbreviations: BMI, body mass index; COVID-19, Corona virus disease 2019; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; NR, not reported; PFTs, pulmonary function tests.

Thomas et al., 2021). In this study, with meta-analysis, we aimed to determine the short (0–3 months), medium (3–6 months) and long (>6 months) respiratory outcomes in patients following COVID-19-related hospitalisation. The findings will instruct appropriate interventions for subsequent increased healthcare utilisation post-COVID-19.

METHOD

Criteria for Inclusion

We included randomised controlled trials (RCTs) and observational studies (cross-sectional, longitudinal, case-control and cohort) of patients with a confirmed diagnosis of COVID-19. The studies included aimed to determine the respiratory outcomes, in particular forced vital capacity (FVC) and diffusing capacity of the lungs for carbon monoxide (DLCO), in patients following COVID-19-related hospitalisation. The selected studies had to follow the ATS / ERS clinical guidelines. The included literatures should be published before May 15, 2021.

Criteria for Exclusion

Study's subjects who were not infected with COVID-19. Studies didn't report the time of hospital discharge or the time was calculated from diagnosis of COVID-19. Studies did not report FVC (% of predicted) or DLCO (% of predicted) or FVC <80% of predicted or DLCO <80% of predicted. Animal experiments,

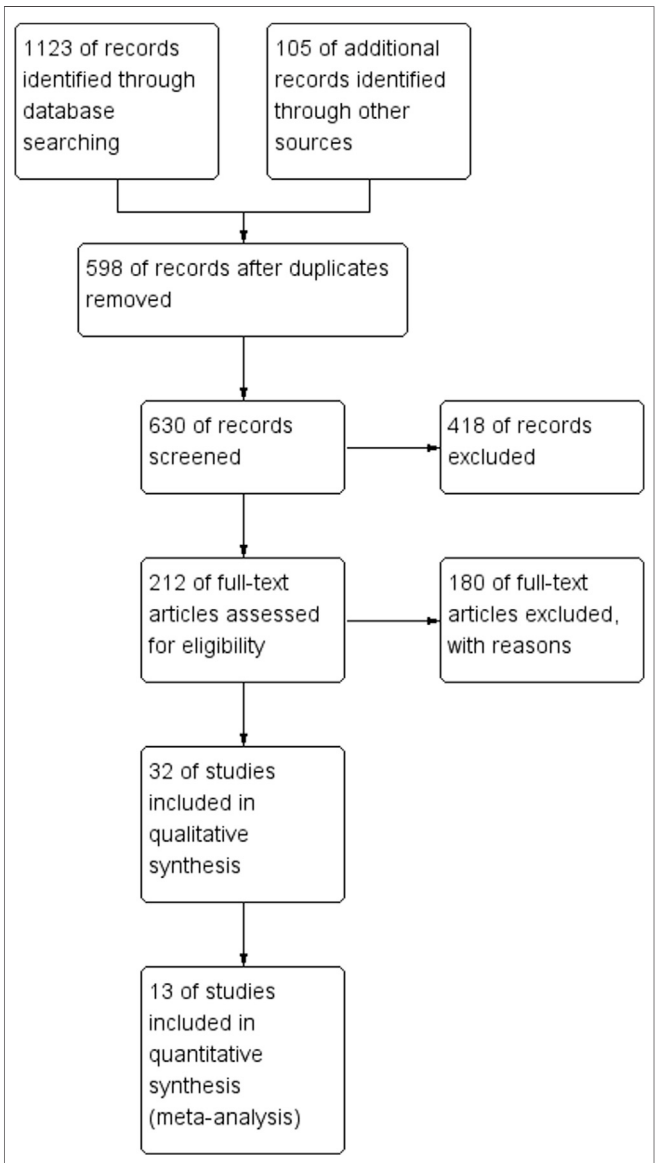

FIGURE 1 | Workflow showing literature extraction (details provided in Methods).

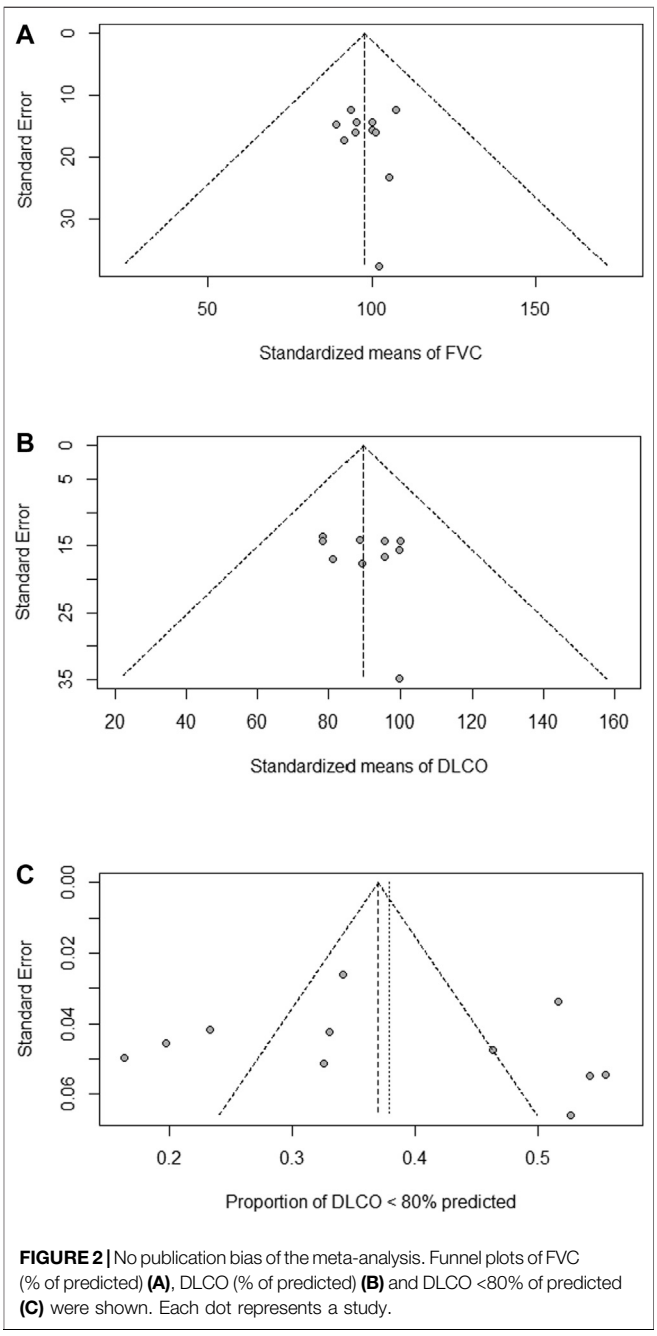

FIGURE 2 | No publication bias of the meta-analysis. Funnel plots of FVC (% of predicted) **(A)**, DLCO (% of predicted) **(B)** and DLCO <80% of predicted **(C)** were shown. Each dot represents a study.

TABLE 1 | Basic characteristics of included studies.

Author	Country	Design	Participants male/female	Age (years)	BMI (kg/m2)	Smoking	Respiratory comorbidities	Time of assessment	Quality rating
Huang et al., (2020)	China	retrospective	57 26M/31F	46.7 ± 13.7	23.9 ± 3.5	History of smoking 9 (15.7%)	No patient was reported having chronic repiratory diseases	30 days after discharge from the hospital	high
Venturelli et al., (2021)	Italy	prospective	767 515M/ 252F	63 ± 13.6	NR	Active smoker 33 (4.3%) History of smoking 179 (23.3%)	NR	81 (66–106) days after hospital discharge	high
You et al., (2020)	China	prospective	18 10M/8F	50.7 ± 12.1	26.4 ± 2.8	NR	No patient was reported having chronic repiratory diseases	38 ± 13.4 days after hospital discharge	high
Lerum et al., (2021)	Norway	prospective	103 54M/49F	59 (49–72)	25.8 (23.8–29.6)	Current smoker 3 (3.4%) previous smoker 34 (39%)	NR	3 months after hospital admission	poor
Daher et al., (2020)	Germany	prospective	33 22M/11F	64 ± 3	28 (24–31)	NR	7 (21%)	6 weeks after hospital discharge	high
Wu et al., (2021)	China	prospective, longitudinal, cohort	83 47M/36F	60 (52–66)	25 (23.5–27.1)	NR	No patient was reported having chronic repiratory diseases	3 months, 6 months, 9 months, 12 months after hospital discharge	high
Liang et al., (2020)	China	Prospective	76 21M/55F	41.3 ± 13.8	23.7 ± 4.5	NR	Cough 45 (60%) Increased sputum production 33 (43%) Activity chest tightness and palpitations 47 (62%)	3 -months follow-up study after discharge	high
Bellan et al., (2021)	Italy	prospective cohort study	238 142M/96F	61 (51–71)	NR	Never 139(58.4%) Former 74(31.1%) Current 25(10.5%) Pack-years, median(IQR) 15(7.25–36)	No patient was reported having chronic repiratory diseases	4 months after discharge	high
Li et al., (2020)	China	a prospective study	18	NR	NR	History of smoking 3(16.6%)	history of tuberculosis 1 (5.5%)	Near discharge and in quarantine period (2 weeks after discharge)	high
van den Borst et al., (2020)	Netherlands	Prospective	124 74M/50F	59 ± 14	NR	Never 48(39%) Former 74(60%) Current 2(2%)	asthma 12 (10%) chronic lung diseases 23 (19%) other lung diseases 4 (3%)	Three months after recovery	high
Mo et al., (2020)	China	Prospective	110 55M/55F	49.1 ± 14.0	23.5 ± 3.0	History of smoking 13 (11.8%)	asthma 1 (0.9%) chronic bronchitis 1 (0.9%) bronchiectasis 1 (0.9%)	At time of hospital discharge	poor
Zhao et al., (2020)	China	retrospective	55 22M/23F	47.7 ± 15.5	NR	active 2 (3.6%) former 2 (3.6%)	cough 7 (43.75%)	3 months after hospital discharge	high
Huang et al., (2021)	China	prospective cohort study	1733 897M/ 836F	57 (47–65)	NR	Never-smoker 1585/1731 (92%) Current smoker 102/1731 (6%) Former smoker 44/1731 (3%)	Chronic obstructive pulmonary disorder 31 (2%)	153.0 (146.0–160.0) days after hospital discharge	high

NR, not reported; BMI, body mass index; M, male; F, female.

TABLE 2 | Summary of studies included pulmonary function test.

	Wu et al., (*n* = 83)			You et al., (*n* = 18)	Zhao et al., (*n* = 55)
FVC, % of predicted	92 (81–99)	94 (85–104)	98 (89–109)	105.1 ± 23.3	NR
DLCO, % of predicted	77 (67–87)	76 (68–90)	88 (78–101)	NR	NR
FVC, < 80% of predicted	19	13	9	3	NR
DLCO, < 80% of predicted	46	45	27	NR	9
Time of assessment	3 months	6 months	12 months	38 ± 13.4 days after hospital discharge	3 months after hospital discharge

	Lerum et al., (*n* = 103)	Borst et al., (*n* = 124)	Li et al., (*n* = 18)	Daher, A et al. (*n* = 33)	Venturelli, S et al. (*n* = 767)
FVC, % of predicted	94 (76–121)	NR	91.5 ± 17.3	NR	95(84–106), *f*
DLCO, % of predicted	83 (72–92)	81 ± 17	NR	65(53–73)	96(81–112), *p*
FVC, < 80% of predicted	7	NR	NR	NR	NR
DLCO, < 80% of predicted	24	41	NR	NR	NR
Time of assessment	3 months after hospital discharge	3 months after recovery	Near to discharge and 2 weeks after	56 days from discharge to follow-up	80(median)days after discharge

	Huang et al. (*n* = 349)	Bellan et al., (*n* = 224)	Liang et al., (*n* = 76)	Huang et al., (*n* = 57)	Mo et al., (*n* = 110)
FVC, % of predicted	NR	98.5 (90–109)	107.1 ± 12.3	100.96 ± 15.93	93.59 ± 12.25
DLCO, % of predicted	NR	79 (69–89), *q*	NR	78.38 ± 13.59	78.18 ± 14.29
FVC, < 80% of predicted	14	NR	NR	6	10
DLCO, < 80% of predicted	114, *l*	113, *q*	15	30	51
Time of assessment	153.0 (146.0–160.0) days after hospital discharge	4 months after hospital discharge	3 months after hospital discharge	1 month after hospital discharge	when discharged from hospital

f: n = 717, p: n = 680, q: n = 219, l: n = 334.

NR, not reported; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide.

TABLE 3 | Summary of re-calculations of median into mean using R studio.

Author	Time	FVC.mean	FVC.sd	FVC.n	DLCO.mean	DLCO.sd	DLCO.n
Frija-Masson	30 days after symptoms onset	91.7	11.14	50	91.27	11.23	50
Daher, A	56 days from discharge to follow-up	NR	NR	NR	88.93	17.67	33
Venturelli, S	80(median)days after discharge	95.02	15.99	717	95.48	16.6	680
Lerum	3 months after hospital discharge	102.1	37.78	103	99.68	34.9	103
Darley,D.R	113(median)days after diagnosis	106.91	15.07	65	106.88	14.79	65
Belan	4 months after hospital discharge	99.9	14.3	224	99.79	14.28	219
Wu	3 months	89.11	14.73	83	88.45	14.13	83
Wu	6 months	95.07	14.3	83	95.26	14.26	83
Wu	12 months	100.19	15.53	83	99.67	15.56	83

FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; NR, Not reported.

medical records, case reports, famous medical experience and review were excluded.

Literature Retrieval and Selection

Firstly, according to the literature inclusion criteria, two researchers independently searched at Pubmed, ScienceDirect, Embase and Web of Science. Secondly, two researchers selected the literature and extracted the data independently in accordance with the standard data extraction table. When it came to divergences, a third researcher did the judgement. After the discussion, researchers reached a consensus. Finally, after the extraction and input of the data, two independent researchers did the subsequent analysis.

Extraction of Data

According to the inclusion criteria, we assessed the design of research, patients, and outcome indicators. First author, published year, number of cases, nationality, ages, body mass index (BMI), smoking status, respiratory comorbidities, time of assessment and, index quantity of FVC, % of predicted, DLCO, % of predicted; FVC <80% of

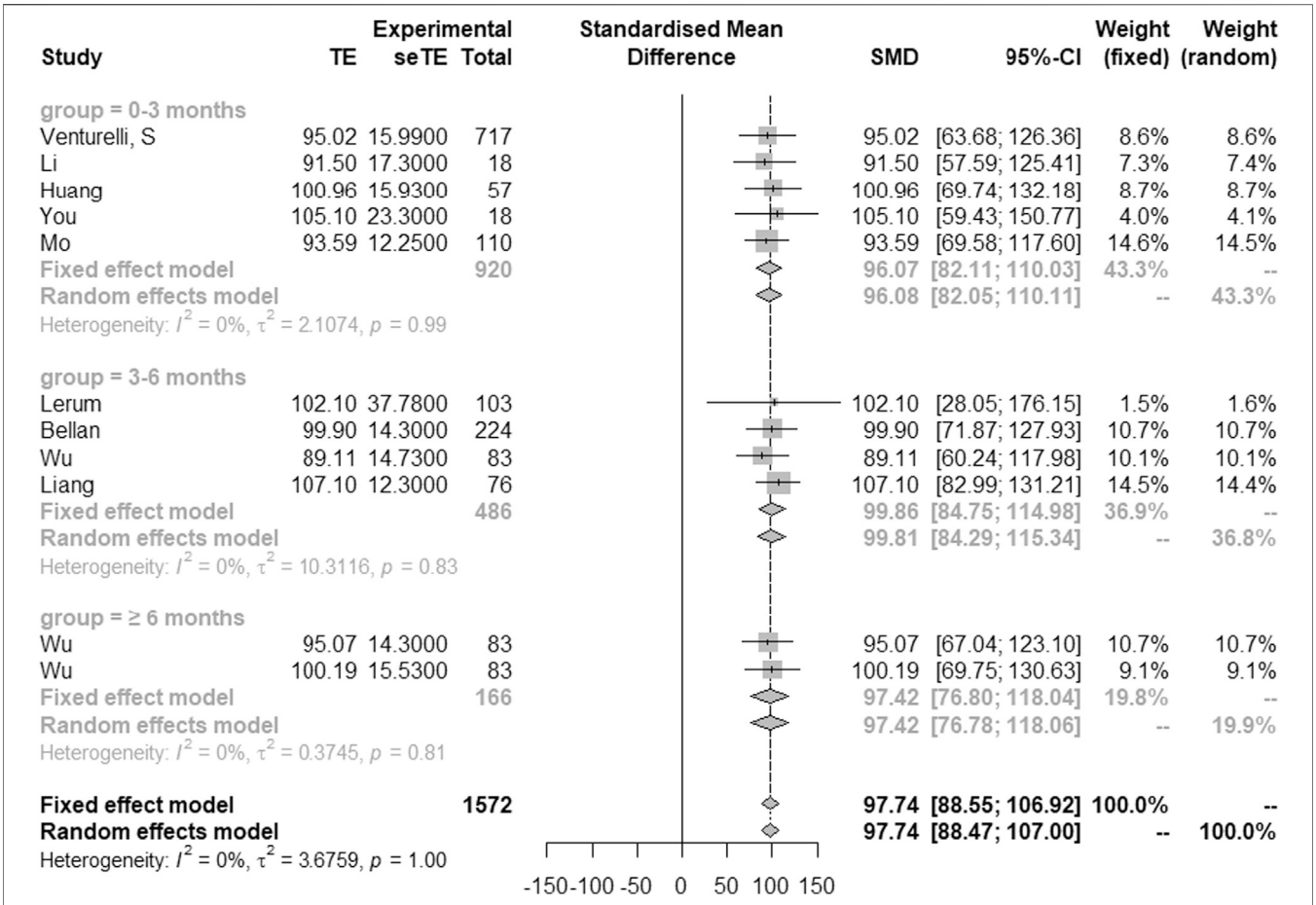

FIGURE 3 | Comparison of longitudinal changes in FVC (% of predicted). Forest plot showing meta-analysis of FVC (% of predicted).

predicted and DLCO <80% of predicted were extracted from eligible studies.

Quality Assessment of Articles

The studies with randomised controlled trials were evaluated by Newcastle-Ottawa Scale (Bellan et al., 2021). As for no controlled trials, it includes the following aspects: 1) selection: Representativeness of the exposed cohort, selection of the non-exposed cohort, Ascertainment of exposure, Demonstration that outcome of interest was not present at start of study; 2) comparability: Research control matched important factors, but also controlled other important factors; and 3) outcome: assessment of outcome, follow-up long enough for outcomes to occur, adequacy of follow up of cohorts.

Synthesis and Analysis of Data

We used package "meta (version 4.18-0)" in R 4.0.1 and R studio to perform meta-analysis of the following pulmonary function tests (PFTs) indexes (1. FVC, % of predicted; 2. DLCO, % of predicted; 3. FVC <80% of predicted; 4. DLCO <80% of predicted.). Patients were divided into three groups: less than 3 months (0–3 months), more than or equal to 3 months and less than 6 months (3–6 months), and more than or equal to 6 months (≥6 months). We re-calculated the median (first quantile, third quantile) to mean ± standard deviation (SD) for FVC (% of predicted) and DLCO (% of predicted) in several studies. Statistical heterogeneity was measured through the I^2 statistic and classified as low (I^2 < 25%), moderate (I^2 25–50%), and high (I^2 > 50%) (Melsen et al., 2014). Subgroup analysis, according to the outcome assessment and severity, was performed. Sensitivity analysis was also employed to assess the change in pooled prevalence due to the selective exclusion of studies.

RESULTS

Literature Extraction

A total of 1,123 articles was retrieved from databases via the retrieval methods. Duplicate literatures were excluded through titles and abstracts. By reading the full text, we excluded 1,110 papers and conference abstracts with incomplete or no specific research method. Finally, 13 papers published in English were included (Liang et al.,

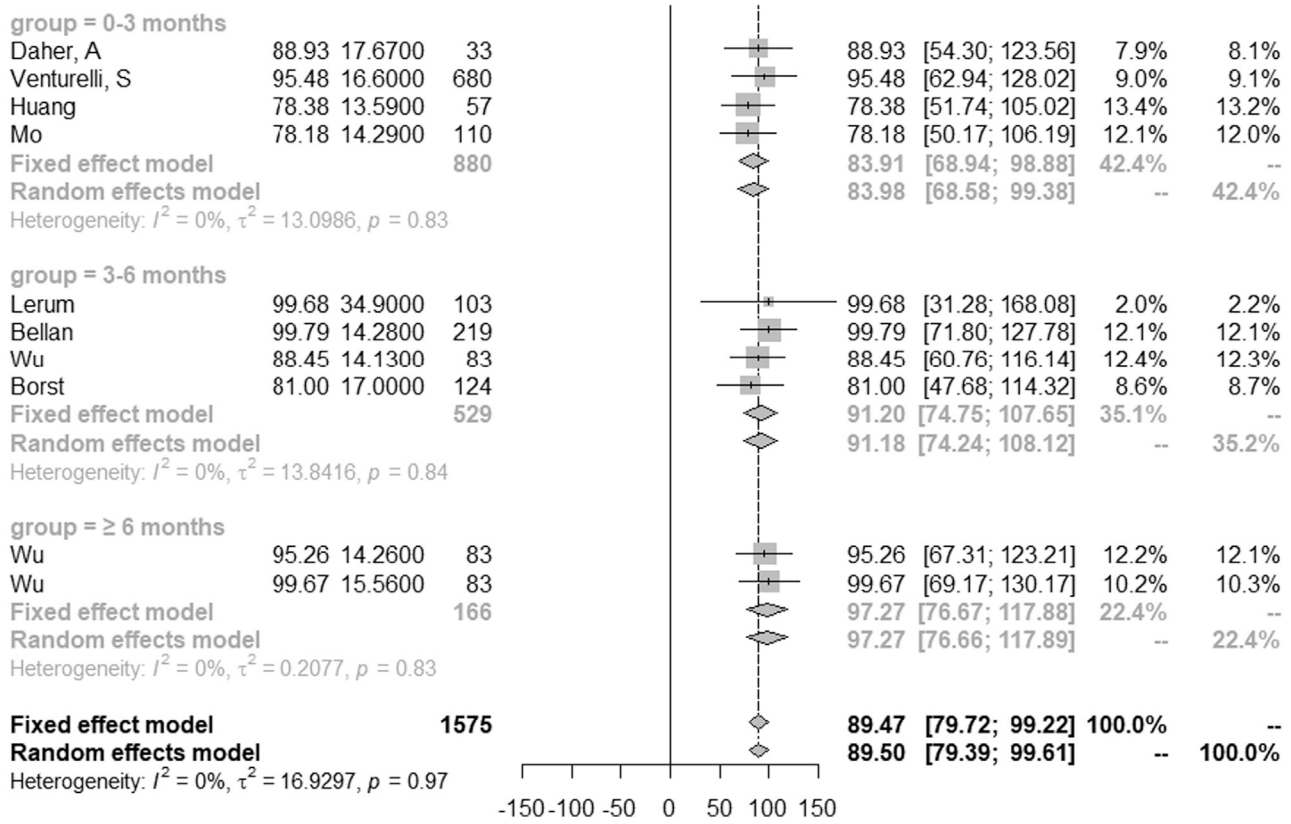

FIGURE 4 | Comparison of longitudinal changes in DLCO (% of predicted). Forest plot showing meta-analysis of DLCO (% of predicted).

2020; Huang et al., 2020; Venturelli et al., 2021; You et al., 2020; Lerum et al., 2021; Daher et al., 2020; Wu et al., 2021; Bellan et al., 2021; Li et al., 2020; van den Borst et al., 2020; Mo et al., 2020; Zhao et al., 2020; Huang et al., 2021), with a total of 3,455 patients. The evaluation of the quality of included studies by Newcastle-Ottawa Scale (NOS) (Stang et al., 2018) showed that two studies had a poor quality and the rest 11 studies passed the quality control. The basic characteristics of the included literatures were detailed in **Table 1** and the procedure of literature retrieval and selection was shown in **Figure 1**.

Among the included studies, 10 studies reported FVC (% of predicted), eight studies reported DLCO (% of predicted), six reported FVC <80% of predicted, and nine reported DLCO <80% of predicted. Wu et al. (2021). reported all the indexes of the patients after the 3, 6 and 12 months following COVID-19-related hospitalisation (**Table 2**). For those data reported in the form of median (first quantile, third quantile), we used R studio to re-calculate them into mean ± SD (**Table 3**).

Publication bias refers to the fact that research results with statistical significance are more likely to be reported and published than those without statistical significance and invalid results (DeVito and Goldacre, 2019). We examined the publication bias of meta-analysis of each indicator. There was no publication bias in FVC (% of predicted; p = 0.93; **Figure 2A**), DLCO (% of predicted; p = 0.54; **Figure 2B**) and DLCO (<80% of predicted; p = 0.94; **Figure 2C**). For FVC <80% of predicted, less than 10 studies were included, so publication bias was not tested.

Comparison of Longitudinal Changes in FVC (% of Predicted)

Nine studies with 11 groups of data showed the results of FVC (% of predicted). Based on the time of patients discharged from hospital, we divided them into three groups: 0–3 months, 3–6 months and ≥6 months. FVC (% of predicted) in 0–3 months, 3–6 months and ≥6 months post discharge were 96.1 (95% CI [82.1–110.0]), 99.9 (95% CI [84.8–115.0]) and 97.4 (95% CI [76.8–118.0]), respectively. In this study, heterogeneity was extremely low (I^2 = 0%), and the overall value of FVC (% of predicted) in all studies was 97.7 (95% CI [88.6–106.9]) (**Figure 3**).

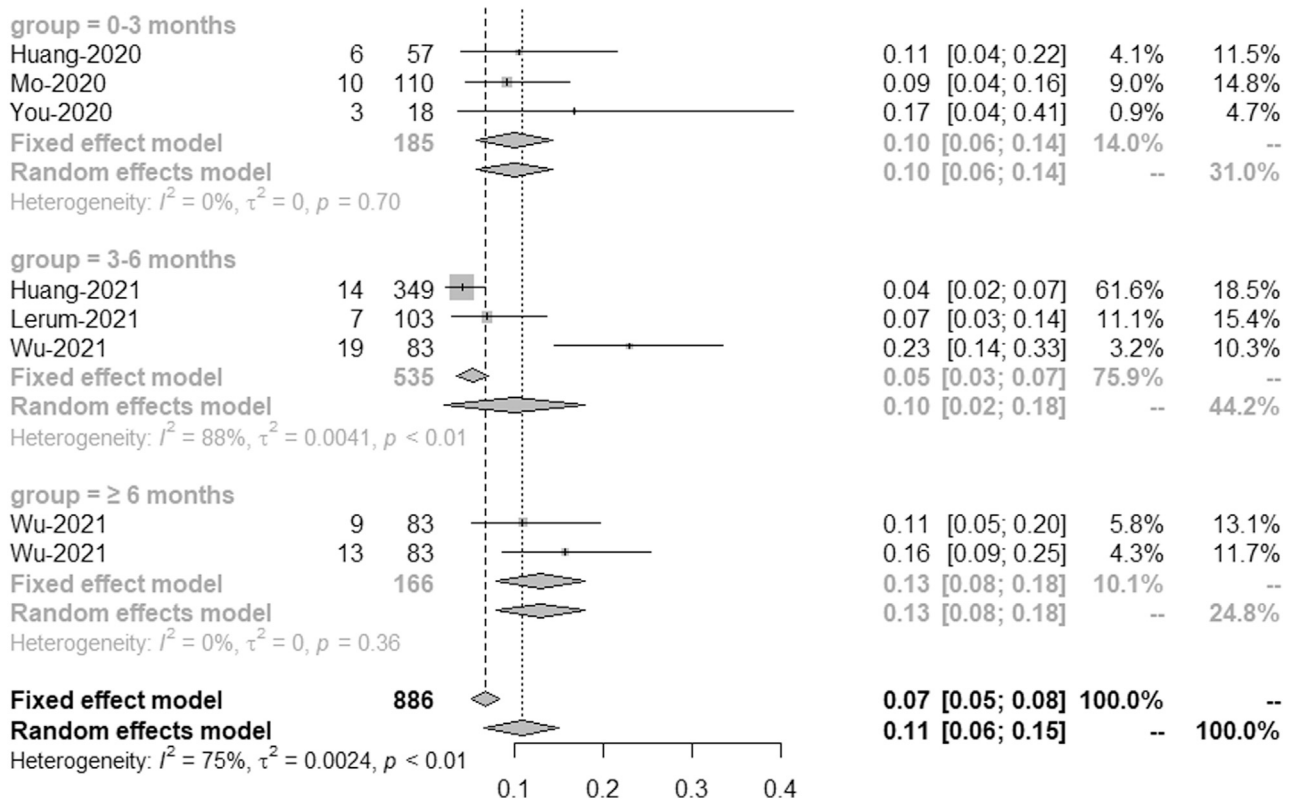

FIGURE 5 | Comparison of longitudinal changes in the percentage of patients with FVC <80% of predicted. Forest plot showing meta-analysis of the percentage of patients with FVC <80% of predicted.

Comparison of Longitudinal Changes in DLCO (% of Predicted)

Eight studies with 10 groups of data showed the results of DLCO (% of predicted). DLCO (% of predicted) in 0–3 months, 3–6 months and ≥6 months post discharge were 83.9 (95% CI [68.9–98.9]), 91.2 (95% CI [74.8–107.7]) and 97.3 (95% CI [76.7–117.9]), respectively. Heterogeneity was considered low (I^2 = 0%) using a fixed effect model (Melsen et al., 2014; Bellou et al., 2016) (**Figure 4**).

Comparison of longitudinal changes in the percentage of patients with FVC <80% of predicted.

These included six studies, which in total have eight groups of data showed the percentage of patients with FVC less than 80% of predicted. Based on the time of patients being discharged from hospital, we divided them into three groups: 0–3 months, 3–6 months and greater than 6 months. Meta-analysis showed that the percentage of patients with FVC less than 80% of predicted in 0–3 months, 3–6 months and ≥6 months post discharge was 10% (95% CI [6–14%]), 10% (95% CI [2–18%]) and 13% (95% CI [8–18%], respectively. The heterogeneity of 3–6 months was large, so the sensitivity analysis was carried out in this study. We removed the study from Wu *et al.* and got the meta-analysis result of this subgroup, which was 4% (95% CI [3–6%]) with I^2 = 6% (**Figure 5**).

Comparison of longitudinal changes in the percentage of patients with DLCO <80% of predicted.

This included nine studies, which have 11 groups of data shows the results of DLCO less than 80% of predicted. Meta-analysis showed a significant and persistent reduction in DLCO over the study period. The percentage of patients with DLCO less than 80% of predicted in 0–3 months, 3–6 months and ≥6 months post discharge was 48% (95% CI [41–56%]), 33% (95% CI [23–44%]) and 43% (95% CI [22–65%]), respectively (**Figure 6**).

DISCUSSION

Post-acute COVID-19 syndrome, also known as long COVID, encompasses a wide range of physical and mental health symptoms that persist after recovery from acute SARS-CoV-2 infections (Nalbandian et al., 2021). Systematic studies of sequelae after recovery from acute COVID-19 are demanded

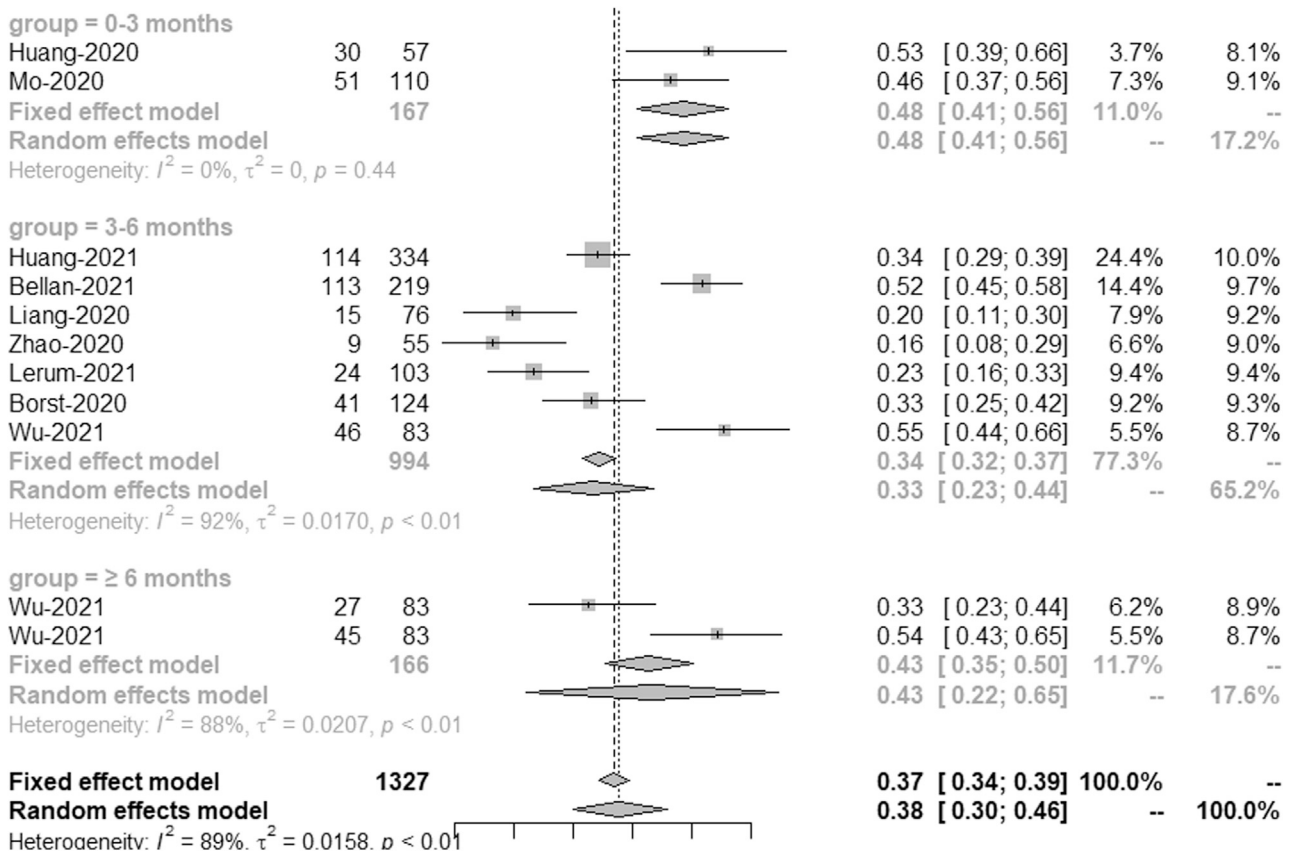

FIGURE 6 | Comparison of longitudinal changes in the percentage of patients with DLCO <80% of predicted. Forest plot showing meta-analysis of the percentage of patients with DLCO <80% of predicted.

to inform effective clinical management for patients suffered from long COVID.

We recently reported the 3 months, 6 months, 9 months, and 12 months respiratory outcomes in patients following COVID-19-related hospitalisation from a relatively small prospective cohort (n = 83) (Wu et al., 2021). In this study, we conducted meta-analysis to determine the short (0–3 months), medium (3–6 months) and long (>6 months) respiratory outcomes in patients following COVID-19-related hospitalisation. Significantly, we found a persistent reduction in DLCO over the study period, consistent with earlier reports (E et al., 2021). Low DLCO could be caused by interstitial changes or pulmonary vascular abnormalities following COVID-19 infections (Lang et al., 2020; Patel et al., 2020; Hanidziar and Robson, 2021). Our study has shown that up to a third of COVID patients still have evidence of defect DLCO 1 year after discharge (Wu et al., 2021), although longer term follow-up with a larger cohort will be required to confirm this observation.

In general, the heterogeneity of the studies included in the meta-analysis was low. However, the heterogeneity of DLCO less than 80% of predicted was higher, which may be caused by different ethnic groups, ages, disease severity, therapies and other factors. In general, the models we used were robust and reliable.

There are several limitations in this study. Firstly, age, sex ratio, nationality and disease severity of the patients included in the study are quite different, which may cause great heterogeneity and affect the final research results. Secondly, we only selected four indicators of lung function, so we cannot investigate the relationship between other indicators and discharge time. To be consistent and comparable with our earlier publication (Wu et al., 2021), we excluded those studies without data on FVC and/or DLCO values <80% of predicted. This might cause some false positive results considering the mean age of included patients is over 50 (van den Borst et al., 2020; Barisione and Brusasco, 2021; Milanese et al., 2021). In addition, pre-existing comorbidities for most COVID-19 patients are not known, which might cause certain

bias of the results. Despite of these limitations, our findings in this meta-analysis are consistent with our previous report (Wu et al., 2021), confirming a high prevalence of persistent lung diffusion impairment in patients following COVID-19-related hospitalisation. Routine respiratory follow-up is thus strongly recommended.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YW conceived and designed the study. TG, FJ, YL, YZ and YL collected the data. TG and FJ performed the data analysis. TG and YL did the evaluation of the quality of included articles. TG and YW wrote the article. All authors are responsible for reviewing data. All authors read and approved the final article.

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Pleural Mesothelial Cells Modulate the Inflammatory/Profibrotic Response During SARS-CoV-2 Infection

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Although lung fibrosis has a major impact in COVID-19 disease, its pathogenesis is incompletely understood. In particular, no direct evidence of pleura implication in COVID-19-related fibrotic damage has been reported so far. In this study, the expression of epithelial cytokeratins and Wilms tumor 1 (WT1), specific markers of mesothelial cells (MCs), was analyzed in COVID-19 and unrelated pleura autoptic samples. SARS-CoV-2 replication was analyzed by RT-PCR and confocal microscopy in MeT5A, a pleura MC line. SARS-CoV-2 receptors were analyzed by RT-PCR and western blot. Inflammatory cytokines from the supernatants of SARS-CoV-2-infected MeT5A cells were analysed by Luminex and ELLA assays. Immunohistochemistry of COVID-19 pleura patients highlighted disruption of pleura monolayer and fibrosis of the sub-mesothelial stroma, with the presence of MCs with fibroblastoid morphology in the sub-mesothelial stroma, but no evidence of direct infection *in vivo*. Interestingly, we found evidence of ACE2 expression in MCs from pleura of COVID-19 patients. *In vitro* analysis shown that MeT5A cells expressed ACE2, TMPRSS2, ADAM17 and NRP1, plasma membrane receptors implicated in SARS-CoV-2 cell entry and infectivity. Moreover, MeT5A cells sustained SARS-CoV-2 replication and productive infection. Infected MeT5A cells produced interferons, inflammatory cytokines and metalloproteases. Overall, our data highlight the potential role of pleura MCs as promoters of the fibrotic reaction and regulators of the immune response upon SARS-CoV-2 infection.

Keywords: SARS-CoV-2, mesothelial cells, inflammatory cytokines, pulmonary fibrosis, mesothelial to mesenchymal transition, WT1

BACKGROUND

Severe acute respiratory syndrome (SARS)-CoV-2 pathogenesis, characterized by clinical phenotypes spanning from asymptomatic infection to mild disease with symptoms related to airways tract implication, severe pneumonia, acute respiratory distress syndrome (ARDS) and multiple organ failure, remains largely obscure (Hu et al., 2021). Asymptomatic, mild, and severe

diseases have been correlated to different cytokine signatures and to differences in innate and adaptive responses to SARS-CoV-2 infection (Bindoli et al., 2020; Carsetti et al., 2020).

It is now well assessed that SARS-CoV-2 first infects epithelial cells of the upper respiratory tract (nasal passages and throat) and especially lungs (bronchi and alveoli), where alveolar type I and type II cells (AT1 and AT2, respectively) are believed to mediate the first encounter with the virus; infection of alveolar macrophages is determinant in mediating the amplification of the inflammatory and immune responses (Grant et al., 2021).

Mechanistically, cell entry is mediated by the engagement of the receptor angiotensin-converting enzyme (ACE2) (Grant et al., 2021). ACE2 is also expressed by cells in the kidney, blood vessels, heart, whose infection by SARS-CoV-2 may mediate the characteristic multi-organ pathology (Grant et al., 2021). Viral uptake is also promoted by transmembrane protease serine (TMPRSS)2, disintegrin and metallopeptidase domain (ADAM)17 cleaving ACE2, in addition to activating the S protein of the virus for membrane fusion (Heurich et al., 2014; Hoffmann et al., 2020). Neuropilin-1 (NRP1) also potentiates SARS-CoV-2 cell entry and infectivity (Cantuti-Castelvetri et al., 2020).

Similarly to the other serosal membranes (i.e., peritoneum, and pericardium), pleura is lined by a monolayer of mesothelial cells (MCs) arranged on a basement membrane, which separates it from the submesothelial stroma (Mutsaers et al., 2015). MCs differentiation is controlled by the transcription factor Wilms' tumor (WT) 1, which is commonly used for lineage-tracing experiments (Gulyas and Hjerpe, 2003; Karki et al., 2014). The main function of MCs resides in the creation of a slippery non-adhesive surface allowing frictionless movements between adjacent parietal and visceral surfaces (Mutsaers et al., 2015). Moreover, MCs favor leukocyte recirculation and regulate the development of immune responses (Terri et al., 2021).

With respect to pathological conditions such as infections, MCs are a key factor in driving the immune response through the production of large quantities of extracellular mediators such as inflammatory cytokines and chemokines (Terri et al., 2021). Moreover, inflammatory and infectious stimuli may promote a complex multistep phenomenon called mesothelial to mesenchymal transition (MMT) (Lopez-Cabrera, 2014). In this context, MCs progressively lose epithelial-like features, acquiring new mesenchymal-like invasive and profibrotic abilities. Lineage-tracing of WT1-positive MCs in a context of fibrotic lung disease provided evidence of MMT induction *in vivo* (Sontake et al., 2018).

Due to its anatomic localization, pleura may be affected by many viral infections of the respiratory tract including influenza, coxsackievirus, respiratory syncytial virus (RSV), cytomegalovirus (CMV) (Nestor et al., 2013).

In spite of the conceivable MCs participation in COVID-19 pathogenesis, so far only indirect evidence has been reported. In this study, we highlight that SARS-CoV-2 causes structural modifications in the pleura with disruption of the mesothelial monolayer and the generation of WT1/cytokeratin-positive cells infiltrating the sub-mesothelial stroma. When analysing cellular/molecular mechanisms underlying this event, we found that MeT5A cells (a pleura non tumorigenic mesothelial cell line) express specific receptors and coreceptors for SARS-CoV-2 and produce infectious viral particles. Moreover, MeT5A cells infection resulted in the production of a broad repertoire of interferons, pro- and anti-inflammatory cytokines, and metalloproteases (MMPs).

Overall, this study provides a first evidence on a specific involvement of pleura MCs in SARS-CoV-2 pathology.

METHODS

Reagents and Antibodies

Polyclonal antibody against WT1 (#12609-2-AP) was from Proteintech (Rosemont, IL); anti SARS-CoV Nucleocapsid (#200-401-A50) for confocal microscopy experiments was from Rockland Immunochemicals, Inc. (Limerick, PA. United States); anti SARS-CoV Nucleoprotein/NP for immunohistochemistry experiments was from Sino Biological, (40143-T62, Beijing, China); anti ACE2 (AB_2792286) was from Invitrogen (Waltham, MA United States); anti ADAM17 (AB_10980438) was from Invitrogen. Monoclonal antibody anti dsRNA (10010200) was from Nordic-MUbio (Rangeerwe, Netherlands), anti-cytokeratin AE1/AE3/PCK26 (760-2595) was from Ventana (Oro Valley, Arizona. United States); anti-hsp90 (sc-13119) was from Santa Cruz Biotechnology (Dallas, TX United States); anti activated caspase 3 (9661) was from Cell Signaling technology (Danvers, MA, United States); anti TMPRSS2 (H-4: sc-515727) was from Santa Cruz Biotechnology; anti-GAPDH (cb1001) was from Calbiochem (Kenilworth, NJ, United States). DRAQ5 staining solution (#130-117-343) was from Miltenyi Biotec (Bergisch Gladbach, Germany). Sodium arsenite was from Sigma-Aldrich (Saint Louis, MO United States).

Cells

The human mesothelial cell line MeT5A (ATCC, Rockville, MD) was cultured in Earle's M199 supplemented with 10% fetal calf serum, 50 U/ml penicillin, 50 µg/ml streptomycin (Sigma-Aldrich).

Vero E6 cells (ATCC) were cultured in Eagle's Minimum Essential Medium supplemented with 10% fetal calf serum, 50 U/ml penicillin, 50 µg/ml streptomycin.

Viral Infection

Subconfluent MeT5A (200.000 cells/well) were incubated with SARS-CoV-2 (SARS-CoV-2 isolate SARS-CoV-2/Huma n/ITA/PAVIA1073 4/2020, clade G, D614G (S) obtained from Dr. Fausto Baldanti, Policlinico San Matteo, Pavia, Italy) in serum-free Eagle's Minimum Essential Medium at a multiplicity of infection (M.O.I) of 1 for 1.5 h at 37°C, 5% CO_2. Then, cells were washed three times with PBS to remove viral inoculum, and complete culture medium was added. Culture supernatants and

cell lysates were collected at 1.5, 24, and 72 h post infection (p.i.). Statistical significance was determined with a nonparametric Wilcoxon signed rank test with GraphPad Prism version 8.0 (La Jolla, CA, United States). Differences were considered significant at $p < 0.05$.

RT-PCR

Viral RNA was extracted from 140 µl of culture supernatant using the Qiamp viral RNA kit (Qiagen, Hilden, Germany), following manufacturers instruction and eluted in 50 µl of elution buffer.

Real time RT-PCR to analyze viral genome was performed on 10 µl of RNA extracted from cell culture supernatant, or 40 ng of cell-associated RNA using the RealStar® SARS-CoV-2 RT-PCR Kit RUO (Altona Diagnostics, Hamburg, Germany), which amplifies the E- and S- viral genes.

Cellular RNA was extracted from cell cultures using TRIzol reagent (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. cDNA synthesis was generated using a reverse transcription kit (A3500) from Promega (Madison, WI), according to the manufacturer's recommendations.

cDNAs were amplified by qPCR reaction using Maxima SYBR Green/ROX qPCR Master Mix (K0253) from Thermo Fisher Scientific (Waltham, MA). qPCR reactions were performed with the Rotor-Gene 6000 thermocycler (Corbett Research, Cambridge, United Kingdom). The primer sequences used in this study are shown in **Table 1**.

Relative amounts, obtained with 2 (−ΔCt) method, were normalized with respect to the housekeeping gene L34. Statistical significance was determined with a *t* test with Prism version 8.0. Differences were considered significant at $p < 0.05$. Values are reported in the graphs.

Western Blotting

MeT5A cells were lysed in CelLytic™ MT Cell Lysis Reagent supplemented with 1 mM PMSF; 1 µg/ml each of aprotinin, leupeptin and pepstatin; and 25 mM NaF (all from Sigma). Equal amounts of protein were resolved by SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Amersham Life Sciences, Little Chalfont, United Kingdom) and probed with primary antibodies using standard procedures. Peroxidase–conjugated secondary antibodies anti-rabbit (711-036-152) and anti-mouse (715-036-150) were from Jackson Immuno Research Laboratories, (West Grove, PA, United States). Nitrocellulose bound antibodies were detected by enhanced chemiluminesence (ECL) Immobilon Classico WBLUC0500 and Immobilon Crescendo Western HRP substrate WBLUR0500 from Millipore (Burlington, MA, United States).

Viral Titration

To estimate the production of infectious SARS-CoV-2, serial dilutions of MeT5A cell culture supernatants were put in contact with sub-confluent Vero E6 cells seeded in 96-well plates. At day 5 after infection, cells were observed for Cytopathic effect (CPE) and tissue culture infective dose (TCID) 50/ml was measured and analyzed by Reed-Muench method.

Confocal Microscopy

72 h after infection, SARS-CoV-2 infected and not infected cells were washed in cold PBS, fixed with 4% paraformaldehyde (Sigma-Aldrich) in PBS and permeabilized with 0.2% Triton X-100 (Sigma-Aldrich) in PBS. Alexa Fluor 488 secondary antibody was from Thermo Fisher Scientific; Cy3-conjugated secondary antibody was from Jackson Immunoresearch (Philadelphia, PA). Coverslips were mounted in Prolong Gold antifade (Life Technologies) and examined under a confocal microscope (Leica TCS SP2, Wetzlar, Germany). Digital images were acquired with the Leica software and the image adjustments and merging were performed by using the appropriated tools of ImageJ software. A minimum of 4 fields per sample (at least 150 total cells per total) from two independent experiments was analyzed.

Viability Assay

Cell viability was evaluated by ViaKrome 808 Fixable Viability Dye (Beckman Coulter) according to manufacturer's instructions. Cells were stained at 24 and 72 h after infection, fixed with 1% Paraformaldehyde (PFA) (Bio-Rad laboratories, Hercules, CA, United States) and washed 1x PBS. Data were recorded with a Cytoflex LX cytometer running CytoExpert Software (Beckman Coulter). Three independent experiments were performed. n.s.: not significant.

Cytokine Detection

Supernatants from SARS-CoV-2 infected MeT5A cell cultures were collected at 24 and 72 h after infection. We performed multianalyte profiling of 37 cytokines, chemokines, and soluble mediators in the supernatants of all samples, using the Luminex based multiplex bead technology (Bio-Plex Pro Human Cytokine Panel group I: APRIL/TNFSF13, BAFF/TNFSF13B, sCD30/TNFRSF8, sCD163, Chitinase-3-like 1, gp130/sIL-6Rβ, IFN-α2, IFN-β, IFN-γ, IL-2, sIL-6Rα, IL-8, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-19, IL-20, IL-22, IL-26, IL-27 (p28), IL-28A/IFN-λ2, IL-29/IFN-λ1, IL-32, IL-34, IL-35, LIGHT/TNFSF14, MMP-1, MMP-2, MMP-3, Osteocalcin, Osteopontin, Pentraxin-3 sTNF-R1 sTNF-R2, TSLP, TWEAK/TNFSF12, Biorad). The assay was conducted accordingly to manufacturer's recommendations. Plates were measured using the Bio-Plex MagPix System and analyzed with the Bio-Plex Manager version 6.0 (BioRad Laboratories).

IL1-β, IL-6, IL-8 and TNF-α were measured in supernatants samples by using an automated ELISA assay (ELLA microfluidic analyzer, Protein Simple, San Jose, CA, United States).

Statistical significance was determined with a *t*-test with GraphPad Prism version 8.0. Differences were considered significant at $p < 0.05$.

Autoptic Lung and Pleura

Lung tissue samples, including pleura, were obtained from post-mortem examination of four SARS-CoV-2-infected patients,

TABLE 1 | List of RT-PCR primers used in this study.

Gene	Forward sequence	Reverse sequence
hACE2	GGGATCAGAGATCGGAAGAAGAAA	AGGAGGTCTGAACATCATCAGTG
hADAM17	GGGCAGAGGGGAAGAGAGTA	GACTTGAGAATGCGAATCTGCT
hIFNα	TGGTGCTCAGCTACAAATCC	CCCATTTGTGCCAGGAGTAT
hIFNβ	TGGGAGGCTTGAATACTGCCTCAA	TCTCATAGATGGTCAATGCGGCGT
hL34	GTCCCGAACCCCTGGTAATAGA	GGCCCTGCTGACATGTTTCTT
hNRP1	AAGGTTTCTCAGCAAACTACAGTG	GGGAAGAAGCTGTGATCTGGTC
hTMPRSS2	AATCGGTGTGTTCGCCTCTAC	CGTAGTTCTCGTTCCAGTCGT

TABLE 2 | Demographic and clinical features of COVID-19 patients.

Patient number	Gender	Age	Comorbidities	Causes of death
1	M	81	Hypertension cardiomyopathy Aortic aneurysm	Myocardial infarction. Diffuse alveolar damage (ARDS). Interstitial pneumonia
2	M	54	None	Interstitial pneumonia. Myocarditis
3	M	82	Not known	Interstitial pneumonia. Cardiorespiratory failure
4	M	74	Hypertension. Knee arthroplasty	Interstitial pneumonia. Cardiorespiratory failure

TABLE 3 | Demographic and clinical features of non-COVID-19 patients.

Patient number	Gender	Age	Comorbidities	Causes of death
1	M	58	Hemicolectomy	H1N1 Pneumonia
2	M	43	Alcoholic cirrhosis	Interstitial pneumonia and pulmonary fibrosis
3	M	47	None	Cardiorespiratory failure
4	F	47	Surgery for frontotemporal meningioma and kidney cancer	Myocarditis and Interstitial pneumonia

performed at the National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS Hospital (Rome, Italy). All patients were diagnosed as COVID-19 by SARS-CoV-2 RT-PCR performed on nasopharyngeal and oropharyngeal swabs. Demographics and clinical course of patients are shown in **Table 2**.

Autopsies were performed according to guidance 167 for post-mortem collection and submission of specimens and biosafety practices to reduce the risk of transmission of infectious pathogens during and after the post-mortem examination (Hanley et al., 2020). Lungs samples, including pleura, of four non-COVID-19 patients were used as comparative controls (**Table 3**).

The study was approved by the local Clinical Research Ethics Committee (approval number: no 9/2020). Written informed consent was waived by the Ethics Commission for both COVID-19 and non COVID-19 patients due to public health outbreak investigation. Specimens from lung tissues were fixed in 10% neutral buffered formalin, and routinely processed to paraffin blocks.

Immunohistochemistry of Pleura

Deparaffinized and rehydrated sections were used for immunohistochemistry. Organ sections were immersed in 10 mM sodium citrate, pH 6.0 and microwaved for antigen retrieval and immunostained on BenchMark ULTRA system fully automated instrument (Roche, Basel, Switzerland). All cases were independently analyzed by two pathologists.

RESULTS

Myofibroblast Transformation of MCs in Visceral Pleura From SARS-CoV-2-Infected Patients

We previously demonstrated that SARS-CoV-2 infection caused a multisystem pathology with a dominant pulmonary and cardiovascular involvement (Falasca et al., 2020). In the present study, autoptic visceral pleura from COVID-19 patients was analyzed and compared to visceral pleura from non-COVID-19 patients.

Masson's trichrome staining revealed the onset of an intense fibrotic response in samples from COVID-19 patients (**Figure 1B**), with respect with non COVID-19 patients (**Figure 1A**). When analyzing pleura cellular components, while the MCs monolayer was maintained in pleura non-COVID-19 patients (**Figures 1C,D**), it appeared

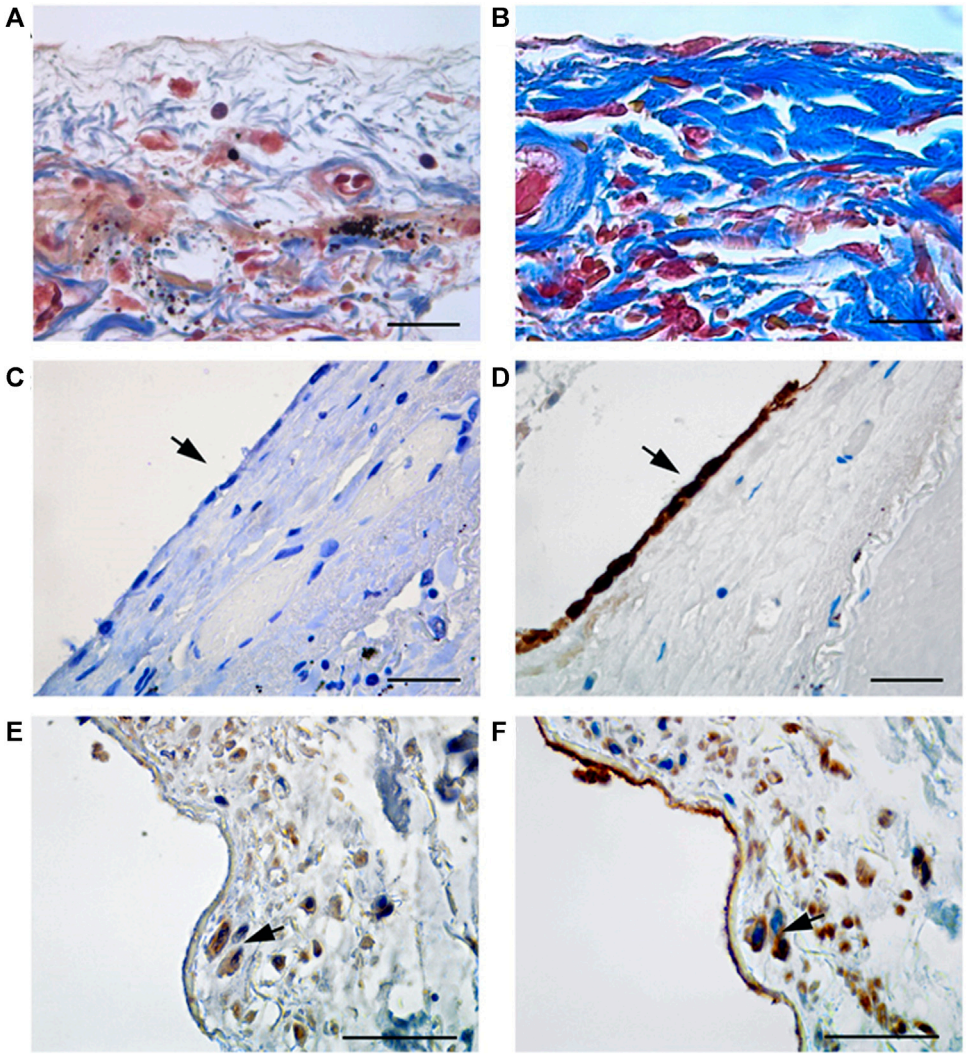

FIGURE 1 | (A) Masson's trichrome staining in tissue from non COVID-19 patients; **(B)** Masson's trichrome staining highlights the presence of collagen fibers (blue stain) in thickened submesothelial layer of visceral pleura from COVID-19 patients. **(C)** Visceral pleura from non COVID-19 patients show absence of staining (arrow) for WT1, a marker of reactive mesothelial cells. **(D)** Keratin AE1/AE3 staining, marker of mesothelial cells, shows a continuous monolayer of MCs in non COVID-19 patients. **(E,F)** Immunohistochemical labeling of WT1 shows positive submesothelial spindle cells in pleura from COVID-19 patients (E, arrow), and Keratin AE1/AE3 staining, performed on a consecutive section, show that the same cells (F, arrows) express both markers. Scale bars = 30 μm.

almost totally lost COVID-19 patients (**Figures 1E,F**), highlighting the specificity of pleural disruption in this disease.

Immunohistochemical labeling with WT1, a MC marker, showed positivity in spindle-like cells infiltrating the sub-mesothelial stroma of COVID-19 patients (**Figure 1E**). Accordingly, staining with anti-cytokeratin AE1/AE3 antibody confirmed the mesothelial origin of these infiltrating cells (**Figure 1F**). The sub-mesothelial stroma in non-COVID-19 samples was devoid of WT1, (**Figure 1C**), or cytokeratin positive cells, (**Figure 1D**), highlighting a specific impact of SARS-CoV-2 infection in promoting the acquisition of invasive ability by MCs. Anti-SARS-CoV immunolabeling did not reveal specific stain in the rare MCs present in visceral pleura from COVID-19 patients (**Supplementary Figure S1A**). However, positivity was found in pneumocytes from the same patients (**Supplementary Figure S1B**), in agreement to previously published data (Falasca et al., 2020).

These results demonstrate that SARS-CoV-2 infection causes the disruption of the monolayer of epithelial-like MCs, which in turn may invade the sub-mesothelial stroma promoting the onset of pleural fibrosis.

MCs Support SARS-CoV-2 Infection/ Replication

To characterize cellular and molecular mechanism underlying the observed lung alterations *in vivo*, we made use of MeT5A, a pleura non-transformed MC line widely used in the study of pleura

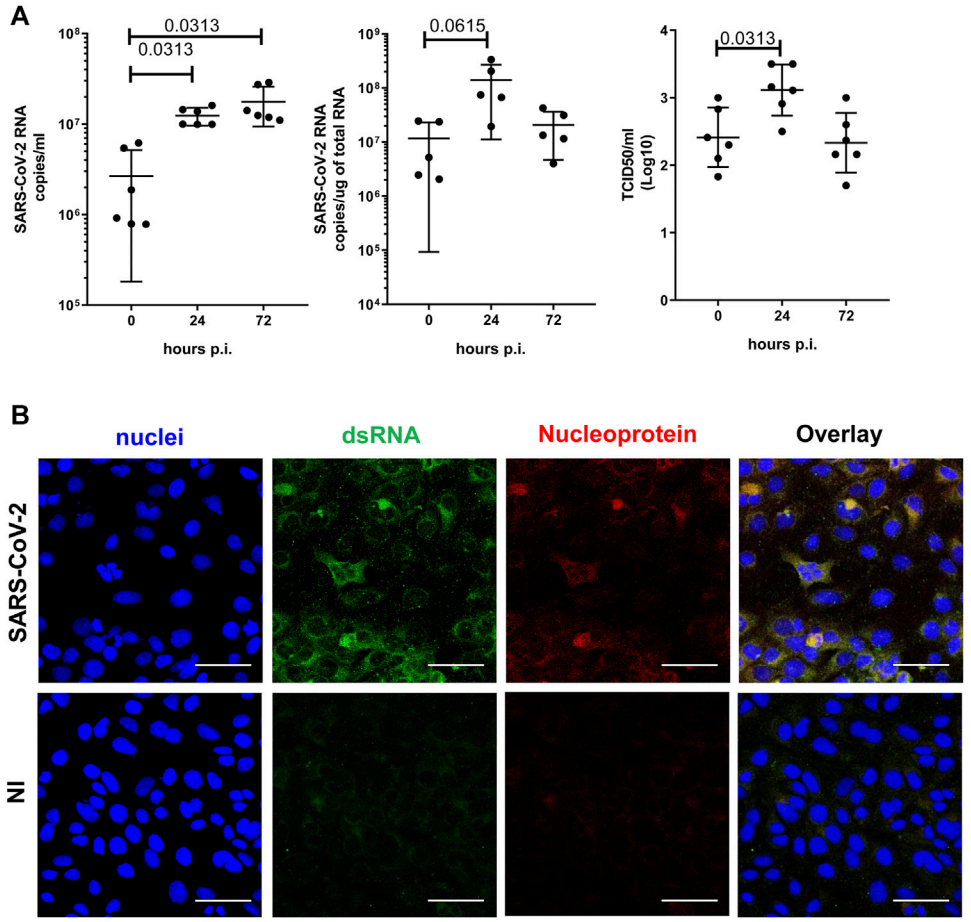

FIGURE 2 | (A) Left: Quantification of SARS-CoV-2 viral RNA expression in culture supernatants of MeT5A cells at 1.5, 24 and 72 h post viral inoculum (M.O.I. of 1). Six independent experiments were performed. Middle: Quantification of SARS-CoV-2 viral RNA expression in total RNA of MeT5A cultured as above. Five independent experiments were performed. Right: A TCID50 (Median Tissue Culture Infectious Dose) assay was performed adding serial dilutions of MeT5A cell culture supernatants to sub-confluent VeroE6 cells seeded in 96-well plates. Six independent experiments were performed. *P* was calculated with respect to time 0 of infection. Differences were considered significant at $p < 0.05$. **(B)** Immunofluorescence of MeT5A cells exposed for 120 h to SARS-CoV-2 (M.O.I. of 1) compared with non-infected (NI) cells. Fixed cells were stained with antibodies against SARS-CoV Nucleocapsid and dsRNA. Nuclei were stained with DRAQ5. A minimum of 150 cells per sample from two independent experiments were analyzed. Scale bar: 50 μm.

pathophysiological functions, such as mesothelial plasticity and fibrosis (Strippoli et al., 2008; Murphy et al., 2012; Rossi et al., 2018; Woo et al., 2021).

SARS-CoV-2 infection (M.O.I. of 1) of MeT5A resulted in a progressive accumulation of viral RNA in the supernatants at 24 (fold increase mean 8.2) and 72 (fold increase mean 16.4) hours p.i. (**Figure 2A**, left). Moreover, intracellular SARS-CoV-2 RNA peaked at 24 and slightly declined at 72 h p.i (**Figure 2A** middle). The presence of infectious SARS-CoV-2 viral particles in MeT5A supernatants was demonstrated by productive infection of Vero E6 cells (**Figure 2A** right). To further confirm viral infection of MeT5A, double-strand (ds) RNA and viral nucleoprotein (N) were detected by confocal microscopy at 72 h p.i. (**Figure 2B**). Exposure of MeT5A cells to SARS-CoV-2 viral particles did not cause an evident cytopathic effect and cell death, as demonstrated by bright-field microscopic analysis (not shown), by a ViaKrome viability assay and by cleaved caspase 3 detection (**Supplementary Figures S2A,B**).

To provide mechanistic evidence on SARS-CoV-2/MC interactions, we analyzed the expression of the plasma membrane receptors implicated in viral entry, namely ACE2, the protease TMPRSS2, and the co-factors NRP1 and ADAM17. As shown in **Figure 3A**, MeT5A cells express ACE2, TMPRSS2, NRP1, and ADAM17. As demonstrated by kinetic infection studies, ACE2 expression has a trend to increase after SARS-CoV-2 infection, whereas no changes in expression of the other receptors were observed. Expression of ACE2, TMPRSS2 and ADAM17 was confirmed at protein level by western blot analysis (**Figure 3B**). Interestingly, we found evidence of ACE2 expression (the main SARS-CoV2 plasma membrane receptor) in pleura MC by IHC (**Figure 3C**).

With respect to cellular specific response, SARS-CoV-2 infection promoted a rapid induction of Type I interferons

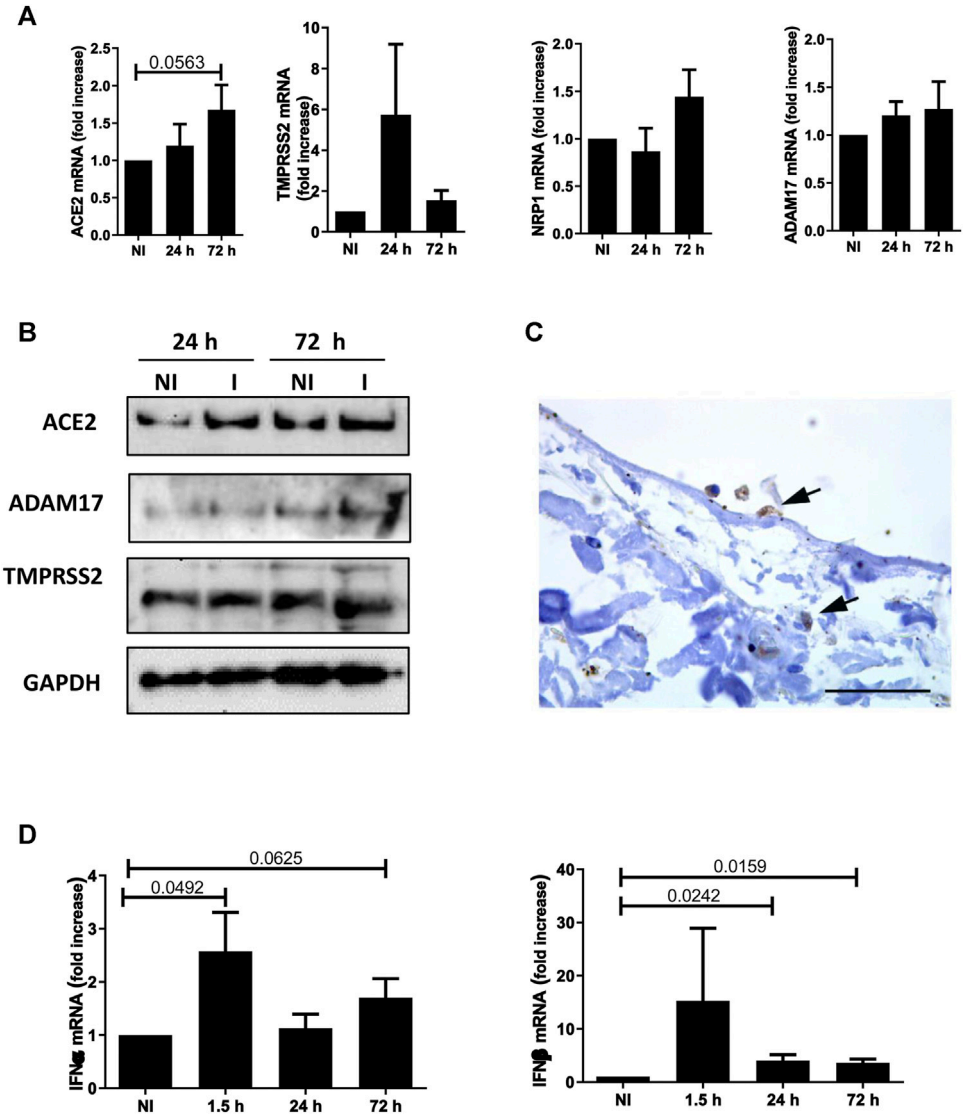

FIGURE 3 | (A) Quantitative RT-PCR expression analysis of *ACE2*, *TMPRSS2*, *NRP1* and *ADAM17* from total RNA of MeT5A cells exposed to SARS-CoV-2 infection (MOI = 1) for 24 or 72 h compared with non-infected (NI) cells. L34 mRNA levels were used for normalization. Bars represent the mean ± SEM of triplicate determinations in at least four independent experiments. *P* was calculated with respect to NI samples. Differences were considered significant at $p < 0.05$. **(B)** Western blot showing the expression of ACE2, TMPRSS2 and ADAM17, SARS-CoV-2 plasma membrane receptors, from total lysates of MeT5A cells treated as in A. I: SARS-CoV-2 infected cells. GAPDH was detected as a loading control. **(C)** Labeling with a specific antibody provides evidence of ACE2 expression (arrows) in MCs from visceral pleura of COVID-19 patients. Scale bar: 30 µm. **(D)** Quantitative RT-PCR expression analysis of *IFNα* and *IFNβ* in MeT5A cells exposed to SARS-CoV-2 for 1.5, 24 or 72 h (M.O.I. of 1) compared with NI cells. Quantitative RT-PCR was performed on total RNA. L34 mRNA levels were used for normalization. Bars represent the mean ± SEM of triplicate determinations in at least four independent experiments. *P* was calculated with respect to NI samples. Differences were considered significant at $p < 0.05$.

(IFN-I), as demonstrated by IFN-α and -β mRNA expression already induced at 1.5 and still significantly expressed at 72 h upon infection (**Figure 3D**).

The Infection of MCs by SARS-CoV-2 Promotes Cytokine Production

The specific contribution of the infected MCs in the modulation of the inflammatory response and extracellular matrix (ECM) remodeling was therefore explored. Supernatants from SARS-CoV-2-infected MeT5A cells were analyzed at 24 and 72 h after infection. 37 extracellular inflammatory mediators were evaluated by Luminex technology. Furthermore, the analysis was extended to another panel of inflammatory cytokines (IL-1β, TNFα, IL-6, IL-8) measured by automatic ELLA assay. The complete list and values of cytokines analyzed, including those not significantly expressed/modulated is shown in

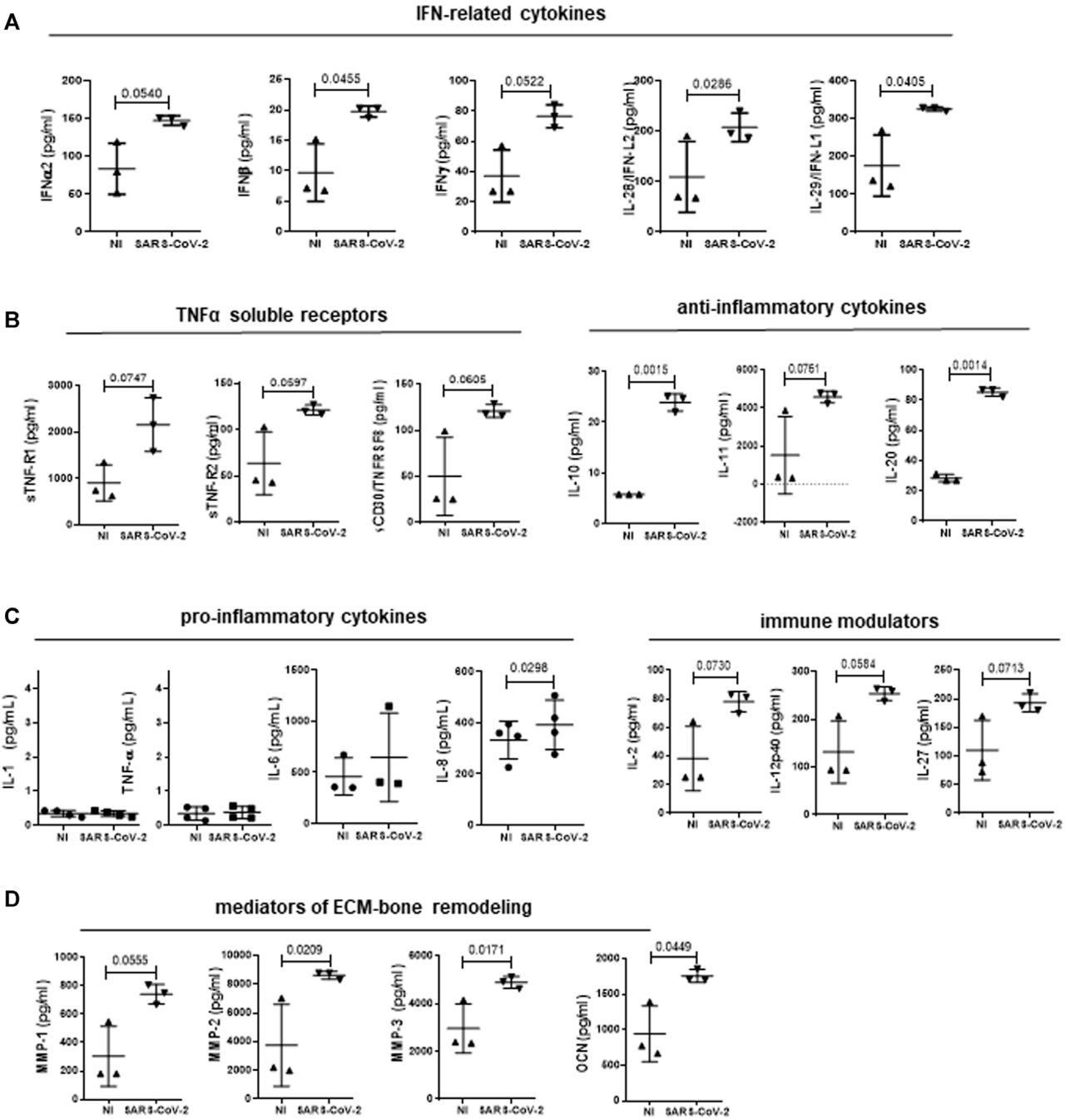

FIGURE 4 | Analysis of cytokine secretion from supernatants of MeT5A cells infected with SARS-CoV-2 or left uninfected for 72 h (M.O.I. of 1). SARS-CoV-2 infection promoted the production of IFN-related cytokines **(A)**; TNFα-soluble receptors **(B, left)**; anti-inflammatory cytokines **(B, right)**; pro-inflammatory cytokines **(C, left)**; immune modulators **(C, right)**; mediators of ECM-bone remodeling **(D)**. *P* was calculated with respect to NI samples. Differences were considered significant at $p < 0.05$.

Supplementary Figure S3, whereas significant induction of cytokines (observed at 72 h after infection) in shown in **Figure 4**.

The induction of an IFN response previously observed at mRNA level was confirmed by the presence of increased levels of IFNα–β–γ and -λ (IL-28-29) (**Figure 4A**). Secretion of cytokines with inhibitory activity belonging to TNF superfamily, (sTNF-R1, sTNF-R2 and sCD30/TNFRSF8) was increased (**Figure 4B**, left). On the other hand, production of TNFα and IL-1β, that are known to be secreted by MCs, was negligible upon SARS-CoV-2 infection (Douvdevani et al., 1994; Raby et al., 2018) (**Figure 4C**, left). Interestingly, the increase in

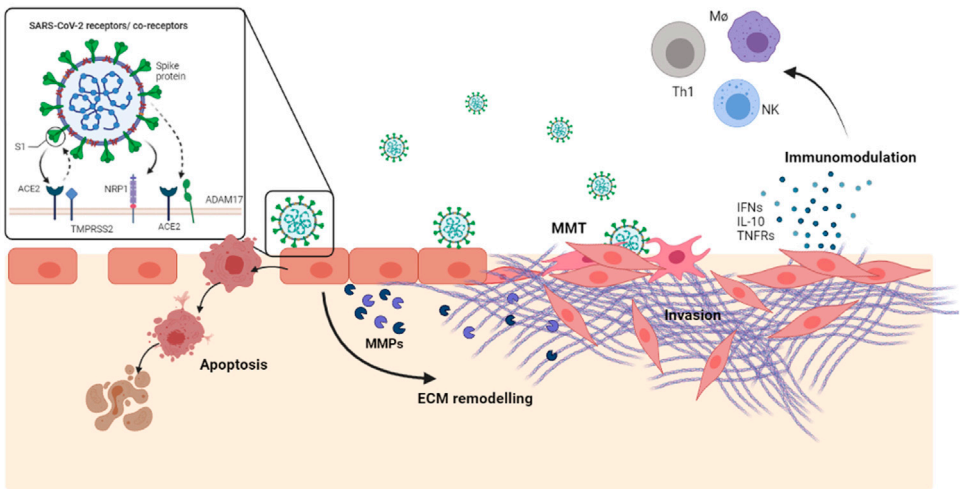

FIGURE 5 | SARS-CoV-2, in combination with other local signals released by the stroma/immune cells, may promote a transient MC infection followed by cell death as well as by induction of MMT. Moreover, infected MCs secrete a number of extracellular mediators active in inflammation, fibrosis immunomodulation.

the production of IL-10 and the structurally related IL-20 was highly significant (**Figure 4B**, right), whereas the abundant production of IL-6, characteristic of these cells, was not significantly increased by SARS-CoV-2 infection (**Figure 4C**, left) (Fujino et al., 1996). Of note, IL-8 production was significantly increased upon SARS-CoV-2 infection (**Figure 4C**, left). Moreover, MCs produced increased amounts of IL-2, IL-12p40 and IL-27, known modulators of innate and adaptive immunity (**Figure 4C**, right). Last, MCs secreted increased levels of MMP1-3, and of osteocalcin (**Figure 4D**). These data suggest that infected MCs may impact both the immune response to SARS-CoV-2 via the predominant production of anti-inflammatory mediators and the modification of the pleural stroma via the production of ECM remodelers.

DISCUSSION

Our observations provide a first evidence of SARS-CoV-2-infection/replication and inflammatory cytokine secretion by pleura MCs. Moreover, immunohistochemical analysis of samples from COVID-19 patients demonstrated a specific alteration of pleura characterized by disruption of the MC monolayer and invasion of the sub-mesothelial stroma by spindle-like MCs.

These data fall in a context where pleura-specific role in SARS-CoV-2 pathology has not yet been clarified. In particular, no evidence so far pointed to a direct role of pleura MCs in the COVID-19 pathogenesis. This in spite of the fact that around 10% of patients develop pleural effusions, have higher incidence of severe/critical illness, mortality rate, and longer hospital stay time compared to their counterparts without pleural effusion (Luo et al., 2020; Mo et al., 2020; Zhan et al., 2021). While there are reports linking fibrosis onset with disease severity, the cellular and molecular mechanisms have been incompletely studied so far.

Indeed, circulating ECM components have been correlated with prognosis (Ding et al., 2020). In COVID-19 patients, radiographic evidence of a wide range of pulmonary alterations associated to fibrotic damage and lung functional impairment are commonly observed (Shi et al., 2020). In fatal cases, increased expression of ECM-specific proteins and ECM regulators has been reported (Skibba et al., 2020). These data suggest that lung fibrosis onset is a pathogenic mechanism of severe SARS-CoV-2 infections. Accordingly, post infectious pulmonary fibrosis is also a known outcome in survivors of SARS, an infection with the closely related virus, SARS-CoV (Hui et al., 2005).

In this study, the observation of MCs loss with disruption of the MCs monolayer in biopsies of visceral pleura from autopsies of COVID-19 patients leads to hypothesize a direct or cell-mediated cytopathic effect of the virus. In particular, WT1-and cytokeratin-positive cells with a fibroblastoid morphology (having undergone bona fide MMT) were found the sub-mesothelial stroma.

Since MCs form in normal conditions a continuous monolayer above the basal membrane, the observation of MCs in the sub-mesothelial stroma during SARS-CoV-2 infection implicates the onset of an invasive program in these cells. However, the limited number of patients analyzed in this study made impossible to make correlations with clinical data and to further characterize the underlying invasive process.

Events linked with MCs plasticity may influence the fibrotic process in different ways. MMT in particular, has been demonstrated as a common mechanism of fibrosis in serosal membranes exposed to biomechanical, inflammatory and infectious stimuli (Ruiz-Carpio et al., 2017; Namvar et al., 2018; Raby et al., 2018; Strippoli et al., 2020).

Once transdifferentiated, MCs produce abundant amounts of TGFβ1, fibronectin, collagens (Mutsaers et al., 2015; Rossi et al., 2018). These cells may also rearrange the ECM through the expression of contractile proteins (i.e., αSMA) as well as the production of MMPs, such as MMP-2, -9 and -14 or MMP inhibitors such as TIMP1 and PAI1 (Ma et al., 1999; Strippoli et al., 2020).

So far, only indirect evidence pointed to an ability of SARS-CoV-2 to infect MCs. Viral load has been occasionally demonstrated in pleural fluid of infected patients; in this case, pleura MCs were found with large multiple nuclei, consistent with a cytopathic effect of the virus, although an infection of MCs by SARS-CoV-2 was not been formally proved (Bennett et al., 2020; Malik et al., 2020; Mei et al., 2020). Furthermore, an indirect evidence of a role of MCs is the fact that pleural effusions are linked with the onset of pleural fibrosis, which, along with pericarditis, is frequently found during COVID-19 (Falasca et al., 2020; Zeng et al., 2020). Indeed, MCs are key determinant of lung fibrotic diseases (Mutsaers et al., 2015; Liu et al., 2020).

In this study, we demonstrated that MeT5A cells express the main entry factors implicated in SARS-CoV-2 infection, i.e. ACE2, TMPRRS2, ADAM17 and NRP1. Cleavage of ACE2 has been demonstrated to impact on viral entry (Hoffmann et al., 2020). While it is already known that MCs express high levels of NRP1, a coreceptor of VEGFR with pro-fibrotic activity, the expression of ACE2, TMPRSS2 and ADAM17 has not been reported so far (Perez-Lozano et al., 2013; Cantuti-Castelvetri et al., 2020).

ACE2 levels in airway epithelial cells have been demonstrated to increase during SARS-CoV-2 infection, potentially rendering these patients even more vulnerable to SARS-CoV-2 (Chua et al., 2020). Other events, such as cigarette smoke may also increase ACE2 levels provoking SARS-CoV-2 increased infectivity in these individuals (Liu et al., 2021).

Our data suggest that ACE2 is already expressed in MCs in basal conditions, and the modulation of its expression is not a major mechanism regulating SARS-CoV-2 infection in these cells; in fact we found an high expression of ACE2 in uninfected MeT5A cells, and a tendential increase upon infection with SARS-CoV-2.

SARS-CoV-2 has a strong specificity in terms of cellular effects. For instance, SARS-CoV-2 promotes apoptosis in VERO3, but not in CALU3 cells (Park et al., 2021). In these cell lines, the induction of apoptosis has been related to high virus production and limited induction of interferon response. Moreover, cell culture conditions are extremely relevant to study SARS-CoV-2 effects in the lung and other organs. For instance, freshly isolated primary alveolar cultures from healthy individuals were found only minimally susceptible to SARS-CoV-2 (Hou et al., 2020).

Our data suggest that apoptosis is not induced in infected MeT5A cells. It seems conceivable that in our experimental conditions MeT5A survival is linked to the induction of Interferon response, which provokes the progressive clearance of infection over time. Apoptosis observed *in vitro* in other cell types (e.g., AT2 cells) may be influenced by specific experimental systems used, such as the use of feeder layer or organoids (Mulay et al., 2021; Katsura et al., 2020; Van Der Vaart et al., 2021). In these latter soft ECM-3D cultures often used to prevent AT2 cell differentiation, the induction of apoptosis may be relatively favored (Zhang et al., 2011; Halder et al., 2012).

By means of multiple approaches, we demonstrated that MCs sustain SARS-CoV-2 infection. Moreover, with respect to the cellular response to infection, we found an early (1.5 h post inoculum) induction of IFN-α and -β mRNA upon treatment with SARS-CoV-2. While the induction of a rapid IFN response is an indirect proof of MC infection, it also witnesses the ability of these cells to effectively clear SARS-CoV-2 infection at later time points. Interestingly, SARS-CoV-2 is sensitive to IFN-β treatment, and it may modulate the onset of the type I IFN response in infected cells through the activity of many viral proteins (Lei et al., 2020).

Differently from *in vitro* studies, autoptic examination of pleura failed to demonstrate infection of MCs by SARS-CoV-2. The almost complete loss of the MC monolayer, the relatively small number of patients examined or the timing of analysis may explain these negative results. To this respect, further study is warranted.

It is known that cytokine production has both a pathogenic and a prognostic role in SARS-CoV-2 infection. Cytokine storm is responsible for multiorgan pathology and eventually death, and inflammatory cytokine signatures may predict COVID-19 severity and patient survival (Del Valle et al., 2020; Mangalmurti and Hunter, 2020).

With respect to cytokine production by MCs, it should be considered these cells are key players in surveying the composition of the fluids covering the serosal membranes and in leukocyte recirculation. In coordination with resident macrophages and other immune cells, MCs secrete inflammatory cytokines and chemokines during bacterial and viral infections due to specific TLR activation, contributing in the shaping of the subsequent immune response (Terri et al., 2021) (Raby et al., 2018).

The analysis of cytokines secreted by SARS-CoV-2-infected MCs highlighted a predominance of anti-inflammatory (i.e., IL-10, sTNF-Rs) over pro-inflammatory (IL-1β, TNFα, IL-6) response. Indeed, while inflammatory cytokine production was negligible (IL-1β, TNFα) or not significantly increased (IL-6), the production of IL-10 and of TNFRs was significantly enhanced upon viral infection. MCs are known to secrete high amounts of IL-6 *in vitro* even in unstimulated conditions, while production of TNF-alpha and IL-1 is more restricted to specific pro-inflammatory stimuli (Topley et al., 1993; Douvdevani et al., 1994; Chen et al., 2015).

Of note, increased expression of sTNF-Rs has been previously reported in septic pleural effusions (Marie et al., 1997). The increase of interferons (IFNα–β–γ and IL-28-29) corresponds to an increase of cytokines with anti-inflammatory/immunomodulatory activity (IL-10, IL-11 and IL-20). During SARS-CoV-2 infection, anti-inflammatory mediators are secreted at the same time with pro-inflammatory mediators, and in particular, IL-10 and IL-6 expression both correlate with disease severity (Han et al.,

2020). However, IL-10 appears to have a "double edge" activity during inflammation: this cytokine is a key negative regulator of T cell mediated responses, but is also endowed with pro-inflammatory effects, including stimulation of IFNγ production (Lu et al., 2021).

Moreover, MCs produced significantly increased levels of IL-2 and IL-12 and IL-27, which may both activate NK and Th1 lymphocytes and promote antigen presentation. Interestingly, IL-27 is both an IFNγ-induced cytokine and an activator of IL-10 synthesis (Murugaiyan et al., 2009; Blahoianu et al., 2014).

The production of MMPs by MCs is potentially relevant for induction of the pleura fibrotic response observed during SARS-CoV-2 infection. Finally, another significantly increased cytokine was osteocalcin. This cytokine implicated in ECM remodeling promotes bone formation and may counteract osteoporosis observed in SARS-CoV-2 patients. A loop with IL-6, abundantly expressed by MCs, promotes osteocalcin expression (Chowdhury et al., 2020).

It is conceivable that due to the considerable extension of pleura surface (2000 cm^2 in an average adult male), the serous fluid recycling and the high vascularization of this organ, the predominant production of cytokines with anti-inflammatory activity by MCs may have a systemic effect of homeostatic dampening of the inflammatory response during infection. On the other hand, pleura may contribute to macrophage/lymphocyte activation and, through the secretion of mediators of ECM remodeling such as MMPs, play a role in lung fibrosis.

Of note, biologic drugs interfering the activity of some of the same cytokines here demonstrated to be produced by infected MCs are being currently analyzed for their therapeutic potential to dampen the noxious inflammatory response (Mangalmurti and Hunter, 2020).

Overall, our results suggest that a transient MCs infection, in combination with other local signals released by stromal/immune cells, may promote both cell death with pleura disruption, and induction of MMT with the acquisition of an invasive abilities. At the same time, SARS-CoV-2 infection promotes the secretion of cytokines and other extracellular mediators which may both modulate the inflammatory response and induce ECM remodeling. A comprehensive experimental model of MCs response to SARS-CoV-2 infection is shown in **Figure 5**.

Thus, MCs are candidates for cellular interventions aimed at restoring the continuity of the monolayer, at modulating the anti-viral immune response and at limiting the insurgence of fibrosis. These discoveries warrant more study to better define the role of MCs and their potential implication for therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Autopsies were performed at the National Institute for Infectious Diseases Lazzaro Spallanzani-IRCCS Hospital, Rome, Italy. The study was approved by the local ethics committee (approval number 9/2020). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

Conceptualization: GM, MTr, RS. Data Curation: VB, MTe, CM. Formal analysis: GM, FT. Funding acquisition: CC, MC, CA, MTr, RS. Investigation: GM, FT, VB, RN, LF, DC, DM. Methodology: GM, CA, RS. Project Administration: RS. Resources: GM, CM, MTe, DC. Supervision: FDN, MC, CA, MTe, RS. Validation: FN, LF. Visualization: GM, FT, RN, LF. Writing-original draft: RS, MTr. Writing-review and editing: GM, FT, FN, RN, LF, CA.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2021.752616/full#supplementary-material

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REVIEW
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The Oral Complications of COVID-19

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Background: COVID-19 is a novel coronavirus infectious disease associated with the severe acute respiratory syndrome. More and more patients are being cured due to the development of clinical guidelines for COVID-19 pneumonia diagnosis, treatment, and vaccines. However, the long-term impact of COVID-19 on patients after recovery is unclear. Currently available reports have shown that patients recovered from COVID-19 continue to experience health problems in respiratory and other organ systems. Oral problem is one of the important complications which has serious impacts on the rehabilitation and future quality of life, such as ageusia and macroglossia, but the oral complication is often being neglected.

Aim of Review: From the perspective of stomatology, we summarized and elaborated in detail the types, pathogenesis of oral complications from COVID-19 patients after rehabilitation, and the reported prevention or treatment recommendations which may improve the COVID-19 patients associated oral diseases.

Key Scientific Concepts of Review: 1) To understand the common oral complications and the mechanisms of the development of oral complications after the COVID-19 recovery; 2) To summary the practical strategies to prevent the oral complications and construct the rehabilitation plans for patients with oral complications.

Keywords: COVID-19, recovery, stomatology, complications, susceptibility

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INTRODUCTION

Corona Virus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an acute respiratory infectious disease with a high infectivity and fatality rate. According to World Health Organization (WHO) data, by October 19, 2021, the cumulative number of confirmed cases reported globally is now over 240 million and the cumulative number of deaths is over 4.8 million.

The main clinical outcomes of COVID-19 patients are acute respiratory infection, with clinical manifestations including fever, fatigue, cough, myalgia, fatigue and other symptoms, and atypical symptoms include expectoration, headache, hemoptysis and diarrhea (Zhu et al., 2020). In clinical treatment, all patients have pneumonia, and about half had dyspnea and lymphocytopenia.

The SARS-CoV-2 can not only cause lung disease, but also a variety of systemic complications. Many patients died due to respiratory and cardiac complications (Chen et al., 2020b). Common complications in patients who died included acute respiratory distress syndrome (ARDS) (113; 100%), type I respiratory failure (18/35; 51%), sepsis (113; 100%), acute cardiac injury (72/94; 77%), heart failure (41/83; 49%), shock (46; 41%), alkali poisoning (14/35; 40%), hyperkalemia (42; 37%), acute kidney injury (28; 25%) and hypoxic encephalopathy (23; 20%). These complications caused

physical injuries that were difficult to recover and seriously affected the lives of patients. In addition, the increased stress caused by COVID-19 and its complications, loss of friends or family members, damage to financial status, work stress and confinement also have a severe impact on patients' mental state.

Angiotensin converting enzyme 2 (ACE2), targeted by SARS-CoV-2, mainly exists on the surface of human epithelial cells, especially type II alveolar epithelial cells, affecting anti-inflammatory, anti-proliferation, anti-fibrosis, anti-apoptosis of alveolar epithelial cells and vasodilator (Zou et al., 2020). ACE2 is also abundant in human oral mucosa and tongue epithelium, and SARS-CoV-2 can also bind to ACE2 receptors from oral tissues to cause the oral complications, including macroglossia, taste disorders, oral mucosa disease and so on (Aziz et al., 2020). As the entrance of digestive tract, oral cavity has a close relationship with human nutrition intake and connects the respiratory tract to assist the respiratory system. In the process of treatment of COVID-19, oral complications should be paid more attention. Meanwhile, some oral lesions may also be a warning signal for COVID-19, such as the peripheral thrombosis. Paying attention to oral lesions can start anticoagulant therapy in time to avoid more serious complications caused by peripheral thromboembolism (Favia et al., 2021).

The purpose of this paper is to summarize and discuss various oral complications of COVID-19 patients, emphasize the importance of prevention and treatment of oral complications in the rehabilitation process of COVID-19 patients, summarize the etiological mechanism to highlight the follow-up study of COVID-19 in dental practice. We also summarize the clinical diagnosis and treatment plan in depth to provide some information in the diagnosis and treatment of COVID-19.

TASTE DISORDERS

Epidemiology

Taste disorders are common in COVID-19. Among COVID-19 positive patients, taste disorders are more prevalent in Europe, North America, and the Middle East, and less prevalent in Asia (Wong et al., 2020). It has been reported that the prevalence of smell and taste disorders in COVID-19 positive patients globally is 34–86% in Europe, 19–71% in North America, 36–98% in the Middle East and only 11–15% in Asia. From the perspective of gender distribution, females are more likely to suffer from taste disorders, and from the perspective of age distribution, young people are more likely to suffer from taste disorders (Lee et al., 2020).

The taste disorder can be simply divided into three types: hypogeusia, dysgeusia, and ageusia. It has been reported (Amorim Dos Santos et al., 2021) that the overall prevalence of taste disorders is 45%, of which 38% are hypogeusia, 35% are dysgeusia, and 24% are ageusia. The final statistical results are different due to different diagnostic methods in different regions and research methods including telephone, online, questionnaire and medical records review, but in general, taste disorders are a common oral complication of COVID-19 infection with a high incidence.

Due to their high incidence and some degree of presentation in the early stages of the disease, taste disorders are now accepted as a diagnostic criterion for COVID-19 in most regions. People with impaired taste are 6–10 times more likely to develop COVID-19 than people with normal taste (Wong et al., 2020). Taste disorders will alert doctors to the possibility of COVID-19 infection and need to seriously consider self-isolating and testing these people.

Clinical Manifestations

Taste disorder is an inability to correctly perceive chemical stimuli such as taste and spiciness. Taste disorders can be roughly divided into the following three types: complete loss of taste (ageusia), reduced taste intensity (hypogeusia), distorted taste (dysgeusia) (Fjaeldstad, 2020).

The diagnosis of taste disorders can be determined by a questionnaire, which is relatively simple but subjective. Alternatively, chemical gustometry can be used.

Both COVID-19 patients and those with the acute cold can have taste disorder, but those with the disease have a harder time distinguishing bitter and sweet, while the ability to distinguish sour and salty is similar to that of those with the acute cold (Huart et al., 2020). Taste strips are also used to help us distinguish COVID-19 patients from those suffering from the acute cold.

Hazards

As one of the five human senses, the loss of taste may seriously affect the quality of life. One effect is that patients who lose their sense of taste are more likely to develop dangerous events such as food poisoning. For example, toxic or spoiled foods often have strange tastes such as bitterness and acid, and sufferers of taste disorders are less likely to taste spoiled food. The inability to taste can lead to anorexia, which can lead to malnutrition, weakened immunity and worsening disease. People who rely on their sense of taste for work, such as chefs, bartenders and pastry chefs, may lose their jobs due to taste disorders and find it difficult to do their jobs after COVID-19 recovery, thus cutting off their financial resources. Studies have shown that taste is associated with depression, but the biological mechanisms are unclear (Yom-Tov et al., 2021). More clinical studies are needed to determine the exact association, pathogenesis, and prognosis.

Pathogenicity Mechanism

The pathogenesis of taste disorders is still unclear, and there are several theories.

Some studies have shown that (Deems et al., 1991; Prescott, 2012) taste disorders may be related to the damage of neuronal cells by viruses, or may be caused by central nervous system ischemia. The peripheral nervous system is affected by the novel coronavirus, as the taste buds are innervated by the cranial nerves, the related functions may be impaired, resulting in taste disorders.

The production of taste mainly comes from taste receptors on the tongue, and sialic acid can protect glycoproteins responsible for the molecular transport of taste stimulation in the taste pore (Witt and Miller, 1992; Pushpass et al., 2019). Loss of taste may be due to SARS-CoV-2 binding to sialic acid receptors, which may be responsible for taste disorders. SARS-CoV-2 interactions with taste components and ACE2 receptors also have a direct impact on COVID-19-related taste disorders (Amorim Dos Santos et al., 2021).

Because taste and smell disorders often occur simultaneously, some research suggests that taste disorders may be related to the loss of smell, because the brain sometimes combines smell and taste together (Prescott, 2012). But this claim is controversial because some patients with the taste disorder COVID-19 do not develop inflammation of the nasal mucosa. Many COVID-19 patients who do not have a sense of smell disorder also develop a taste disorder (Ibekwe et al., 2020). In some patients with both taste disorder and smell disorder, the taste disorder may appear before smell disorder (Ibekwe et al., 2020).

Recent studies have also shown that taste cells can be induced to produce a large number of inflammatory cytokines, such as interleukin 6, under inflammatory stimulation (Cazzolla et al., 2020). Elevated levels of inflammatory cytokines can cause cells in taste buds to die and inhibit cell renewal, which can also lead to taste disorders (Cazzolla et al., 2020).

Treatment

Most taste loss patients will recover within 3 weeks, and most recovery time is 7 days (Lee et al., 2020). It shows that most mild COVID-19 patients can recover their subjective sense of taste without direct medical intervention (Levy, 2020). However, nearly a year after COVID-19 patients were cured, some patients still did not recover these feelings. Alternatively, some patients regained their sense of taste, but their sense of taste was reversed and disordered, accounting for 29% of the patients according to the data reported in the study (Nouchi et al., 2021). In Nguyen's study (Nguyen et al., 2021), 38.5% of patients had only partial taste recovery after 6 months, while 11.5% had no recovery at all after 6 months of treatment. Women are also less likely to be cured than men, accounting for 73.3% of patients who were not cured after 6 months.

SARS-CoV-2 is similar to other coronaviruses, exhibiting neural invasion (Hu et al., 2020). Neurostimulants acting on the nervous system, such as steroids, B vitamins and ATP, have been shown to be promising in treating taste disorders in COVID-19 patients (Okada et al., 2021). In addition, zinc may play a potential role in the treatment of taste disorders (2021). Zinc protects natural tissue barriers such as the epithelium, prevents entry of pathogens, balances the immune system and the redox system. As zinc is related to the action of carbonic anhydrase, lack of zinc will significantly reduce taste sensitivity and impair salivary secretion (Goto et al., 2000; Goto et al., 2001; Tanaka, 2002). Therefore, zinc supplementation can improve taste disorders (Heckmann et al., 2005). However, the use of zinc varies from person to person, and some patients with taste disorders have no effect after zinc supplementation (Sturniolo et al., 1992), even using very high zinc concentration (Lyckholm et al., 2012).

ORAL MUCOSAL LESIONS

Epidemiology

Oral mucosal lesions are also a common complication of COVID-19 patients. Compared to taste disorders, oral mucosal lesions are rarely reported.

Patients with oral mucosal lesions present with a variety of oral pathologies. The most common continuous lesions accounted for 73.85%, including ulcer 55.38%, aphtous lesions 12.31%, erosion 6.15%, followed by macula 6.15%, petechiae 4.61%, plaque 4.61%, bullae 3.08%, the least is gingival abnormalities. For example, peeling and necrotizing gingivitis accounted for 1.54%, and blister and pustules accounted for 1.54% (Egido-Moreno et al., 2021).

Among the patients with oral mucosal lesions, the most common site is tongue (52.56%), followed by palate and lip (16.67%), gingival (7.69%), buccal mucosa (3.85%), and connective site (2.56%) (Egido-Moreno et al., 2021).

Clinical Manifestations

Oral mucosal lesions caused by SARS-CoV-2 have a variety of clinical manifestations, and the mucous membranes of the tongue, palate, lip, gingival and buccal of patients are affected, with varying amounts, colour and appearance. Specific symptoms include irregular ulcers, small blisters, petechiae, erythematous plaques, and desquamative gingivitis. In addition, these oral mucosal lesions can be co-infected with other viruses, and few patients are caused by a single virus (Chen et al., 2020d; Galvan Casas et al., 2020). Patients with oral mucosal lesions are usually accompanied by other skin lesions. Currently, the characteristics of oral mucosal lesions induced by COVID-19 are similar to those of non-COVID-19 patients, so the diagnosis should be combined with epidemiological history.

Hazards

Oral mucosal lesions can cause pain and easily induce concurrent infection of other bacteria, fungi and viruses, such as *Candidiasis* and HSV-1 (Riad et al., 2020a; Tang et al., 2020). Oral mucosal lesions seem to develop into secondary manifestations and co-infection-related debilitating systemic conditions in patients (Amorim Dos Santos et al., 2021).

Pathogenicity Mechanism

At present, there is controversy about whether oral mucosal lesions are directly caused by SARS-CoV-2 or are secondary manifestations.

The cells with ACE2 receptors are distributed on the tongue mucosa and salivary glands, these cells may become the host cells of the virus, thus causing inflammatory reactions in oral organs and tissues (Riad et al., 2020b; Amorim Dos Santos et al., 2021). Recently, Xu et al. (2020a) demonstrated the sensitivity of SARS-CoV-2 to tongue mucosa and salivary glands, and oral mucosa may be one of the targets of virus infection in humans. Therefore, oral mucosal lesions may be directly related to COVID-19 infection (Brandão et al., 2021).

However, some researchers believe that the oral symptoms are due to opportunistic or secondary infections caused by decreased immunity in the course of COVID-19 treatment after coronavirus infection. From a large number of reported medical records, many patients are co-infection and secondary manifestations. Patients' immune status is altered during treatment, and many COVID-19 cases are fatal due to bacterial and fungal co-infection (Chen et al., 2020a; Zhou et al., 2020). In some patients, the suppressed immune system triggers reactivation of herpes

simplex virus or varicella-zoster virus, leading to viral co-infection and illness. Meanwhile, oral mucosal lesions may also be induced by the immune system deterioration or disease treatment. The host produces cytokine storms during treatment for COVID-19, which can induce drug eruptions and lead to drug allergies and hives (Izquierdo-Domínguez et al., 2021).

The causes of oral mucosal lesions are complex, and many other non-viral factors can also lead to oral mucosal lesions. For example, during the COVID-19 pandemic lockdown, restrictions on social life can lead to work stress, anxiety about survival, inability to visit dental clinics, and possibly poor oral hygiene, all of which can lead to oral mucosal lesions (Guo et al., 2021). In order to reduce oral viral load after going out, some patients frequently use oral disinfectants such as hydrogen peroxide mouthwash, which may also cause oral ulcers (Hasturk et al., 2004; Petrescu et al., 2020). After the occurrence of oral mucosal lesions, the causes should be considered from various aspects.

Treatment

For all COVID-19 patients with oral mucosal lesions, the affected site is usually cured within 3–21 days through local treatment and oral hygiene (Amorim Dos Santos et al., 2021). For patients suspected of co-infection, when conditions are available, *Saccharomyces cerevisiae* tongue scraping culture can be carried out to determine the patient's fungal infection, and biopsy can also be used. Antifungal agents such as intravenous fluconazole and oral nystatin may be used in the treatment of these patients (Amorim Dos Santos et al., 2020). Some alcohol-free antibacterial mouthwashes can also be used, such as 0.12% sodium dichloroglyconate chlorhexidine or 1% hydrogen peroxide (Amorim Dos Santos et al., 2020).

At the same time, since oral mucosal lesions of COVID-19 patients are often co-infected, dentists should pay attention to the infection of patients from multiple aspects in the process of treatment and treat patients for different causes. Oral mucosal lesions can be accompanied by other skin lesions. In the treatment process, patients should be also observed for skin lesions during a comprehensive physical examination. In addition, dentists should explain to the treatment team the importance of maintaining oral hygiene to avoid oral mucosal lesions in COVID-19 patients.

MACROGLOSSIA DISEASE

Epidemiology

The incidence of macroglossia is relatively small. In a review of 210 patients with severe COVID-19 who had been admitted to intensive care unit in 14 clinical studies, only one (0.5%) developed macroglossia (Hocková et al., 2021).

Clinical Manifestations

Macroglossia is a clinical diagnosis and its etiology is very complicated. A few cases of macroglossia had been reported before the COVID-19 outbreak. The clinical definition of macroglossia is that the tongue usually extends beyond the tooth or alveolar crest in its natural state. According to the different pathological types, macroglossia can be divided into true macroglossia and relative macroglossia. The main difference between the two is that true macroglossia has definite histopathological abnormalities, while relative macroglossia may have corresponding clinical symptoms, but its histological structure is normal. Some patients with macroglossia may develop different complications, such as keratinised tongue plaques or infection (Sharma et al., 2021). The most common cause of macroglossia in non-COVID-19 patients is congenital lingual vein malformation or lymphatic vascular malformation, which is more common in young children, while macroglossia caused by COVID-19 is more common in adults, most of whom have been on ventilators (Andrews et al., 2020; Sharma et al., 2021).

Hazards

As the tongue grows, thickens and stiffens, the tongue cannot be retracted back into the mouth for a long time, which will make the tongue lose a lot of water and cause pain. A large tongue can cause tongue dysfunction and all kinds of complications. Patients will have speech disorders such as unclear speech, digestive disorders such as dysphagia, airway obstruction and other respiratory disorders. Severe cases will be completely unable to eat or speak (Brockerville et al., 2017).

Pathogenicity Mechanism

At present, the occurrence of macroglossia in COVID-19 patients is still a rare phenomenon. There is no literature or definitive study showing the correlation between SARS-CoV-2 and macroglossia. Before COVID-19, macroglossia was caused by congenital diseases such as vascular malformations and muscle hypertrophy, or acquired diseases such as amyloidosis, hypothyroidism, endocrine disorders, metabolic disorders, tumor infiltration, viral infections, inflammatory diseases and trauma (Weiss and White, 1990).

One of the underlying causes of macroglossia is lymphatic congenital malformations (Yesil et al., 2015; Chen et al., 2020c). When the tongue is infected by bacteria or viruses, its immune system attacks the bacteria or viruses through the lymphatic system (Cheng et al., 2013). This swelling occurs in the lymphatic system to activate the inflammatory response. During the recovery of the normal inflammatory response, lymphatic will naturally detumescence back to the normal state. But the lymphatic swelling reflux obstacles can occur in some people with lymphatic congenital malformation (Horasanli et al., 2010). The lymphatic long-term blockage will result in the phenomenon of "giant tongue". In addition, acquired angioedema can be caused by co-infection of viruses or bacteria, drug treatment, and idiopathic non-histamines (Vogel et al., 1986; Kutti Sridharan and Rokkam, 2021), all of which may be the cause of macroglossia in COVID-19.

A large number of COVID-19 patients are treated with mechanical ventilation (Azmy et al., 2020; Akhavan et al., 2021). It is also believed that the patients who recovered from COVID-19 are suffering from "macroglossia" because of

mechanical ventilation in the prone position (Hocková et al., 2021). To help COVID-19 patients breathe normally, doctors may choose to strengthen their lungs by placing them prone to intubated breathing in a hospital bed. This treatment may exacerbate the blockage of lymph flow, which may lead to enlargement of the tongue (Hocková et al., 2021). This may also be related to the patient's autonomic nervous function instability (Berger, 2020). Given that autonomic dysfunction is common in severe COVID-19 patients, the risk of macroglossia is likely to be further amplified when patients receive mechanical ventilation in the prone position (Dani et al., 2021).

Others believe that the etiology of macroglossia may also be genetic, because according to the current reports, the patients with macroglossia are mainly black race (Andrews et al., 2020; Azmy et al., 2020; Nuño González et al., 2021). According to epidemiological studies, the incidence of symptomatic angioedema in the black population is much higher due to a genetic predisposition to angioedema (Montinaro and Cicardi, 2020). However, since macroglossia is so rarely reported, more evidence is needed to construct the relationship between macroglossia and genetic predisposition.

Treatment

To avoid severe water loss from the tongue, the tongue can be loosely wrapped with a wet saline gauze and then wrapped with compressed Coban from the distal end to the proximal end (Andrews et al., 2020). To relieve the symptoms of dry mouth, moisturizing mouthwash can also be used to moisturize (Sharma et al., 2021).

For patients with mild symptoms, local compression therapy can be used to subside the edema and allow smooth recovery (Andrews et al., 2020). Corticosteroids and bite blocks can also be used for conservative treatment (Andrews et al., 2020). For patients with severe macroglossia, surgery is the most effective way to remove the tongue hanging out of the mouth and unable to retract (Dolan et al., 1989). Because the tongue is so important for both taste and speech, the operation is delicate and difficult. When part of the tongue is removed, other parts of the tongue will replace the removed taste buds (Zhang et al., 2019). However, after partial tongue removal, the patient's language ability may be affected (Andrews et al., 2020).

For most people with macroglossia, an oversized tongue can completely block the airways, leading to suffocation and sudden death (Alonso-Rodriguez et al., 2018). Patients with macroglossia need closely monitored and lifesaving measures such as tracheotomy (Junghaenel et al., 2012). Because mechanical ventilation in the prone position may be associated with macroglossia, some physicians also recommend other measures to improve oxygenation in patients with macroglossia to avoid further progression of the disease (DePasse et al., 2015).

OTHER ORAL COMPLICATIONS

In addition to the above oral complications, there are still some other oral complications, which are less reported, or indirectly related to oral.

Loose Teeth

Some patients may experience symptoms such as tooth loss, gingival sensitivity, or gray or brittle teeth (Abdalla and Zwaid, 2021). Currently, the number of patients with this symptom is very small (Sirin et al., 2021). It may occur as the SARS-CoV-2 can irritate the gums, then cause sudden teeth loss.

Oral Diseases in Patients With Mechanical Ventilation

Severe COVID-19 patients require long-term mechanical ventilation, and these patients will suffer from a large number of oral complications (Silva et al., 2020), such as bad breath, secretions retention in the mouth, mucosal damage, etc. Long-term intubation can easily lead to oral and laryngeal muscle injury, resulting in dysphonia and dysphagia after extubation (Castillo-Allendes et al., 2021).

DISCUSSION

Here we described in detail the common oral complications of COVID-19 which may be served as a kind of early warning sign of COVID-19 infection, as it can be used to screen quickly and initially for COVID-19 infection when. Taste disorders, oral mucosal lesions, and other clinical manifestations often occur in other diseases, but as oral complications of COVID-19, they will have some unique characteristics, once these characteristics are discovered, it is more necessary to arouse people's vigilance. For example, patients with cold sometimes have symptoms similar to taste failure. In the clinical process, the two are easily confused, but due to the different etiology between COVID-19 complications and the common cold, there are some characteristics that can distinguish the two diseases in the practice of diagnosis and treatment. Patients with a taste deficit caused by COVID-19 have a harder time tasting bitter and sweet tastes than those suffering from a cold (Huart et al., 2020). Although both types of taste loss are often accompanied by smell loss. People with colds often have runny noses or stuffy noses, which are less common in patients with COVID-19, but COVID-19 patients have a worse ability to distinguish smells (Huart et al., 2020). This may be because mucosal congestion and edema are common in people with cold, but in COVID-19 patients, the virus attacks the chemosensory receptors, causing damage to receptors in pathways and higher cortical areas. In addition, senile taste bud sensory function degradation is also a common cause of taste disorders, in the process of diagnosis and treatment, the patient's age is also considered. There are many causes of oral mucosal lesions, for example, immune diseases or fungal infections. Therefore, whether this symptom is a complication caused by the COVID-19 can be effectively judged by combining the simultaneous occurrence of cough, fever, dyspnea, and other symptoms. For macroglossia, the common clinical etiology is due to tongue tumors, such as hemangioma, lymphangioma, Beckwith-Wiedemann syndrome, etc. However, these patients are younger and can

TABLE 1 | The guidelines for diagnosis and treatment of the oral complications of COVID-19 patients.

	Clinical manifestations	Distinguish from non-COVID-19 patients	Treatment
Taste disorders	Inability to correctly perceive chemical stimuli	Easily confused with the acute cold. Harder to distinguish bitter and sweet than acute cold patients. Often have smell loss, but runny noses or stuffy noses is uncommon	Mild COVID-19 patients can heal themselves. Others can use neurostimulants such as steroids, B vitamins and ATP. Zinc supplementation can be used, but need to pay attention to the zinc concentration
Oral mucosal lesions	Irregular ulcers, small blisters, petechiae, erythematous plaques, and desquamative gingivitis, etc. Co-infection with other pathogens. Accompanied by other skin lesions	Covid-19 patients often combining with cough, fever, dyspnea	Oral local treatment. Antimicrobial treatment. Keep oral microorganisms ecological balance
Macroglossia disease	Tongue extends beyond its natural state	Macroglossia caused by COVID-19 are mostly adults. Most were on ventilators	Avoid severe water loss. Mild patients can use local compression therapy. Severe patients need surgery. Tracheotomy can be used for patients with dyspnea

be diagnosed by pathology, CT, and other methods, while the patients with macroglossia caused by COVID-19 are mostly adults. And most of them had an important characteristic, that is, they had been seriously infected with COVID-19 and had received mechanical ventilation. These features of oral complications of COVID-19 are summarized in **Table 1** for diagnostic reference.

The human microbiome also plays an important role in human health. The oral cavity is a key part of the body's microbial communication with the outside world, connecting the respiratory tract and digestive tract. In normal conditions, there exists a layer of barrier dominated by beneficial bacteria on the surface of oral mucosa tissue, which can prevent the invasion of pathogenic microorganisms and activate the human immune system to eliminate relevant pathogens and limit the pathogens remaining in the human body to the range of harmless to the human body. If the oral microecology is out of balance, the prevention of oral mucosa is easy to be breached by pathogens, and all systems of the human body will be mercilessly attacked. SARS-CoV-2 entry into the human body causes a decrease in oral microbial diversity and breaks the balance of oral microecology (Ma et al., 2021), which may make it easier for pathogens to enter the lungs from the mouth and lead to lung co-infection and may directly or indirectly affect the severity of COVID-19. The use of high doses or long-term antibiotics, especially broad-spectrum antibiotics, in the treatment of severe COVID-19 patients, often accompanied by secondary bacterial infections, undoubtedly contributes to the development of COVID-19 complications (Chen et al., 2020). The harm of the abuse of antibiotics not only includes liver and kidney, nerve, and blood system damage. More importantly, antibiotics kill or inhibit pathogenic sensitive bacteria, while other insensitive bacteria take the opportunity to turn over, a large number of growth and reproduction. The severe COVID-19 patients have significantly increased antibiotic resistance genes in their oral microbiome (Ma et al., 2021), which can lead to more severe infections. Therefore, the ecological balance of oral microorganisms should be considered when treating oral complications of COVID-19.

CONCLUSION

Here we summarized various oral complications associated with confirmed and suspected COVID-19 patients. Among them, taste disorders have now been listed by the World Health Organization as a symptom of COVID-19 (World Health Organizatio, 2021). Oral mucosal lesions have also been shown to be associated with COVID-19. Oral complications can result from improper medication use, weakened immunity, vascular damage, local and systemic inflammation, and neglect of oral hygiene during COVID-19 treatment (Botros et al., 2020; Iranmanesh et al., 2021). For patients with COVID-19, the oral signs and symptoms should be paid more attention during the treatment, including taste disorders, pechymosis, Candidiasis, traumatic ulcers, HSV-1 infection, geographic tongue, thrush, etc (Arastehfar et al., 2020; Xu et al., 2020b; Nejabi et al., 2021). A multidisciplinary approach to oral health care and clinical oral examinations for COVID-19 patients will be benefit for the treatment and recovery. This paper highlights the importance of integrating stomatology into the COVID-19 treatment to improve the oral health and rehabilitation of COVID-19 patients (Al-Awfi, 2021).

AUTHOR CONTRIBUTIONS

XZ: methodology, formal analysis, data curation, writing—original draft, and writing—review and funding acquisition. JD: validation, writing—original draft, and writing—review and editing. QG: methodology, data curation, and writing—original draft. ML: methodology, data curation, and writing—original draft. YL: methodology and data curation. LC: conceptualization, methodology, formal analysis, writing—review and editing. BR: conceptualization, methodology, writing—review and editing. All authors contributed to the article and approved the submitted version.

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CRISPR Technology in Gene-Editing-Based Detection and Treatment of SARS-CoV-2

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Outbreak and rapid spread of coronavirus disease (COVID-19) caused by coronavirus acute respiratory syndrome (SARS-CoV-2) caused severe acute respiratory syndrome (SARS-CoV-2) that started in Wuhan, and has become a global problem because of the high rate of human-to-human transmission and severe respiratory infections. Because of high prevalence of SARS-CoV-2, which threatens many people worldwide, rapid diagnosis and simple treatment are needed. Genome editing is a nucleic acid-based approach to altering the genome by artificially changes in genetic information and induce irreversible changes in the function of target gene. Clustered, regularly interspaced short palindromic repeats (CRISPR/Cas) could be a practical and straightforward approach to this disease. CRISPR/Cas system contains Cas protein, which is controlled by a small RNA molecule to create a double-stranded DNA gap. Evidence suggested that CRISPR/Cas was also usable for diagnosis and treatment of SARS-CoV-2 infection. In this review study, we discoursed on application of CRISPR technology in detection and treatment of SARS-CoV-2 infection. Another aspect of this study was to introduce potential future problems in use of CRISPR/Cas technology.

Keywords: coronaviruses, SARS-CoV-2, CRISPR/Cas9, gene editing, ACE-2 receptors

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INTRODUCTION

Coronavirus disease (COVID-19) was spread in December 2019 and was recognized as a zoonotic disease (Drosten et al., 2017; Andersen et al., 2020). Severe acute respiratory syndrome (SARS) virus was detected in sputum samples in 2003, and advanced stages in fecal samples may have been transmitted to humans by an intermediate host such as bats and civets (Wang and Eaton, 2007; Graham and Baric, 2010). Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can be transmitted from an unknown carrier to a healthy person who could infect many people. SARS-CoV-2 resulted in pneumonia in Wuhan, China, with various symptoms reported. The disease has developed into a pandemic (Wu C. et al., 2020; Wu D. et al., 2020; Guan et al., 2020). Appropriate methods could treat and control the disease. CRISPR/Cas9 was first recognized as a microbial immune system through which these organisms acquire immunity to invading viruses and plasmids (Garneau et al., 2010). When the invaded foreign DNA enters the bacteria, it is cleaved by cas nuclease enzymes. A portion of the cleaved DNA is then placed between two repeating sequences at the CRISPR site. Here, it is called a spacer (Barrangou and Horvath, 2017; Shmakov et al., 2017). The spacer sequences are used as templates to generate short RNA sequences. These sequences direct the

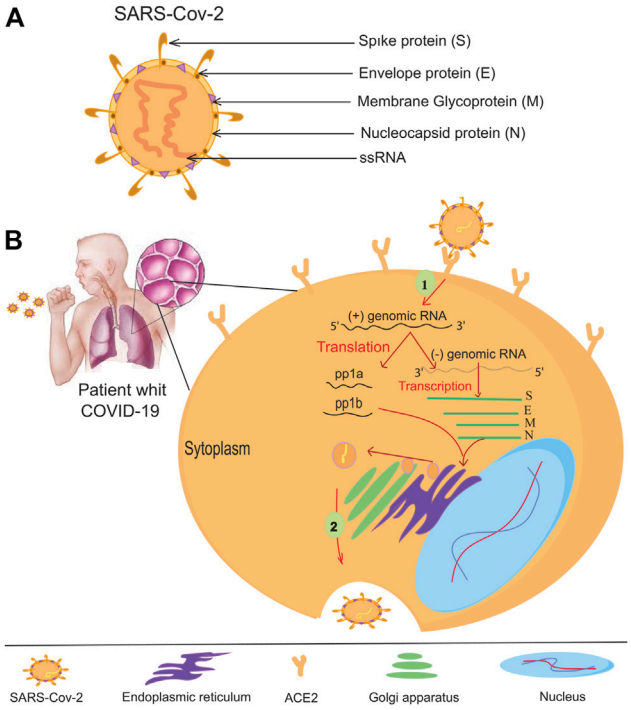

FIGURE 1 | The coronavirus virion and its life cycle. **(A)** Typical structure and proteins of the coronavirus virion. The coronavirus genome encodes an (S) spike glycoprotein, an (E) envelope glycoprotein, an (M) membrane glycoprotein, and an (N) nucleocapsid protein. **(B)** To enter the host cells, the SARS-CoV-2 virus binds to the ACE -2 receptors of the host cell helped by protein (S), then the RNA of the virus enters the host cell. All viral products are provided at the expense of the host cell, so many viruses are produced by virus-infected cells.

Cas protein to the invasive DNA. Once the Cas protein binds to the invasive DNA, the enzyme cuts the outer DNA sequence into both strands, creating the region of double-strand breaks (DSB) (Jinek et al., 2012; Wei et al., 2015; Brinkman et al., 2018) and the nucleotides at the DSB position change the structure or end codon in the gene. Non-homologous end-joining repair (NHEJ) or homology-directed repair (HDR) systems are induced to edit the genome to cut and cleave external DNA. These deletions and additions lead to a permanent change in the open reading frame (Hsu et al., 2014; Klein et al., 2019).

Some model systems, including mammalian cells, can efficiently cleave any complementary sequence to the gRNA and target and cleave the RNA. Targeted genome editing, often called CRISPR/Cas9, is increasingly recognized as an effective tool in medicine (Bawage et al., 2018; Freije et al., 2019). It can inactivate the SARS-CoV2 virus in mammalian cells by truncating the specific sequence of the virus. Cas proteins appear to help in detection and treatment of viral infections. They are introduced into the viral genome via guide RNAs and destroy it in the target regions (Aman et al., 2018; Xiao et al., 2018; Dolan et al., 2019). However, more regular interaction and collaboration between virology and molecular biology is needed to achieve substantial results in the treatment of SARS-CoV-2 (Rodrí guez-Rodr í guez et al., 2019). In this review, we first introduce general concept of CRISPR/Cas9. Then, we discuss potential challenges for treatment, and finally, we address prospects for CRISPR/Cas9-based antiviral strategies for SARS-CoV-2.

The coronavirus family (CoVs) contains many virus species, and these viruses can cause various diseases in birds, livestock, and humans (Miłek and Blicharz-Domanska, 2018). Members of the family are spherical and have an approximate diameter of 125 nm. The spines protrude from the surface of the virion and give the virus a particular crown-like appearance (Neuman et al., 2006). There are four main structural proteins in coronavirus: Membrane (M), spikes (S), envelope (E), and nucleocapsid (N) proteins (**Figure 1A**). The spikes bind the virus to the host cell receptor (Beniac et al., 2006; Wrapp et al., 2020). Researchers have discovered human coronavirus receptors, such as angiotensin-converting enzyme 2 (ACE2) for SARS-CoV (Li et al., 2005) and HCoV-NL63 (Wu et al., 2009) or aminopeptidase N (APN) for HCoV-229E (Yeager et al., 1992; Li et al., 2005). ACE2, APN, and DDP4 are ectopeptidase enzymes with different functions expressed on the surface of various cell types, including those of the human respiratory tract. MERS-CoV has been shown to require a dipeptidyl peptidase-4 (DPP-4) surface receptor to enter the host cell. SARS-CoV-2 uses the endogenous enzyme angiotensin-converting enzyme 2 (ACE2) to enter host cells (Raj et al., 2013; Monteil et al., 2020). After binding to the appropriate receptor, a fusion of the virus and cell membrane occurs, and the viral genome is transferred to the host cell's cytoplasm. In the host cell, the viral products are produced and assembled (Belouzard et al., 2009). After the virus particles are assembled, they are transported to the cell surface by vesicles and released by exocytosis (Fehr and Perlman, 2015). SARS-CoV-2 mainly infects epithelial cells in the lung but can also invade macrophages and dendritic cells (Weinheimer et al., 2012; Zhou et al., 2015). The exact mechanism of lung injury caused by SARS-Cov-2 is still unknown (Zhou et al., 2020). Serologic evidence of SARS-CoV-2 infection was observed in some individuals at animal markets before the disease outbreak. Some animals have also been infected with SARS-CoV-2 viruses isolated from camels, Himalayan palm civets, and raccoon dogs (Azhar et al., 2014; Fung et al., 2020). Further evidence from phylogenetic analysis suggests that SARS-CoV-2 is bat-derived (Lau et al., 2005; Zheng, 2020), as the genetic similarity between SARS-CoV-2 and bat-SARS is greater than 95%. Bat-SARS has the potential to be transmitted to humans. Although the risk of transmission is lower, it is affected by bat SARS in an area (Cook et al., 2021). Although one study has shown that SARS-CoV-2 is not a mosaic (Paraskevis et al., 2020), there is speculation about its heritability. The UK Medicines Agency has approved molnupiravir to treat mild to moderate COVID-19 in people with at least one risk factor for severe disease. Molnupiravir could cut the number of people who need to go to the hospital in half and reduce the number of deaths. However, the supply would not last long if it were given to everyone who is sick because the daily caseload is high. Use of the drug would likely be limited to those at the highest risk for disease complications, such as older adults with heart, lung, or kidney disease, diabetes, or cancer (Wu F. et al., 2020; Mahase, 2021). Vaccination is the most important method

of epidemic control. The emergence of numerous SARS-CoV-2 variants that are less prone to disease- and vaccine-induced immunity threatens progress. Despite these ongoing threats, the efficacy of the SARS-CoV-2 vaccine provides a reason for optimism for 2021 (Creech et al., 2021). SARS-CoV-2 neutralizing antibodies in the serum of cured patients can be recovered and reused if SARS-CoV-2 recurs (Zhou and Zhao, 2020). Such antibodies will help protect individuals at high risk.

APPLICATION OF CRISPR/CAS9 TECHNOLOGY IN CURRENT VIROLOGY

Host-virus conflict is a dynamic process. Viruses use host factors to complete their life cycle, and the host uses the body's immune system to fight off the viral infection, so it does not waste energy. The virus must enter the host cell to replicate its genome and complete its life cycle (Sicard et al., 2019) (**Figure 1B**). Theoretically, antiviral treatments can prevent the virus from entering the host cell or destroy the genetic elements of the virus. Targeting host factors can help the virus become resistant to antiviral drugs (Lin and Gallay, 2013). However, this hypothesis needs further investigation to identify its weaknesses and exploit them after these weaknesses have been addressed. The CRISPR/Cas9 system could be useful because it targets viral nucleic acid and host material quickly and conveniently (Lino et al., 2018). Cas9 is known in CRISPR/Cas systems as a DNA endonuclease directed from a guide RNA (sgRNA) to the target DNA to alter the genome of the target region (Kennedy and Cullen, 2015; Ishino et al., 2018). This genome editing leads to an antiviral status within the host cell. CRISPR/Cas9 was the first system studied in HIV-1 gene therapy research (Xiao et al., 2019). Host cell receptors, CCR5 and CXCR4, help HIV enter the host cell (Wilen et al., 2012; Santos-Costa et al., 2014). Therefore, one of the antiviral candidates to treat HIV is suppressing these receptors. Researchers have successfully suppressed the expression of CCR5 in primary CD4 T cells at an appropriate level using the CRISPR/Cas9 gene-editing system. These cells develop resistance to HIV-1 and do not cause extracellular toxicity (Hou et al., 2015; Li et al., 2015).

Host cell factors such as the Apo-Lipoprotein B Editing Complex (APOBEC3) and Tripartite Motif Containing 5 (TRIM5) have been identified as viral limiters for HIV infection (Bogerd et al., 2015). APOBEC3 is thought to act as an antiviral agent by causing mutations in the viral genome. CRISPR/Cas9-based regulation of the host APOBEC3 factor reduces HIV reporter gene expression and provides antiviral effects (Jern et al., 2009; Bogerd et al., 2015). Two specific amino acids in TRIM5 have made it an actual antiviral agent against HIV-1 infection. This antiviral candidate can induce cleavage of viral capsid proteins, demonstrating its antiviral properties (Sastri and Campbell, 2011; Weatherley et al., 2017). Therefore, TRIM5 may be a suitable target for the CRISPR/Cas9 system. Eliminating microRNA-146 by CRISPR/Cas9 resulted in a significant increase in HIV-1 limiting factors (Teng et al., 2019).

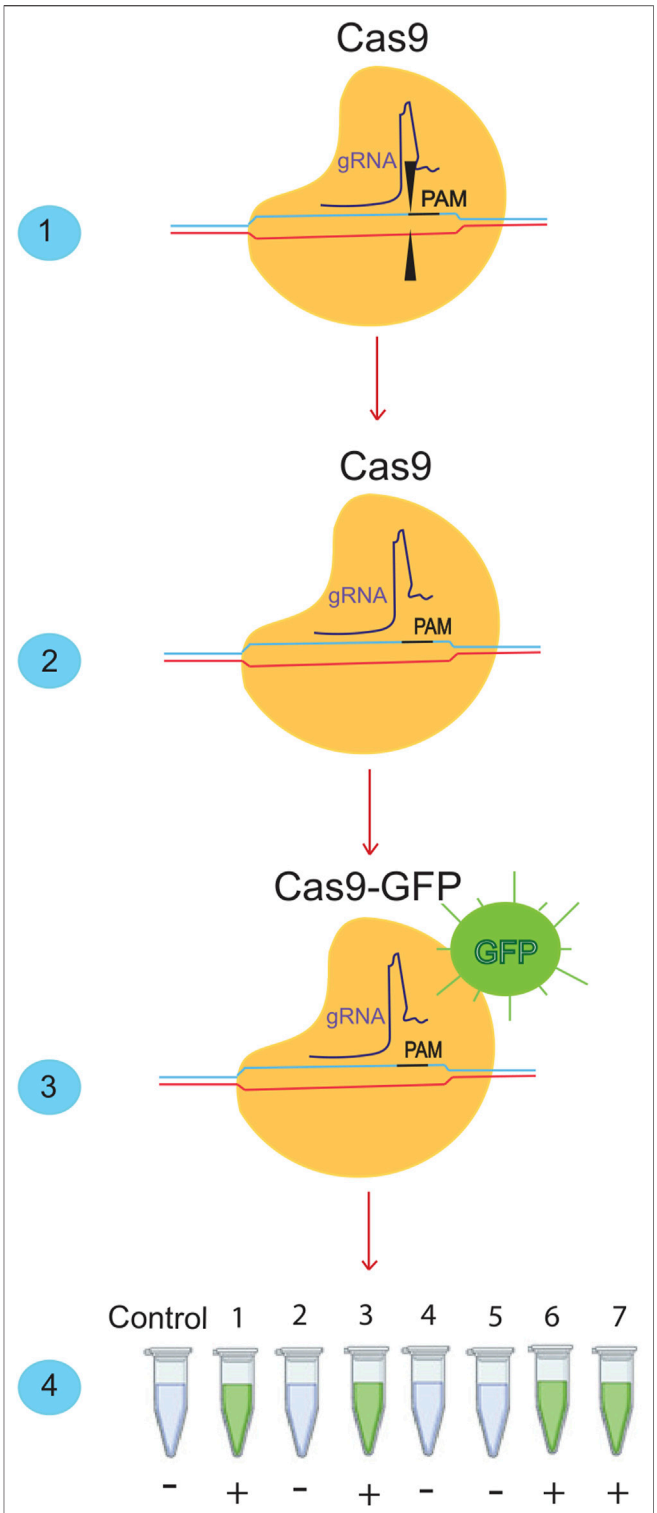

FIGURE 2 | Detection of SARS-cov-2 using the Crisper/Cas system. (1) The Crisper/Cas9 system binds to specific sequences using gRNA. (2) If we remove the cutting effect from this system and (3) add a fluorescent dye, we can bind it to the desired sequences. When it is bound to the desired sequence, it will produce a green color. (4) With this ability of the CRISPR/CAS9 system, many SARS-CoV-2 samples can be detected in a short time.

Some host factors are essential for virus replication, assembly, and budding. Therefore, knocking out their genes could be an alternative to preventing HIV-1 infection (Lin and Nagy, 2013; Xu and Nagy, 2015); such gene deletions can be performed using the CRISPR/Cas9 system (Gilani et al., 2019). Interestingly, these gene deletions have no significant effects on host cells (Chen J. S. et al., 2018). These results demonstrate the antiviral potential of CRISPR/Cas9-based therapies, as it is possible to disrupt key host factors essential for HIV infection.

APPLICATION OF CRISPR/CAS TECHNOLOGY IN SARS-COV-2

The worldwide pandemic of SARS and CoV-2 poses a significant threat to global public health and societal stability and has become a significant global public health problem. Regrettably, current diagnostic and therapeutic methods to prevent and control SARS-CoV-2 have many limitations. CRISPR/Cas technology has emerged as a potential complement to conventional methods in recent years. Biomedicine has extensively used biological tools based on the CRISPR/Cas systems. They are helpful in pathogen detection, clinical antiviral treatment, and drug and vaccine discovery. Therefore, CRISPR/Cas technology could be promising in preventing and treating SARS-CoV-2 and other emerging infectious diseases.

APPLICATION OF CRISPR IN THE DETECTION OF SARS-COV-2

CRISPR-Cas systems could be used for molecular diagnosis of nucleic acids (**Figure 2**) (Jia et al., 2020:; Kaminski et al., 2021). CRISPR-Cas-based diagnostic methods have the same sensitivity and specificity as conventional PCR. However, their cost is low because they do not require complex or expensive technology (Ayanoğlu et al., 2020). The application of CRISPR-Cas in molecular diagnostics could transform global diagnostic and healthcare systems (Gootenberg et al., 2017). The Cas proteins used in CRISPR-Cas systems vary depending on the DNA or RNA targeted and the intended applications (Makarova et al., 2015). Following the global spread of the COVID-19 pandemic, rapid and straightforward diagnostic techniques are in high demand. CRISPR-based methods, which have demonstrated superior detection capability in as little as 30–60 min, could overcome this obstacle. In addition, a CRISPR/Cas9-mediated lateral flow nucleic acid assay (CASLFA) has been developed to identify infections using the CRISPR/Cas system (Wang et al., 2020). However, FDA approval is still pending. Regarding "collateral breast activity," CRISPR-based diagnostic techniques have been developed using the Cas12a or Cas13 nuclease. The Cas12a/Cas13 nuclease, a component of the CRISPR tool, is activated after CRISPR RNA binds to the target bosome (crRNA). When produced, it non-specifically cleaves ssDNA/RNA particles in the vicinity and explicitly acts as a collateral bosome or transbosome. Researchers took advantage of this property to develop fluorescently labeled ssDNA/RNA press reporter probes capable of detecting visible bands in a paper strip via a side-stream assay, enabling the development of a novel nucleic acid-based diagnostic test (Chen S. et al., 2018). Viral RNA targeting crRNA can activate Cas protein, resulting in collateral cleavage of press reporter probes and a helpful band on the paper strip (Chen J. S. et al., 2018). In the newly developed Specific High-sensitivity Enzymatic Reporter Unlocking (SHERLOCK) technology, the activity of the crRNA-Cas13a protein complex is used to recognize RNA molecules and cut collateral RNA near the target RNAs. Metsky et al. have developed a website with CRISPR-Cas13-based assay designs for the detection of 67 diseases, including SARS-CoV-2, Zika virus, and dengue fever, with a choice of single or multiple panels (Metsky et al., 2020). The comprehensive SARS-CoV-2 diagnostic test is based on advanced technology from SHERLOCK. This technique uses fluorescently identified, non-targeted press reporter RNA (Kellner et al., 2019). SHERLOCK test for detecting SARS-CoV-2, which has a sensitivity of 10 copies per microliter and can be fluorescently confirmed, has been validated with counterfeit RNA fragments. This molecular analytical test should be inexpensive and provide rapid results. The DETECTOR is a similar technique used to amplify pathogenic DNA with RPA, and reverse transcription to identify RNA viruses is also used as a SHERLOCK system in this procedure. Cas12a-crRNA identifies the target and activates the Cas12a nuclease, which cleaves fluorescently labeled reporter sDNA without discrimination. In less than an hour, DETECTOR distinguished between human papillomavirus 16 (HPV16) and human papillomavirus 18 (HPV18) in pure DNA from cultured human cells and professional samples (Myhrvold et al., 2018). Broughton et al. diagnosed COVID -19 using two specific crRNAs targeting genes E and N and a discovery series ranging from 70 to 300 copies per microliter of sample material. They used LAMP with reverse transcription instead of RPA-based amplification to identify COVID -19 in less than 30 min. These CRISPR-Cas-based nucleic acid detection methods require independent amplification of nucleic acids. They require human activity, complicating detection and increasing the risk of spreading contamination (Broughton et al., 2020a). Ding et al. developed the AIOD-CRISPR (All-In-One Dual CRISPR-Cas12a) assay method, which enables rapid visual detection of viral nucleic acids with high sensitivity and accuracy. In this article, all materials required for viral nucleic acid detection are incubated in a single pot at 37°C, which simplifies the process and minimizes the risk of contamination. With high sensitivity, SARS-CoV-2 genomic RNA was detected with the AIOD-CRISPR assay (Ding et al., 2020). LED assay uses blue light illumination to image the tubes instead of a paper dipstick with sidestream detection. Scientists in India have identified the Francisella novicida Cas9 orthology (FnCas9) as sensitive to nuclear differences, and the Linked Attire Discovery Assay (FELUDA) for FnCas9 has been developed as a low-cost point-of-care (LCC) assay for identifying SARS-CoV-2 infections in the clinical setting (Azhar et al., 2020).

CRISPR'S ADVANTAGES IN DETECTION OF SARS-COV-2

"Metagenomics" and "qRT-PCR" are two widely used molecular methods for identifying novel viruses (Gu et al., 2019; Corman et al., 2020). Current qRT-PCR-based SARS-CoV-2 diagnostic methods are efficient and accurate for virus detection. Where qRT-PCR technology is not available, the virus can spread globally (Lucia et al., 2020). Finally, the diagnostic accuracy of many molecular methods has not yet been clarified. Although CRISPR/Cas has been a widely used gene-editing strategy since 2013, the simultaneous promiscuous cleavage tasks of a specific collection of Cas nucleases were discovered later and used to detect nucleic acids from artificial insemination (Chen S. et al., 2018; Harrington et al., 2018). Due to its exceptional sensitivity, specificity, and reliability, RNA-directed nucleic acid detection based on CRISPR/Cas nuclease has recently shown significant potential for developing next-generation molecular diagnostic technology (Ding et al., 2020; Lucia et al., 2020). For example, AIOD-CRISPR can detect only 1.2 copies of DNA targets and 4.6 documents of RNA targets in 40 min of incubation without preamplification when detecting SARS-CoV-2 (Lucia et al., 2020). CRISPR-nCoV has the same sensitivity and uniqueness as next-generation metagenomic sequencing (mNGS) in less than 40 min (Hou et al., 2020). Some of the major advantages of this approach over existing techniques such as qRT-PCR are 1) uniformity of signal in a nucleus (e.g., SARS-CoV-2 guide RNAs can be distinguished from SARS-CoV and MERS-CoV at the N_2 site) and 2) integration with low-cost portable reporting sheets and side streptometers. 3) isothermal signal amplification for rapid target detection in the absence of termocycling. CRISPR-based assays are more accessible and convenient than RT-PCR viral RNA identification assays because the CRISPR system does not require bulky instrumentation or complicated processes (Ganbaatar and Liu, 2021). Most CRISPR-Cas-based detection methods require pre-amplifying a specific nucleic acid combination and manual procedures. These modifications will undoubtedly complicate procedures and impose costs on the environment. The following table summarizes the current studies on the diagnosis of SARS-CoV-2.

Sensitive and Specificity

CRISPR-based diagnostic assays show excellent clinical sensitivity and specificity (Huang W. et al., 2020; Joung et al., 2020a; Patchsung et al., 2020). To improve the sensitivity and specificity of CRISPR-based SARS-CoV-2 detection, different models were created. Selection of two crRNAs improved sensitivity (Huang Z. et al., 2020) and increased resistance to viral RNA changes (Ooi et al., 2020).

In addition, several improvements have been made to increase the sensitivity of CRISPR-based SARS-CoV-2 detection experiments. These include modifying crRNA (Nguyen et al., 2020), incorporating small particles to improve action kinetics (Joung et al., 2020b), improving reagent ratios (Huang W. et al., 2020), increasing reagent concentration through careful focusing (Ramachandran et al., 2020), and increasing RNA input besides RNA quantity. Computational techniques ensure the sensitivity and specificity of amplification primers and crRNAs to detect SARS-CoV-2 (Ackerman et al., 2020; Arizti-Sanz et al., 2020; Metsky et al., 2020). Researchers developed customized CRISPR-based assays with sensitivity and specificity comparable to quantitative real-time PCR (qPCR) based on these findings. Incorporating the methods described above could help improve the overall sensitivity and specificity of COVID -19 CRISPR diagnostic tools.

Turn-Around Time

Several CRISPR-mediated COVID -19 diagnostic studies have used the same techniques as RT -qPCR to recover viral RNA, consistent with previous findings (Ali et al., 2020; Huang Z. et al., 2020). Unlike rapid RNA extraction techniques needed for point-of-care diagnostics, these methods are time-consuming. Therefore, several researchers have investigated whether CRISPR-based assays are feasible using rapid viral RNA extraction methods. When Joung and colleagues mixed the clinical samples with the Quick Extract solution, they incubated them at 95°C for 5 min before assaying them for viral RNA. Heat treatment and chemical reduction were performed as a 10-minute technique lysed the viral particles and inactivated the nucleases (Arizti-Sanz et al., 2020). In one study, Ramachandran et al. used electric field-driven microfluidics to recover viral RNA in less than 5 min (Ramachandran et al., 2020). RT-qPCR to detect SARS-CoV-2 takes approximately 45 min if RNA extraction is omitted (Corman et al., 2020). This result, considering that RT-qPCR is compatible with rapid RNA extraction methods, suggests that the time required for RNA extraction in CRISPR-based research equals that of RT-qPCR (Ladha et al., 2020). The time required for an essay varies depending on the subject. However, specific efficient procedures can be completed in less than 30 min (Broughton et al., 2020b). Others require 40, 45, or 50–60 min without RNA extraction (Arizti-Sanz et al., 2020; Patchsung et al., 2020). One study found that an automated CRISPR-based assay can be performed in 30 min (Ramachandran et al., 2020). CRISPR-based SARS-CoV-2 detection methods equal RT-qPCR in terms of assay time. RT-qPCR analyses require sending samples to a central laboratory. However, CRISPR-based diagnostics allow on-site detection, which drastically reduces reporting time.

Ease of Use

RT-qPCR experiments are performed as one-step reactions using master mixes to simplify them. A master mix containing both RT-LAMP and Cas12a-based detection reagents was developed by Joung and colleagues and proved stable after six freeze-thaw cycles (Joung et al., 2020a). Most CRISPR-based SARS-CoV-2 detection assays require two phases, but researchers have also developed one-step methods that require less time and effort (Arizti-Sanz et al., 2020; Joung et al., 2020b). A wide range of CRISPR-based assays can be performed using a single method, with RT-qPCR requiring less time or the same time as RT-PCR due to preparing the required reagents in master mixes. CRISPR-based assays are comparable to RT-qPCR in terms of ease of use. However, point-of-care assays require fewer manual activities

and a lower level of technical skill. CRISPR-based assays are being developed that are both automated and sample-to-result (Ramachandran et al., 2020).

Requirement of Equipment

Since most CRISPR-based research uses isothermal techniques, a thermocycler is not required. So, it's possible to use a normal heating block or water bath to perform the tests (Ali et al., 2020; Metsky et al., 2020). When using a lateral flow readout technique, no signal detection equipment is required. After DNA amplification, the reaction tube must be opened, which can lead to contamination and false-positive results in subsequent tests. Therefore, reading lateral flow strips requires a specific position or a closed cartridge. A fluorescence readout would be more appropriate. Although a plate reader primarily detects the fluorescent signal, many studies have shown that it can also be identified by eye examination under blue light (Ding et al., 2020; Wang et al., 2020).

Viral RNA can be isolated from clinical samples using techniques that do not require complex or lengthy equipment for the CRISPR-based identification of SARS-CoV-2. Compared with traditional, labor-intensive RNA extraction techniques, these rapid extraction methods have the same (Joung et al., 2020b) or slightly lower efficiency (Guo et al., 2020).

Microfluidic devices that extract viral RNA and enable CRISPR-based detection can be used for automated or sample-to-result assays (Ramachandran et al., 2020). Researchers used a battery-powered, portable thermal cycler and fluorescence reader (Rauch et al., 2020). Finally, these examples demonstrate that tests to detect SARS-CoV-2 with CRISPR do not require expensive or complicated equipment. These tests can be performed in locations other than a central laboratory, such as airports, clinics, and other locations with limited resources.

Cost per Test

Although using lateral flow strips increases the cost per assay (Ooi et al., 2020), the total material cost for fluorescence-based CRISPR-mediated SARS-CoV-2 detection assays is lower than the material cost for RT -qPCR-based SARS-CoV-2 detection assays (Hou et al., 2020; Ooi et al., 2020). For example, the CRISPR-COVID assay costs less than $3.50 for a single reaction, depending on the assay technique (Hou et al., 2020). On an industrial scale, the cost can be as low as $0.6 per pound (Gootenberg et al., 2020; Kellner et al., 2019). In addition, CRISPR-based screening has reduced the cost of the first tool (Guo et al., 2020). (Guo et al., 2020). Fluorescence-based CRISPR-mediated COVID–19 analysis assays are less costly than RT–qPCR.

APPLICATION OF CRISPR IN SARS-COV-2 TREATMENT

SARS-CoV-2 is a novel coronavirus of the positive-sense RNA virus family that infects the respiratory tract and causes disease through direct cytotoxic effects and the production of host cytokines (Liu et al., 2020). The life cycle of SARS-CoV-2 is like that of other strongly associated coronaviruses, such as the virus that causes SARS. The virus spreads its RNA genome in the cell, synthesizes the genomic and subgenomic negative sense RNAs used in the viral mRNA, and produces a new copy of the viral positive sense genome (Du et al., 2009; Mocarski et al., 2020). While conventional vaccines recognize viral proteins or viruses by activating the human immune system and limiting viral entry into the cell (Rappuoli, 2018), the CRISPR-based approach is an alternative antiviral strategy to recognize and eliminate the viral genome and mRNAs within the cell. It should be possible to restrict viral replication to specific positives and viral mRNAs while destroying viral genome replication and gene expression templates. Therapeutic applications of CRISPR are on the rise. Technology plays an essential role in exploring potential therapies for various genetic diseases through direct modification of the genome (Straiton, 2019).

Besides DNA targeting Cas9, RNA targeting CRISPR-Cas13 is an antiviral approach against single-stranded RNA viruses such as lymphocytic choriomeningitis virus (LCMV), influenza A

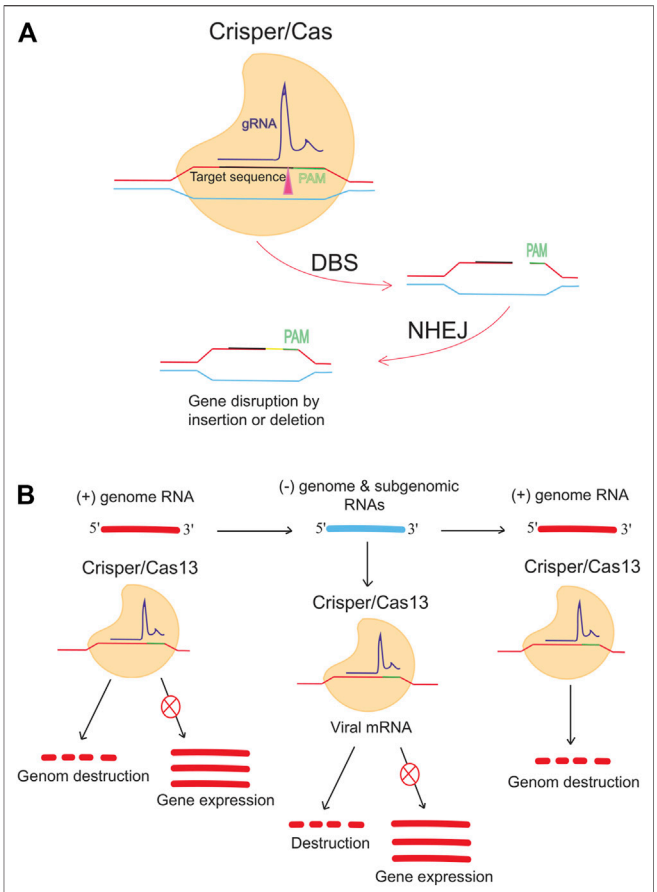

FIGURE 3 | Anti-COVID-19 genome editing was performed using the Crisper/Cas system and PAC-MAC function. **(A)** A mutation in the target gene is caused by the CRISPR/CAS9 system, which is supported by the NHEJ repair mechanism and causes a change in the gene. The gene expression is reduced or stopped depending on the target. **(B)** Cas13d can inhibit viral activity and replication. Cas13d targets and cleaves 100% of the positive sense RNA produced by viruses.

TABLE 1 | Some CRISPR-based SARS-CoV-2 diagnostic studies.

CRISPR/System	Sample type	Number of samples	Assay time	Platform	Specific/ Sensitive	Country	References
CRISPR–Cas12a	respiratory swab	36	<40 min	DETECTR	—	United States	Broughton et al. (2020b)
CRISPR–Cas13a	nasopharyngeal swabs	154	>60 min	SHERLOCK	100%/96%	Thailand	Patchsung et al. (2020)
CRISPR–Cas13a	nasopharyngeal swabs	1808	110 min	CREST	100%/88.8%	United States	Rauch et al. (2020)
CRISPR–Cas13a	nasopharyngeal swabs	50	50 min	SHINE	100%/90%	United States	Arizti-Sanz et al. (2020)
CRISPR–Cas12b	nasopharyngeal or anterior nasal swab	202	<60 min	STOPCovid	98.5%/ 93.1%	—	Joung et al. (2020a)
CRISPR-Cas3 And CRISPR–Cas12a	nasopharyngeal and oropharyngeal swab	31	40 min	CONAN	95%/90%	Japan	Yoshimi et al. (2020)
CRISPR–Cas12a	nasopharyngeal swabs, sputum, BAL	378	30 min	DETECTR	95.5%/93%	Dutch	Brandsma et al. (2020)
CRISPR/Cas12a	Clinical sample	31	45 min	CRISPR/ Cas12a-NER	100%/100%	China	Wang et al. (2020)
CRISPR/Cas12a	raw nasopharyngeal swab	8	35 min	ITP-CRISPR	100%/75%	United States	Ramachandran et al. (2020)
CRISPR/Cas12a	Pharyngeal swab, nasopharyngeal swabs	295	60 min	SENA	100%/100%	China	Huang et al. (2020a)
CRISPR–Cas13a	nasopharyngeal swab, bronchoalveolar lavage fluid specimens	114	40 min	CRISPR-COVID	100%/100%	China	Hou et al. (2020)

BAL, broncheo-alvealar lavage.

virus (IAV), and vesicular stomatitis virus (VSV) in human cells (Freije et al., 2019). Conversely, Stanford College (CA, United States) researchers are working on CRISPR-based therapies for infectious diseases, using a different method and going beyond the human genome. When researchers worked on the flu virus, they followed in the footsteps of many others. They shifted the focus of their gene-targeted antiviral drug to COVID-19 and the pandemic (Abbott et al., 2020a; Abbott et al., 2020b). It was reported that the prophylactic CRISPR antiviral approach in human lung epithelial cells (PAC-MAN) was identified as a potentially helpful new technique to stop viral traits and replication and that the PAC-MAN approach was identified as a type of genetic intervention to target SARS-CoV-2 and potentially all sequenced coronaviruses (**Figure 3**). Interestingly, a pool of crRNA suppressed about 70% of the reporter signal, demonstrating the potential of CRISPR PAC–MAN technology to degrade viral genetic material. In addition, several crRNAs targeting the entire conserved region of the SARS-RdRP CoV-2 and N-protein genes caused RNA degradation of over 80 and 90%, respectively.

To reprogram CRISPR-Cas13b against the genomic and subgenomic RNAs of SARS-CoV-2, we performed genome-wide computational predictions and screens at single-nucleotide resolution. Cas13b effectors reprogrammed to target accessible segments of spike and nucleocapsid transcripts had silencing efficiencies greater than 98 percent in virus-free animals. Tailored and multiplexed Cas13b CRISPR RNAs (crRNAs) inhibit viral replication in mammalian cells infected with replication-competent SARS-CoV-2, including novel dominant variants. CRISPR-Cas13-based viral suppression strategy is readily adaptable and can be extended to harmful viruses other than SARS-CoV-2 and, therefore, could provide an effective platform for antiviral treatments (Fareh et al., 2021). However, it is critical to identify and study the deleterious effects of using single-guide RNAs (sgRNAs), the CRISPR/Cas system, or PAC-MAN on host physiology. Although the Cas method seems to have a high chance of successfully identifying therapies, it needs further investigation.

However, it is critical to identify and investigate the deleterious effects of using single-guide RNAs (sgRNAs), the CRISPR/Cas system, or PAC-MAN on host physiology. Although the Cas method seems to have a high chance of successfully identifying therapies, it needs further investigation.

LIMITATION OF CRISPR TECHNOLOGY

CRISPR technology is advancing rapidly. Although recently discovered and new, CRISPR/Cas is a tool with multiple genome engineering capabilities. Because of its ability to edit genomes in such a user-friendly way, it has attracted the attention of biomedical researchers. CRISPR can appropriately solve various viral diseases. In cell-based and animal studies, successful results have been achieved in several human viral infections (Scheufele et al., 2017; Brokowski, 2018; Li et al., 2019). The therapeutic use of CRISPR/Cas to treat human viral diseases has generally gained great importance (Scheufele et al., 2017). However, gaining expertise in the diagnostic and therapeutic use of CRISPR/Cas in viral infections is associated with potential risks. Because this is a new science, we will briefly describe the limitations of CRISPR/Cas. We hope these limitations will be addressed, and an appropriate therapeutic and diagnostic system for SARS-CoV-2 will be developed. There are several legitimate concerns about CRISPR technology's

efficacy and technical limitations. According to research, both target and off-target editing provide limited and partial results, and CRISPR studies in animals and human cells have demonstrated these limitations (Guo and Li, 2015; Peng et al., 2016; Bohaciakova et al., 2017; Zischewski et al., 2017).

Although few studies have shown off-target editing and most studies support CRISPR/Cas, one of the major concerns associated with the CRISPR/Cas system is the possibility of off-target activity and mutant viruses. Viral escape mutations are caused by deletions (indels) at the Cas9 segregation site (Guo and Li, 2015). Using NHEJ repair system, Cas9 causes a mutation and renders the virus ineffective. Typically, NHEJ repair system repairs the damage (Ingram et al., 2019). However, a subset of these mutations can cause the virus to survive and escape, and viruses with such mutations are no longer interested in the original gRNA (De Silva Feelixge et al., 2018). Thus, inappropriate mutations can occur with any virus. If these cells are infected with the mutant virus, they might resist CRISPR/Cas treatment. Therefore, the desired results may not be achieved. Non-mutated virus-infected cells may provide a viral reservoir for disease spread in subsequent disease episodes (Allocati et al., 2016). Therefore, CRISPR/Cas system is risky and not profitable in the antiviral market.

Transferring CRISPR/Cas9 to virus-infected cells is another limitation of this new method. The success of this technology in the clinical setting is necessary to control the most severe viral diseases, including SARS-CoV-2 (Rath et al., 2015). As CRISPR becomes more efficient and sensitive, these concerns may become obsolete. Technology is advancing at an unprecedented pace.

CONCLUSION

Emerging viruses such as SARS-CoV-2 are responsible for hundreds of thousands of illnesses and deaths worldwide each year. The disease is spreading everywhere and destroying the economies of affected populations. Genome editing strategies to deactivate the viral genome could be a suitable way to treat such diseases. The lack of effective drugs and vaccines may be contributing to so many SARS-CoV-2 samples being collected for rapid diagnosis. Containing and preventing further spread of the virus appears to be critical, and rapid detection of infection in organisms may prevent further spread of the disease. CRISPR/Cas is a solution for treating virus-related diseases with many future applications. Therefore, the CRISPR/Cas9 system could be helpful, especially if some valuable and specific sgRNAs are developed. If the CRISPR/Cas system leads to therapeutic and diagnostic solutions, the financial burden will be reduced because CRISPR/Cas-based therapies will eliminate the need for drugs. In addition, the treated individual will not suffer repeated disease relapses. Therefore, advances in the CRISPR/Cas system and the success of clinical trials in animal models are critical **Table 1**, Guanghui et al., 2020.

AUTHOR CONTRIBUTIONS

BS, CBA, and AN developed the concept and designed the study. MAO, SH, and AI did a systematic search and prepared the first draft. All authors participated in the revising of the manuscript before submission.

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Cancer Occurrence as the Upcoming Complications of COVID-19

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Previous studies suggested that patients with comorbidities including cancer had a higher risk of mortality or developing more severe forms of COVID-19. The interaction of cancer and COVID-19 is unrecognized and potential long-term effects of COVID-19 on cancer outcome remain to be explored. Furthermore, whether COVID-19 increases the risk of cancer in those without previous history of malignancies, has not yet been studied. Cancer progression, recurrence and metastasis depend on the complex interaction between the tumor and the host inflammatory response. Extreme proinflammatory cytokine release (cytokine storm) and multi-organ failure are hallmarks of severe COVID-19. Besides impaired T-Cell response, elevated levels of cytokines, growth factors and also chemokines in the plasma of patients in the acute phase of COVID-19 as well as tissue damage and chronic low-grade inflammation in "long COVID-19" syndrome may facilitate cancer progression and recurrence. Following a systemic inflammatory response syndrome, some counterbalancing compensatory anti-inflammatory mechanisms will be activated to restore immune homeostasis. On the other hand, there remains the possibility of the integration of SARS- CoV-2 into the host genome, which potentially may cause cancer. These mechanisms have also been shown to be implicated in both tumorigenesis and metastasis. In this review, we are going to focus on potential mechanisms and the molecular interplay, which connect COVID-19, inflammation, and immune-mediated tumor progression that may propose a framework to understand the possible role of COVID-19 infection in tumorgenesis and cancer progression.

Keywords: COVID-19 sequelae, SARS-CoV-2, cancer, long COVID-19, immune homeostasis

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INTRODUCTION

Over the previous decades, viral infections have posed significant challenges for cancer management. Several oncogenic viruses are known to cause cancer. However, there is no evidence linking between cancer subtypes and Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) infections in clinical and pre-clinical studies. Cancer patients are more susceptible to SARS-CoV-2 infection with possible poor prognosis than normal population due to their systemic immunosuppressive state caused by the cancer and anticancer treatments, such as active chemotherapy. The perpetually growing numbers of

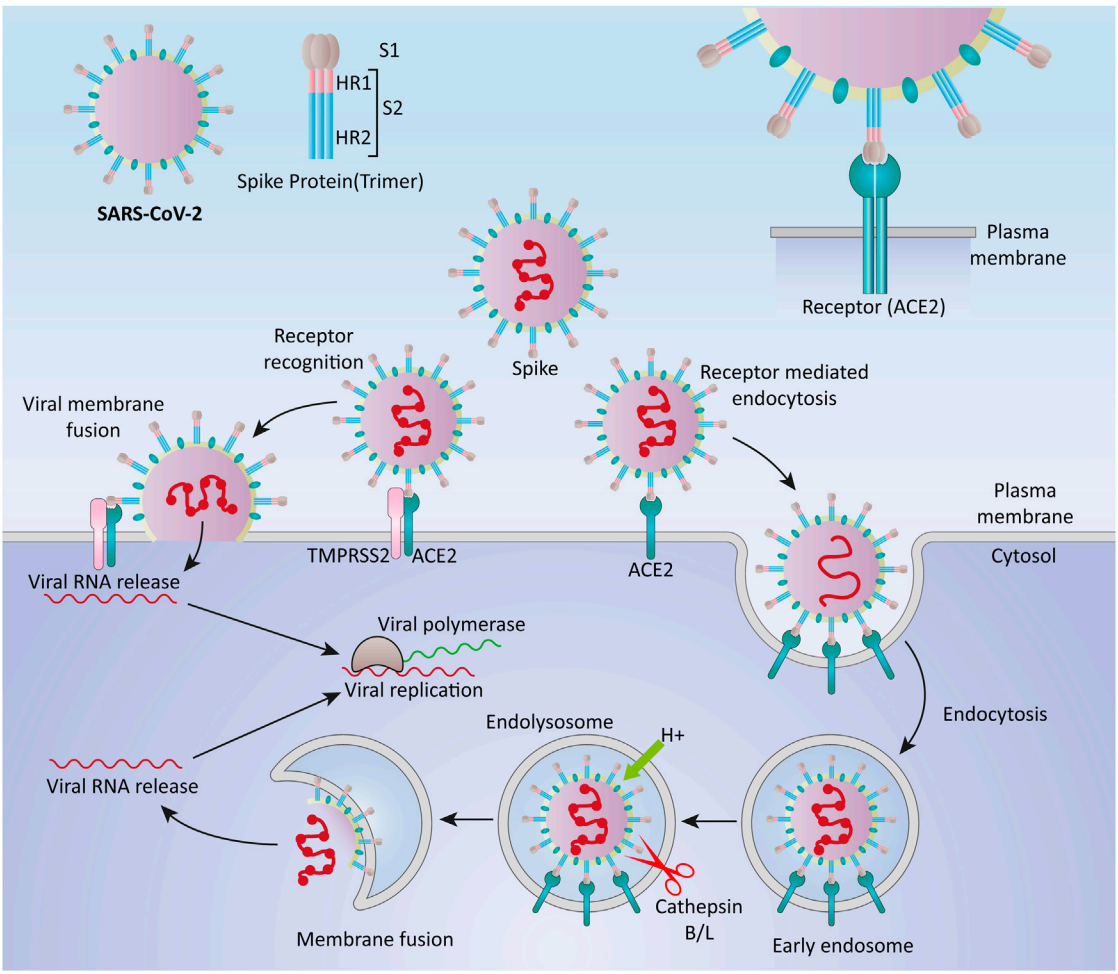

FIGURE 1 | Cell entry mechanisms of SARS-CoV-2. Coronaviruses are believed to enter the host cells *via* two main routes, through direct delivery of the genome into the cytosol *via* fusion with the host cell membrane, and through endocytosis.

COVID-19 infections and the increase in numbers of diagnosed and undiagnosed cancer patients have warranted apprehending the interrelationship between COVID-19 and cancer. COVID-19 is concomitant with activation of oncogenic pathways, including The Janus kinase signal transducer and activator of transcription)JAK-STAT (, mitogen-activated protein kinase (MAPK), and Nuclear factor kappa B (NF-κB), which potentially can increase the risk of cancer (Li et al., 2020a).

On the other hand, COVID-19 and cancer look alike in several ways such as inappropriate T-cell responses. Another similarity is antigenic stimulation caused by damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) molecules occurs in both cancer and infectious disease (Hotchkiss and Moldawer, 2014). DAMP and PAMP figure out inflammation, leading to the release of various cytokines, increased levels of reactive oxygen and nitrogen species, tissues damage, and apoptosis. Moreover, hypoxia as well as hypoxic microenvironment secondary to inflammation or virus-induced angiotensin-converting enzyme 2 depletion provoke oxidative stress and probable malignant transformation (Saini and Aneja, 2021). In addition, the hypoxic microenvironment results in lysyl oxidase (LOX) production, which increases tumor cells invasion and facilitates migration and metastasis (Ye et al., 2020).

Immune responses in acute phase of COVID-19 patients, called cytokine storm are arranged by proinflammatory cytokines, which are also recognized to promote tumorigenesis (Del Valle et al., 2020).

Furthermore, the consecutive low-grade inflammation seen in COVID-19 patients after acute phase may result in a constant cycle of inflammation-induced organ injury and injury-induced inflammation. Although, cancer development is never the consequence of an insulated event; it could be hypothesized that COVID-19 may predispose the human to cancer development and accelerate cancer progression and metastasis. In this review, we discuss the pathways and hypotheses that may expose patients with COVID-19 to cancer in the future.

EPIDEMIOLOGY AND CLINICAL CHARACTERISTICS OF COVID-19

The recent coronaviruses epidemics have rapidly spread with irreparable consequences (Guan et al., 2020; Sohrabi et al., 2020). As of 28 October 2021, the World Health Organization (WHO) reported a total of 245M confirmed cases with 4.97M deaths, making it as one of the deadliest crisis in history (Dikid et al., 2020).

Coronaviruses can cause multiple organs infection especially respiratory tract infection. The clinical signs and symptoms are typically including fever, dry cough, muscle pain, diarrhea, and breathing difficulties (Abdel-Moneim and Hosni, 2021). Sever cases of COVID-19 represent acute respiratory distress syndrome (ARDS), sepsis and septic shock, multiorgan failure leading to death (Zafer et al., 2021). Indeed, in these cases, viruses can evade the immune system and spread to other organs, such as cardiovascular system, gastrointestinal system, central nervous system, liver and kidney, where they cause a variety of serious diseases (Bajaj et al., 2021; Soltani et al., 2021). Apart from the consequences created by the coronaviruses, underlying medical conditions such as cancer, cardiovascular diseases, renal diseases, and type I/II diabetes, increase the risk for severe COVID-19 infection (Haybar et al., 2020).

STRUCTURE, LIFE CYCLE AND PATHOGENESIS OF SARS-COV-2

SARS-CoV-2 is a positively-sensed, and single-stranded RNA-enveloped virus with spherical capsids (120–160 nm) (Hoffmann et al., 2020; Nakagawa and Makino, 2021). The coronaviruse genome (GenBank no. MN908947) is about 26.4–31.7 kb long, making the largest among RNA viruses encoding 9,860 amino acids (Woo et al., 2010). Both structural and nonstructural proteins are found in gene fragments. Non-structural proteins such as 3-chymotrypsin-like protease, papain-like protease, and RNA-dependent RNA polymerase that are encoded by open reading frame (ORF) region, while structural proteins such as spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid protein (N).

SARS-CoV-2 surface is covered by the S protein, a large glycosylated transmembrane protein (1,160–1,400 aa) that binds to the host cell receptor angiotensin-converting enzyme 2 (ACE2) and mediates viral cell entrance (de Wit et al., 2016; Wu et al., 2020). Since the pathogenesis of coronaviruses has not been completely understood, the precise molecular mechanism of entry of viruses into cells remains unclear (Glebov, 2020). Coronaviruses are thought to enter the host cells by two main routes: direct genome into the cytosol *via* fusion with the host cell membrane, and endocytosis (Pelkmans and Helenius, 2003) (**Figure 1**). Typically, when the S protein attaches to the receptor, proteases on the host cell membrane, such as transmembrane protease serine 2 (TMPRSS2) and airway trypsin-like protease TMPRSS11D, promote virus entry into the cell by activating the S protein. The viral RNA genome is translated into two polyproteins and structural proteins in the cytoplasm once the virus enters the cell, facilitating the construction of virus progeny (Perlman and Netland, 2009). Replication and transcription of the viral RNA genome occur through protein cleavage with continuation/discontinuation of RNA synthesis that is mediated by a replicase-transcriptase complex. Eventually, structural proteins are synthesized, assembled, and packaged in the endoplasmic reticulum-Golgi intermediate compartment of the host cell (Li et al., 2020b).

Furthermore, it has been proposed that SARS-CoV-2 entered cells through clathrin-mediated endocytosis (Bayati et al., 2020). SARS-CoV-2, like SARS-CoV and MERS-CoV, may use numerous pathways to successfully enter the cytosol of the host cell (Hartenian et al., 2020; Yang and Shen, 2020).

COVID-19 INFECTION AND THE IMMUNE SYSTEM RESPONSES

The immune system responses to SARS-CoV-2 are considered to be critical in controlling the pathogenicity and clinical symptoms of the patient (Yang et al., 2020). After virus infection, the immune system uses multiple mechanisms for recognition and defends against viruses (Koch et al., 2013). The body uses both the immune responses (innate and adaptive immunity), to eliminate viral infection (Ye et al., 2021) (**Figure 2**). During the first hours and days, the innate immune response to viruses begins with innate immune cells, like phagocytic cells (neutrophils, macrophages) and dendritic cells (Zhou and Ye, 2021). These cells express different types of Pattern-recognition receptors (PRRs) to recognize DAMPs and PAMPs (Li and Wu, 2021). In COVID-19 patients, NK cells can kill virus-infected cells by releasing cytotoxic granules or through participating in antibody-dependent cellular cytotoxicity and producing different cytokines and chemokines (Ma et al., 2021).

After the fusion of the virus into the cells, viral genome starts replication and transcription (Astuti and Ysrafil, 2020). Then immune system recognized RNA viruses by cytosolic and endosomal RNA receptors, such as RIG-I-like receptors and toll-like receptors (TLR3, TLR7, and TLR8) (Hosseini et al., 2020). Within hours, RNA virus recognition by these receptors can activate some of the transcription factors like nuclear factor kappa-light-chain- enhancer of activated B cells (NF-κB), to produce pro-inflammatory cytokines and chemokines and activation of immune cells (Kircheis et al., 2020). The result of these processes is the production of interferons -λ (type III IFN) and type I IFN, pro-inflammatory cytokines (for example, interleukin-6, interleukin-18, and interleukin-1) and some chemokines such as chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-C motif) ligand 7 (CCL7) by immune cells (Stetson and Medzhitov, 2006).

Moreover, the infected cells may undergo the process of inflammatory cell death (pyroptosis) and release DAMPs, such as viral nucleic acids and oligomers (Yap et al., 2020).

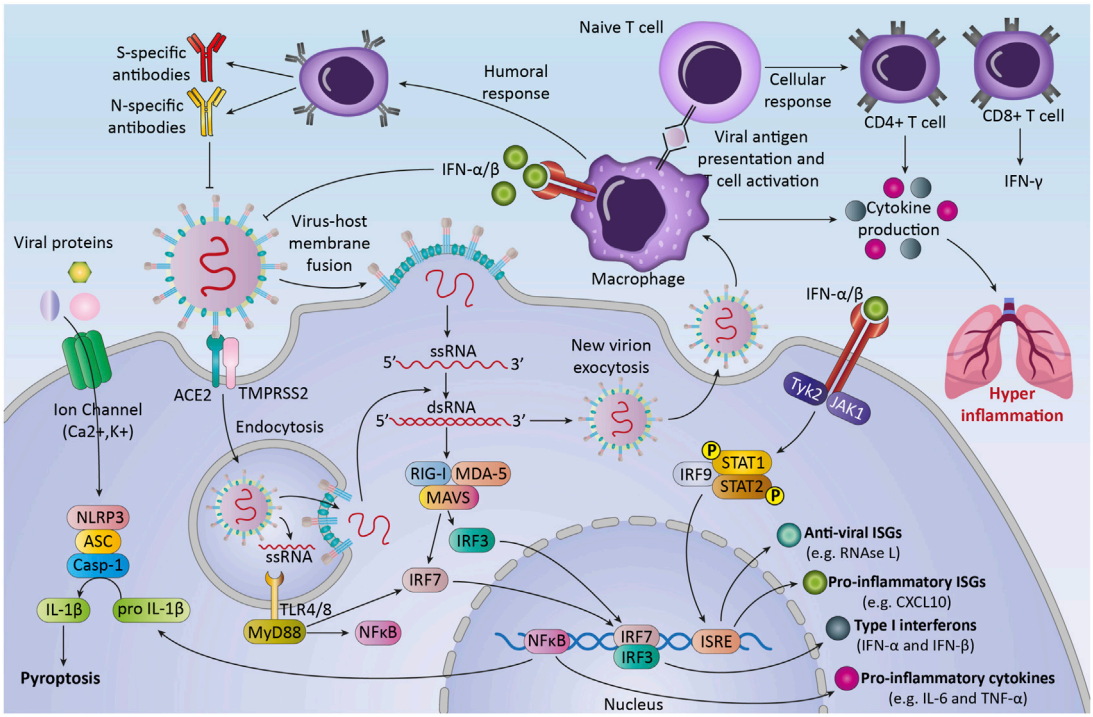

FIGURE 2 | The immune response against SARS-CoV-2.The first line of host defense against SARS-CoV2, innate immunity, is the response against the virus by recognizing Pattern associated molecular patterns (PAMPs) through transmembrane or intracellular pattern recognition receptors (PRRs). Recognition of viral components leads to the activation of immune cells and transcription factors that lead to the production of different cytokines, chemokines, and anti-viral proteins. These processes can lead to activation of adaptive immune response that consists of three major cell types (B cells, $CD4^+$ T cells, and $CD8^+$ T cells). After pathogen elimination, adaptive immunity regulates innate immunity to avoid unnecessary host cell damage. Unbalanced response and immune system overactivation can cause collateral damage to host tissues and exacerbate disease severity.

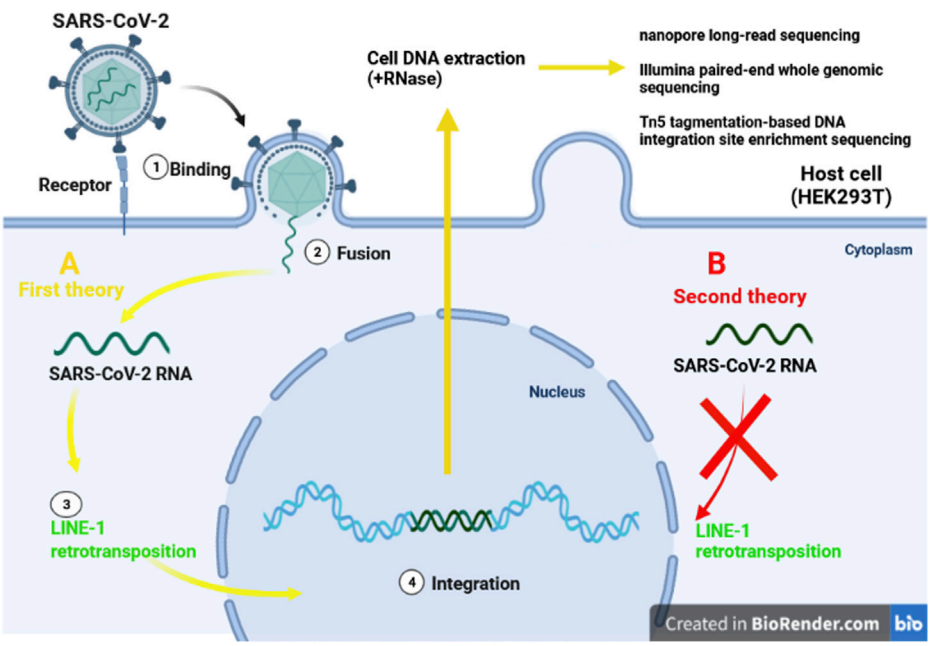

FIGURE 3 | Two different theories of integration SARS-CoV-2 RNA. The first idea claims that LINE-1 retrotransposon proteins (A/yellow lines) can incorporate SARS-CoV-2 into human DNA. Despite the presence of the LINE-1 protein machinery (B/red line), the second explanation finds no evidence of virus genomic integration.

The second line of defense, the adaptive immune response, happens several days later after the innate immune response occurs. T cells and B cells, two classes of lymphocytes, carry out the adaptive immune response against COVID-19. In the majority of infected patients, B cells at first produce IgM and IgA antibodies and then IgG, that is measurable between weeks two and five after infection (Emmerich et al., 2021). Various structural proteins of the virus can be targeted with these antibodies (Pan et al., 2021).

The other adaptive immune cells, $CD8^+$ T cells, directly kill cells that are infected with the virus and stop virus spreading further (de Candia et al., 2021). The other type of T cells, $CD4^+$ T cells, produce different types of cytokines and chemokines that influence the function of B and T cells, and other immune cells such as macrophages (Chen et al., 2010).

After pathogen elimination, adaptive immunity regulates innate immunity to avoid unnecessary host cell damage (Zhou et al., 2020). Notably, while a highly organized immune response to viral infection is important, an unbalanced response and immune system overactivation inadvertently targeting healthy cells and lead to host tissues damage (Catanzaro et al., 2020). The imbalance of the immune system in fighting against SARS-CoV-2 can induce inflammation, lymphopenia, and cytokine storm in patients and follow that septic shock, acute lung injury, ARDS, and multi-organs failure may occur, that increases patient's mortality rate (Jamal et al., 2021). Early studies indicated that COVID-19 patients have higher neutrophil and lower lymphocyte counts in blood compared with healthy controls (Eslamijouybari et al., 2020). Also, $CD8^+$ lymphocytes and NK cells show an exhausted phenotype and significant reduction in severe COVID-19 patients compared with patients with mild SARS-CoV-2 -infection and healthy controls (Zheng et al., 2020). These changes can disrupt the immune system and the antiviral effects of immune cells. On the other hand, cytokine storm can occur as a result of hyperactivity of infected macrophages and other immune cells that cause the production of various inflammatory cytokines (Zheng et al., 2020).

Increasing NF-κB has an important effect on cytokine storms created by an acute respiratory RNA virus. Activation of TLRs by recognition of virus PAMPs leads to downstream signaling pathways like NF-κB and interferon regulatory factor 3 (IRF3) (Hariharan et al., 2021). NF-κB plays key roles in regulating different cellular functions, such as cell growth and cell death, immune responses, and inflammation (Barnabei et al., 2021). It has been shown that COVID-19 is able to activate TLR4-mediated NF-κB signaling (Kircheis et al., 2020). Overactivation of NF-κB targets genes in the context of inflammation and affect the activation of adhesion molecules and inflammatory cells that all can cause or worsen lung disease and is a sign of chronic inflammation (Liu et al., 2017). A growing body of evidence suggests that infection and chronic inflammation as a risk factor can lead to cancer and develop some other diseases (Rossi et al., 2021). Therefore, further investigation about the interaction among the immune system and coronavirus can be effective to reduce its adverse consequences.

DOES IMMUNE DYSREGULATION PREDISPOSES INDIVIDUAL TO CANCER

IL-6/JAK/STAT Signaling Pathway

Infection with SARS-CoV-2 causes innate immune responses, which result in a diverse set of immune mediators and the activation of some signaling pathways, including Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor kappa B (NF-κB), interferon response factor 3 (IRF3), and IRF7. The signaling cascade that results from these pathways in infected cells increases the production of pro-inflammatory cytokines (Li et al., 2020c; Prompetchara et al., 2020).

In COVID-19 related cytokine storm syndrome (COVID-CSS), different inflammatory cytokines such as Interleukin-1 (IL-1), Interleukin-10 (IL-10), and tumor necrosis factor (TNF)-α are elevated about 2-100 fold above normative values. In some patients with COVID-19, IL-6 levels rise more than 1,000 fold above normal range (Blanco-Melo et al., 2020; Herold et al., 2020; Laing et al., 2020; Al-Baadani et al., 2021). IL-6 signals through both classic and trans-signaling pathways, activates JAK-STAT3 signaling. The IL-6/JAK/STAT3 pathway is also abnormally activated in many types of cancer, which is generally associated with a poor clinical prognosis (Johnson et al., 2018). In the tumor microenvironment, IL-6/JAK/STAT3 signaling pathway plays a pivotal role in regulating the growth, survival, invasiveness, metastasis, and development of many cancers. In addition, the IL-6/JAK/STAT3 acts as a key component in suppressing the antitumor immune response (Bromberg and Wang, 2009; Kumari et al., 2016). Previous research identified the role of IL-6 as a driver of tumorigenesis and anti-apoptosis signaling and a vital biomarker of cancer diagnosis, risk, and prognosis (Vargas and Harris, 2016). Also, IL-6 enhanced the metastasis rate in breast cancer through the positive effect on tumor stem cell self-renewal and epithelial-to-mesenchymal transition (EMT) process (Bharti et al., 2016; Xiao et al., 2017).

STAT3 hyperactivation in tumor cells can occur as a result of increased IL-6 levels in the serum and/or the tumor microenvironment, or as a result of loss-of-function mutations affecting STAT3 negative regulators. IL-6 is produced by different cell types including stromal cells, tumor-infiltrating immune cells, and the tumor cells (Nozawa et al., 2006; Walter et al., 2009; Nagasaki et al., 2014). IL-6 directly stimulates the expression of STAT3 downstream targets in tumor cells. STAT3 signaling controls the expression of genes involved in tumor growth, survival, invasion, and angiogenesis. The ability of STAT3 to promote IL6 gene expression is *via* binding to the IL6 promoter, which results in a positive autocrine feedback loop (Chang et al., 2013). STAT3 also promotes angiogenesis, invasiveness, and/or metastasis, as well as immunosuppression (Teng et al., 2014; Huynh et al., 2017).

In addition to direct effects on tumor cells, IL-6 and JAK/STAT3 signaling can have a profound effect on tumor-infiltrating immune cells. STAT3 is often hyperactivated in tumor-infiltrating immune cells and negatively regulates natural killer (NK) cells, neutrophils, effector T cells, and dendritic cells (DCs). Taken together, these data suggest that, STAT3 activation in immune cells probably leads to down-modulation of antitumor immunity response (Harris et al., 2007; Yu et al., 2007; Herrmann et al., 2010; Iwata-Kajihara et al., 2011). Collectively, IL-6/JAK/STAT signaling pathway plays a fundamental role in tumorgenesis and immunosuppressive tumor microenvironment (Kortylewski et al., 2005; Yu et al., 2009).

NFκB Pathway

The hyper-activation of NF-κB pathway has been implicated in the pathogenesis of the severe/critical COVID-19 phenotype (Hirano and Murakami, 2020). The excessive NF-κB activation subsequent to viral proteins detection *via* the innate immune system, probably has a causative role in covid-19 cytokine storm, extrapulmonary manifestations of COVID-19, and fatality rate (Liao et al., 2005; Oeckinghaus and Ghosh, 2009; DeDiego et al., 2014). This 'rapid acting' primary transcription factor seems to exert its effects by stimulating the gene expression of a wide variety of cytokines, adhesion molecules, chemokines, acute phase proteins, and inducible effector enzymes (Liang et al., 2004; Zhang et al., 2017).

In a similar way, NFκB is an important signaling pathway involved in the pathogenesis of tumors, and the potential role of excessive activation of this signaling pathway in oncogenesis has been confirmed. In various tumor types, intervention in this signaling pathway for targeted cancer therapy has been reported. NFκB signaling is involved in cell biogenic activities such as inflammation, cellular immunity, and stress (Salazar et al., 2014; Sau et al., 2016; Song et al., 2017). In lymphatic cancer, breast cancer, and colon cancer, the excessive activation of the NFκB-signaling pathway leads to uncontrolled and abnormal cell proliferation, differentiation, and apoptosis, as well as metastasis, and treatment resistance. The NFκB pathway is often altered in both solid and hematopoietic malignancies, promoting tumor-cell proliferation and survival (Hayden and Ghosh, 2008; Walther et al., 2015; Forlani et al., 2016; Sau et al., 2016). Therefore, the activation of NFκB pathway, as a shared pathway between COVID-19 and some cancers, plays an important role in disease progression.

IFN-I Signaling

IFNs are members of a large family of cytokines that are currently divided into three groups (type I, II, and III IFNs) (Negishi et al., 2018). Type I IFNs are involved in the development of innate and adaptive immune responses against both cancer and infectious diseases (Bonjardim, 2005). Similar to IL-6, IFN-Is signaling activates the JAK/STAT pathway (Schindler et al., 2007). Recent studies have demonstrated that IFN signaling could be a key mechanism in tumor proliferation. Inhibition of IFN pathway induction and dysregulated IFN signaling promote tumor cell senescence and death (Sangfelt et al., 1999; Fuertes et al., 2011; Schreiber et al., 2011). Type I interferons are crucial for restricting responses during the early stage of SARS-CoV-2 infection, however, IFN-I signaling was dampened in patients with severe COVID-19 (Blanco-Melo et al., 2020), and low level of IFNs along with an increase in IL-6, have been detected in the peripheral blood samples or lungs of patients (Hadjadj et al., 2020). These data illustrate the possible role of impaired INF-I signaling induced by SARS-CoV-2 infection, in inefficient antitumor response, which leads to tumor progression. On the other hand, lymphopenia, functional exhaustion, and impaired cellular immunity responses are associated with IFN production suppression. This notion is supported by evidence, which highlights the role of IFNs in effective T cell proliferation and survival (Marrack et al., 1999). The continued production of pro-inflammatory mediators due to viral persistence has a negative effect on natural killer cells and $CD8^+$ T-cell activation in the case of COVID-19 infection. The $CD8^+$ T-cell population undergoes quantitative and qualitative changes. Decreased in NK cells, $CD4^+$ T cells, and $CD8^+$ T cell population and impaired activation phenotypes are frequently observed, particularly in the severe form of COVID-19 disease (Ai et al., 2020; Pedersen and Ho, 2020). In COVID-19 patients specific cell population, the cytotoxic $CD3^-CD56dimCD16+$, was significantly reduced, and more importantly, elevated expression of regulatory molecules such as CD244 and programmed death-1 (PD-1) on NK cells and T cells, as well as decreased serum cytotoxic effector molecules such as perforin and granzyme A, were noticed. Previous research has also found exhaustion phenotypes of $CD8^+$ T cells in patients with severe COVID-19, which is caused by the upregulation of inhibitory receptors (IRs), which may impair host defences and lead to poor disease outcomes (Caruso et al., 2020; De Biasi et al., 2020; Diao et al., 2020; Laing et al., 2020; Schultheiß et al., 2020; Song et al., 2020; Zheng et al., 2020).

However, immune dysregulation may result in the COVID-19 severity, this functional exhaustion and subset alteration of NK and T cells may have a negative effect on efficient cell function during anti-tumor immune responses (McKinney and Smith, 2016; Wang et al., 2017; He et al., 2019).

Although the role of immune dysregulation and robust production of cytokines in COVID-19 pathogenesis has been well documented, future studies will be vital to investigate the shared molecular pathways and possible interaction between COVID-19 and the development and progression of cancer.

INTEGRATION OF SARS-COV2 GENOME AND CANCER INDUCTION; BE OR NOT TO BE

The World Health Organization, considers the infections as inducers for 15.4% of all cancers, with the viruses constitute up to 9.9% of all cancer causig infectious agents. The most prominent viral carcinogenic agent known to be hepatitis B virus, hepatitis C virus, human papillomavirus, and Epstein-Barr virus (Plummer et al., 2016). Integration of the viral genome into the host genome has been illustrated to be an unintended mechanism that may lead to the clonal growth and cancer develpemnt. In head-and-neck, cervical cancer, and

liver cancer, the integration of viral genome into the host genom has been proven. Most of the geneome integrations were observed to be distributed across chromosomes, and a significant number of viral genome integrations occurred in the intronic (40%) regions, whereas only 3.4% of integrations were recognized in gene coding regions (Zapatka et al., 2020). In RNA viruses, the RNA genome is reverse-transcribed by the viral reverse transcriptase which subsequently integrates into the host chromosome shortly after infection, and expressed under the control of viral transcriptional regulatory elements. Following integration process, oncogenesis occurs due to activation or inactivation of the host genes, oncoproteins, and tumor suppressors (McLaughlin-Drubin and Munger, 2008). Several hypotheses have been put forward regarding integration of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) into the human genome (Dai et al., 2020; Zhang et al., 2021) (**Figure 3**). Prolonged SARS-CoV-2 RNA shedding, and recurrence of PCR-positive tests, 60 days after the initial positive PCR test, have been widely demonstrated in non-infectious patients. These patients, howevere, do not appear to shed any infectious viruses (Bullard et al., 2020; Li et al., 2020d; Hosseini et al., 2020; Mina et al., 2020). Consequently, researchers have investigated the capability of SARS-CoV-2 as positive-stranded RNA viruses, for integration into human genome subsequent to reverse-transcription (RT). Hence, the transcription of the integrated sequences, provides logical evidence fo recurrence of PCR-positive tests (Dai et al., 2020). However, several investigations refuse the integration of SARS-CoV-2 into the human genome (Briggs et al., 2021; Kazachenka and Kassiotis, 2021; Parry et al., 2021; Smits et al., 2021).

To investigate the integration of SARS-CoV-2 RNA into the genome of infected human cells *in vitro,* Zhang et al. applied three different procedures: nanopore long-read sequencing, Illumina paired-end whole genomic sequencing, and Tn5 tagmentation-based DNA integration site enrichment sequencing (Zhang et al., 2020).

Recent studies provided enough evidences for the possibility of SARS-CoV2 integration into the human genome. The viral RNA found to be reverse transcribed in human cells by RT from LINE-1 elements or by HIV-1 RT. It has found the endogenous LINE-1 in human cells was induced to be overexpressed upon SARS-CoV-2 infection or following SARS CoV2-associated cytokine storm *in vitro*. The upregulation of host cell LINE-1 RT seems supportive for long-term positivity of PCR tests, even after recovery (Kazazian et al., 2017; Zhang et al., 2021). Although, Smits et al. applied long-read DNA sequencing to cultured HEK293T cells infected with SARS-CoV2 which provided no evidence for genomic integration of the SARS-CoV-2 into the host genome (Smits et al., 2021).

In another experiment, Briggs et al. examined 768 COVID patient's nasal swabs in order to evaluate the positive role of viral genome integration on diagnostic PCR test for COVID-19. In this study, LINE-1-mediated retrotranscription of SARS-CoV2 RNA genome into the host DNA found to be a rare event with no practical impact on RT-PCR-based diagnostic capability. These data indicated the supposed SARS-CoV-2 integrations are likely artefactual, stemming from amplicon DNA contaminations or other unintended processes (Briggs et al., 2021).

As a consequence, the correlation between the integration of SARS- CoV-2 into the host genome, and cancer has remained unknown. Moreover, further studies on different species of SARS-CoV-2 are needed for more comprehensive conclusion.

COVID-19 DIAGNOSTIC TESTINGS AND RISK OF CANCER

In clinically COVID-19 suspected patients, computed tomography (CT) is the preferred imaging modality, and it is useful for monitoring patients during treatment. A dose reduction procedure is an essential requirement given the increased number of chest CT scans requested by referring physicians and the need to reduce the possible dangers posed to patients by ionizing radiation (Radpour et al., 2020; Azadbakht et al., 2021). Several works reported that the chest CT had higher sensitivity (around 97%) for COVID-19 pneumonia diagnosis compared to standard methods such as real-time PCR and NGS (Ai et al., 2020; Caruso et al., 2020; Fang et al., 2020). One of the major concerns is that CT ionising radiation may increase the risk of leukaemia and solid tumors incidence (Power et al., 2016). Some organ systems are highly radiosensitive, whereas others have stronger defences against the effects of ionizing radiation. Organs like the oesophagus, breast, and bladder, for example, are particularly vulnerable, although the rectum, pancreas, and prostate are far less so (Power et al., 2016). A study from February 24th to April 28th 2020 including 3,224 high-resolution thorax CT showed a very low risk estimation of cancer despite the impressive increment in thoracic CT examinations due to COVID-19 cases (Ghetti et al., 2020).

It has been suggested that one CT scan is equivalent to 300-400 chest X-rays, and that having multiple CT scans at a young age may raise the risk of cancer later in life. CT scans have two major dangers, according to the FDA. First, the test findings reveal a benign or coincidental finding, prompting unnecessary, perhaps invasive follow-up examinations that could pose further dangers. Second, x-ray radiation exposure increases the risk of cancer induction. The infection risk from contamination of surface, aerosolization during the CT acquisition of a Covid-19 patient, putting healthcare workers working in the CT suite at an increased risk of contracting this infection, and the CT imaging facility becoming a Covid-19 infection source to other patients undergoing CT examination are some of its limitations.

FUTURE PERSPECTIVE

Future studies should focus on the disposition of patients recovering from the novel coronavirus for cancer. This increased risk to cancer could be related to the cumulative effect of different distinct aspects of coronavirus infection yet to be elucidated. Some of these aspects include the immune dysregulation, intensive production of cytokines, organ

damage secondry to infection or other factors such as CT scans, and induced mutations as the result of the viral genome integration into the host genome.

While both innate and adaptive immune systems are indispensible to combat SARS-CoV-2 infection, uncontrolled host immune responses are among main COVID- related pathogenicity. Therefore, these two opposite aspects of the immune system should be addressed before putting forth fututre solutions to reduce post-infection side effects.

Future studies are vital to investigate shared molecular pathways and possible interactions between COVID-19 and the development and progression of cancer and to investigate whether the virus can be regarded as an etiological factor in the development of cancer. Our knowledge about coronavirus is relentlessly being updated and this review should be interpreted in light of future reliable findings.

AUTHOR CONTRIBUTIONS

SH and LS contributed in the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. IR, ND, GV, and YE contributed in the providing and revising the draft and agreed for all aspects of the work.

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REVIEW
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Susceptibility to Metabolic Diseases in COVID-19: To be or Not to be an Issue

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Despite the passage of more than 17 months from the beginning of the COVID-19 pandemic, challenges regarding the disease and its related complications still continue in recovered patients. Thus, various studies are underway to assay the long-term effects of COVID-19. Some patients, especially those with severe symptoms, experience susceptibility to a range of diseases and substantial organ dysfunction after recovery. Although COVID-19 primarily affects the lungs, multiple reports exist on the effect of this infection on the kidneys, cardiovascular system, and gastrointestinal tract. Studies have also indicated the increased risk of severe COVID-19 in patients with diabetes. On the other hand, COVID-19 may predispose patients to diabetes, as the most common metabolic disease. Recent studies have shown that Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) binds to Angiotensin-Converting Enzyme 2 (ACE2) receptors, which are expressed in the tissues and organs involved in regulating the metabolic status including pancreas, adipose tissue, gastrointestinal tract, and kidneys. Therefore, SARS-CoV-2 may result in metabolic disturbance. However, there are still many unknowns about SARS-CoV-2, which are required to be explored in basic studies. In this context, special attention to molecular pathways is warranted for understanding the pathogenesis of the disease and achieving therapeutic opportunities. Hence, the present review aims to focus on the molecular mechanisms associated with the susceptibility to metabolic diseases amongst patients recovered from COVID-19.

Keywords: COVID-19, recovered, susceptibility, metabolic diseases, molecular mechanisms

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INTRODUCTION

Investigation of the history of medicine indicates that the outbreak of viral respiratory infections is not a new phenomenon. During the past century, there have been several reports on the prevalence of respiratory infections including Severe Acute Respiratory Syndrome Coronavirus-1 (SARS-CoV-1) (Tratner 2003) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Lancet 2013). Since December 2019, the Coronavirus Disease-19 (COVID-19) pandemic has become the most challenging issue throughout the world. Many studies have been done on the nature of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and its therapeutic strategies (Cardamone and Donatiello 2020; Schlosser et al., 2020).

Although COVID-19 has been primarily recognized as a respiratory infection, clinical evidence has demonstrated that SARS-CoV-2 affects multiple organs (de-Madaria et al., 2020; Ridruejo and

TABLE 1 | Diagnostic criteria for metabolic syndrome.

Obesity	Central obesity as defined by ethnicity/race-specific waist circumference, but can be assumed if Body Mass Index (BMI) ≥ 30 kg/m^2 and two or more of the following
Fasting plasma glucose	≥100 mg/dl or being on treatment for diabetes mellitus
Blood pressure	≥130/85 mmHg or being on anti-hypertensive drugs
Triglycerides	≥150 mg/dl or being on lipid lowering agents
High density lipoprotein-cholesterol	<40 mg/dl for males, < 50 mg/dl for females, or being on treatment for dyslipidemia

Soza 2020; Sharma et al., 2020; Hunt et al., 2021; Nalbandian et al., 2021). Therefore, post- COVID-19 complications are predictable in recovered patients. For instance, it has been hypothesized that COVID-19 may result in metabolic syndrome. Metabolic syndrome refers to a cluster of conditions that rises the risk of cardiovascular disorders and type II diabetes (Eckel et al., 2005). The International Diabetes Federation (IDF) reported the metabolic syndrome criteria in 2006 (Zafar et al., 2018), which have been summarized in **Table 1**.

Recent studies have indicated that SARS-CoV-2 binds to Angiotensin-Converting Enzyme 2 (ACE2) receptors. These receptors mediate the transmission and infection processes of the virus. The virus has spike (S) glycoprotein on its surface, which can attach to the cell surface in order to turn this glycoprotein into two domains (S1 and S2) and exert its effect. This separation is allowed by FURIN and Transmembrane Serine Protease (TMPRSS2). In this way, S1 binds to ACE2 receptors and S2 binds to the cell membrane, allowing the virus to enter the cell through endocytosis (Ding and Liang 2020; Mönkemüller et al., 2020). In fact, after the virus binds to ACE2, the receptor's ectodomain is cleaved. Then, the transmembrane domain is internalized by endocytosis. On the other hand, the whole ACE2 molecule can enter the cell with the virus (Li et al., 2005). ACE2 is expressed in the tissues and organs involved in regulating the metabolic status including pancreas (Liu et al., 2020), gastrointestinal tract (Qi et al., 2020; Zhang et al., 2020), liver (Chai et al., 2020), and kidneys (Qi et al., 2020). Thus, metabolic syndrome may be a potential complication in patients recovered from SARS-CoV-2 (Lee et al., 2021; Sathish et al., 2021).

Zhang et al. reported abnormal plasma metabolomic profiles in patients recovered from COVID-19 without any underlying diseases. They found that some clinical indicators were abnormal in patients recovered from moderate and severe COVID-19 3 months after discharge. These parameters included taurine, succinic acid, hippuric acid, some indoles, and lipid species that were associated with the functions of kidneys, lungs, heart, liver, and the coagulation system (Zhang et al., 2021). Moreover, Holmes et al. referred to the post-acute COVID-19 syndrome in non-hospitalized patients with COVID-19 in the post-acute phase of the disease. They analyzed the blood samples of 27 patients 3 month after acute COVID-19 infection. The findings indicated disruptions in several parameters including taurine and glutamine/glutamate ratio. These results demonstrated the possibility of damages to the liver and muscles in non-hospitalized patients with COVID-19 in the post-acute phase (Holmes et al., 2021). On the other hand, the results of a systematic review and meta-analysis revealed that severe COVID-19 was associated with elevated blood glucose levels and a slight increase in HbA1c levels (Chen Juan et al., 2020). Although limited studies have been conducted on patients' follow-up after COVID-19 infection, the results obtained so far have shown the fluctuation of multiple metabolic indicators in patients recovered from COVID-19, especially its severe form.

While a variety of studies have shown that several underlying diseases including diabetes (Gupta et al., 2020), kidney diseases (Rabb 2020), and cardiovascular disorders (Li Jialing et al., 2020) increase the severity and mortality in SARS-CoV-2 infection, the association between COVID-19 and the onset of some diseases needs to be determined. Hence, the present review aims to investigate the mechanisms involved in the susceptibility of recovered patients to metabolic syndrome. In this regard, the organs involved in metabolic syndrome including pancreas, gastrointestinal tract, kidneys, and liver (**Figure 1**) have been discussed and the relationships between these organs and COVID-19 have been addressed.

The Effect of COVID-19 on the Pancreas

There is a growing body of evidence on the bidirectional relationship between diabetes and COVID-19. This implies that while patients with diabetes experience more severe forms of COVID-19, patients with COVID-19 may develop metabolic complications and diabetes (Rubino et al., 2020).

Diabetes has been introduced as a risk factor for higher morbidity and mortality in patients with COVID-19. Evidence has revealed that COVID-19 may affect the pathophysiology of diabetes through increased ACE-2 expression, increased paired basic amino acid cleaving enzyme (FURIN), impaired T-cell function, and increased interleukin-6 (Singh et al., 2020). Therefore, patients with diabetes need special clinical management to avoid the progressive damage. On the other hand, due to the diabetogenic nature of COVID-19, evaluation of recovered patients is essential. In this regard, investigation of the underlying mechanisms that lead to the onset of diabetes in patients with COVID-19 can be beneficial for the prevention and management of the issue.

It seems that the most important mechanism related to the COVID-19 complications is the expression pattern of the Receptor-Binding Domain (RBD) of ACE2 on cells, as a binding site for SARS-COV-2 (El-Huneidi et al., 2021). Studies have shown that ACE2 is expressed in the β-cells of the pancreas. Thus, SARS-COV-2 may exert direct effects on pancreatic β-cells, subsequently resulting in glucose and metabolic instability. Analysis of the GTEx database revealed a higher expression of

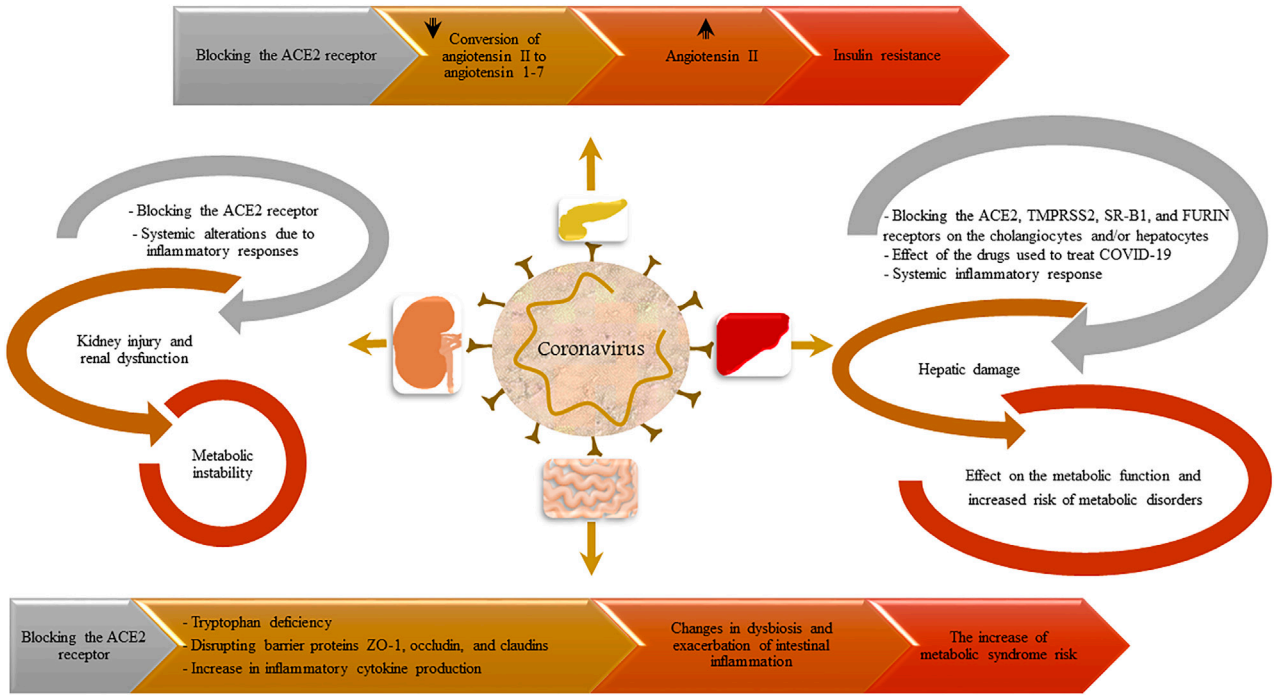

FIGURE 1 | A summary of the possible effects of COVID-19 infection on the development of metabolic syndrome.

ACE2 in the pancreas than in lungs. Moreover, evaluation of ACE2 distribution in the pancreas showed the expression of this receptor in both exocrine and endocrine parts (Liu et al., 2020). Liu et al. evaluated pancreatic injury after COVID-19 infection in a cohort study on 121 patients. They found that pancreatic injury occurred mainly in severe COVID-19 infection, which was suggested to be associated with ACE2 expression in the pancreas (Liu et al., 2020). Researchers also focused on the potential of COVID-19 to affect the endocrine pancreas through the activation of NHA2 as a Na+/H+ Exchanger (NHE) (Cure E. and Cumhur Cure M., 2020). Based on the previous studies, NHA2 is present in human pancreatic β-cells. Additionally, *in vitro* and *in vivo* assays have indicated that NHA2 is critical for insulin secretion in β-cells (Deisl et al., 2013). Accordingly, NHA2 deficiency is accompanied by a decrease in insulin secretion. On the other hand, increased NHA2 activity leads to insulin resistance. In COVID-19 infection, conversion of angiotensin II to angiotensin 1–7 decreases after blocking the ACE2 receptors by the virus. The elevated level of angiotensin II leads to the increase of insulin resistance. Cure et al. also suggested that the chymase pathway was probably involved in the formation of angiotensin II. Chymase is a major non-ACE pathway in the generation of angiotensin II. Through this alternative pathway, angiotensin-(1–12) and angiotensin I are converted to angiotensin II by the proteolytic enzymatic activity of chymase. Although the role of this pathway in COVID-19 disease is not well known, chymase may be a potential mediator. Garvin et al. proposed the positive interplay between the kallikrein-bradykinin system and chymase in COVID-19 patients (Garvin et al., 2020).

Activation of NHE by angiotensin II results in the increase of Na+ and H+ exchange as well as Na+ and Ca2+ exchange. In this regard, H+ ions accumulate out of the cell and make the microenvironment acidic and hypoxic. Subsequent to hypoxia, reactive oxygen radicals increase. Oxidative stress plays a fundamental role in metabolic syndrome. Besides, accumulation of reactive products can develop insulin resistance. Increased levels of Ca2+ and Na+ also damage cells (Cure E. and Cumhur Cure M., 2020).

Hypoxia is yet another factor that can affect the function of the endocrine pancreas. In hypoxia due to COVID-19 infection, the lactate level elevates. The increased level of lactate causes the increment of H^+ ions, which move inside cells and make NHE overactive (Cure Erkan and Cumhur Cure Medine, 2020). Moreover, there is a dynamic relationship between lactate and glucose. Lactate has been introduced as the major gluconeogenic precursor that increases glucose production (Bergman et al., 2000; Emhoff et al., 2013). This situation can impose an additional burden on the pancreas and can be destructive in severe and prolonged COVID-19 infection.

In conclusion, the new onset of diabetes in non-diabetic patients and exacerbation of diabetes in those suffering from the disease have been reported subsequent to COVID-19 infection. According to the abovementioned information, paying attention to the basic mechanisms involved in the interaction between COVID-19 infection and diabetes is a useful guide for taking preventive and therapeutic measures. Considering the high expression of ACE2 receptors in pancreatic β-cells, they may be damaged by SARS-CoV-2. Thus, early diagnosis and careful management of patients with

COVID-19 are needed to avoid further complications related to hyperglycemia and metabolic imbalance. Moreover, targeting ACE2 has been proposed to mitigate infectivity (Jia et al., 2021). Recently, strategies related to ACE2 targeting such as decreasing Ang II to decoy receptor (Haschke et al., 2013), blocking ACE2 (Huentelman et al., 2004), enhancing ACE2 shedding (Jia et al., 2009), and facilitating ACE2 internalization (Touret et al., 2020) have been used for the prevention and treatment of COVID-19. All in all, the diabetogenic potential of COVID-19 requires further studies to determine its main characteristics and persistence, especially in cases with new onset diabetes.

The Effect of COVID-19 on the Gastrointestinal Tract

The primary function of the gut is to supply the body with energy via building elements and water/electrolytes. Energy homoeostasis consists of two principal functional states; i.e., catabolic state and anabolic state. The switch between these two metabolic conditions is related to meal ingestion and digestion/absorption and is thus dependent on the functional state of the gastrointestinal tract (Fändriks 2017). The gut microbiota plays a pivotal role in maintaining the homeostasis of human health (Festi et al., 2014). The human intestinal microbiota composition is the result of a bilateral interaction between the host and its microbial consortium. In fact, the composition and stability of the intestinal microbiota are determined by nutrition, drugs, diseases, etc. (Hur and Lee 2015). Dysbiosis of the gut microbiota caused by various factors leads to extensive physiological changes and increases the risk of metabolic syndrome (Festi et al., 2014).

It mainly infects lung cells, but may involve the gastrointestinal tract that has ACE2 receptors in the epithelial cells of the intestinal mucosa, but not in the esophagus and stomach (He et al., 2006; Gu and Korteweg 2007; Xiao et al., 2020). In the gastrointestinal tract, ACE2 has been described as a key regulator of dietary amino acid homeostasis, expression of antimicrobial peptides, local innate immunity, and gut microbial ecology (Hashimoto et al., 2012). In fact, ACE2 is associated with the amino acid carrier, B0AT1, which regulates the intestinal flora. This occurs because this transporter allows the absorption of tryptophan, which stimulates the mTOR path to produce antimicrobial peptides. Thus, the SARS-CoV-2 infection changes and blocks the ACE2 receptors in the brush edge, causing tryptophan deficiency and lower production of antimicrobial peptides, which can in turn cause changes in the intestinal microbiota and result in inflammation (Ding and Liang 2020; Mönkemüller et al., 2020). On the other hand, the interaction between SARS-CoV-2 and ACE2 in the gastrointestinal tract may lead to damage to the barrier function *via* disrupting barrier proteins ZO-1, occludin, and claudins as well as an increase in inflammatory cytokines production, which may in turn lead to dysbiosis and exacerbation of intestinal inflammation (Fernández-Blanco et al., 2015; Chen Li et al., 2020). Besides, intestinal inflammation may augment dysbiosis and damage the intestinal mucosal barrier function. Moreover, intestinal lymphocytes, dendritic cells, and macrophages may perpetuate the cytokine storm (Fernández-Blanco et al., 2015).

Preventing interaction between the SARS-CoV-2 RBD and ACE2 may be an effective strategy to prevent viral infections. In this way, ACE2 inhibitors can affect intestinal amino acid metabolism, antibacterial peptide secretion, intestinal microbial homeostasis, and innate immunity through the mTOR pathway (Hashimoto et al., 2012; Li et al., 2020). Furthermore, metabolic syndrome can be improved through the alteration of intestinal microbiota via the administration of probiotics and prebiotics that increase the short chain fatty acid production as well as by using a diet with 30% fat content and a high fruit and vegetable content such as the Mediterranean diet (Santos-Marcos et al., 2019).

The Effect of COVID-19 on Kidneys

Evidence has revealed an association between Chronic Kidney disease (CKD) and the metabolic syndrome (Wu et al., 2021). A significant correlation has also been reported between kidney insufficiency and diabetes, obesity, and hypertension (Culleton et al., 1999). The metabolic syndrome related to renal dysfunction results from various factors (Peralta et al., 2006) including hyperinsulinemia (insulin resistance), activation of the renin-angiotensin system, abnormal production of growth factors, inflammation, and oxidative stress. Among these factors, hyperinsulinemia is the most important risk factor for the metabolic syndrome associated with CKD, which induces renal damage mechanisms (El-Achkar et al., 2005). On the other hand, kidney is a major organ in the regulation of glucose and plays a key role in glucose reabsorption and filtration (Rabkin et al., 1984). Furthermore, kidneys primarily metabolize insulin, resulting in the degradation of circulating insulin (Mather and Pollock 2011). Considering renal function in the clearance of insulin, renal insufficiency affects glucose homeostasis and alters insulin metabolism (Duckworth et al., 1998; Garla et al., 2017). According to these findings, CKD and diabetes are complicatedly intertwined. This implies that the management of metabolic abnormalities due to CKD is a major challenge for clinicians (Garla et al., 2017). Therefore, any reason for renal complication may lead to the disturbance of glucose metabolism and, subsequently, the development of metabolic syndrome.

Renal injury results from viral infections through direct and indirect invasion by offending viruses, leading to cytopathic injury followed by renal cells injury (Prasad and Patel 2018). Re-emergence of different viral infections has been a recent pattern associated with kidney diseases (Morens et al., 2004). In this context, the consequences of the COVID-19 pandemic have been overrunning, being a challenge facing healthcare systems worldwide (Cheng et al., 2020). Acute Kidney Injury (AKI), as a major complication of COVID-19, arises from multifactorial mechanisms and increases the risk of mortality in these patients (Lili et al., 2020; Gupta et al., 2021). Furthermore, CKD develops in AKI-recovered patients as a long-term renal dysfunction. Notably, kidney transplant patients as well as individuals with CKD are at an increased risk of severe COVID-19 (Jager et al., 2020), and patients with severe

COVID-19 are at a high risk of AKI as a frequent complication (Ng et al., 2021). It has been revealed that podocytes and proximal tubular epithelial cells are present as a kidney tropism upon SARS-CoV-2 infection. Progression of the renal injury initiates through changes in the metabolism of kidney cells, resulting in metabolic diseases (Yan et al., 2018; Legouis et al., 2020). Generally, two scenarios can be considered for kidney injury and decreased function of this organ in patients with COVID-19. The main scenario is the direct infection of renal cells with SARS-CoV-2, which is in accordance with the fact that kidney is one of the particular targets of COVID-19 through ACE2 receptors. In the second scenario, systemic alterations in metabolism stemming from the immune system response may lead to AKI in patients with COVID-19 (Andrade Silva et al., 2021a). Based on a follow-up study on COVID-19-recovered patients, the kidney expresses the ACE2 receptors like the heart, lung, and gut (Diao et al., 2020). Moreover, the Proximal Convoluted Tubule (PCT) of the host renal cells consists of two important receptors, namely ACE2 and Transmembrane protease Serine 2 (TMPRSSs), for viral entry (Pan et al., 2020). After entry, accumulation of the extracellular matrix occurs and causes diuresis and proliferation of kidney cells, finally leading to AKI (Balachandar et al., 2020). Intriguingly, a recent investigation found that AKI following COVID-19 was associated with the increased binding of the virus to the ACE2 receptors (Perico et al., 2020). Moreover, the SARS-CoV-2 entry process was promoted through the proteolytic activity of TMPRSS (Shen et al., 2017). After SARS-CoV-2 enters renal cells, in case the cells' inflammatory system can pass the threshold for activating the Nucleotide-binding Domain (NOD)-like Receptor Protein 3 (NLRP3) inflammasome, cell death will occur via pyroptosis (Qing et al., 2020). The NLRP3 inflammasome is a cytosolic protein complex, which is a crucial regulator of the inflammation pathway and the innate immune response (Martinon et al., 2009). Hence, inflammasome has a central role in the pathogenesis of several inflammatory disorders such as diabetes, atherosclerosis, and arthritis (Schroder et al., 2010; Davis et al., 2011). There are three pathways for this intracellular protein complex to become activated. In the first pathway, the spike protein SARS-CoV-2 directly stimulates the NLRP3 inflammasome by binding to renal cells via the ACE2 receptors. In the second pathway, activation of the Renin–Angiotensin–Aldosterone System (RAAS) increases the level of angiotensin II, which induces the NLRP3 inflammasome after attaching to the angiotensin I receptor. In the third pathway, some cleavage fragments including C3a and C5a as anaphylatoxins and C5bC9 as a membrane attack complex are released through the direct interaction between the Complement Cascade (ComC) and SARS-CoV-2, which may lead to the activation of the NLRP3 inflammasome (Ratajczak and Kucia 2020). It should be noted that the hyper-activation of the NLRP3 inflammasome is a significant problem for human host cells, resulting in the perturbation of mitochondrial function, cell death, and severe kidney tissue injury (van den Berg and Te Velde 2020). This results from the fact that NLRP3 activation leads to caspase-1 cleavage, induction of other Damage-Associated Molecular Patterns (DAMPs), and production of pro-inflammatory cytokines IL-1β and IL-18 (Freeman and Swartz 2020). Therefore, this activation should be downregulated through increasing antibodies and adequate adaptive responses (Ros et al., 2020). The overstimulation of the NLRP3 inflammasome and cell death pathways can influence renal functionality, leading to inflammation and kidney damage, triggering alterations in tubular epithelial cell metabolism, and finally resulting in metabolic disturbance (Li et al., 2019). Yet, further studies are required to clarify the molecular mechanisms involved in metabolic dysfunction during COVID-19 progression.

Toll-Like Receptors (TLRs) are a type of pattern recognition receptors, which have been revealed as another potential receptor binding to the S protein of SARS-CoV-2 (Choudhury and Mukherjee 2020). Based on the recent studies, the interaction between TLR-4 receptors and SARS-CoV-2 results in the induction of inflammatory responses and profoundly affects the metabolism of mitochondrial, lipid, and glycolytic homeostasis in antigen presenting cells including macrophages and dendritic cells, eventually affecting the systemic metabolism (Perrin-Cocon et al., 2018; Lauterbach et al., 2019). Furthermore, activation of TLR4 leads to a severe inflammation pathway in kidneys, thereby causing AKI (Andrade-Silva et al., 2018; Andrade Silva et al., 2021b). However, it remains unknown whether metabolic dysfunction after recovery from COVID-19 can be correlated to TLRs signaling in kidneys. Therefore, recovered patients are recommended to be followed up to ensure the absence of underlying conditions stemming from COVID-19. In this context, creatinine levels have to be monitored frequently for acute kidney damage after recovery from COVID-19. Now, the key question rises whether such compounds as NLRP3 inflammasome inhibitors and ComC inhibitors that mitigate the innate immunity activation can be considered a therapeutic option. In this regard, mesenchymal stromal cells can be nominated as the potential modulators of the immune system.

The Effect of COVID-19 on the Liver

Liver is known as a key organ in the regulation of necessary routs for the maintenance of systemic metabolic hemostasis. The regulation of lipid and glucose hemostasis is orchestrated by hepatocytes. Following metabolic imbalance between glucose and lipid metabolism, hepatic steatosis occurs. In fact, whenever the amount of liver fat reaches more than 5% of the liver weight, hepatic steatosis occurs, irrespective of alcohol consumption (Rosselli et al., 2014). Accordingly, Nonalcoholic Fatty Liver Disease (NAFLD) has been identified as the hepatic manifestation of metabolic syndrome, because it is closely related to the impaired metabolism of fatty acids, lipoproteins, and glucose (Rosselli et al., 2014; Godoy-Matos et al., 2020). NAFLD is in fact the most common liver disease that is associated with metabolic and cardiovascular disorders including insulin resistance, hypertension, dyslipidemia, and type II diabetes. Recently, a number of international experts have suggested definition criteria for the metabolic dysfunction-associated fatty liver disease (MAFLD). The definition of MAFLD includes hepatic steatosis confirmed through histological,

imaging, or blood biomarkers together with at least one of the three main criteria of metabolic disorder; i.e., overweight/obesity, type II diabetes, and the presence of metabolic dysregulation. According to epidemiological assessments, the highest prevalence of MAFLD has been detected in Middle East and South America where almost half of the population have the metabolic syndrome. It is noteworthy that advanced hepatic fibrosis is observed in about 7% of people with hepatic steatosis and its incidence is almost doubled in the presence of metabolic syndrome (Godoy-Matos et al., 2020).

The prevalence of liver injury following COVID-19 has been reported to be 21.5–46%. Additionally, it has been found to be associated with multifactorial and heterogeneous reasons that are required to be monitored closely and continuously. Thus, in the context of COVID-19, it should be explored whether the liver damage is related to the side effects of the drugs used for COVID-19 treatment, the direct effect of the virus, an underlying liver disease, and/or combination in the disease course. Liver damage has been supposed to be more severe in patients with NAFLD. Up to now, different mechanism of liver injury caused by COVID-19 infection have been proposed (Yu et al., 2021). The first mechanism may be a direct damage to the liver via ACE2 receptors. ACE2 is widely expressed in hepatobiliary system cells including hepatocytes and cholangiocytes (Alqahtani and Schattenberg 2020). Two other receptors called TMPRSS2 and FURIN are also important for infection in hepatic cells (Marjot et al., 2021). The expression of ACE2 is higher in cholangiocytes than in hepatocytes. TMPRSS2 and FURIN are expressed in many liver cells types. However, very few hepatocytes express both ACE2 and TMPRSS2. Cholangiocytes play a key role in liver regeneration and immune response, and their dysfunction can lead to hepatic damage. In a recent study, Chai et al. evaluated the expression of ACE2 in healthy human liver tissues and demonstrated that viruses might directly bind to ACE2-positive cholangiocytes, but not to hepatocytes. Thus, they suggested that the liver damage in patients with SARS and COVID-19 might not be associated with the direct infection of hepatocytes. Cholangiocyte dysfunction by viral infection as well as by other causes like the drugs used for treatment or the systemic inflammatory response induced by pneumonias could lead to liver injury. Alkaline Phosphatase (ALP) and Gamma Glutamyltranspeptidase (GGT), as cholangiocytes injury markers, increased in some patients with COVID-19, supporting the role of cholangiocytes dysfunction in liver injury in these patients (Alqahtani and Schattenberg 2020; Marjot et al., 2021). Recently, Zhao et al. created a human liver ductal hepatocyte organoid model that was permissible to SARS-CoV-2 infection and replication. They observed that SARS-CoV-2-infected cholangiocytes directly impaired their barriers and bile acid transporting functions that resulted in hepatobiliary injury, confirming the strong role of cholangiocytes in the liver injury induced by SRAS-CoV-2 (Marjot et al., 2021).

Apart from the specific role of ACE-2 receptors, additional receptors have been found to play roles in virus entry. One of these receptors is the high-density lipoprotein Scavenger Receptor B-type 1 (SR-B1), which has been reported to help facilitate ACE2-dependent coronavirus attachment *in vitro*, reminiscent of hepatitis C virus infection. In addition, treatments targeting SR-B1 decrease the lipoprotein-mediated increment of SARS-CoV-2 infection (Wei et al., 2020).

In the second possible mechanism of liver injury, hypoxia may occur in hepatocytes following severe lung damage with SARS-CoV-2 infection, leading to an increased expression of ACE2 receptors and Hypoxia-Inducible Factors (HIFs). HIFs are one of the factors involved in hepatocyte metabolism whose increase can result in hepatic steatosis and liver injury (Li et al., 2021).

The third possible mechanism can be Drug Induced Liver Injury (DILI). Many drugs are currently used to treat patients with COVID-19, some of which may have side effects on the liver, leading to hepatocyte toxicity (Li et al., 2021). As an instance, Cai et al. disclosed that the use of lopinavir/ritonavir compounds significantly caused liver damage (Cai et al., 2020), suggesting the need for paying more attention to this drug.

Cytokine storm may be the next possible mechanism of liver injury (Alqahtani and Schattenberg 2020; Ahmed and Ahmed 2021). Cytokine storm involves the initiation of an immune response cascade due to a severe inflammatory response after COVID-19 infection. Elevated inflammatory markers such as C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), D-dimer, IL-6, IL-2, and serum ferritin lead to cytokine storms in patients with severe COVID-19, which can be followed by the sudden deterioration of the condition towards multi-organ failure and severe liver injury.

Another suggested pathway is aggravated liver damage in patients with NAFLD. Monocyte Chemotactic Protein-1 (MCP-1), also called C-C chemokine motif ligand 2 (CCL-2), was enhanced after SARS-CoV-2 infection, which aggravated steatohepatitis (Xie et al., 2016). IL-6, as the most important cytokine in the cytokine storm, also enhanced significantly in individuals with fatty liver, which could activate the innate immune response in the liver and promote the progression of liver failure (Portincasa et al., 2020b). Overall, studies have demonstrated that the upregulation of MCP-1 in COVID-19 might aggravate the progression from NAFLD to nonalcoholic steatohepatitis (Gao and Tsukamoto 2016). Hence, COVID-19 might accelerate the progression of MAFLD (Portincasa et al., 2020a; Prins and Olinga 2020). In patients suffering from severe COVID-19, a reduction was observed in visceral and peripheral blood flow, which led to hypoxia in hepatocytes (Dunn et al., 1973). Then, HIFs could further worsen MAFLD (Chen et al., 2019; Gonzalez et al., 2019). Considering the close association between COVID-19 and MAFLD, patient management is necessary during the pandemic. Lifestyle modifications such as weight loss and nutritional training may mitigate the chance and severity of COVID-19 infection and decelerate the progress of liver damage. Therefore, timely and standard diagnosis and treatment for patients with MAFLD and COVID-19 should be taken into account.

CONCLUSION

Based on the recent studies, the effects of SARS-COV-2 are not limited to the respiratory system, and the disease may involve a wide range of organs. In this regard, ACE2 receptors play a critical role. The coronavirus can affect the organs involved in metabolic disorders including pancreas, liver, kidney, and gastrointestinal tract through these receptors. Subsequent to the blocking of ACE2 receptors by SARS-COV-2, a cascade of molecular pathways occurs that possibly leads to metabolic instability and the metabolic disturbance syndrome in patients as well as in recovered individuals (Chen Juan et al., 2020; Zhang et al., 2021). Therefore, patient management in terms of preventing the involvement of these organs by disrupting the pathogenesis of the virus can prevent further complications.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Three Month Follow-Up of Patients With COVID-19 Pneumonia Complicated by Pulmonary Embolism

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Background: Previous studies have demonstrated persistent dyspnoea and impairment of respiratory function in the follow-up of patients who have recovered from COVID-19 pneumonia. However, no studies have evaluated the clinical and functional consequences of COVID-19 pneumonia complicated by pulmonary embolism.

Objective: The aim of our study was to assess the pulmonary function and exercise capacity in COVID-19 patients 3 months after recovery from pneumonia, either complicated or not by pulmonary embolism.

Methods: This was a retrospective, single-centre, observational study involving 68 adult COVID-19 patients with a positive/negative clinical history of pulmonary embolism (PE) as a complication of COVID-19 pneumonia. Three months after recovery all patients underwent spirometry, diffusion capacity of the lungs for carbon monoxide (DLCO), and 6 minute walk test (6MWT). In addition, high-resolution computed tomography (HRCT) of the lung was carried out and CT-pulmonary angiography was conducted only in the PE+ subgroup. Patients with a previous diagnosis of PE or chronic lung diseases were excluded from the study.

Results: Of the 68 patients included in the study, 24 had previous PE (PE+) and 44 did not (PE–). In comparison with the PE– subgroup, PE+ patients displayed a FVC% predicted significantly lower (87.71 ± 15.40 vs 98.7 ± 16.7, $p = 0.009$) and a significantly lower DLCO % predicted ($p = 0.023$). In addition, a higher percentage of patients were dyspnoeic on exercise, as documented by a mMRC score ≥1 (75% vs 54.3%, $p < 0.001$) and displayed a SpO_2 <90% during 6MWT (37.5% vs 0%, $p < 0.001$). HRCT features suggestive of COVID-19 pneumonia resolution phase were present in both PE+ and PE– subjects without any significant difference ($p = 0.24$) and abnormalities at CT pulmonary angiography were detected in 57% of the PE+ subgroup.

Conclusion: At the 3 month follow-up, the patients who recovered from COVID-19 pneumonia complicated by PE showed more dyspnoea and higher impairment of pulmonary function tests compared with those without PE.

Keywords: COVID - 19, pulmonary embolism, lung function, spirometry, DLCO, KCO, six minute walk test

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the novel Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2), can involve multiple organs, though the lungs' involvement plays the key role in all the most severe clinical manifestations. After SARS-CoV-2 accesses human cells by the angiotensin-converting enzyme 2 (ACE2) receptors, mostly expressed by type 2 pneumocytes, an acute systemic inflammatory response may occur, followed by several lung pathological events (Mo et al., 2020). The extensive injury of alveolar epithelial cells and endothelial cells can elicit a fibroproliferative response. Chronic alveolar and vascular remodeling can also, in turn, evolve either in lung fibrosis and/or pulmonary hypertension (Frija-Masson et al., 2020; Venkataraman and Frieman, 2017). In addition, the interplay between inflammation and thrombosis, known as thrombo-inflammation, may contribute to a procoagulant state, which can be responsible for the vascular thrombosis frequently detected in small caliber pulmonary vessels (Bikdeli et al., 2020; Goeijenbier et al., 2012; Klok et al., 2020). Venous thromboembolism has been reported particularly in severe COVID 19 patients, with a prevalence ranging from 17 to 69%, and genetic risk factors seem to play a pathogenetic role (Calabrese et al., 2021). While the clinical manifestations of patients affected by COVID-19 during the acute phase of the disease have been largely described, the consequences after recovery from SARS-Cov-2 infection still need to be further investigated.

The assessment of lung injury in COVID-19 survivors includes different types of functional respiratory evaluations, among which the most commonly used are spirometry, diffusing capacity of the lung for carbon monoxide (DLCO), and 6-minute walk test (6MWT) (British Thoracic Society Guidance, 2020). In accordance with the most recent guidelines of the British Thoracic Society, a face-to-face review is suggested 12 weeks after discharge in patients with severe COVID-19 pneumonia. Several studies, performed either at discharge or several months after recovery, have shown that the most common respiratory functional abnormality is reduced DLCO, followed by a restrictive ventilatory defect at the spirometry (Mo et al., 2020; Torres-Castro et al., 2021). In addition, an impairment of exercise capacity has also been described in a small percentage of patients post COVID-19 (Vitacca et al., 2021). However, no study has focused yet on the respiratory functional consequences occurring in patients surviving COVID-19 pneumonia complicated by pulmonary embolism, except for Mendez et al. (2021) who observed only fifteen patients with worse DLCO values.

The present study aimed to assess pulmonary function and exercise capacity in COVID-19 patients 3 months after recovery from pneumonia, either complicated or not, by pulmonary embolism.

MATERIALS AND METHODS

We conducted a retrospective, single-centre, observational study involving adult patients who received a diagnosis of SARS-CoV-2 pneumonia confirmed by real time reverse transcription polymerase chain reaction (RT-PCR) on naso-pharyngeal swab and high resolution computed tomography (HRCT) of the lung. All enrolled patients had either severe COVID-19 pneumonia (in the presence of fever, cough, dyspnea, fast breathing, one among respiratory rate >30 breaths/min, severe respiratory distress, or SpO_2 <90% on room air) or critical with mild ARDS (P/F between 200 and 300 mmHg, with either PEEP or cPAP ≥5 cm H_2O) (SARI Guidelines, 2019).

Patients included in the study were divided into two subgroups based on the positive/negative clinical history of pulmonary embolism (PE) as a complication of COVID-19 pneumonia, i.e. PE+ and PE−, respectively. PE diagnosis was confirmed by CT pulmonary angiography. All patients were treated with the best of care according to the NIH COVID 19 guidelines (NIH Covid-19 Guidelines, 2019), including prophylactic dose anticoagulation unless contraindicated. Patients with COVID-19 who experienced an incident thromboembolic event were managed with therapeutic doses of anticoagulant therapy. After discharge, PE+ patients were treated with direct oral anticoagulants (DOACs) for at least 6 months. Following hospital discharge till follow-up visit, all patients did not need any clinical visits or hospital admission. Three months after recovery from SARS-CoV-2 infection, all patients performed pulmonary function tests at the outpatient service of the Department of Respiratory Pathophysiology, Monaldi Hospital, Naples (Italy), including spirometry, DLCO, and 6MWT. In addition, HRCT of the lung was carried out and CT pulmonary angiography was performed only in the PE + subgroup.

Based on the clinical data collected from medical records about all clinical comorbidities and ongoing therapies, confirmed by lung HRCT, we excluded from the study all patients with a previous diagnosis of chronic lung diseases (i.e., chronic obstructive pulmonary disease, bronchial asthma, diffuse parenchymal lung disease) or PE.

All subjects provided written informed consent to participate in the study, which was approved by the local ethics committee of the University of Campania Luigi Vanvitelli and A.O.R.N. Ospedali dei Colli, in accordance with the 1976 Declaration of Helsinki and its later amendments (AOC-0020053-2020).

Data Collection

Baseline demographic and anthropometric characteristics (sex, age and body mass index), history of smoking, and comorbidities were collected from clinical medical records.

Spirometry and single-breath DLCO test were performed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (Graham et al., 2017, 2019), using Vyntus BODY (Vyaire Medical).

The following spirometric parameters were measured: forced expiratory volume in the first second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio and forced expiratory flow at 25, 50, 75% of the forced vital capacity (FEF 25, FEF 50 and FEF 75, respectively). DLCO, alveolar volume (V_A), and transfer coefficient of the lung for carbon monoxide (KCO) were measured by the single-breath DLCO test.

All parameters were expressed as absolute values and percentages of the predicted value (%)and considered reduced if below the lower limit of normality (LLN), according to the

Global Lung Function Initiative 2012 reference equations for spirometry (Quanjer et al., 2012).

An obstructive ventilatory defect was defined by a FEV_1/FVC ratio lower than LLN. A flow-volume curve displaying an FVC % <80% and an FEV_1/FVC ≥70% was considered suggestive of a restrictive ventilatory defect (Soriano et al., 2012). Reduced DLCO % and KCO % were defined as lower than 80% of the predicted value, according to the Global Lung Function Initiative 2017 reference equations for DLCO (Stanojevic et al., 2017).

Proper performance of spirometry and single-breath DLCO test was ensured by medical personnel and all the measures suggested by local national guidelines to avoid the risk of SARS-CoV2 infection were adopted (Tognella and Piccioni, 2021). In particular, the measurement of lung volumes by plethysmography, due to the objective difficulties in the disinfection of the chamber in the post-pandemic phase 2, was not allowed. All enrolled patients had to exhibit a negative nasopharyngeal swab for the molecular detection of SARS-CoV-2 RNA before pulmonary functional exams.

The 6 minute walk test (6MWT) was performed according to ATS/ERS guidelines (Holland et al., 2014). The distance walked during 6 minutes was measured and compared with 6MWT predictive values according to the reference equation published by ENRIGHT and SHERRILL (Enright and Sherrill, 1998) and considered reduced if lower than LLN. The dyspnea intensity was assessed by the modified Medical Research Council (mMRC) dyspnea scale (range from 0- dyspnea only with strenuous exercise to 4- too dyspneic to leave the house or breathless when dressing) (Fletcher et al., 1959; Williams, 2017). Finally, the Borg dyspnea scale score (range from 0 – nothing at all to 10 very severe) was assessed before and after 6MWT. During the test, peripheral capillary oxygen saturation (SpO_2) was measured by pulse oximetry on the index finger and SpO_2 levels below 90% were considered pathological.

Statistical Analysis

All variables included in the study were first analyzed by descriptive statistics techniques. In depth, qualitative data were expressed as number and percentage, while quantitative variables either median and interquartile range (IQR) or mean and standard deviation (SD), based on their distribution, were assessed by the Shapiro-Wilk test. Between the groups, the differences at baseline were tested either by the parametric paired Student *t* Test or by the non-parametric Wilcoxon signed rank test, as appropriate, whilst qualitative data were analyzed either by the Chi Square test or the Fisher Exact test. The most significant findings were further exemplified by box-plots and bar diagrams.

A p-value <0.05 was considered statistically significant. Data were analyzed using SPSS Software, Version 26 (IBM, Armonk, New York, United States), and STATA 16 software (StataCorp. 2019: StataCorp LP, College Station, TX, United States).

RESULTS

In total, 68 COVID-19 patients were enrolled in the study, 24 with PE (PE+) and 44 without (PE−). All baseline clinical and functional characteristics of the whole study population are described in **Table 1**. The mean age was 54.9 (±12.8) years and the majority of patients were males (73.5%), nonsmokers (61.8%), and overweight (median BMI 28). The most frequent comorbidities were systemic arterial hypertension (45.6%) and obesity (41.2%) and 39 (57.4%) patients showed more than one comorbidity. The median time from the recovery from SARS-CoV-2 infection to the clinical follow-up visit was 90 days (IQR 60–120).

Spirometry and DLCO were uneventfully completed in all subjects. Abnormalities at spirometry were detected in a small percentage of patients (14.7%), with 4 (5.9%) showing an obstructive ventilatory defect and 6 (8.8%) displaying a flow-volume curve suggestive of a restrictive ventilatory defect. A reduction of DLCO <80% predicted was instead observed in 27 (39.7%) patients and 7 (10.3%) showed a concomitant reduction of KCO <80%, while 20 (29.4%) had a normal KCO.

The 6MWT was performed in all study population. Most patients (37, 54.4%) complained of dyspnea, assessed by an mMRC grade ≥1. Moreover, 13.2% of patients displayed a SpO_2 <90% during the test, and 25% had a distance walked below the LLN. All patients, except for three, complained of increased dyspnea after the test, as rated by the Borg dyspnea scale.

Altogether, 57 of the 68 patients performed HRCT of the lung. Radiological changes suggestive of a resolution phase of COVID-19 pneumonia, represented by residual areas of ground glass opacity (GGO) and/or consolidations and/or linear bands, were present in 78.9% of patients.

The comparison of PE+ and PE− subgroups did not disclose any significant difference for sex and comorbidities. PE+ patients, indeed, were significantly older than PE− patients (61 ± 11.1 vs 51.5 ± 12.5, p = 0.003) and also had a higher prevalence of smoking habits (p = 0.015) (**Table 2**). In addition, we did not observe any significant difference between PE+ and PE− subgroups in the prevalence of patients with severe or critical with mild ARDS COVID-19 pneumonia (8 vs 15% and 92 vs 85%, p = 0.476, respectively).

The results of the pulmonary functional tests in the two subgroups of patients are described in **Table 2**. At the spirometry, PE+ patients displayed a FVC% predicted significantly lower than PE− (87.71 ± 15.40 vs 98.7 ± 16.7, p = 0.009). In particular, a higher percentage of PE+ patients had a FVC% <80% in comparison with PE− (29.1 vs 6.8%, p = 0.033) (**Figure 1**, left panel). Moreover, the flow volume curve suggestive of a restrictive ventilator defect was more prevalent in PE + compared to PE− subgroup, although without reaching a statistical significance (16.7% vs 4.5%, p = 0.212). PE+ patients also showed a significantly lower DLCO % predicted (p = 0.023) (**Figure 1**, right panel). Instead, no statistical difference in KCO% was observed between PE+ and PE−, though a higher percentage of PE+ patients had a concomitant reduction of DLCO% and KCO%.

The main symptom complained by all COVID-19 survivors was persistent dyspnea, which was significantly more prevalent in PE+ as compared to PE− subgroup, as demonstrated by the

TABLE 1 | Baseline characteristics of the study population (n = 68).

Age (yrs) (mean ± SD)	**54.9 (12.8)**
Sex (M/F), *n* (%)	50 (73.5)/ 18 (26.5)
BMI, median [IQR]	28 [25–31]
Smoking habit, *n* (%)	
Yes	2 (2.9)
No	42 (61.8)
Ex	24 (35.3)
Comorbidities, *n* (%)[a]	
Arterial hypertension	31 (45.6)
Cardiomyopathy	5 (7.4)
Diabetes Mellitus	10 (14.7)
Obesity	28 (41.2)
GERD	15 (22.1)
Spirometry, *n* (%)	
FEV_1% (mean ± SD)	94.5 (17.9)
FVC % (mean ± SD)	94.8 (17)
FEV_1/FVC (mean ± SD)	81.8 (6.7)
FEF 25% (mean ± SD)	100.2 (26)
FEF 50%, median [IQR]	95.5 [76–110]
FEF 75%, median [IQR]	73.5 [53.5–89.5]
Interpretation of Spirometry, *n* (%)	
Normal	58 (85.3)
Restrictive deficit	6 (8.8)
Obstructive deficit	4 (5.9)
DiffusionCapacity Test	
DLCO %, median [IQR]	82 [72.3–93]
DLCO % < 80, *n* (%)	27 (39.7)
KCO % (mean ± SD)	99.9 (19.2)
KCO% < 80%, *n* (%)	8 (11.8)
DLCO % < 80% + KCO <80%, *n* (%)	7 (10.3)
DLCO % < 80% + KCO >80%, *n* (%)	20 (29.4)
6MWT	
mMRC ≥1, *n* (%)	37 (54.4)
SpO_2 <90% 6MWT, *n* (%)	9 (13.2)
Walk distance<LLN, *n* (%)	17 (25)
Lung HRCT, *n* (%)	
Normal	12 (17.6)
Pathologic	45 (66.3)
Not performed	11 (6.1)
Interval discharge and respiratory function test (days), median (IQR)	90 (60–120)

SD, standard deviation; M, Male; F, Female; BMI, Body Mass Index; IQR, Interquartile range; GERD, Gastroesophageal Reflux Disease; FEV1, Forced expiratory volume in one second; FVC, Forced Vital Capacity; FEF 25, FEF 50 and FEF 75, forced expiratory flow at 25, 50, 75% of the forced vital capacity; DLCO, diffusion capacity of the lungs for carbon monoxide; KCO, transfer coefficient of the lung for carbon monoxide; mMRC, modified Medical Research Council Dyspnea scale; 6MWT, 6-minute walk test; SpO_2, peripheral capillary oxygen saturation; LLN, lower limit of normality; HRCT, high resolution computed tomography.

[a]*Comorbidities prevalence was computed considering singularly each comorbidity.*

mMRC dyspnea score ≥1 (75% vs 54.3%, $p < 0.001$) (**Figure 2**, upper panel).

We also observed a higher prevalence of PE+ patients with a SpO_2<90% during 6MWT (37.5 vs 0%, $p < 0.001$) (**Figure 2**, lower panel). The percentage of patients with a distance walked at 6MWT< LLN, indeed, did not significantly differ between the two subgroups. Finally, HRCT features suggestive of COVID-19 pneumonia resolution phase were present in 90% of PE+ patients and 73% of PE− subjects ($p = 0.24$), whilst abnormalities at CT pulmonary angiography were detected in 57% of PE+.

DISCUSSION

The main objective of the present study was to assess potential differences in pulmonary functional impairment in COVID-19 survivors with pneumonia either complicated or not by PE. At the 3 month follow-up, COVID-19 survivors with previous PE showed a significantly lower FVC% and DLCO%. Although a higher percentage of PE+ patients had a concomitant reduction of DLCO% and KCO%, the difference between the two subgroups did not reach statistical significance. Recently, several authors have discussed the clinical significance of the reduction of DLCO and whether it is associated with a decrease in KCO among COVID-19 survivors.

DLCO represents the lung's ability to exchange gas and may occur with various combinations of KCO and VA, each suggesting different underlying pathological modifications. KCO, the transfer coefficient of the lung for carbon monoxide, is a measure of CO uptake from alveolar gas and is affected by the thickness and area of the alveolar -capillary membrane, blood volume, and hemoglobin concentration/ properties in capillary vessels supplying ventilated alveoli. A reduced DLCO with either normal or near normal KCO might be related to a reduced alveolar volume caused by changes in the mechanical properties of the chest wall and respiratory muscles. Conversely, when both DLCO and KCO are reduced, we might suspect either membrane or pulmonary capillary abnormalities (Nusair, 2020). Unexpectedly, in the PE+ subgroup, we did not find any significantly higher prevalence of patients showing a reduction of both DLCO % and KCO %, although abnormalities suggestive of pulmonary embolism at CT pulmonary angiography were present after 3 months in this subset of patients. This finding might be due to the relatively small sample size of our study population, with only 10% of them showing functional abnormalities. In addition, Laveneziana et al. (2021) have suggested that reduction of both DLCO and KCO could be in favor of lesions involving alveolar membrane and/or pulmonary capillaries susceptible of some recovery. On the contrary, the reduction of the only DLCO with normal KCO suggests definitive alveolar loss destruction with no perspective of recovery. Of note, in the majority of both PE+ and PE− subjects the two functional parameters were within the normal ranges, whilst about one-third displayed a reduction of only DLCO with normal KCO. Similarly, Mo X and colleagues, who evaluated at the discharge 110 COVID-19 survivors classified into three groups of severity, observed a higher reduction of both DLCO and total lung capacity in the most severe cases. In about half of patients with reduced DLCO, KCO remained within the normal ranges (Mo et al., 2020).

The most frequently observed ventilatory deficit, though in a small percentage of patients, was the restrictive, with a higher percentage of PE+ patients displaying a reduced forced vital capacity. However, a restrictive ventilatory deficit might be associated with the condition of obesity, which was frequently observed in COVID-19 patients and documented in the present study in more than 40% of both PE+ and PE− patients. Moreover, it is of note that the majority of COVID-19 pneumonia survivors did not have any clinically significant changes in spirometry at 3 month follow-up.

Regarding the exercise test, indeed, we observed a significantly higher prevalence of PE+ patients complaining of dyspnea on

TABLE 2 | Comparison of baseline characteristics between PE+ and PE– subgroups.

	PE+ (*n* = 24)	PE– (*n* = 44)	*p*
Age (yrs) (mean ± SD)	61 (11.1)	51.5 (12.5)	0.003
Sex (M/F), *n* (%)	19(79.2)/5(20.8)	31(70.5)/13(29.5)	0.436
BMI, median [IQR]	28.5 [26–32.5]	27.5 [25–31]	0.624
Smoking habit, *n* (%)			0.015
Yes	2 (8.3)	—	
No	10 (41.7)	32 (72.7)	
Ex	12 (50)	12 (27.3)	
Comorbidities, *n* (%)			
Arterial hypertension	14 (58.3)	17 (38.6)	0.119
Cardiomyopathy	3 (12.5)	2 (4.5)	0.475
Diabetes Mellitus	6 (25)	4 (9.1)	0.158
Obesity	10 (41.7)	18 (40.9)	0.952
GERD	4 (16.7)	11 (25)	0.627
Spirometry, *n* (%)			
FEV_1% (mean ± SD)	91.1 (15.5)	97.9 (18.9)	0.140
FVC % (mean ± SD)	87.7 (15.4)	98.7 (16.7)	0.009
FVC % <80% (%)	29.1	6.8	0.033
FEV_1/FVC (mean ± SD)	83.6 (7.9)	80.9 (5.8)	0.117
FEF 25% (mean ± SD)	100.2 (25.3)	100.3 (26.7)	0.989
FEF 50%, median [IQR]	99.5 [79.5–108.5]	89.5 [69.3–112]	0.458
FEF 75%, median [IQR]	76.5 [59–82.3]	72 [52.5–94]	0.940
Interpretation of spirometry, *n* (%)			0.091
Normal	20 (83.3)	38 (86.4)	
Restrictive deficit	4 (16.7)	2 (4.5)	
Obstructive deficit	—	4 (9.1)	
Diffusion capacity test			
DLCO %, median [IQR]	79.5 [61–89.5]	86.5 [75.3–95.5]	0.023
DLCO % <80, *n* (%)	12 (50)	15 (34.1)	0.200
KCO % (mean ± SD)	95.2 (18.2)	102.3 (19.4)	0.160
KCO% < 80%, *n* (%)	4 (16.7)	4 (9.1)	0.354
DLCO% <80% + KCO<80%, *n* (%)	4 (16.7)	3 (6.8)	0.390
DLCO% <80% + KCO>80%, *n* (%)	8 (33.3)	12 (27.3)	0.600
6MWT			
mMRC ≥1, *n* (%)	18 (75)	19 (54.3)	<0.001
SpO_2 <90% 6MWT, *n* (%)	9 (37.5)	0	<0.001
Walking distance<LLN, *n* (%)	3 (16.7)	14 (40)	0.088
Lung HRCT, *n* (%)[a]			0.244
Normal	2 (10)	10 (27)	
Pathologic	18 (90)	27 (73)	

SD, standard deviation; M, Male; F, Female; BMI, Body Mass Index; IQR, interquartile range; GERD, Gastroesophageal Reflux Disease; FEV1, Forced expiratory volume in one second; FVC, Forced Vital Capacity; FEF 25, FEF 50 and FEF 75, forced expiratory flow at 25, 50, 75% of the forced vital capacity; DLCO, diffusion capacity of the lungs for carbon monoxide; KCO, transfer coefficient of the lung for carbon monoxide; mMRC, modified Medical Research Council Dyspnea scale; 6MWT, 6-minute walk test; LLN, lower limit of normality; HRCT, high resolution computed tomography; SpO_2, peripheral capillary oxygen saturation.

[a]*We considered only the real number of patients (57) who performed the exam.*

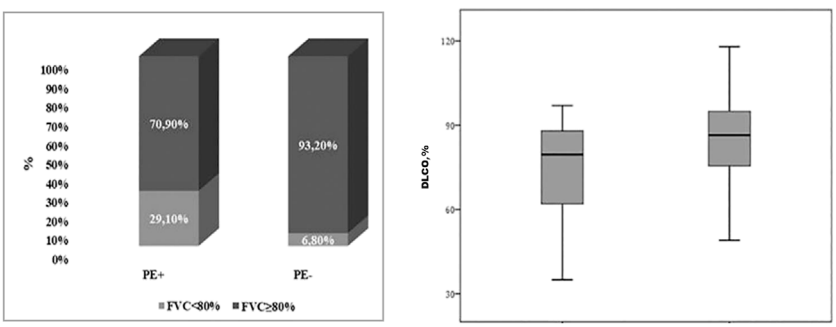

FIGURE 1 | Box-plot showing differences between PE+ AND PE– in terms of FVC% <80% (29.1 vs 6.8%, $p = 0.033$) (on the left) and in terms of DLCO% [79.5 (61–89.5) vs 86.5 (75.3–95.5); $p = 0.023$] (on the right). PE, Pulmonary Embolism; FVC, Forced Vital Capacity; DLCO, Diffusion capacity of the lungs for carbon monoxide; LLN, Lower Limit of Normality.

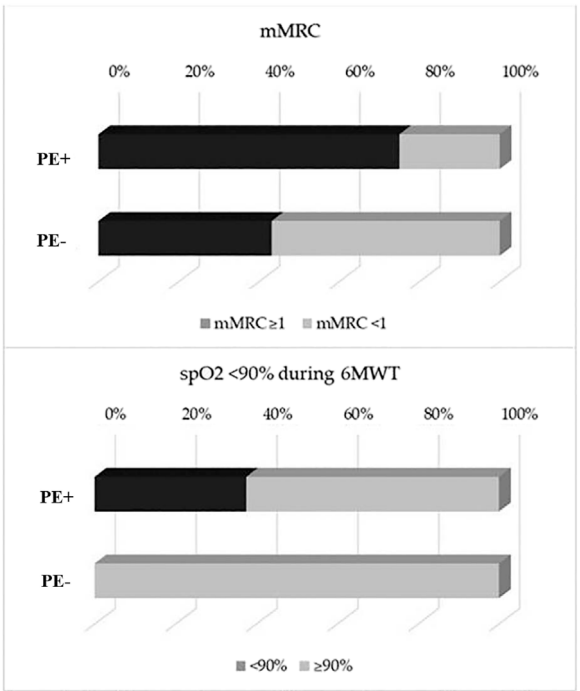

FIGURE 2 | Bar diagram showing differences between PE+ AND PE− in terms of mMRC ≥1 (75 vs 54.3%, $p < 0.001$) (upper panel) and for SpO_2 <90% during 6-minute walk test (6MWT) (37.5 vs 0%, $p < 0.001$) (lower panel). PE, Pulmonary Embolism; mMRC, modified Medical Research Council Dyspnea scale; 6MWT, 6-minute walk test.

exertion and peripheral oxygen desaturation during the test. Similarly, another study showed, in COVID-19 survivors with persistent dyspnea, a lower % predicted walked distance and oxygen saturation during the 6MWT, alongside higher ratings of dyspnea and leg fatigue during the exercise test (Cortés-Telles et al., 2021). According to the inclusion criteria, all patients enrolled in the study did not have any previous respiratory diseases, which could have affected their pulmonary function and/or exercise capacity, even though smoking history was more prevalent in PE+ patients, also older than PE−. In addition, no findings suggestive of concomitant lung diseases were observed at lung HRCT both in PE+ and PE− patients. All COVID-19 survivors showed residual imaging abnormalities of an ongoing resolution of COVID-19 pneumonia at lung HRCT, though without differences between PE+ and PE− subgroups. These data suggest that the higher clinical and functional impairment of PE+ patients could be a consequence of a pulmonary perfusion defect persisting 3 months after the recovery rather than to pulmonary parenchymal alterations. Findings from CT pulmonary angiography demonstrated perfusion defect in 57% of PE + patients.

Frija-Masson et al. (2020) found abnormal lung function tests in more than 50% of patients with a mix of restrictive ventilatory defects and low diffusion patterns. In about one third of patients, the authors observed that a decreased DLCO was not associated with chest CT abnormalities, thus leading to the hypothesis of vascular damage induced by SARS-CoV-2. Huang et al. (2021) further observed no significant correlation between total severity score at chest CT and pulmonary functional parameters during follow-up visits. The authors also observed a reduction of DLCO in more than 50% of patients, with a higher incidence of DLCO impairment associated with a lower percentage of predicted TLC and 6MWD in severe as compared to moderate and mild disease. The authors further observed that a small percentage of patients with no residual abnormalities presented with a slight decrease in DLCO.

Our study is of course characterized by several limitations. First, the relatively small sample size and the short follow-up mean that the results need to be interpreted carefully. As previously stated, the cost of the nasopharyngeal swab for the molecular detection of SARS-CoV-2 RNA, which was needed to perform the pulmonary function tests, limited the number of patients who agreed to the study participation. As for follow-up, we performed further PFTs 18 months after the recovery from SARS-Cov2 infection in 10 PE+ and PE− patients of the 27 who displayed a reduction of FVC% and/or DLCO at 3 month follow-up. Overall, seven patients saw an improvement of DLCO, which reached normal percentage predicted values in four cases, while the impairment remained stable in three patients. One patient also had a reduction of FVC% predicted value, which returned at normal values at 18-month follow-up (data not published). Based on these findings, we can hypothesize that most patients should experience recovery from functional impairment. Finally, the diagnosis of restrictive pattern exclusively performed on the reduced FVC associated with either a normal or higher FEV_1/FVC is questionable. However, the measurement of TLC by plethysmography was avoided due to the restrictions imposed by local national guidelines regarding pulmonary function tests during the COVID-19 pandemic (Tognella and Piccioni, 2021).

These limitations suggest the need for larger study populations and longer follow-up studies to better establish the characteristics and trends of modification of lung function and exercise tolerance in COVID-19 survivors complicated by PE. However, based on our findings, which depict a higher pulmonary functional impairment at 3-month follow-up among patients with a history of pulmonary embolism complicating SARS-CoV2 pneumonia, we suggest using diagnostic exams to assess the presence of pulmonary embolism in COVID-19 survivors with persistent dyspnea, DLCO impairment, and peripheral oxygen desaturation during the 6MWT.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study was approved by University of Campania "Luigi Vanvitelli" and A.O.R.N Ospedali dei Colli. The patients provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GF, CC, and AA contributed to conception and design; AC, IM, and MF contributed to experimental procedures; DM, VA, and MP were involved in the acquisition of data; PP was involved in data analysis; PP and CC interpreted the data; CC, PP, and AA drafted the article; GF and CC critically revised the article for important intellectual content; all authors approved the final version to be published.

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REVIEW
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Significance of Cardiac Troponins as an Identification Tool in COVID-19 Patients Using Biosensors: An Update

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Coronavirus disease 2019 (COVID-19) has rapidly developed as a global health emergency. Respiratory diseases are significant causes of morbidity and mortality in these patients with a spectrum of different diseases, from asymptomatic subclinical infection to the progression of severe pneumonia and subsequent acute respiratory distress syndrome. Individuals with cardiovascular disease are more likely to become infected with SARS-CoV-2 and develop severe symptoms. Hence, patients with underlying cardiovascular disease mortality rate are over three times. Furthermore, note that patients with a history of cardiovascular disease are more likely to have higher cardiac biomarkers, especially cardiac troponins, than infected patients, especially those with severe disease, making these patients more susceptible to cardiac damage caused by SARS-2-CoV. Biomarkers are important in decision-making to facilitate the efficient allocation of resources. Viral replication in the heart muscle can lead to a cascade of inflammatory processes that lead to fibrosis and, ultimately, cardiac necrosis. Elevated troponin may indicate damage to the heart muscle and may predict death. After the first Chinese analysis, increased cardiac troponin value was observed in a significant proportion of patients, suggesting that myocardial damage is a possible pathogenic mechanism leading to severe disease and death. However, the prognostic performance of troponin and whether its value is affected by different comorbidities present in COVID-19 patients are not known. This review aimed to assess the diagnostic value of troponin to offer insight into pathophysiological mechanisms and reported new assessment methods, including new biosensors for troponin in patients with COVID-19.

Keywords: cardiovascular disease, troponin, COVID-19, biosensor, SARS-CoV-2, diagnostic value

1 INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an emerging outbreak from Wuhan City, China, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Malik et al., 2020). In 15% of infected patients, the clinical course of this pathology is complicated by the development of severe forms of interstitial pneumonia, which may lead to acute respiratory distress syndrome (ARDS), multi-organ failure (MOF), and death (Mattiuzzi and Lippi, 2020). Although the cardiovascular system did not appear to be particularly affected by the virus at the start of the pandemic, other studies found that patients with a history of cardiovascular disease or cardiovascular risk factors had higher mortality rates than those without a history of cardiovascular disease (Akhmerov and Marbán, 2020; Bansal, 2020).

Emerging literature has reported that 7%–28% of COVID-19 patients had developed an acute cardiac injury, eventually causing more complications and mortality (Wang et al., 2020a; Guo et al., 2020). In addition, several studies have shown that COVID-19 patients who did not have heart disease prior to infection may have heart problems (Kim et al., 2020; Zeng et al., 2020; Zhou, 2020). Early diagnosis of heart disease in these patients is possible by measuring cardiac troponin as the gold standard marker of myocardial damage (Park et al., 2017). The most recent international guidelines recommend cardiac Troponin I (hs-cTnI) and T (hs-cTnT) testing, which are particularly sensitive for diagnosis of myocardial injury and acute myocardial infarction (MI). Cardiac troponins have gradually gained greater clinical importance in the diagnosis, treatment, and prognosis of patients with cardiovascular disease (Park et al., 2017; Clerico et al., 2019). However, there are few studies that focused on the role and concentration of highly sensitive cardiac troponins in patients diagnosed with COVID-19.

This study aimed to evaluate the diagnostic value of troponin to offer insight into pathophysiological mechanisms and reported new assessment methods, including new biosensors for troponin in patients with COVID-19.

2 OVERVIEW OF SARS COV-2

Over the past two decades, outbreaks of different viral diseases have occurred, including the human immunodeficiency virus (HIV/AIDS) from 1981 to present, severe acute respiratory syndrome coronavirus (SARS-CoV-1) during 2002–2004, influenza A virus subtype H1N1 (A/H1N1) during 2009–2010, the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012-present, Ebola virus disease (EVD) [which was known as Ebola Hemorrhagic Fever (EHF)] during 2013–2016, Zika virus (ZIKV) in 2015, and the most recent and important outbreak in 2020, which is called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Definitively, SARS-CoV-2 has an outstanding genetic symmetry (96.2%) with the bat coronavirus RaTG13, which was secured from bats in Yunnan in 2013, but there are some discrepancies in the origin of SARS-CoV-2, which needs to be further investigated (Paraskevis et al., 2020). Incipiently, SARS-CoV-2 prompted a series of acute abnormal respiratory diseases in Wuhan, Hubei Province, China, in December 2019. After a few months, the World Health Organization (WHO) termed the SARS-CoV-2 infection disease as coronavirus disease 2019 (COVID-19) and declared this ongoing outbreak on January 30, 2020 (Majumder and Minko, 2021). COVID-19, the cause of the current global pandemic, is an infectious disorder caused by a lately discovered RNA virus called SARS-CoV-2 (Basiri et al., 2021). The main concerns of COVID-19 are its rapid spread and high mortality rate (Basiri et al., 2021). Many countries have also been forced to adopt a virtual work environment and to impose repeated lockdowns. Based on the Worldometer site data, since August 9, 2021, more than 203,500,000 coronavirus cases worldwide have been reported (https://www.worldometers.info/).

After the COVID-19 outbreak, the race among different companies to promote effective vaccines and therapeutics to treat this mysterious disease, as well as diagnostic tests, has begun, with many trials underway (Basiri et al., 2021; Golchin, 2021). Reputably, fast and reliable detection of SARS-CoV-2 in potential patients is crucial to control this outbreak in hospitals and societies (To et al., 2020; Zhai et al., 2020). The current main screening tests for COVID-19 patients include real-time (r) reverse-transcription (RT) polymerase chain reaction (PCR) (rRT-PCR) and similar modified molecular tests to detect the presence of specific genetic material of the virus (To et al., 2020; Zhai et al., 2020). The positive rate of rRT-PCR for oropharyngeal swabs of COVID-19 patients has been reported to be 53.3%–71% in different studies (Fang et al., 2020; Zhang et al., 2020). However, the RT-PCR results ordinarily after 2–8 days become positive (Huang et al., 2020). However, several studies emphatically suggest utilizing viral Ig M and Ig G serological tests and computerized tomography (CT) scans of the chest to confirm PCR results (Fang et al., 2020; Zhai et al., 2020; Zhang et al., 2020). Different therapeutic approaches referring to scientific reports have been considered. To date, there are no prevailing data from randomized controlled clinical trials to support any particular anti-SARS-CoV-2 agents for COVID-19 patients; however, lopinavir (LPV), favipiravir, remdesivir, chloroquine, and hydroxychloroquine as antiviral agents, convalescent plasma and CR3022 as antibodies, and dexamethasone, prednisone, methylprednisolone, and hydrocortisone as corticosteroids have been considered in COVID-19 treatment guidelines (Zhai et al., 2020; Golchin, 2021). Moreover, several cell-based therapy products such as mesenchymal stem cells, natural killer cells, dendritic cells, and exosomes have been reported to have positive results for treating severe COVID-19 patients in some clinical trials (Basiri et al., 2021; Golchin, 2021). Furthermore, several vaccines have been developed based on conventional and innovative vaccine development models that demonstrate promising antibody responses to help prevent people from getting infected with SARS-CoV-2 (Soiza et al., 2021). Several vaccines have been approved for emergency use authorization, such as Pfizer-BioNTech, Moderna, Johnson and Johnson's Janssen, Sinopharm, and AstraZeneca.

TABLE 1 | The future Biomarkers and diagnostic utility against COVID-19.

Biomarkers	Organ/System involved	Type of biomarkers	Role/Effect	Step of disease (mild-severe-critical)	Ref
1-cytokines:(IL-6, IL-10, IL-1R, MCP-1, TNF-alpha)	Inflammation system (serum)	Immunological	Role in severity:(IL-6, IL-1R, TNF are increased)	Severe	Lippi and Plebani (2020a), Hu et al. (2020)
2-chemokines (CXCL8, CXCL9, CXCL10)			GFs were significantly higher in fatal than severe and/or mild but not correlated to disease severity	Fatal	Xu et al. (2020)
3-procalcitonin			Prognosis role: risk factor of in-hospital mortality	Severe	Lippi and Plebani (2020a), Hu et al. (2020)
4-neopterin			Prognostic role: higher in severe COVID-19 disease patients	Severe	Ozger et al. (2021)
1-lymphocyte counts (LYM)	Hematological (serum)	Immunological	Predictor of prognosis: LC decrease	Severe	Tan et al. (2020)
2-neutrophil counts (NØ)			Neutrophilia-induced lung injury in severe patients	Severe	Wang et al. (2020b)
3-neutrophil-to-lymphocyte ratio (NLR)			An independent risk factor of the in-hospital mortality, NLR increases	moderate-severe ARDS in severe COVID-19	Liu et al. (2020b), Ma et al. (2020)
4-neutrophil-to-$CD8^+$ T cell ratio (N8R)			Powerful prognostic factors	Severe	Liu et al. (2020c)
5-eosinophil counts (EØ)			Was generally very low in the early stages of the disease in severe patient	Early stages/severe	Zheng et al. (2020)
6-platelet counts (PLT)			PLT decrease	Severe	Zheng et al. (2020)
7-platelet-to-lymphocyte ratio (PLR)			Had higher levels on admission	Severe	Simadibrata et al. (2020)
D-dimer levels	Coagulation (serum)	Biochemical	D-dimer increase (≥0.5 mg/L)		Garcia-Olivé et al. (2020)
Serum ferritin			Ferritin increase severity in hospitalized patients	Severe	Kappert et al. (2020)
Aspartate aminotransferase (AST)	Hepatic and metabolic	Biochemical	Severity and mortality diagnostic	Severe	Malik et al. (2021)
Alanine aminotransferase (ALT)			Elevated ALT (>40 IU/L)	Severe	Malik et al. (2021)
Lactate dehydrogenase (LDH)			LDH increase	Unclear	Yuan et al. (2020), Serrano-Lorenzo et al. (2021)
C-reactive protein			CRP increase	Severe	Kermali et al. (2020)
Cardiac troponin (cTn)	Cardiac Muscle	Biochemical	Severity and mortality increase		Henry et al. (2020b)
Creatinine proteinuria	Renal	Urine sample	Severity: Urea and creatinine increase	1. Severe 2. Moderate to severe	Cheng et al. (2020), Ouahmi et al. (2021)

The COVID-19 outbreak is a potent warning of the current challenge of emerging and re-emerging infectious pathogens and the demand for regular surveillance, rapid diagnosis, and extensive investigation to explain the basic biology of viruses and the physiopathology of their causative diseases.

3 BIOMARKER CHANGES IN COVID-19

Detection of biomarkers is important in classifying patients at risk of developing COVID-19, and their molecular classification is vital to improve treatment and diagnosis. In this part, an updated summary of routine biomarker levels in COVID-19-positive and -negative patients is provided with an in-depth look at the potential application of these biomarkers for diagnosis, prognosis, and treatment. Besides, a classification of different biomarkers based on the organs involved is shown in **Table 1**.

Biomarkers are measurable parameters. For example, levels of gene expression and protein are evaluated as common or pathological diagnostic factors, whether available or mediated (Kraus, 2018; Caruso et al., 2021). Depending on the application, biomarkers can be classified as hazards that cause long-term sensitivity, pharmacodynamics/response, diagnosis, recurrence, analysis, monitoring, evaluation of the most effective biotherapeutic, predictive and safety markers. It is essential to differentiate between prognostic biomarkers that are valuable to recognize patients more likely to have a particular result individually from treatment and predictive biomarkers that contain a relationship of a treatment to control in patients with and without the biomarker.

Numerous prognostic biomarkers in COVID-19 that predict disease intensity have already been confirmed in clinical situations (Kermali et al., 2020; Caruso et al., 2021). A recent retrospective cohort study concluded that among biomarkers that

discriminate between severe and non-severe patients are those related to changes in immune responses and its imbalance (Liu et al., 2020a; Qin et al., 2020a). Studies that review the role of cytokines in SARS and MERS have also specified an association between cytokine release syndrome (CRS) and their severity. Realizing their function in COVID-19 may support simplifying the plan of unique immunotherapies (Mahallawi et al., 2018).

Biomarkers associated with infectious diseases, such as inflammatory factors IL-2R, TNFα, and IL-6 and factors related to cell count, are evident in high proportions in more severe patients than milder ones (Qin et al., 2020b). In contrast, platelet count decreases in severe cases only (Lippi et al., 2020). The results of a meta-analysis reviewing six studies showed that interleukin-6 levels were significantly increased (2.9-fold) in severe COVID-19 cases compared to mild cases (Coomes and Haghbayan, 2020).

Factors related to inflammation as biomarkers may be beneficial in recognizing COVID-19 and distinguishing it from other viral pneumonia diseases. C-reactive protein (CRP) is an acute phase reactant protein that is increased in response to inflammation, simultaneously with a boost of other inflammatory cytokines associated with severity and mortality of COVID-19 patients (Ali, 2020). Another study conducted in Wuhan showed significantly higher CRP levels in severe COVID-19 patients than others (Qin et al., 2020c).

Procalcitonin, a factor formed by many kinds of cells in the body, is an extra inflammatory factor thought to be more particular for most bacterial infections. Some papers reported that levels of procalcitonin were related to the severity of COVID-19-positive individuals and can, as such, help to confirm COVID-19 in some cases (Hu et al., 2020). The results of a meta-analysis study showed that people with high procalcitonin had a five times higher risk of severe SARS-CoV-2 (Lippi and Plebani, 2020a).

Gene sequencing studies have shown that genetic changes related to chromosome 3 have been closely associated with the severity of infection to SARS-CoV-2 and hospitalization (Group, 2020; Initiative, 2020). Additional biomarkers associated with SARS-CoV-2 that are related to severity and mortality include cardiovascular biomarkers, of which cardiac troponin (cTn) is important (Zhou et al., 2020), or to the rate of chronic kidney diseases where a rise of creatinine amounts is detected in severe patients (Cheng et al., 2020). In addition to these medical biomarkers, there is now extensive literature on molecular factors that identifies the disease related to SARS-CoV-2 infection and that can be used to identify therapeutic targets.

Other studies have revealed considerably higher levels of kidney markers such as serum urea, creatinine, and markers of glomerular filtration rate in severe patients. In a study with a large sample (n = 701), it was found that raised serum creatinine levels on admission correlated with severity due to significant abnormalities in the coagulation pathway (Cheng et al., 2020).

Acute kidney damage (AKI), coronary artery and cerebrovascular disease, and lung tissue disorders such as COPD all occurred in severe COVID-19 cases and they were linked to leukocytosis, high creatinine kinase, and raised LDH and PT. Additionally, AKI is viewed as a critical sign of disease severity and is assessed using prognostic variables such as serum creatinine (sCr), urea, and cystatin C (Rasmi et al., 2021).

The results of this study and previous studies show that no particular biomarker will have the sensitivity and specificity to identify or reject COVID-19. There is a plan for sharing biomarkers and combining a merged reference standard for analyzing COVID-19 (Graziadio, 2020). This decision looks sensible when bringing up the biomarkers recognized by the current study, where low lymphocyte counts (LYM), eosinophil counts (EØ), neutrophil-to-lymphocyte 3-ratio (NLR), platelet counts, and platelet-to-lymphocyte ratio (PLR) are unlikely to segregate between respiratory infections and COVID-19. On the other hand, values of C-reactive protein, IL-6, and other biochemical factors such as lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase exhibited high specificity against COVID-19 (Cheng et al., 2020; Kermali et al., 2020; Rasmi et al., 2021) (**Table 1**).

Angiotensin-converting enzyme 2 (ACE2) is a transmembrane receptor that converts angiotensin II to angiotensin (Wiese et al., 2021). It has been reported that ACE2 is expressed by different tissues, such as small intestine, colon, kidney, and heart. It is proved that ACE2 is expressed by cardiomyocytes, cardiac fibroblasts, and coronary artery endothelial cells (Herman-Edelstein et al., 2021). Notably, ACE2 is associated with developing hypertension and COVID-19 and has a harmful effect on renal tissues (Li et al., 2017; Bourgonje et al., 2020). Many studies have shown that SARS-CoV-2 can bind with ACE2 through the spike (S) protein and then enter host cells and subsequently infect cardiac cells, resulting in myocardial injury or death (Ni et al., 2020). However, acute renal damage and/or acute tubular necrosis may occur during SARS-CoV-2 infection, and based on the results mentioned in this section, it can be said that hypertension is the most common accompanying symptom observed in patients with COVID-19 (Rasmi et al., 2021). In this regard, another critical element is transmembrane serine protease 2 (TMPRSS2), which belongs to the serine protease family. TMPRSS2 is mainly expressed in the salivary gland, lung, thyroid, gastrointestinal tract, pancreas, kidney, and heart muscle (48). It seems that TMPRSS2 plays a vital role in coronavirus disease. Indeed, TMPRSS2 cleaves spike glycoprotein, facilitating viral entry (Peacock et al., 2021).

4 TROPONIN STRUCTURE AND FUNCTION

Cardiac troponins have three subunits (Troponin C, Troponin T, and Troponin I). Troponin C, namely, TN-C or TnC, is categorized as a calcium-binding protein expressed from the TNNC1 gene in cardiac and skeletal muscle (Cheng and Regnier, 2016; Marston and Zamora, 2020a). Troponin I is known as the inhibitory subunit, which inhibits the interaction of myosin with actin. There are three different isoforms (the fast and slow skeletal isoforms and the cardiac-specific isoform) for Troponin I. Troponin T, the largest subunit including 288 amino acids (36 kDa), is responsible for cardiac contraction. Troponin T can be divided into different functional regions, such as the N-terminus, also known as the T1 region (interacts with tropomyosin), and the C-terminus, also known as the T2 region (Cheng and Regnier, 2016).

Cardiac Troponins I and T are considered biomarkers for myocardial injury expressed in cardiomyocytes. In physiological conditions, troponin is presented in very small to undetectable quantities in the blood; however, in pathological conditions such as myocardial injury, troponin is increased in the blood (Maynard et al., 2000; Silva et al., 2010). Based on findings, it seems that cardiac troponin is released into blood circulation during the first 24 h and mostly subside within 2 weeks (Katus et al., 1991). Several studies showed that cardiac troponins are the more sensitive and more specific marker for diagnosis of myocardial injury compared to other makers, such as CK-MB (Al-Hadi and Fox, 2009).

In humans, Troponin I is extracted in three isoforms: specific cardiac and fast and slow isoforms (Wade et al., 1990; Tiso et al., 1997). The cardiac isoform of TnI is stated absolutely in the heart (Bodor et al., 1995; Bhavsar et al., 2000; Dellow et al., 2001). The gene of cardiac TnI (TNNI3) is placed on the 19th chromosome (19q 13.4) and involves seven introns and eight exons (Bhavsar et al., 1996). The cTnI consists of five domains: C-terminal mobile domain, regulatory domain, inhibitory domain, IT-arm, and N terminal domain (Katrukha, 2013). At high levels of Ca^{2+}, this domain interacts with a hydrophobic cleft on the surface of the TnC N-terminal domain, creating the third link region of hcTnI with TnC interaction of the regulatory domain with TnC guiding the separation of the TnI inhibitory domain from actin and the shift of tropomyosin that permits the creation of the actomyosin compound (Vassylyev et al., 1998; Li et al., 1999; Wang et al., 2002). The mobile domain of hcTnI comprises the C-terminal and the H4 α-helix part of the molecule (Takeda et al., 2003). It was verified that at a low level of Ca^{2+}, the mobile domain of TnI cooperates with tropomyosin and the C-terminal part of actin. These relations are believed to play a vital role in the adjustment of Ca^{2+}-dependent contraction and stabilization of the troponin complex on the surface of the thin filament (Galińska-Rakoczy et al., 2008; Galinska et al., 2010).

To secure the troponin complex on the actin filament, Troponin T plays a leading role in the arrangement of complex subunits and muscle contraction regulation (Tobacman, 1996; Perry, 1998). The N-terminal variable domain of the hcTnT molecule maintains central and C-terminal domains (Katrukha, 2013). Based on the latest studies on different chimeric proteins and deletion mutants of TnT, the N-terminal part of the protein manipulates the conformation of the complex and the interaction of the troponin complex with actin and tropomyosin (Wang and Jin, 1998; Chandra et al., 1999; Biesiadecki et al., 2007) and imitates the Ca^{2+} sensibility of the muscle (Sumandea et al., 2009; Gollapudi et al., 2012; Mamidi et al., 2013) and the development of maximum force of contraction (Gollapudi et al., 2013; Mamidi et al., 2013). Interacting with tropomyosin and probably actin, this C-terminal part of the molecule secures the troponin complex in the non-active state owing to the deletion of the last 14 amino acid residues hcTnT caused in the improvement of actin-activated ATPase activity *in vitro* (Franklin et al., 2012).

Troponin C consists of a short N-terminal domain formed by the first α-helix (N-α-helix) and four Ca2+ binding EF-hands that combined pairwise into the N-terminal C-terminal globular domains (Katrukha, 2013). Each EF-hand consists of two α-helices (α helices A-H) located in the Ca2+ binding loop (Takeda et al., 2003).

While the extracted troponin molecule is a moral issue for delegated structural analysis, *in vivo* troponin is a fully incorporated content of the thin filament along with tropomyosin and actin. Troponin's collaborations with actin and tropomyosin are the basis of Ca2+-dependent regulation of the thin filament (Gordon et al., 1997), and all the mensuration of troponin regulatory function involves the whole thin filament interacting with myosin. Two essential mechanisms in the regulation by troponin components are the inhibition of the contractile interaction of myosin–actin–tropomyosin by the dissuasive activity of Troponin I and the release of the inhibition by Troponin I through the binding of Ca2+ to Troponin C. Troponin T functionally acts as an integrating component necessary for the Ca2+-regulatory action of troponin (Ohtsuki et al., 1986)

The mechanism by which troponin switches the thin filament activity in response to Ca2+ has been established for some time. At low cytosolic Ca2+ levels, the formation of the actomyosin complex is sterically inhibited by Troponin I (TnI), through its C terminus binding to actin and locking tropomyosin in a blocking position such that strong binding of actin to myosin is not allowed. Because of enhancement of the Ca2+ level, a single Ca2+ ion binds to the regulatory N-terminal cTnC site II. It initiates an intramolecular conformational shift, revealing a hydrophobic patch of NcTnC and exposing it for interaction with the cTnI switch peptide, which is also linked with a joint motion of the N-terminal TnC domain relative to the IT domain. The binding of the TnI switch peptide to the hydrophobic patch drags the C terminus of TnI afar from actin. This action permits a cooperative shift of the tropomyosin molecule across the actin surface, encountering all the myosin-binding sites on actin and thus permitting cross-bridge cycling (Marston and Zamora, 2020b). The various kinds of cTn studies are shown in **Table 2**.

Based on the latest international guidelines, classical methods are clinically dependent on cardiac troponin testing for identification and diagnosis of myocardial damage (MD) and risk stratification. However, cardiac troponin overexpressed in patients with positive risk factors for MD, and characterized by high-sensitivity immunoassays cTns (hs-cTnI and T) as gold standard laboratory techniques in adults and pediatric age (Clerico et al., 2021a). Additionally, hs-cTn approaches are capable of monitoring myocardial renewal and remodeling mechanisms, and can be used to quickly identify individuals who are mostly at risk of developing symptomatic heart failure, perhaps leading to earlier diagnosis and a better prognosis (Clerico et al., 2021b).

5 DIAGNOSTIC VALUE OF TROPONIN IN COVID-19

Cardiac injury is frequently encountered in patients with COVID-19 and is associated with increased risk of death (Wibowo et al., 2021). Cardiac complications include the development of incident heart failure, acute coronary syndrome (ACS), and arrhythmia, all of which are associated with elevation in cTn (Park et al., 2017). Elevated troponin may

TABLE 2 | Several studies for Cardiac troponins.

Type of study	Number of patients	Finding	References
Retrospective	187	Elevated TnT levels in 52 patients	Guo et al. (2020)
Case Report	1	Raised serum creatinine and Troponin I level	Alhogbani (2016)
Case Report	1	Enhanced serum creatinine and Troponin T level Troponin T was more than 10,000 ng/L. Creatine kinase isoenzyme CK-MB 112.9 ng/L	Hu et al. (2021)
Case Report	1	Troponin I level was 1.26 ng/ml (<0.3 ng/ml) and NT-proBNP was 1,929 pg/ml (<125 pg/ml)	Kim et al. (2020)
Retrospective	25	Elevated CRP, cTnI, D-dimer, LDH, and lactate levels	Kermali et al. (2020)
Retrospective	14,855	cTn-negative = 13,828 (N), cTn-positive = 1027 (N)	Tanboğa et al. (2021)
Retrospective	49	12% Elevated TnT levels	Zhu et al. (2020)
Retrospective	101	Almost half of whom had aN hs-TnT value fivefold more than the normal upper limit	Wei et al. (2020)
Retrospective	466	High cTnI level *N* = 168 (36.05%)	Ali et al. (2021)
Prospective	207	Elevated TnT levels, was significantly correlated with native T1	Puntmann et al. (2020)
Case Series	187	Elevated TnT levels, patients with high TnT levels had more severe respiratory dysfunction	Shi et al. (2020)
Case Report	1	Elevated levels of markers of myocyte necrosis (Troponin T level)	Inciardi et al. (2020)

signify myocardial damage and is predictive of mortality. However, the prognostic performance of troponin and whether its value is affected by various comorbidities that may be present in patients with COVID-19 are not known (Wibowo et al., 2021). In addition to causing pneumonia (the main complication of the infection), SARS-CoV-2 may induce a direct damage to the heart: on the one hand, causing a myocardial infection (myocarditis), with significant impairment of cardiac contractility; on the other hand, it might affect the pericardium (pericarditis) with the formation of an effusion that may also impair cardiac function (Chen et al., 2020). In case of viremic phase, the mechanism by which the virus might attack heart cells could be related to the elective affinity between the viral spike proteins of SARS-CoV and type 2 angiotensin-converting enzyme receptor (ACE-2), which is well represented on myocardial cells. Another hypothesis is that the virus may migrate from the lung with infected macrophages to the myocardium (Piccioni et al., 2020). ACE-2 is also present on the endothelial cells of the vessels, so theoretically, acute vasculitis (inflammation of the vessels) of the intra-myocardial vessels could also occur, which would end up causing ischemic damage (Hanff et al., 2020) .

Myocardial damage could also be caused by severe general inflammation. This leads to the release of abundant quantities of inflammatory substances (cytokine storm), with a toxic effect on the heart muscle, thus compromising its function (Tveito, 2020). It is also possible that, in some cases, the adrenergic hyperactivation following respiratory distress and, possibly, the psychic stress related to the condition cause a ventricular dysfunction typical of the Tako-Tsubo syndrome, or an acute myocarditis that presents itself as a Tako-Tsubo syndrome (Sala et al., 2020).

Finally, it has been shown that cardiac function can be seriously compromised due to the severe infectious state in patients with known heart failure, myocardiopathy, or serious valvular diseases (Dong et al., 2020). In all these manifestations of heart damage, the evaluation of cardiac biomarkers such as troponin is essential, above all to highlight an early diagnosis of cardiac involvement, to guide a possible prognosis, and to present a helpful follow-up (Piccioni et al., 2020). As mentioned above, troponin increase correlates with the severity of infection (Metkus et al., 2017).

A meta-analysis carried out calculating the standardized mean difference (SMD) and 95% confidence interval (95% CI) of cTnI or cTnT values in COVID-19 patients with or without severe disease has shown that cTnI concentration is only marginally increased in all patients with SARS-CoV-2 infection, whereby values exceeding the 99th percentile in the upper reference limit (URL) can only be observed in 8%–12% of positive cases (Lippi and Plebani, 2020c).

In an observational cohort study of patients with COVID-19, all of the following biomarkers were measured: Troponin I, B-type natriuretic peptide, C-reactive protein, ferritin, and d-dimer. Among the tested biomarkers, Troponin I ≥0.34 ng/ml was the only independent predictor of 30-day mortality. Use of a simple risk score, which incorporates troponin levels, age, and presence of hypoxia on presentation, can help stratify patients at risk for in-hospital mortality associated with COVID-19 (Manocha et al., 2021).

A retrospective analysis was carried out on 54 subjects admitted to Tongji hospital in February 2020. Patients with or without myocardial damage, defined with three times higher serum cardiac troponin value, were analyzed and compared. During hospitalization, 44% of cases (n = 24) were complicated by myocardial damage and 48% (n = 26) died in the hospital. Mortality was significantly higher in patients with myocardial damage than in patients without myocardial damage, and this correlated with the values of troponin, C-reactive protein, and pro-BNP. This study also confirms that the involvement of myocardial tissue in COVID-19 disease correlates with the severity of the clinical picture. COVID-19 patients with severe respiratory failure and myocardial damage have a significantly higher risk of in-hospital mortality. In addition, the study suggests that it is important to monitor patients with high troponin values at the first check with the serial dosages of this biomarker to understand the evolution of the myocardial injury during hospitalization for COVID-19 patients (Piccioni et al., 2020) .

Evidence of COVID-19-associated increases in circulating cardiac Troponin T (cTnT) and cardiac Troponin I (cTnI)

above the 99th percentile reference limit is emerging in the literature (Wang et al., 2020a; Liu et al., 2020d; Ruan et al., 2020).

Elevated serum troponin levels on admission statistically correlated with mortality in COVID-19 patients (Al-Zakhari et al., 2021). In a retrospective cohort analysis, cTnI was significantly elevated in 54 subjects who died compared with 137 survivors (median [IQR] cTnI 22 [5.6–83.1] ng/L vs. 3 [1.1–5.5] ng/L, $p \leq 0.0001$) (Zhou et al., 2020).

The principal pathophysiology indicates a cardio-inflammatory response, as many significantly ill COVID-19 patients determine concomitant elevations in acute phase reactants such as CRP and the natriuretic peptides. This action may present clinically as fulminant myocarditis (Gaze, 2020).

In one case report study, a 37-year-old man presented with a 3-day history of chest pain and dyspnea. Electrocardiographic data implied an ST-segment elevation acute MI, and cTnT was substantially elevated at >10,000 ng/L (99th percentile reference limit <14 ng/L), with simultaneous elevations in CK and B-type natriuretic peptide. The appropriate working diagnosis was ACS. Subsequent CT coronary angiography discovered no verification of coronary stenosis. A sputum sample was assessed for 13 viral nucleic acids, of which only coronavirus was positive. The diagnosis changed to coronavirus fulminant myocarditis with cardiogenic shock and pulmonary infection. The patient was successfully handled with glucocorticoid and human Ig; cTnT diminished to 220 ng/L from 1 to 3 weeks (Hu et al., 2021).

6 DEVELOPMENT OF BIOSENSORS FOR CARDIAC TROPONIN IN COVID-19

Biosensors are devices designed to detect and quantify target biomarkers in certain diseases and thus aid in the early prediction of these complicated disorders (Nakamura and Karube, 2003; Wang et al., 2005). Their prime principle is similar to antigen–antibody interactions, whereby agents of interest like an enzyme, antibody, or DNA/RNA molecules are coated over the transducer surface, and upon interaction with the target substance or biological elements of interest, they generate quantifiable signals (Prieto-Simón et al., 2014; E Wang et al., 2011; Pei et al., 2013). However, both antibody and antigen provided high specificity, sensitivity, and applicability in humans (Sivasubramanian et al., 2009). The tendency for fabrication of biosensors is a vital needed process to resolve limitations encounter classical bioassays, critical care overload with a wide limit of detection (LOD) range, rapid turnaround time, high stability, reliable detectability for undiagnosed or asymptomatic cases, low costs and compatible with sustained lab quality schemes.

Quantification of cardiac troponins is among the most vital biomarkers of cardiovascular disorders especially MI. Due to their high specificity and sensitivity, cardiac troponins are considered vital markers of coronary events (Spyracopoulos et al., 1997; Takeda et al., 2003; Babuin and Jaffe, 2005). Usually, the circulating troponin level is very low in normal patients and is not usually detectable; however, in acute MI, its level increases within 24 h of myocardial events (Antman et al., 2000). Biosensor technologies are capable of early detection of troponin in human biological fluids and thus are very important tools in survival against cardiovascular fatalities (Abdolrahim et al., 2015).

6.1 Types of Cardiac Troponin Biosensors

Many developed troponin biosensors are different from each other according to transduction mechanisms and broadly grouped into electrochemical, optical, and acoustic detectors. Electrochemical detectors include amperometric, impedimetric, potentiometric, as well as conductance-dependent detectors as in **Figure 1**. Likewise, optical detectors are exemplified by fluorescence- and chemiluminescence-dependent detectors, surface plasmon resonance (SPR)-based detectors, and surface-enhanced Raman spectroscopy (SERS) detectors. Yet, another group of acoustic detectors includes surface acoustic wave (SAW)-dependent detectors and quartz crystal microbalance (QCM)-dependent sensors, as summarized in **Table 3**.

6.2 Electrochemical Sensors

The basic principle of these detectors is based on a change in the potential difference or current/impedance after immunological reactions taking place at the electrode surface. So, these types of biosensors have numerous biological molecules like various metabolites, enzymes, nucleic acid, and DNA acting as detection probes. These sensors are highly sensitive, cost-effective, selective, as well as reliable regarding their results. They are further grouped into the following:

(1) Impedimetric Sensors

These sensors quantify the change in resistivity value after potential is applied across the electrodes. In the presence of some electrolytes at the surface of the specialized impedimetric sensor, upon application of potential to the electrode, polarization is observed. Subsequently, charges move to the electrode surface, thus forming an electric double layer. This double layer is essential since it is a pocket of charges and substantially changes upon antigen–antibody interaction at the electrode surface. Various cross-linking polymers are used to adequately immobilize the antibodies at the electrode surface (Lomant and Fairbanks, 1976). These polymers contain the R-S-H group upon which they bind with the gold electrode whereas another CO-NHS group is available for making bonds at the other end. When interacting with an antibody, the bond breaks and an amine link is formed with the antibody. Various types of linkers are used based on the type of electrode and type of substrate (Choi et al., 2000; Swaim et al., 2004). For instance, ZnO nano-sensor utilizes an R-S-H linker and α-cTnT antibodies *via* EIS to detect cardiac troponins. This detector can measure both the Troponin I and Troponin T in picograms inside the human blood (Shanmugam et al., 2017). A similar detector using porous graphene oxide substrate was developed by Kazemi et al. (2016).

(2) Amperometric Sensors

The device works on the same basic principle as the previous one, whereby specific antibodies are immobilized at the surface of electrodes, and upon reaction with antigen, changes in the current

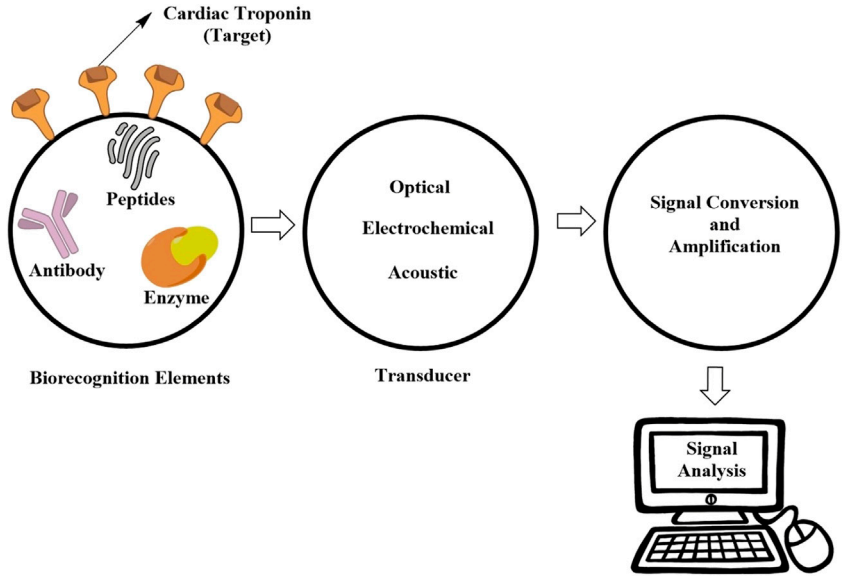

FIGURE 1 | Mechanisms underlying the primary function of biosensors.

are measured (Stetter and Li, 2008). These sensors are different from the previous one because impedimetric sensors record resistivity changes. In amperometric sensors, change in current is measured after antibody reaction with a specific antigen or biomolecule. The electrodes also contain redox probe or chemical mediator-labeled antibodies, which assist in quantifying changes in current at working electrodes and thus provide beneficial information about the antigen–antibody binding mode after applying a specific potential bias to the electrode. Subsequently, alteration in the current relative to the reference electrode is calculated. In these sensors, signal detection is usually done through cyclic voltammetry. Most commonly, sensing electrodes are made of carbon nanotubes owing to their flexible electrochemical properties including better electrical conductance, coherence with other materials, and mechanical strength (Gomes-Filho et al., 2013). Having a high electron transport rate, the reaction rate for the majority of antigen–antibody reactions is faster with higher sensitivity and better performance. Typically, i-STAT handheld is an amperometric sensor developed by Abbot United States and worked on the same immunoassay-dependent approach. This sensor can detect cTnI from blood samples (17 μl) in concentrations as low as 0–50 ng ml^{-1} in a few minutes *via* a disposable cartridge. This biosensor is very useful clinically for detecting MI and the prognosis of the disease (Steinfelder-Visscher et al., 2008).

(3) Potentiometric Sensors

These sensors assess any potential difference between the set of electrodes separated from each other *via* a semi-permeable membrane. Changes in potential difference escalate due to either pH changes or oxidation–reduction reactions pertinent to the electrode surface (Bakker and Pretsch, 2005). When interactions happen between antigen adsorbed at the electrode–electrolyte interface and immobilized captured probe, relevant changes in the potential difference arise (Novell et al., 2012). The change in potential is proportional to the extent of antigen binding with capture probe. Light addressable potentiometric sensors (LAPSs), pH electrode-reliant glass ion-selective electrodes (ISEs), and solid-state ion-selective field-effect transistors (ISFETs) are examples of this type of sensor. The modified versions of ISE sensors are field-effect transistor sensors and are mostly preferred owing to their better immuno-sensing capabilities.

Further, Cambridge researchers developed a new polypyrrole-coated sensor as an example of ISFET capable of detecting cardiac troponins (Purvis et al., 2003). This sensor is superior for having the ability to immuno-separate during analysis; the pyrrole layer at the electrode surface is polymerized *via* potentiodynamic electropolymerization cascade, making the sensor more robust and highly sensitive. Potential difference develops as a result of receptor–target interactions, and a complex is formed with the polypyrrole layer found on the electrode surface and is proportional to the amount of cardiac Troponin I molecules binding to its monoclonal antibodies (Palan et al., 1999).

(4) Conductance-Dependent Sensors

These types of sensors measure biological molecules on the basis of changes in the conductance values of solutions. Firstly, specific voltage is applied to a sensor that acts as a microfluidic channel coated with biomolecules like antibodies, thus generating a baseline. This baseline provides initial measurement values for later comparison when the conductance changes after reaction with an antigen-containing solution (Lee et al., 2009). Antigen–antibody interactions considerably change the quantity of current required for detection, which is

TABLE 3 | A detailed comparison between cardiac tropobiosensors used in lab diagnosis.

Biosensor name	Used technique	Detection limit	Pros	Cons
ZnO or porous reduced-graphene oxide (rGO) nano-sensor utilizes R-S-H linker and Troponin antibodies *via* EIS. Liu et al. (2020d), Manocha et al. (2021)	Electro-impedimetric sensors (EIS)	0.07 ng/ml	• Detection of both Troponin I and Troponin T in pico-amounts • Highly sensitive, cost-effective, and selective	• The biocompatibility and non-toxicity of graphene nanomaterials are still not confirmed • Impedimetric sensors suffer from hesitations in resistivity
• *Graphene quantum dots (GQDs) and polyamidoamine (PAMAM)-modified gold electrodes.* Ruan et al. (2020)	Electro-amperometric sensors (EAS) based on voltammetry	20 fg/ml	• Highly stable and sensitive electrodes • Assimilation of targeted immunoreactions without interferences	Testing errors in the preclinical stages
		50 ng/ml	• Uses tiny amounts of patient serum sample and gives results in a few minutes	
• *Abbott- immuno i-STAT handheld.* Al-Zakhari et al. (2021)			• Widely used and considered as one of the successful POC tool for detection of cTns	
Polypyrrole-coated ISFET sensor. Gaze (2020)	Electro-potentiometric sensors (EPS)	0.01 ng/ml	Robust and ultrasensitive with wide detective range	Delayed results up to 20 min
Silicon nanowire-based sensor. Nakamura and Karube (2003)	Electro-conductometric sensors (ECS)	1 ng/ml	• Detect serum cTns up to 1 fg/ml	Less stable due to the effect of salt concentration in the buffer and the length of the wire used
TiO_2 nanotube array (TNTA). Wang et al. (2005)	Fluorescence-based sensors (FBS)	0.1 pg/ml	• Cheaper, sensitive, increased surface area with high compatibility and applicability • Detect serum cTns up to 100 pg/ml	• Needs high temperature • TNTA affected by presence of impurities and changes in pH • The power of detection is a function on nanotube length and thickness • Applied only on 10% diluted serum
Gold nanoparticles (AuNPs)-modified TiO_2 nanotube array (TNTA). Prieto-Simón et al. (2014)	2.2 pg/ml	Swift detection with accuracy	Low detection limit than ELISA	
Classical sandwich ELISA. E Wang et al. (2011)	Chemiluminometric immunosensors (ELISA)	0.02 ng/ml	Reliable assays	• Slow turnaround time about 20 min • Not cheap
ELISA-on-chip biosensor based on cross-flow chromatography for detection of antibodies. Pei et al. (2013)		0.01 ng/ml	• Swift detection up to 30 s • Suitable for POC • Cheaper and more sensitive than conventional colorimetric assays	• Not commonly used • The cost per test is still not cheap enough
Poly(dimethylsiloxane) (PDMS)–AuNPs composite-based biosensor. Sivasubramanian et al. (2009)		0.01 ng/ml	Precise, easy fabrication, and high stability	• Difficulty in labeling, expensive, and bulky • Detection time not less than 20 min • Still under experiment
Ru-PAMAM/AuNPs-based electrochemiluminescence (ECL). Spyracopoulos et al. (1997)	Electrochemiluminescence (ECL)	12 fg/ml	• Better sensitivity, specificity, stability, and reproducibility • Label-free method	Still being tested
Surface enhanced Raman spectroscopy (SERS)-based competitive immunoassay. Babuin and Jaffe (2005)		33.7 pg/ml	• Total detection time is 7 min • High specificity and stability with sharp bands	• Metallic coated nanoparticles may be toxic *in vivo* overtime • Imaging problems due to insufficient light wavelength used to penetrate body tissues • Low precision due to scattering possibilities
Localized surface plasmon resonance (LSPR)-based nanosensor. Antman et al. (2000)		250 × 10¯6 ng/L	• High sensitivity, applicability, and reproducibility • Easily detect cTnT in asymptomatic cases • Low cost • Label-free method	• Presence of any other analytes in the solution may lead to overlapped peaks in the infrared region • Limited penetration to 100 nm makes it a bad choice for large molecules • Unable to detect cTnT from cell extracts
Quartz crystal microbalance (QCM) -based sensor. Abdolrahim et al. (2015) Surface acoustic wave (SAW)-based sensors. Lomant and Fairbanks (1976)	Acoustic sensors	5 ng/ml	Sensitive compared to EIS for detection of cTnI	Needs delicate control in pressure and temperature Still under evaluation

subsequently construed to measure the solution conductance. Researchers developed a silicon nano-wire-dependent sensor working on the same principle and capable of detecting cardiac Troponin T in blood as low as 1 ng ml^{-1} (Chua et al., 2009).

6.3 Optical Sensors

The basic principle of these biosensors is to detect any changes in the input light frequency or changes in its polarization phase after immunological reaction. Hence, they are also named as fluorescence, luminescence, surface plasmon resonance (SPR), and colorimetric sensors (Fan et al., 2008).

(1) Fluorescence-Reliant Sensors

The first-ever fluorescence biosensor was reported in 1941 (Coons et al., 1941), where the target analyte labeled with fluorescent dye or probe is allowed to react with a biomolecule leading to a change in the fluorescence intensity. Later on, a more efficient and modified "point-of-care" biosensor using TiO_2 nanotube arrays was developed by Kar et al. (2012). Though having high sensitivity, these sensors are associated with a tedious labeling process and difficult quantitative analysis due to signals emitted from fluorophores on molecules (Cox and Singer, 2004). Because of this, ellipsometry-based biosensors are usually preferred. A silicon optical sensor with a dielectric spin applied over the top of the silicon substrate and subsequently functionalized for cardiac Troponin I detection is also developed. However, the LOD of these sensors is relatively low compared to electrochemical detectors and cannot quantify cardiac Troponin I in pure serum (Diware et al., 2017a). Further, photo-electrochemical sensors also have the same basic working principle, but they are coupled with a photo-responsive device in addition to the normal immuno-sensing components. The target antibody is usually immobilized at the electrode surface and response is observed *via* laser light. Photo-current is usually detected proportionally to antigen (troponin) concentration. When an immunological reaction between antigen and antibody occurs, this leads to decline in photo-current. The LOD of these sensors is quite high and comparable with electrochemical sensors (Tan et al., 2017).

(2) Chemiluminometric Sensors

In these calorimetric sensors, the quantity of target biomolecules is assessed from the quantity of light absorbed by the chromogenic agent at a specific wavelength. ELISA is a typical example of these calorimetric sensors that again work on immunological reactions' principles (Cho et al., 2009). Initially, a biomolecule acting as antigen is complexed with a primary antibody that is subsequently linked to an enzyme-linked secondary antibody. The biomolecule of interest is quantified from the activity of the conjugated enzyme generated by the product. A modified version of this concept is "ELISA on chip" for the quantification of cardiac Troponin I (Cho et al., 2009). Among the novel strategies is the use of nano-materials whereby nano-carriers are loaded with an extra quantity of enzymes, which enormously increase the quantity of signal-emitting molecules and thus improve signal amplification (Li et al., 2013). For instance, a PDMS–gold composite biosensor was reported by Wu et al. in 2010 for the detection of cardiac Troponin I (Wu et al., 2010). Additionally, these nano-particles (i.e., AuNPs) are well-functionalized substrates for biological target molecules including antibodies, antigens, and enzymes. So, the combination of polydimethylsiloxane (PDMS) with $AuNP_S$ is ideal for developing calorimetric sensors.

(3) Surface-Enhanced Raman Spectroscopy-Dependent Sensors

The multiplex and highly sensitive SERS biosensors have significantly improved the issues associated with the primitive Raman spectroscopy method like low accuracy due to less scattering efficiency (Campion and Kambhampati, 1998). The basic principle of SERS is excitation of electrons from a roughened metallic surface *via* laser or other electromagnetic radiation, which subsequently starts oscillation, having the same wavelength of the incident radiation. Thus, enrichment in electric field occurs due to addition of a secondary electric field to the already present electric field. When the electrons' movement is restricted at a particular oscillation frequency, resonance is experienced in the incident field. The resulting resonance is vital in the detection of biological molecules of interest (Vo-Dinh, 2008). Signal detection for biological targets can be further improved *via* addition of nano-scale roughness and specialized coating of the metallic surface. One SERS biosensing technique for cardiac Troponin I utilizes a magnetic bead surface coated with immobilized antibodies having nano-tags for binding with target molecules. This SERS device has comparatively high sensitivity (LOD 33.7 pg ml^{-1}) than other techniques (Zhang et al., 2011).

(4) Surface Plasmon Resonance-Dependent Sensors

Since its inception 1983 by Liedberg, SPR-based sensors have been widely used to quantify biological molecules including detection of cardiac troponins (Liedberg et al., 1983). These sensors are capable of quantifying equilibrium constants for reactions like protein–protein interactions, protein interactions with DNA molecules, and other ligands. The sensor consists of a glass prism coated with immobilized biological molecules like antibodies and the molecules to be detected allowed to flow through it. Subsequent to immunological reaction (i.e., antigen–antibody reaction), the light intensity changes proportional to the amount of target molecules (Englebienne et al., 2003). Researchers in United States developed an extremely sensitive SPR-type sensor using LSPR (localized surface plasmon resonance) capable of detecting very minute quantities (10^{-18} M) of cardiac troponins in biological fluids like blood, urine, and serum (Liyanage et al., 2017). A modified LSPR using human Troponin I binding peptide, applied on gold nano-rods was also developed (Tadepalli et al., 2015).

6.4 Acoustic-Dependent Sensors

These sensors are constructed on the basic theory of mass evaluation *via* piezoelectric crystals. For instance, when immunological reactions occur causing the formation of

complex at the electrode surface, the mass changes cause a shift in the crystal frequency, thus generating an electrical signal.

(1) Quartz Crystal Microbalance

The QCM contains quartz crystal and the device utilizes the piezoelectric properties of the same crystal and thus measure variation in mass as a result of changes in resonant frequency as explained by the Sauerbrey formula; $\Delta f = -2f^2\Delta m/A\rho\upsilon$.

QCM sensors are in clinical use to quantify cardiac troponins. The sample is applied at the sensor and the surface of electrodes pre-loaded with the target immobilized antibodies causing mass loading of the sensor after the reaction. Researchers have developed a cost-effective QCM sensor for the analysis of cardiac Troponin T. When a shift occurs in the frequency, it generates electric signals corresponding to the mass accumulating at the electrode surface (Wong-ek et al., 2010).

(2) Surface Acoustic Wave-dependent Sensors

These sensors also operate based on the same principle of QCM, where interdigitated transducer electrodes are coupled with piezoelectric crystal surface. The electrical signals are converted by transducers to polarized transversal acoustic waves that move across the piezoelectric crystal. Accumulation of mass over the piezoelectric crystal causes a shift in frequency of the acoustic waves generated by subsequent biosensing (Pohl, 2000). Examples of these sensors are Rayleigh-SAW, Love-wave, and Lamb-wave sensors, which are commonly used to assess cardiac Troponin I in biological samples (Priya et al., 2015). A Love-wave-type SAW sensor was reported previously to detect cardiac Troponin I, whereby immobilized antibodies on AuNPs react with cardiac Troponin I antigens and thus a response is sent to the sensor (Lee et al., 2011).

6.5 Tailoring Cardiac Troponin Detectors for COVID-19 Patients

Baker et al. developed the LOCKR system to produce a biosensor that utilizes light to indicate the presence of the target molecule. Initially, LOCKR proteins are present in a "closed" state and unable to emit light. When, the target molecule is present, it will bind with a specific region of the sensor, switching the LOCKR to the "open" state and emit light, which is easily recorded. The key feature of the LOCKR system is that it can easily be adapted to sense a range of targets, because the target binding region can be swapped without affecting the rest of the system. The biosensor was reliable in detection of SARS-CoV-2 virus in tiny amounts as 15 p.m. in 2 min and may able to detect a range of targets dependent on the benefit of the LOCKR system (Quijano-Rubio et al., 2021).

Surprisingly, a newly sensitive microfluidic biosensor with mesoporous nickel vanadate hollow-nanosphere modified chitosan (Ch-$Ni_3V_2O_8$) template was designed for detection of cTnI levels in patient serum samples depending on the high redox activity and biocompatibility of the Vanadium. However, this matrix worked against cTnI antibodies with a detection limit to 5 pg/ml with high sensitivity, selectivity, and reproducibility (Singh et al., 2019).

Xian et al. built a hand-made modularized Si MOSFET-based biosensor able to detect SARS-CoV-2 spike proteins in saliva to 100 Fg/ml and cTnI levels down to 100 pg/ml. This platform is recommendable for POC at emergent events and pandemic crises due to its cheaper cost and disposable sensor units (Xian et al., 2020). Recently, a smartphone app joined with an autonomous capillary microfluidic chip (ACMC) and self-aligned on-chip focusing (SOF) lenses to quantify serum cTnI levels in 12 min with 78–94 pg/ml detection limits using 100 μl of sample, based on the sandwich immunofluorescence principle. However, this creative platform is possibly used for POCT field in resource-limited settings (Liang et al., 2019). Boonkaew et al. designed a successful model of graphene oxide-modified carbon electrode stencils printed on an ePAD for detecting CRP, cTnI, and procalcitonin (PCT) using the square wave voltammetry (SWV) principle. Their results showed significant LOD values for cTnI about 0.16 ng/ml with $R^2 > 0.99$ and RSD < 5% (Boonkaew et al., 2021). Synergistically, a ZnO-NPs-based FET biosensor showed a diagnostic efficiency with LOD of 3.24 pg/ml in the detection of serum cTnI (Boonkaew et al., 2021).

Another fascinating model that consists of a Tetrahedral DNA (TDs) aptamer built in HCR and Au/Ti3C2-MXene amplified units on EC/ECL biosensors detected cTnI levels in blood samples of COVID-19 critical cases with LODs of 0.04 or 0.1 fM (Sandoval et al., 2020). Indeed, reaching out to models of biosensors of sufficient sensitivity and reliability will contribute to predict CVD-associated COVID-19 complications at a time early enough for clinical interventions.

7 CHALLENGES AND PERSPECTIVE

As new cases are being recognized during the COVID-19 pandemic, identification of clinical and diagnostic presentations is being refined. Cardiac biomarkers, specifically natriuretic peptides and cTn, are usually promoted in COVID-19 patients. In pathologies of majority of diseases, the elevation of cTn is related to disease severity and poor prognosis (Fang et al., 2020). The method of using sequential cardiac troponin can make risk classification easy, helps make decisions about how and when to use preclinical manifestations, and informs stage grouping and disease phenotyping along with hospitalized COVID-19 patients (Sandoval et al., 2020).

The principally significant roles of the cardiac troponins, cTnI and cTnT, are as diagnostic gold standard and the most specific and sensitive biomarkers for cardiac injury and acute MI and in categories threats in acute coronary syndrome and myocardial necrosis (Apple et al., 2012). Recent studies have revealed that sequential analysis of cT in patients with chronic heart failure is able to give more sufficient prognostic data. Patients with a high level of troponin concentrations have a worse prognostic fate than others (Masson et al., 2012), for instance, higher decreasing ejection fraction (cardiac output), lower systolic blood pressure, higher rate of in-hospital mortality and acute decompensated heart failure, and higher re-hospitalization rate

in both ischemic and non-ischemic heart failure. Regardless of the cause of heart failure, increased cTn is an autonomous predictor of poor consequences in individuals with heart failure outside of the setting of acute MI. In clinical studies, the influence on therapy and follow-up has yet to be determined (Peacock et al., 2008; Catarino et al., 2021).

The release of cTn in the peripheral blood starts in 3–24 h, and maximum rates of up to 10–20 h for cTnI and more than 15 to 120 h for cTnT return to original levels after 10 days (cTnI) and 14 days (cTnT) (Danese and Montagnana, 2016). The European Society of Cardiology (ESC) and the American College of Cardiology (ACC) both consider troponin increases to be critical in the diagnosis of AMI. To eliminate false-positive outcomes, the ESC/ACC set a cutoff value for diagnosing acute MI as an increased value of cardiac biomarkers over the 99th percentile of the upper reference limit. This percentile can be changed depending on the patient's age, sex, or nationality (Catarino et al., 2021). A retrospective study on diverse analytic situations showed that gender can affect the results of cTnI, which may boost the sensitivity in women and specificity in men. Finally, their data confirm that a diagnosis of MI still relies on clinical opinion and evaluating cTn levels should be seriously assessed and should consider the clinical manifestations (Eidizadeh et al., 2021). Clinicians should be able to interpret cardiac biomarkers in the same manner as they comprehend blood count reference ranges, in order to get the most out of greater sensitivity, quicker decision paths, and improved risk prediction (Alaour et al., 2018).

Notice to importance of cTn, researchers have been following the safe, noninvasive and more sensitive and specific techniques with the least cost, time consuming and commercially accessible for a minimal detection amount of troponin in the blood and recently in saliva. Nowadays, there are numerous high-sensitivity biosensors published in the literature for measuring troponins in serum or whole blood within 30 min, with LOD as low as a few pg/ml (Liu et al., 2016) or even less than 1 pg/ml (Diware et al., 2017b), and only three biosensors were effectively defined in literature for recognition and quantification of troponins in saliva. All three salivary troponin biosensors are detected as type cTI: bead-based ELISA with spectrophotometric detection at 450 nm (Park et al., 2012), electrochemical–differential pulse voltammetry (DPV) (Chekin et al., 2018), and fluorescence spectroscopy in the 220–350 nm range (Rezaei and Ranjbar, 2017).

Furthermore, the pathophysiology of cardiovascular disease (CVD) and COVID-19 in acute steps is similar and driven by several biological procedures, including oxidative stress, inflammation, interaction between hormone and nervous system activation, or myocyte injury. However, the collective use of various biomarkers may obtain complementary prognostic information and may provide a better device to reduce mortality rates of COVID-19 based on coronary artery disease (CAD) involvement. In a patient with high-troponin levels besides increased levels of CRP, adiponectin and soluble intercellular adhesion molecule (sICAM)-1 were significantly raised in both blood and saliva after acute myocardial infraction (AMI). In conclude, all of the world healthcare services is attempting to prevent and early diagnosis of CAD and AMI for well prognostic future and effective treatment specially during the COVID-19 pandemic. Therefore, the early detection of AMI has a critical and cost-effective role in patient survival. The ability to detect a TN quickly and accurately in a small volume of body fluids (serum and saliva) and at an extremely low concentration (less than 24 pg/ml in a blood sample) is required for the development of a POCT TN detection device. Various biosensors and devices have been presented so far to detect TNs. These biosensors will create new chances for rapid recognizing with low LOD and high precision, relying on similar pathophysiology of cTn in COVID-19 and CAD (Henry et al., 2020a; Lippi and Plebani, 2020b).

AUTHOR CONTRIBUTIONS

YR designed the investigation; AG and NH divided each author's responsibilities; OM and SA prepared the figures and revised the work; SG managed the article writing and submission; OM and FJ worked on language editing.

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REVIEW
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Multisystem Inflammatory Syndrome and Autoimmune Diseases Following COVID-19: Molecular Mechanisms and Therapeutic Opportunities

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), has led to huge concern worldwide. Some SARS-CoV-2 infected patients may experience post–COVID-19 complications such as multisystem inflammatory syndrome, defined by symptoms including fever and elevated inflammatory markers (such as elevation of C reactive protein (CRP), erythrocyte sedimentation rate, fibrinogen, procalcitonin test, D-dimer, ferritin, lactate dehydrogenase or IL-6, presence of neutrophilia, lymphopenia, decreased albumin, and multiple organ dysfunction). Post–COVID-19 complications may also manifest as autoimmune diseases such as Guillain-Barré syndrome and systemic lupus erythematosus. Signaling disorders, increased inflammatory cytokines secretion, corticosteroid use to treat COVID-19 patients, or impaired immune responses are suggested causes of autoimmune diseases in these patients. In this review, we discuss the molecular and pathophysiological mechanisms and therapeutic opportunities for multisystem inflammatory syndrome and autoimmune diseases following SARS-CoV-2 infection with the aim to provide a clear view for health care providers and researchers.

Keywords: autoimmune disease, COVID-19, SARS-CoV2, multisystem inflammatory syndrome, multisystem inflammatory syndrome in children (MIS-C)

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, which was first reported in the city of Wuhan, China. The virus spread globally, and at the time of writing, more than 410 million cases and about 5 million deaths have been reported (Dong et al., 2020). Analysis of SARS-CoV-2 in terms of structural biology has categorized it into ORF6, 7a, 8, ORF3, E, M, ORF1ab, S, and ORF10. The spike protein enables the virus to adhere to the

host cell membrane and the nucleocapsid protein (N protein) holds the RNA genome of the virus. The membrane and envelope along with spike protein constitute a viral envelope (Rota et al., 2003). The nonstructural RNA genome of ORF6, 7a, 8, ORF3, ORF1ab, and ORF10 consist of conserved information for replication of the genome to a great extent (Andersen et al., 2020).

Although SARS-CoV-2 is primarily known to cause substantial pulmonary disease, studies indicate that it can affect multiple organs and cause gastrointestinal, hepatic, cardiovascular, renal, neurological, ophthalmic, and cutaneous manifestations of COVID-19 (Johnson et al., 2020). Some patients experience post-acute COVID-19, extending beyond 3 weeks from the onset of the first symptoms, and chronic COVID-19, extending beyond 12 weeks (Greenhalgh et al., 2020). Post-acute COVID-19 is associated with complications such as dyspnea, decreased exercise capacity, thromboembolic events, palpitations, chest pain, fatigue, anxiety, depression, resolution of acute kidney injury (AKI), prolonged viral fecal shedding, hair loss, and multisystem inflammatory syndrome (MIS) both in children and adults (Nalbandian et al., 2021). MIS is defined by symptoms including fever and elevated inflammatory markers (more than two of the following: elevation of C reactive protein (CRP), erythrocyte sedimentation rate, fibrinogen, procalcitonin test, D-dimer, ferritin, lactate dehydrogenase or IL-6, presence of neutrophilia, lymphopenia, decreased albumin, multiple organ dysfunction, and exclusion of other plausible diagnoses) (Rodríguez et al., 2020; Tenforde and Morris, 2021).

SARS-CoV-2 infection may alter the immunologic system, resulting in states ranging from abnormal cytokine or chemokine production and maladaptive immune response to an increased number of activated macrophages, monocytes, and neutrophils and hyperactivated T cells (Rodríguez et al., 2020). Several studies reported autoimmune diseases such as immune thrombocytopenic purpura (ITP), Guillian-Barrė syndrome (GBS), Graves' disease, systemic lupus erythematosus (SLE), antiphospholipid antibodies, and thrombosis associated with COVID-19 (Ehrenfeld et al., 2020; Rodríguez et al., 2020); however, MIS and autoimmune diseases can occur both para- and post-COVID-19 (Wang et al., 2015; Santos et al., 2021). Although the mechanism is unclear, some factors such as pro-inflammatory cytokines and chemokines, damage-associated molecular patterns (DAMPs), molecular mimicry, cross-reactive antibodies, and auto-antibodies may attribute to post-COVID-19 autoimmune diseases (Liu et al., 2021).

This review aims to provide a clear view for health care providers and researchers regarding the molecular and pathophysiological mechanisms and therapeutic opportunities.

Post COVID-19 Multisystem Inflammatory Syndrome in Children (MIS-C)

MIS-C is an uncommon but serious medical condition which is associated with SARS-CoV-2 (Hasan et al., 2021). The disease seems to develop from a dysregulated immune response, resulting in endothelial dysfunction and a hyperinflammatory state, which eventually causes capillary leak, followed by multiorgan failure (Panaro and Cattalini, 2021). MIS-C is characterized by inflammation in multiple organs such as the brain, kidneys, heart, eyes, lungs, skin, or gastrointestinal system (Hasan et al., 2021). Therefore, the clinical picture can be wide, depending on the severity and organ manifestation (Panaro and Cattalini, 2021). According to a recent systematic review, the most common symptoms were gastrointestinal symptoms (71%), with the incidence of vomiting (25%), diarrhea (27%), and abdominal pain (36%), followed by mucocutaneous manifestations (conjunctivitis, strawberry tongue, skin rash, and dried-cracked lips). Fever, as a key criterion of MIS-C diagnosis, was present in all of the evaluated cases (Panaro and Cattalini, 2021; Radia et al., 2021). Furthermore, cardiac complications including acute myocardial injury, myocarditis, decreased ejection fraction, coronary artery aneurysm, pericarditis, valve dysfunction, pericardial effusion, arrhythmia, ventricular dilation, and tachycardia are reported (Panigrahy et al., 2020; Pouletty et al., 2020; Simpson and Newburger, 2020). Many gastrointestinal signs and symptoms of MIS-C overlap with those of acute appendicitis (Nakra et al., 2020; Belay et al., 2021; Martin et al., 2021).

Clinical criteria defined by the CDC (Center for Disease Control and Prevention) for MIS-C includes 21 years of age or less presenting with fever, laboratory findings in favor of inflammation, involvement of more than two organs; with no other differential diagnosis; and positive SARS-CoV-2 IgG or RT-PCR, definite exposure to SARS-CoV-2 within 4 weeks of symptom onset (Holstein, 2021). There is a 2- to 4-week delay in developing MIS-C after COVID-19 infection, and the peak of MIS-C lags behind the peak of acute SARS-CoV-2 infection (Feldstein et al., 2020; Verdoni et al., 2020). Serology tests have a higher probability of identifying MIS-C than reverse transcription polymerase chain reaction (RT-PCR), since MIS-C is a late form of the disease that occurs when the antibody rates are increasing, and the presence of the virus is no longer expected (Toubiana et al., 2020).

Laboratory findings have shown elevated levels of cytokines including IL-6, IL-8, IL-10, TNF-α, IFN-ɣ, sC5b-9 (associated with endothelial damage), IL-18, sIL-2R, CXCL9, elevated levels of acute-phase reactants including CRP and procalcitonin (PCT), and elevated vascular injury markers such as d-dimers and B-natriuretic proteins (Diorio et al., 2020; García-Salido et al., 2020). In addition, several factors are associated with cardiac injury, such as D-dimer, BNP, N-terminal -proBNP (NT-proBNP), and troponin-T are elevated in MIS-C patients (Diorio et al., 2020; García-Salido et al., 2020; Simon Junior et al., 2021). Also, increased levels of ferritin and erythrocyte sedimentation rate (ESR), lactic dehydrogenase and triglycerides, prolonged prothrombin time, lymphopenia, thrombocytopenia, neutrophilia, and hypoalbuminemia are seen (García-Salido et al., 2020; Lee et al., 2020; Panigrahy et al., 2020; Pouletty et al., 2020; Abdel-Haq et al., 2021; Simon Junior et al., 2021).

MIS-C shares some clinical features with Kawasaki disease (KD), which is a paediatric, self-limited, systemic inflammatory vasculitis, and macrophage activation syndrome (MAS); however, it is considered a different condition with a wide variety of clinical presentation (Al Maskari et al., 2021;

Appleberry et al., 2021; Haoudar et al., 2021; Hasan et al., 2021; Jain et al., 2021; Tolunay et al., 2021). It is reported that 40% of patients with MIS-C met the criteria for either incomplete or complete KD (Feldstein et al., 2020). KD is typically known as a disease of young children < 5 years old, while MIS-C has been widely reported in a wide age range (from 1.6 to 20 years, with a median age of 6–11 years) (Abrams et al., 2020; Ouldali et al., 2020) In addition, the ethnicity of the MIS-C patient population is predominantly African-American/Hispanic while KD mostly affects the Asian population (Bukulmez, 2021). The pattern of coronary artery dilation in the two conditions is different from each other. The coronary involvement in MIS-C is usually mild and is detectable during the early phase of febrile illness, which rapidly resolves on short-term follow up in the majority of cases (Farooqi et al., 2021; Feldstein et al., 2021). On the other hand, the coronary artery dilation usually peaks after remission of the febrile illness in KD (McCrindle et al., 2017). The difference in severity and timing of coronary artery dilation in the two diseases can be justified with different pathologic mechanisms. It is assumed that coronary dilation in MIS-C is due to rising levels of circulating cytokines which is accompanied by endothelial cell dysfunction and probably edema resulting in mild dilation of the coronary arteries, and inflammatory cells infiltrate the coronary arteries in KD, resulting in disruption of elastin and collagen fibers and structural integrity loss, which eventually leads to aneurysms of the arteries (Orenstein et al., 2012). Autoantibody profiles have also been compared in patients with MIS-C and KD (Consiglio et al., 2020). The levels of antibodies to vascular endothelial cell proteins, like endoglin, were found to be higher than in healthy controls in both groups of patients; however, some autoantibodies (including that to discoidin I-like domain-containing protein 3 and EGF-like repeat) were overexpressed in KD compared with MIS-C. Moreover, plasma levels of endoglin were reported to be elevated in both groups of patients compared with healthy individuals, which raises the possibility that antibodies to endothelial cells might be the result, rather than the cause, of vascular damage. One other possibility is that the S protein superantigen of SARS-CoV-2 can cause aberrant activation of B cells (Bar-Meir et al., 2021).

MAS is characterized by unremitting fever, hepatic dysfunction, hyperferritinemia, pancytopenia, and coagulopathy, and it is commonly associated with systemic juvenile idiopathic arthritis (sJIA) and SLE (Crayne et al., 2019). Laboratory findings suggest that cytokine storm in MIS-C is relatively similar to that in MAS. However, MIS-C patients have higher fibrinogen, CRP, ESR, and prohormone-B-type natriuretic protein (proBNP) levels and lower ferritin levels and lymphocyte counts than MAS (Aydın et al., 2021; Otar Yener et al., 2021). In addition, MAS patients have higher IL-12, L-18, and CXCL9 than MIS-C patients (Lee et al., 2020). MIS-C patients have a significantly lower left ventricular ejection fraction, indicating a more severe disease than MAS (Aydın et al., 2021).

Pathophysiology

Cytokine Storm

MIS-C patients show elevated levels of IL-6 (Kaushik et al., 2020; Lee et al., 2020; Evans and Davies, 2021). IL-6 functions through two signaling pathways termed classic-cis-signaling and trans-signaling. In classic-cis signaling, IL-6 binds to its transmembrane receptor (mIL-6R), which is expressed on hepatocytes, megakaryocytes, and several immune cells, resulting in dimerization of gp130, phosphorylation of STAT3, and also activation of Akt/mTOR and MAPK signaling pathways (Tanaka et al., 2014; Patra and Ray, 2021). In the trans-signaling pathway, IL-6 binds to soluble IL-6 receptor (sIL-6R), forming a complex that binds to gp130, which is ubiquitously expressed. Binding to gp130 activates the JAK/STAT3 pathway in cells lacking mIL-6R such as endothelial cells, vascular smooth muscle cells (VSMCs), and fibroblasts which further triggers the production of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), vascular endothelial growth factor (VEGF), and the reduction of E-cadherin expression on endothelial cells (Tanaka et al., 2016).

TNF-α is also increased in the acute phase of MIS-C (Carter et al., 2020). However, Consiglio et al. reported that TNF-α levels were significantly lower in patients with MIS-C than in adults with acute COVID-19 and TNF-α levels were relatively similar to those in healthy children (Consiglio et al., 2020). TNF-α is a proinflammatory cytokine. The binding of TNF-α to TNF-R1 induces recruitment of TRADD (TNF-R1-associated death domain protein), and TRADD further recruits FADD/MORT1, TRAF2, and death domain kinase RIP. FADD/MORT1 induces TNF-associated cell death, and RIP and TRAF2 are involved in the activation of NF-κB and JNK (Liu, 2005). NF-κB can induce several proinflammatory gene expressions and elevation of cytokines and chemokines and participates in inflammasome regulation (Liu, 2005).

SARS-CoV-2 infection can mediate the secretion of IL-6 and TNF-α *via* several mechanisms. For example, attachment of SARS-CoV-2 spike protein to Angiotensin-converting enzyme 2 (ACE2) receptors on respiratory epithelial cells and entry results in inflammatory cytokine production and a weak IFN response. Membrane-bound immunologic receptors and downstream signaling pathways mediate the proinflammatory response of pathogenic Th1 cells and intermediate $CD14^+CD16^+$ monocytes and subsequently cause cytokine storm by the infiltration of neutrophils and macrophages into the lung tissue (Hussman, 2020). Activated pathogenic Th1 cells release granulocyte-macrophage colony-stimulating factor (GM-CSF), which further stimulates $CD14^+CD16^+$ monocytes to secrete IL-6 and TNF-α (Zhou et al., 2020a).

In addition, SARS-CoV-2 viral genomic single-stranded RNA or other RNA compositions may act as pathogen-associated molecular patterns (PAMPs) and bind to pathogen recognition receptors (PRRs) such as TLRs and RLRs (Khanmohammadi and Rezaei, 2021). PAMP recognition leads to activation of IRF3/7 and NF-κB downstream signaling pathways resulting in the secretion of IFN-I and proinflammatory cytokines (Yang et al., 2021). Also, Hirano and Murakami indicated that activation of the NF-κB pathway leads to occupation and reduction of ACE2 surface receptors (Hirano and Murakami, 2020). Reduction of ACE2 expression results in an increase in angiotensin II, which binds to angiotensin receptor I and the complex through disintegrin and metalloprotease 17 (ADAM17) and induces TNF-α and sIL-6R production (Eguchi et al., 2018).

Kang et al. reported that IL-6 is positively correlated with plasminogen activator inhibitor-1 (PAI-1) and, through the trans-signaling pathway, can induce endothelial damage and coagulopathy in patients with COVID-19–related cytokine release syndrome (CRS) (Kang et al., 2020). Also, IL-6 can increase tissue factors on monocytes triggering the coagulation cascade and thrombin activation (Kang and Kishimoto, 2021). In addition, IL-6 is related to vascular damage through C5a expression and VE-cadherin disassembly (Kang and Kishimoto, 2021). MIS-C patients show elevated levels of IL-8 (Carter et al., 2020; Kaushik et al., 2020; Riollano-Cruz et al., 2021).

Cellular Immunity

Neutrophils play an essential role in the innate immune response. Carter et al. reported increased neutrophil CD64 median fluorescence intensity (MFI), a neutrophil activation marker, in the acute phase of MIS-C. Activated neutrophil levels are normalized in the resolution phase. Also, they reported decreased CD10 MFI on neutrophils, which implies decreased mature neutrophils (Carter et al., 2020). Neutrophils are capable of ferritin secretion, and elevated ferritin levels are seen in MIS-C patients (Simon Junior et al., 2021). Ferritin has an immunosuppressive and proinflammatory function. The immunosuppressive role includes suppressing the delayed type of hypersensitivity, suppressing antibody production, regulating granulomonocytopoiesis, and reducing phagocytosis by granulocytes through H-ferritin signaling pathways on lymphocytes, downregulation of CD2 and CXCR4, and inducing the production of IL-10 (Rosário et al., 2013). The proinflammatory role of ferritin is proposed by Ruddell et al., in which ferritin activated the TIM-2-independent pathway and further leads to the activation of NF-κB and production of proinflammatory cytokines such as IL-1β (Ruddell et al., 2009).

MIS-C patients have elevated levels of fibrinogen and D-dimer, indicating abnormal coagulopathy. Neutrophils can form neutrophil extracellular traps (NETs) that are associated with thrombosis and may play a role in MIS-C (Jiang et al., 2020a; Middleton et al., 2020). In the conventional NETosis pathway, activation of TLRs, receptors for IgG–Fc, complement, or cytokines lead to increased cytoplasmic calcium and elevated calcium levels activate protein kinase C (PKC) and phosphorylation of gp91phox (Kaplan and Radic, 2012). The phosphorylation of gp91phox results in the activation of phagocytic oxidase and production of reactive oxygen species (ROS) and rupture of granules and the nuclear envelope along with chromatin decondensation. NET release occurs after the rupture of the plasma membrane (Papayannopoulos et al., 2010). NETs can promote thrombosis through platelet and red blood cell adhesion and aggregation. DNA, histones, and proteases in NETs have procoagulant properties (Yang et al., 2016). NETs are also involved in morbid thrombotic events in patients with COVID-19 (Zuo et al., 2021). However, Seery et al. reported that NET production was similar in children with COVID-19 and healthy controls (Seery et al., 2021).

T cells may be involved in the pathogenesis of MIS-C. Consiglio et al. reported that in patients with MIS-C, total T cell frequencies were lower than in healthy controls and $CD4^+$ distribution was similar between children with MIS-C and mild COVID-19. Central memory (CM), effector memory (EM), and terminally differentiated effector $CD4^+$ T cells were higher and naïve $CD4^+$ and follicular helper T cells were lower in MIS-C and COVID-19 patients than in children with KD. Compared to children with mild COVID-19, children with MIS-C had significantly lower $CD4^-$ (mostly $CD8^+$) T cells (Consiglio et al., 2020). Carter et al. observed decreased helper ($CD4^+$), cytotoxic ($CD8^+$), and γδ T cells in the acute phase of MIS-C. Levels of CD4+CCR7+ T cells (primarily naive T cells and a small proportion of CM T cells) were high and, during the acute phase, had higher HLA-DR MFIs, which is indicative of activation. Also, γδ T cells with antiviral properties were decreased in the acute phase (Carter et al., 2020).

Noval Rivas et al. proposed a superantigen hypothesis in which SARS-CoV-2 spike protein encodes a high-affinity superantigen-like sequence motif near the S1/S2 cleavage site of the spike protein that can bind to the T-cell receptor (TCR). This may result in excessive T-cell activation and proliferation due to its similarity to superantigenic Staphylococcal Enterotoxin B (SEB) (Noval Rivas et al., 2021).

Antibodies and Immune Complexes

Autoantibodies are involved in the pathogenesis of MIS-C. Consiglio et al. detected antibodies against endoglin in MIS-C patients. Endoglin is a glycoprotein expressed by endothelial cells, and it is crucial for structural integrity and is predominantly seen in the vascular endothelium and heart muscle. They reported that autoantibodies are possibly a consequence of tissue damage due to elevated plasma endoglin levels (Consiglio et al., 2020). Autoantibodies against the MAP2K2 and three members of the Casein kinase family (CSNK1A1, CSNK2A1, and CSNK1E1) are seen explicitly in MIS-C patients. The casein-kinase 2 pathway is involved in viral replication, and antibodies produced against the component of the mentioned pathway may attribute to the development of MIS-C (Consiglio et al., 2020).

Mechanism of Organ Damage

It has been proposed that the major mechanism of organ damage in individuals with MIS-C is antigen–antibody–mediated cytokine storm (Haslak et al., 2021). The underlying mechanism of myocardial injury in patients with MIS-C is not clearly understood. However, it has been assumed that acute viral myocarditis, systemic inflammation, hypoxia stress cardiomyopathy, and, less commonly, ischemia, which is the result of coronary involvement, may play a role in causing the damage (Sperotto et al., 2021). It is also not clear how exactly MIS-C is related to neurological involvement. It has been suggested that cellular edema of neurons, which is the subsequent result of immune-mediated neuronal damage and inflammatory response, may be the cause for neurological involvement in patients with MIS-C (Lin et al., 2021). Cross-reaction of the infectious agent with ocular-specific antigens is a proposed theory explaining the pathogenesis of uveitis in children with MIS-C (Wildner and Diedrichs-Möhring, 2020).

TABLE 1 | Summary of studies on the therapeutic options for multi-system inflammatory syndrome following SARS-CoV2 infection.

Author	Year	Type of study	Patient(s)	Clinical manifestations of MIS	Intervention/drugs	Outcomes of treatment	References
Hasan et al	2021	Case series	6-male 2-female	Fever, rash, tachycardia, hypotension, abdominal pain, diarrhea, vomiting, decreased oral intake, cough, sore throat, conjunctivitis	IVIG, corticosteroids, antibiotics, anticoagulants, epinephrine/ norepinephrine, aspirin, Interleukin-1ra inhibitor	Survived	Hasan et al. (2021)
Pouletty et al	2020	Cohort	8-male 8-female	Skin rash, hands and feet erythema/oedema, conjunctivitis, dry cracked lips, cervical lymphadenopathy	Intravenous immunoglobulin, steroids, Anti–IL-1 treatment, Anti–IL-6 treatment, hydroxychloroquine	Survived	Pouletty et al. (2020)
Appleberry et al	2021	Case report	Male	Fever, seizures	Steroids, IVIG, midazolam	Survived	Appleberry et al. (2021)
Diorio et al	2020	Cohort	2-male 4-female	Fever, rash, cracked lips, conjunctivitis, myocardial dysfunction, shock, requiring intubation and vasoactive, severe abdominal pain, diarrhea	IVIG, aspirin, steroids	Survived	Diorio et al. (2020)
Abdel-Haq et al	2021	Case series	15-male 18-female	Fever, vomiting, diarrhea, abdominal pain, respiratory distress, skin rash, neck tenderness, lymphadenopathy, chest pain, hypotension, cardiac involvement, coronaries dilated, ejection fraction	IVIG, infliximab	Survived	Abdel-Haq et al. (2021)
Lee PY et al	2020	Case series	16-male 12-female	Fever, conjunctivitis, hypotension/shock, skin rash, extremity swelling/erythema, acute kidney injury	IVIG, methylprednisolone, anakinra, remdesivir, antibiotics, aspirin, enoxaparin	Survived	Lee et al. (2020)
Riollano-Cruz et al	2021	Case series	11-male 4-female	Rash, conjunctivitis, swollen hands and feet, tachycardia, hypotension, abdominal pain, emesis, diarrhea, myalgia, chest pain, nausea, sore throat, headache, neck stiffness	Vancomycin, cefepime, metronidazole, ivig, tocilizumab, enoxaparin, clindamycin, norepinephrine, vasopressin, amiodarone, lidocaine, vancomycin, meropenem, anakinra, remdesivir, linezolid, cefepime, dobutamine	Survived	Riollano-Cruz et al. (2021)
Consiglio et al	2020	Case control	8-male 3-female	Encephalitis, headache, conjunctivitis, rash, swollen hands and feet, abdominal pain, myocarditis, sore throat, lymphadenopathy, vomiting, cough	L-1RA (anakinra), hydroxychloroquine, IVIG, steroids	Survived	Consiglio et al. (2020)
Guanà, Riccardo et al	2021	Case report	7-year-old boy	Low-grade fever, conjunctivitis, gastroenteritis-like symptoms, hyperpyrexia associated with asthenia, and anorexia	2 mg/kg/day intravenous methylprednisolone for 1 week, followed by 1.5 mg/kg/day oral prednisolone for an additional 1 week	Survived	Guanà et al. (2021)
Sweeny, Katherine F et al	2021	Case report	16-year-old boy	Abdominal pain, diarrhea, hematochezia, severe active gastro-duodenitis, patchy colitis	Steroids, intravenous immunoglobulin, and infliximab	Survived	Sweeny et al. (2021)
Esteve-Sole, Ana et al	2021	Cohort	18-female 19-male	Increased gastrointestinal and neurological symptoms, increased lymphopenia and thrombopenia, and decreased neutrophilia	Intravenous immunoglobulin [IVIG], steroids, tocilizumab, anakinra	Survived	Esteve-Sole et al. (2021)

(Continued on following page)

TABLE 1 | (*Continued*) Summary of studies on the therapeutic options for multi-system inflammatory syndrome following SARS-CoV2 infection.

Author	Year	Type of study	Patient(s)	Clinical manifestations of MIS	Intervention/drugs	Outcomes of treatment	References
Almoosa, Zainab A et al	2020	Case series	5-female 5-male	High grade fever, GI symptoms, diarrhea, abdominal pain and emesis, conjunctivitis, lymphadenopathy, irritability, shock	Ventilatory support, vasoactive support, IVIG, antibiotic, steroids, antiviral (favipiravir), heparin, aspirin	8 patients survived	Almoosa et al. (2020)
J. J. Rodriguez-Smith rt al	2021	Cohort	8-female 11-male	Lymphopenia, thrombocytopenia, marked elevation of inflammatory markers, hyperferritinaemia, elevated cardiac biomarkers, and acute renal injury	Intravenous immunoglobulin, corticosteroids, IL-1 receptor antagonist anakinra	Survived	Rodriguez-Smith et al. (2021)
Rojahn, Astrid Elisabeth et al	2020	Case report	Child - unknown sex and age	Piperacillin/tazobactam and intravenous fluids, vasopressor therapy (noradrenaline)	Abdominal pain, nausea, vomiting, frontal headache, and reduced general condition	Survived	Rojahn et al. (2020)
Karthika IK et al	2021	Case report	14-year-old girl	Headache, fever, bilateral uveitis, unilateral cervical lymphadenopathy, oral mucosal changes, and abdominal pain	Intravenous immunoglobulin (IVIG) and oral steroids	Survived	Karthika et al. (2021)
Shahein AR et al	2021	Case report	6-year-old boy	Phlegmonous ileocolitis, myocarditis, shortness of breath, fatigue, tachypnea	Intravenous ceftriaxone and metronidazole, immunoglobulin, and methylprednisolone	Survived	Shahein et al. (2021)
Garcia-Dominguez M et al	2020	Case series report	1 boy and 3 girls	Fever, gastrointestinal involvement, general malaise, asthenia, and adynamia	Vasoactive therapy, fluid resuscitation, and also, 3 of them received IVIG	Survived	Garcia-Dominguez et al. (2020)
Balasubramanian S et al	2020	Case report	8-year-old boy	Fever, respiratory symptoms	IVIG, tocilizumab, ceftriaxone, and azithromycin	Survived	Balasubramanian et al. (2020)
Whittaker et al	2020	Case series	58 children (20 female)	Fever, vomiting, abdominal pain, diarrhea, rash, conjunctival injection, inflammation, myocardial injury, shock, and coronary artery aneurysms	Inotropic support, IVIG, corticosteroid, anakinra, infliximab	57 patients survived	Whittaker et al. (2020)
Dufort et al	2020	Case report	95 patients (53 male)	Fever, chills, tachycardia, gastrointestinal symptoms, rash, conjunctival injection, mucosal changes	Vasopressor support, ICU, mechanical ventilation, IVIG, systemic glucocorticoids, vasopressor support, echocardiogram	94 patients survived	Dufort et al. (2020)
Godfred et al	2020	Case report	570 patients (254 female)	Fever, rash, conjunctivitis, peripheral edema, gastrointestinal symptoms, shock, and elevated inflammation markers, cardiac damage	IVIG, steroids, antiplatelet and anticoagulation and vasoactive medication, respiratory support, ventilation, dialysis, immune modulators	560 patients survived	Godfred-Cato et al. (2020)

COVID-19 activates the thrombosis cascade through different mechanisms. It also leads to overexpression of PAI-1 (Ahmed et al., 2020). It is found that overexpression of PAI-1 is related to coronary artery aneurysm development in patients with KD (Senzaki et al., 2003), which can explain the risk of development of the condition on follow-up of patients with MIS-C (Patnaik et al., 2021).

Treatment

The aim of the multidimensional approach toward MIS-C's treatment is to modulate cytokine storm and inflammation. Treatment for fever, dehydration, stress ulcer prophylaxis, and hypercoagulability are considered the standard treatment choices (Beroukhim and Friedman, 2020). **Table 1** summarizes the findings of studies on the potential treatments for MIS following COVID-19.

Fluid Resuscitation and Antibiotic

It is vital to administer antibiotic and hydrate the patients who are septic and hypotensive during physical exam for MIS-C. Furthermore, it is sometimes necessary for the patients to receive inotropes until bacterial infection has been ruled out (Jiang et al., 2020a).

Intravenous Immune Globulin (IVIG)

IVIG is considered a first-line treatment in patients with KD and can reduce the risk of coronary artery lesion (CAL) (Newburger et al.,

TABLE 2 | Summary of molecular mechanisms of and therapeutic options for autoimmune diseases following SARS-CoV2 infection/vaccination.

Diseases	Molecular mechanisms	Therapeutic option	References
Mucous membrane pemphigoid (MMP)	• Subepidermal autoimmune blistering disease • Oral-pharyngeal erosions • Severe ocular scarring	• Methylprednisolone • Dapsone • Azathioprine	Drenovska et al. (2021)
Autoimmune bullous dermatoses (AIBD): Pemphigus vulgaris (PV)	• Autoantibodies (desmoglein 1 and desmoglein 3) • Painful mucosal and cutaneous erosions • Flaccid bullae	• Systemic corticosteroids • Antibiotics • Acyclovir • IVIG	Drenovska et al. (2021)
Autoimmune bullous dermatoses (AIBD): Bullous pemphigoid (BP)	• Subepidermal autoimmune blistering disease • Pruritus and blister formation on an erythematous base	• Topical and systemic corticosteroids • IVIG • Deoxycycline and dapsone	Drenovska et al. (2021)
Long COVID: an estrogen-associated autoimmune disease	• Autoantibodies • Sex hormones	• Personalized medicine based on the sex and appearance of autoantibodies	Ortona et al. (2021)
Myasthenia gravis	• Autoantibodies against acetylcholine receptor • Increased inflammatory markers: interleukin-6, CRP, ferritin, fibrinogen, D-dimer • MG composite score = 3	• Pyridostigmine bromide • Prednisone • IVIG	Restivo et al., 2020; Sriwastava et al. (2021b)
Hashimoto's thyroiditis	• Increased TSH and thyroid peroxidase antibody levels • Low free thyroxine	• Levothyroxine	Tee et al. (2021)
Systemic lupus erythematosus (SLE)	• Elevation of LDH, CRP, and ferritin • Proteinuria • Thrombocytopenia • Motor and sensory polyneuropathies • Pleural effusion • Low complement • Increased anti-La/SSB, anti-SSA/Ro, anti-cyclic citrullinated peptides (anti-CCP) and anti-double-stranded deoxyribonucleic acid antibody (anti-dsDNA) antibodies, anticardiolipin immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies, anti-β2-glycoprotein I IgG, and immunoglobulin A • (IgA) antibodies • Lupus nephritis class I	• Methylprednisolone • Hydroxychloroquine • Cyclophosphamide • Gabapentin • Vitamin B	Bonometti et al. (2020); Slimani et al., 2020; Zamani et al. (2021)
Varicella-like rash	• Mild thrombocytopenia • Pathophysiological mechanism remains unknown	• No specific treatment	Genovese et al. (2020); Slimani et al. (2020)
Graves' disease	• Suppressed TSH • Free thyroxine (normal/increased) • Elevated free triiodothyronine • + TSH receptor antibodies • + Thyroperoxidase and thyroglobulin antibodies • Increased thyroid iodine uptake	• Thiamazole • Propranolol	Mateu-Salat et al. (2020)
Generalized pustular psoriasis	• Mild hypocalcemia • Neutrophilia • Elevation of creatinine level	• Oral acitretin • Tapered oral prednisolone	Shahidi Dadras et al. (2021)
Autoimmune disease following Covid-19 vaccination			
Autoimmune hepatitis	• Biopsy consistent with autoimmune hepatitis • ALT/AST increasing in blood	• Prednisolone	Bril, (2021)

TSH = thyroid-stimulating hormone, RBC= red blood cell, LDH= Lactate Dehydrogenase, MG= myasthenia gravis, ALT= alanine aminotransferase, AST= aspartate aminotransferase

2004). The mechanism of IVIG is not fully understood. However, it has been suggested that IVIG is involved in the blockage of the Fc receptor, neutralization of pathogenic products, immune-modulation, regulation of T-cell activity, and cytokine production (Kuo et al., 2016). Therefore, the American Academy of Pediatrics and the American Heart Association guideline suggested the use of high doses of immunoglobulins (2 g/kg) within 8–12 h with high doses of aspirin for the treatment of KD (Newburger et al., 2004). Several studies reported the use of IVIG for the treatment of MIS-C either alone or in combination with other therapies (Consiglio et al., 2020; Diorio et al., 2020; Garcia-Dominguez et al., 2020; Kaushik et al., 2020; Appleberry et al., 2021; Hasan et al., 2021; Jonat et al.,

2021; Karthika et al., 2021; Shahein et al., 2021; Yasuhara et al., 2021). For example, in a study by Pouletty et al. on 16 patients with MIS-C, 15 patients received IVIG, and only 5 (31%) showed remission after single IVIG treatment and others required second-line treatment (Pouletty et al., 2020).

IL-6 Inhibitors

Tocilizumab is a recombinant humanized monoclonal IgG1κ antibody and binds to IL-6R and inhibits cis-signaling, trans-signaling, and trans-presentation by preventing IL-6 attachment. Tocilizumab has been approved for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis (Pelaia et al., 2021). In addition, a systematic review and meta-analysis by Wei et al. on 26 studies showed that tocilizumab is associated with a lower risk of mortality and the need for mechanical ventilation in COVID-19 patients (Wei et al., 2021).

In a case report of a child with hyperinflammatory syndrome and COVID-19, tocilizumab (8 mg/kg IV over 2 h) was used 72 h after IVIG infusion, resulting in settled fever spikes and reduction of inflammatory parameters to normal (Balasubramanian et al., 2020a).

IL-1 Inhibitors

IL-1 may play a significant role in the MIS-C pathology. IL-1α and IL-1β are the two cytokines which mediate inflammatory response to lung injury. Inflammation is caused by the production of IL-1α by injured epithelium and endothelial tissues, while IL-1β is released by invading myeloid cells. The IL-1 receptor antagonist (IL-1Ra) is the main mechanism that prevents excessive inflammation caused by either cytokine. IL-1Ra inhibits the receptor that transmits the pro-inflammatory effects of both IL-1α and IL-1β. Anakinra is the recombinant form of the naturally occurring IL-1 receptor antagonist (IL-Ra), which prevents the binding of IL-1α and IL-1β to IL-1R (Cavalli and Dinarello, 2018; Pasin et al., 2021). A meta-analysis by Pasin et al. involving a total of 184 COVID-19 patients showed that anakinra was associated with decreased mortality rate and requirement of mechanical ventilation (Pasin et al., 2021). Lee et al. used anakinra (doses ranging from 5 to 13 mg/kg/day) in five patients with MIS-C and reported that adding anakinra was associated with improvement of the inflammatory process in patients. Clinical improvement was seen in all cases, with resolution of fever, cessation of inotrope treatment, and improvement of inflammatory markers. CRP, d-dimer, and ferritin were also decreased (Lee et al., 2020).

TNF Inhibitors

Infliximab is a recombinant DNA-derived chimeric human-mouse IgG monoclonal antibody, which binds to the soluble and membrane form of TNF and blocks TNF signaling and biological activities (Guo et al., 2013). Administration of infliximab was associated with the reduction of inflammatory markers and cytokine concentrations in patients with COVID-19 (Robinson et al., 2020).

Dolinger et al. reported a child with Crohn's disease, MIS-C, and COVID-19 treated with infliximab (10 mg/kg). The treatment resulted in resolved fever, tachycardia, and hypotension within hours. Also, IL-6 and IL-8 concentrations decreased with TNF-α normalization (Dolinger et al., 2020).

Abdel-Haq et al. used high-dose infliximab (10 mg/kg) as the second-line treatment in 12/13 patients with MIS-C. The results showed that infliximab was associated with the resolution of fever, improvement of cardiac function, and improvement of coronary artery dilatation (Abdel-Haq et al., 2021).

Corticosteroids

Various studies reported using corticosteroids, including methylprednisolone, prednisolone, hydrocortisone, and dexamethasone for the treatment of MIS-C (Godfred-Cato et al., 2020; Jiang et al., 2020a; Cheung et al., 2020; Diorio et al., 2020; Lee et al., 2020; Panigrahy et al., 2020; Pouletty et al., 2020; Zou et al., 2021).

Anticoagulation

Low dose of aspirin (3–5 mg/kg/day; maximum 81 mg/day) should be used in MIS-C patients and continued until the platelet count is normalized and normal coronary arteries are confirmed at ≥4 weeks subsequent to diagnosis. The treatment should be avoided in patients with active bleeding, significant bleeding risk, and/or a platelet count of ≤80,000/μl. It is also highly recommended to prescribe enoxaparin for patients who suffer from coronary artery aneurysms with a z-score of ten or higher, patients with an ejection fraction less than 35%, and patients with a documented thrombosis. In other patients, decisions about prescribing anticoagulation should be made based on the individual risk factors of patients (Henderson et al., 2021).

Supportive Care

Patients with MIS-C who rapidly deteriorate need more intensive care. Extra corporeal membrane oxygenation is such a piece of equipment which may need to be used (Belhadjer et al., 2020).

Figure 1 summarizes the underlying mechanisms of post COVID-19 MIS-C.

Post COVID-19 Multisystem Inflammatory Syndrome in Adults (MIS-A)

MIS can occur at any age and can arise synchronously with SARS-CoV-2 infection or as a postinfectious phenomenon (Dabas et al., 2021). MIS is a new disease related with SARS-CoV-2 that has also been seen in adults (Mieczkowska et al., 2021). According to CDC, MIS-A is defined by the following criteria:

(1) severe illness requiring hospitalization in a person aged ≥21 years;
(2) a positive test result for current or previous SARS-CoV-2 infection at admission or during the previous 12 weeks;
(3) severe dysfunction of one or more extrapulmonary organ systems;
(4) laboratory evidence of severe inflammation; and
(5) absence of respiratory illness.

Molecular Mechanisms

From a pathophysiological perspective, virus-infected cells undergo pyroptosis. This process involves the release of both

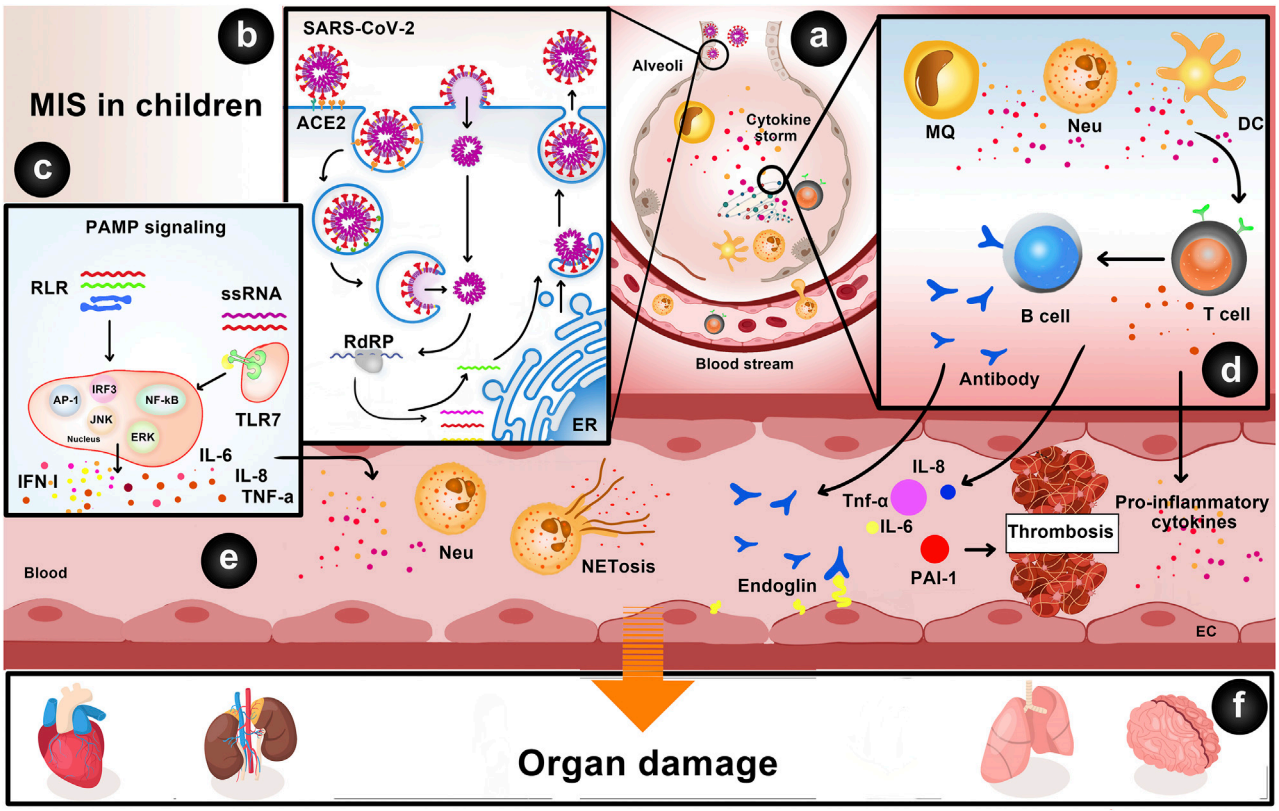

FIGURE 1 | Underlying mechanisms of multisystem inflammatory syndrome in children. **(A,B)** The binding of SARS-CoV-2 spike protein to ACE2 receptors on respiratory epithelial cells leads to its cell entry and viral genome replication via RNA-dependent RNA polymerase (RdRP). Membrane-bound immunologic receptors and downstream signaling pathways mediate the pro-inflammatory response resulting in cytokine storm through the infiltration of neutrophils and macrophages into the lung tissue. **(C)** SARS-CoV-2 viral genomic ssRNA or other RNA compositions may act as PAMPs and bind to TLRs and RLRs, leading to the activation of IRF3, NF-κB, MAPK/ERK, AP-1, and JNK signaling pathways and secretion of IL-6, IL-8, TNF-α, and IFN-I. **(D)** Cytokines produced by macrophages, neutrophils, and dendritic cells cause T cell–dependent activation of B cells and production of antibodies and autoantibodies (e.g., anti-endoglin). **(E)** Neutrophils can form NETs, which are released subsequent to the rupture of the plasma membrane. NETs, IL-6, IL-8, TNF-α, and PAI-1 induce endothelial damage and thrombosis. **(F)** Cytokine storm, autoantibodies, and activation of immune cells lead to multiple organ damage, including the lungs, brain (e.g., cellular edema of neurons), heart (e.g., acute viral myocarditis), and kidneys (e.g., acute kidney injury).

cellular and viral components collectively called DAMPs and PAMPs (Freeman and Swartz, 2020; Shenoy, 2020). In the context of acute illness, DAMPs and PAMPs are recognized by the components of the innate immune system and the antigen-presenting cells (APCs) resulting in the release of interleukin (IL)-1β from the activated neutrophils and macrophages (Leyfman et al., 2020). IL-1β initiates local inflammation, which further stimulates the recruitment and activation of neutrophils, lymphocytes, and macrophages that release cytokines such as IL-6, interferon-γ, inducible protein-10, and monocyte chemoattractant protein-1 (MCP-1) (Leyfman et al., 2020). Pulmonary tissue damage secondary to SARS-CoV-2 pneumonia also contributes to asymptomatic or minimally symptomatic hypoxemia (Galwankar et al., 2020), which itself is a potent inducer of IL-6 and other cytokines (Leyfman et al., 2020). All these processes combine to produce a CRS potentiating the inflammatory damage in the lungs, kidneys, heart, brain, and gastrointestinal tract and leading to AMIS-COVID-19 (Leyfman et al., 2020). Nuclear factor-kappa B (NF-κB) plays an important role in cellular synthesis and development of CRS (Hirano and Murakami, 2020). Inactivation of NF-κB has been shown to effectively dampen CRS and prevent the development of AMIS-COVID-19 (DeDiego et al., 2014). The first step in the COVID-19–related inflammatory cascade is IL-1β production that is initiated upon recognition of PAMPs and DAMPs by a multiprotein cytosolic complex called the inflammasome. The inflammasome activation is known to be inhibited by colchicine, an agent used to treat acute attacks of gout and familial Mediterranean fever (Molad, 2002; Leung et al., 2015).

Although the exact mechanism of MIS-A is unknown, it appears by reason of a delay in the cytokine storm associated with the initial infection (Parpas et al., 2021). Viral infections are caused by a mechanism considered antibody-dependent enhancement (ADE), which increases the level of neutralizing antibodies and may lead to pathogenicity, while SARS-CoV-2 antibodies are supposed to be have protective and neutralizing properties. The exact

cause of MIS-A is not known, but it may be due to a malfunction of the innate and adaptive host immune system that causes multi-organ failure, or it may be by reason of homology between SARS-CoV-2 spike protein and staph enterotoxin B super-antigen structure and sequence resulting in a hyper-inflammatory state (Al-Falahi et al., 2021).

Treatment

Until now, there have been no widely accepted guidelines for the ideal therapeutic approach to adults with MIS. According to pathophysiological and clinical resemblance between MIS and incomplete KD and the effectiveness of IVIG in TSS, the literature indicates that MIS similar to KD should be cured with immune-modifying agents, first-line glucocorticoids, and IVIG to invert the inflammatory response. Moreover, current reports indicate that the combination of IVIG and steroid therapy may have better outcomes for treatment than IVIG monotherapy in KD (Henderson et al., 2020). Consequently, the value of glucocorticoids as first-line treatment for hyperinflammatory syndromes remains incontestable, despite the role of biologicals remaining unclear so far. Despite the fact that the exact efficiency in the long term remains unclear, patients in limited case series demonstrate hopeful results (Godfred-Cato et al., 2020; Jiang et al., 2020; Dufort et al., 2020; Henderson et al., 2020; Riphagen et al., 2020; Verdoni et al., 2020; Whittaker et al., 2020). The suggested treatment guideline for patients identified by MIS related to SARS-CoV-2, according to The American College of Rheumatology, consists of the following.

IVIG

The suggested dose of IVIG for patients with KD-like features is similar to what is used for KD, 2 g/kg (up to 70–80 g) body weight over a period of at least 8–12 h. In patients who have no or poor response, the administration of a second dose of IVIG can be regarded (Henderson et al., 2020).

Glucocorticoids

Severe cases with cardiac involvement, TSS, or hemophagocytic lymphohistiocytosis (HLH)-like course of the disease should be treated with a combination of IVIG and high doses of glucocorticoids. For the suggested dose of glucocorticoids, methylprednisolone during the early life-threatening stage in a regimen of 1 mg/kg body weight daily, or in more severe cases based on clinical characteristics and laboratory findings, methylprednisolone 30 mg/kg pulse therapy once daily during 1–3 days, and in cases with secondary HLH or central nerve system involvement, dexamethasone 10 mg/m2 once daily seems to be helpful. When the disease has reached the final stages and the patient is going to be dismissed from hospital, the oral dose of prednisolone can be decreased over a period of weeks to minimize the risk of relapse. The supporting evidence for using immune-modifying therapy is from previous case series, describing similar patient populations in the same health conditions, like KD, HLH, and TSS. In these case series, 75% of the cases were treated alike with IVIG, and they demonstrated clinical and cardiac recovery after treatment (Godfred-Cato et al., 2020; Dufort et al., 2020; Riphagen et al., 2020; Verdoni et al., 2020; Whittaker et al., 2020). In other limited case series, about 55% of the patients were treated with glucocorticoids in different doses. Before administrating IVIG in these patients, it is essential to obtain blood for blood cultures in analysis of possible pathogens and serologic SARS-CoV-2 test.

Biologicals

The biological anakinra (interleukin-1 receptor antagonist) is advised for patients with uncontrolled growing disease activity, severe secondary HLH, or shock by cardiac involvement in spite of the started therapeutic process according to steps 1 and 2—suitable because of its safety profile and short half-life.

Downing, S., et al. (2020) reported a case of successful combined pharmacotherapy for a patient with MIS-A and COVID-19 using colchicine, aspirin, and montelukast. The studied patient indicated remarkable recovery within 24 h of the starting of a colchicine-based regimen. Aspirin is the second in the suggested regimen that inhibits COX1&2 irreversibly and acts as an anti-inflammatory drug. The antiplatelet action of aspirin is mediated through the deterrence of TXA2 generation. Aspirin also has demonstrated antiviral effects against RNA viruses of the respiratory tract including influenza A viruses and rhinoviruses by its action on the regulation of the NF-κB pathway (Kopp and Ghosh, 1994; Yin et al., 1998; Glatthaar-Saalmüller et al., 2017). The third agent is montelukast, a cysteinyl leukotriene 1 receptor antagonist that is used to decrease bronchial inflammation in asthma (Pizzichini et al., 1999). Montelukast can also regulate the generation of IL-6, TNF-α, and MCP-1 through deterrence of NF-κB (Maeba et al., 2005). Montelukast may also have a direct antiviral effect on the SARS-CoV-2 main protease enzyme. Computer modeling studies propose that montelukast should have high-affinity binding to the active pocket of the main protease enzyme (Wu et al., 2020). Consequently, montelukast may have a bimodal action as a leukotriene antagonist and a protease inhibitor (Downing et al., 2020).

Autoimmune Diseases Following COVID-19 Infection

Autoimmune diseases constitute a wide range of diseases characterized by the disruption of tolerance to self-antigens resulting in pathological changes and disruption of the target tissue's function. Both genetic and environmental factors can trigger autoimmune diseases. Environmental factors include nutrition, microbiota, infections, xenobiotics, pharmaceutical agents, hormones, ultraviolet light, silica solvents, collagen or silicone implants, heavy metals, and vaccines (Wang et al., 2015). In addition, viruses are a major environmental factor associated with autoimmune diseases such as autoimmune hepatitis (Epstein–Barr virus), autoimmune myocarditis (Coxsackie virus), GBS (Zika virus), and multiple sclerosis (Epstein–Barr virus, Theiler's virus, Varicella-zoster virus, Measles virus, and Cytomegalovirus) (Smatti et al., 2019).

It has been suggested that SARS-CoV-2 can trigger autoimmune diseases. In a systematic review of 64 articles, Shaikh et al. concluded that GBS is recognized as one of the presentations of the COVID-19 disease (Sheikh et al., 2021). In a systematic review by Saad et al. about 33 patients with

autoimmune diseases after COVID-19, there were sixteen cases of GBS, eight cases of autoimmune hemolytic anemia, three cases of ITP, two cases of KD, and one case of subacute thyroiditis. They concluded that COVID-19 is involved in the development of various autoimmune diseases (Saad et al., 2021). In a review of 57 patients, Alonso-Beato et al. reported that ITP could occur both in mild and severe COVID-19 and during the course of the disease, and patients showed higher bleeding rates than in other ITP series (Alonso-Beato et al., 2021). In a prospective cohort study, Garjani et al. reported that in 57% of the patients (230 of 404 patients), COVID-19 infection leads to exacerbation of multiple sclerosis (MS) symptoms (Garjani et al., 2021). Also, several studies reported SLE following COVID-19 infection (Bonometti et al., 2020; Slimani et al., 2021; Zamani et al., 2021; Gracia-Ramos and Saavedra-Salinas, 2021).

The mechanism of post-COVID-19 autoimmunity is unclear; however, several factors may attribute to the condition. The third phase of COVID-19 infection is associated with acute respiratory failure, shock, immunothrombosis, multiorgan dysfunction or failure, and death (Berlin et al., 2020). The immune response in the third phase is characterized by hyperinflammation and cytokine storm (Wilson et al., 2020). The hyperinflammation mechanism can be associated with defects in type 1 and 3 IFN and dysregulated innate and adaptive immunity and can be stimulated by pathogen-driven factors and damage-associated markers (Winchester et al., 2021). A recent study by *Pan* et al. showed that SARS-CoV-2 N protein (involved in virus replication, assembly, and immune regulation) could interact with NLRP3 inflammasome resulting in cytokine storm and lung damage in mice (Pan et al., 2021).

Autoantibodies can be involved in the autoimmunity following COVID-19 infection. Several studies reported the presence of autoantibodies such as antinuclear antibodies (ANA), anti-SSA/Ro antibodies, and anti-IFN-I antibodies (Bastard et al., 2020; Zhou et al., 2020b). Antiphospholipid antibodies such as lupus anticoagulant, anti-cardiolipin, and anti-β2-glycoprotein I are correlated with immunocoagulopathy and thrombosis. They are detected in several cases of COVID-19 (Abdel-Wahab et al., 2016; Harzallah et al., 2020). Zuo et al. suggested that higher levels of antiphospholipid antibodies are related to the release of NETs, higher platelet counts, and more severe respiratory symptoms. They also reported that IgG extracted from patients with the antiphospholipid syndrome (APS) can initiate NET release from isolated neutrophils, and injection of the IgG to mouse models promotes venous thrombosis (Zuo et al., 2020). **Table 2** summarizes the potential molecular mechanisms of and therapeutic options for post-COVID-19 autoimmune diseases.

Similarities and Differences Between MIS-C and Autoimmune Diseases

Autoantibodies are involved in the pathogenesis of various autoimmune diseases such as Graves' disease, myasthenia gravis, and SLE (Ludwig et al., 2017). As mentioned earlier, MIS-C patients show elevated levels of autoantibodies against endoglin, MAP2K2, and three Casein kinase family members, including CSNK1A1, CSNK2A1, and CSNK1E1 (Consiglio et al., 2020).

Moreover, a recent study identified 189 and 108 peptide candidates for IgG and IgA autoantigens, respectively. Some of the significant autoantibodies in MIS-C patients were anti-La (also seen in SLE and Sjogren's disease) and anti-Jo-1 (also seen in idiopathic inflammatory myopathies) (Gruber et al., 2020). Also, Porritt et al. identified eight autoantigens in MIS-C that were previously seen as autoantigens in autoimmune diseases, including TROVE2 (SLE and Sjogren's syndrome), KLHL12 (primary biliary cirrhosis and Sjogren's syndrome), HK1 (primary biliary cirrhosis), ATP4A (type I diabetes and corpus atrophic gastritis), and FAM84A (inflammatory bowel disease) (Porritt et al., 2021). Bastard et al. reported that at least 10% of the patients with severe COVID-19 have autoantibodies against type I IFNs. Neutralizing autoantibodies against type I IFNs are also seen in patients of autoimmune polyendocrinopathy syndrome type I and SLE and may attribute to the pathogenesis of MIS-C (Bastard et al., 2020). However, several autoantigens in MIS-C were not associated with autoimmune diseases, and some had a tissue-specific expression, including endothelial and cardiac tissue (P2RX4, ECE1, and MMP14) and gastrointestinal tract (MUC15, TSPAN13, and SH3BP1) (Gruber et al., 2020).

The immune cell profiles of MIS-C patients show characteristics like autoimmune diseases. For example, Expansion CD11c + B cells expressing TBX1, along with decreased expression of CXCR5, CD21, CD24, and CD38, are seen in patients with MIS-C and SLE (Hoste et al., 2022). Also, Porritt et al. reported a high expression of IGHV4-39, a gene associated with autoreactive B cells, in an RNA cluster of MIS-C patients. IGHV4-39 expression was previously seen in autoreactive B cells of MS patients (Porritt et al., 2021). Furthermore, Ki67 + $CD4^+$ T cells with high expression of ICOS, PDCD1, MAF, and IL-21 and low CXCR5 expression are seen in MIS-C and rheumatoid arthritis (Rao et al., 2017; Ramaswamy et al., 2021). Neutrophils are involved in various autoimmune diseases such as MS, SLE, inflammatory bowel disease, rheumatoid arthritis, and type I diabetes (Wang et al., 2018). Similarly, in MIS-C patients, FcγR and complement pathways of neutrophil activation are seen along with pathogenetic mechanisms such as NETosis, tissue damage caused by ROS and protease production, and cytokine and chemokine expression (Porritt et al., 2021).

Activation of complement system proteins by autoantibodies can result in leukocyte activation, cytotoxicity, and tissue damage, and they play a significant role in the pathogenesis of autoimmune diseases (Thurman and Yapa, 2019). Severe MIS-C patients show high expression of C1qA, C1qB, and C1qC involved in classical complement activation mediated by the abundance of autoantibody immune complexes (Porritt et al., 2021). However, in SLE, C1q deficiency is considered a risk factor that leads to reduced efficiency of apoptotic cell removal. Also, anti-C1q autoantibodies are seen in SLE patients and amplify local complement activation (Trouw et al., 2017).

Similarities Between COVID-19 Infection and Autoimmune Diseases

There are some similarities in the pathogenesis and treatment of COVID-19 infection and autoimmune diseases. COVID-19 infection can result in cytokine storm with increased IL-1b,

IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, G-CSF, GM-CSF, IFN-γ, TNFα, IP10, MCP1, MIP1A, and MIP1B (Huang et al., 2020). In SLE and COVID-19 patients, increased IL-17 induces G-CSF resulting in kidney tissue damage. Also, IL-22 produced by Th17 cells is associated with SLE and COVID-19 pathogenesis by regulating antiapoptotic proteins, serum amyloid A (SAA) level, and fibrinogen production (Tse et al., 2004; Wu and Yang, 2020). Increased SAA is associated with higher COVID-19 severity and mortality, so anti-SSA drugs can be beneficial (Chen et al., 2020; Zinellu et al., 2021). Procoagulant changes and abnormal coagulation tests are seen in COVID-19 and SLE patients, which can be associated with the role of IL-6 in increasing fibrinogen levels (Hadid et al., 2021).

Also, Woodruff et al. focused on similarities between B-cell immunophenotypes in COVID-19 infection and SLE. They characterized a specific type of IgD and CD27 double-negative (DN) B cells in SLE that correlated the CXCR5-CD21^{-}CD11c+ (DN2) group with disease severity (Woodruff et al., 2020). These B cells have extra-follicular that is consistent with the study of Kaneko et al., reporting the loss of germinal centers in the lymph nodes and spleens of acute COVID-19 patients as a result of irregular TNF production (Kaneko et al., 2020). In addition, the cell population is correlated with elevated inflammatory markers such as IL-6 and CRP and can produce autoantibodies (Woodruff et al., 2020).

ACE2, which converts angiotensin II to angiotensin-1-7, is a receptor of SARS-CoV-2 on epithelial cells (Rezaei et al., 2021). The binding of angiotensin II to the AT1 receptor triggers increased oxidative stress, inflammation, fibrosis, and vasoconstriction; however, angiotensin-1-7 stimulates vasodilation, antioxidant, and antiproliferative effects (Beyerstedt et al., 2021). Monteil et al. showed that human recombinant soluble ACE2 (hrsACE2) could protect from lung injury and prevent SARS-CoV-2 from entering the cell (Monteil et al., 2020). Inflammation in RA patients may lead to the involvement of endothelial cells and the development of atherosclerotic lesions, so lack of angiotensin II production by inhibiting ACE, an enzyme converting angiotensin I to angiotensin II, can improve vascular endothelial function (Sattar et al., 2003).

MS patients show elevated inflammatory cytokines such as IFN-γ, IL-12, TNF-α, and IL-17, along with the migration of Th1 and Th17 across the blood–brain barrier (Maxeiner et al., 2014). IL-6 and TGF-β stimulate STAT3 activation, leading to Th17 activation along with IL-17 and IL-21 secretion (Yang et al., 2007). MS patients have higher levels of osteopontin (OPN) in serum and cerebrospinal fluid (CSF). OPN is produced by T cells, B cells, macrophages, NK cells, dendritic cells, and neutrophils and acts as a pro-inflammatory cytokine by inducing the production of IL-12 in macrophages and IFN-γ in T cells and inhibition of IL-10 production in macrophages (Niino and Kikuchi, 2011). Also, increased IFN-γ and IL-12 lead to the upregulation of Th1 cells in the brain (Khaibullin et al., 2017). Th17 cells and IL-17 play an essential role in the pathogenesis of COVID-19 and MS (Dos Passos et al., 2016; Martonik et al., 2021).

Due to the similarities in pathogenesis, related therapeutic options can be proposed. For instance, fedratinib is a JAK2-specific inhibitor that inhibits Th17 cytokine production. Anifrolumab is an IFN-I signaling inhibitor that showed promising results in SLE patients. Also, chloroquine and hydroxychloroquine can bind to the virus and interfere with the glycosylation of the ACE2. Also, ACE inhibitors such as quinapril can inhibit TNF-α production and has an anti-inflammatory effect (Najafi et al., 2020).

Autoimmune Bullous Dermatoses (AIBDs)

AIBDs include heterogeneous disorders which mainly consist of two groups of disorders: the subepidermal pemphigoid group which is named bullous and mucous membrane pemphigoid (MMP), and the intraepidermal pemphigus group which is named pemphigus vulgaris (PV) and foliaceus. These disorders have a common clinical feature, which is skin and mucosal blistering resulting in significant cutaneous damage accompanied by vast erosion formation (Drenovska et al., 2021). It is assumed that the underlying mechanisms in COVID-19, such as acute respiratory distress syndrome, extensive lung damage, and cytokine release storm, such as IL-1, IL-6, and TNF-α leading to interstitial pulmonary inflammation (Wang et al., 2020), could have impacts on these autoimmune disorders.

PV, as an autoimmune bullous disease affecting the mucosa and skin, is induced by autoantibodies against desmoglobin 1 and desmoglobin 3, which are adhesion proteins of the epidermis (Stanley and Amagai, 2006; Abdollahimajd et al., 2020). An approach for treatment of new cases of PV who are proven cases of COVID-19 has been suggested by Abdollahimajd et al. (Abdollahimajd et al., 2020). Intralesional or topical corticosteroid and dapsone should be considered in mild cases of PV (Abdollahimajd et al., 2020). IVIG as a therapeutic option could be administrated in severe cases of PV (Brown et al., 2018; Abdollahimajd et al., 2020). Not only may IVIG be the safest immunomodulatory for the long term in all age groups (Zhang and Liu, 2020) but it has also been suggested as a therapeutic option for COVID-19 (Wang et al., 2020). In cases with unavailability or unaffordability of IVIG, rituximab, which is a chimeric mouse/human anti–CD-20 monoclonal antibody, should be considered with patient monitoring and caution (Abdollahimajd et al., 2020).

Bullous pemphigoid (BP) is considered as subepidermal autoimmune disease, which is characterized by blister formation on an erythematous base and pruritis (Schmidt and Zillikens, 2013). Drenovska et al. reported a suspicious case of COVID-19 presented with newly diagnosed BP. He received a treatment course of systemic and topical corticosteroid which resulted in rapid control of BP (Drenovska et al., 2021). It should be considered that topical corticosteroids are safer than systemic therapy, particularly for extensive forms of BP (Joly et al., 2002). It has also been suggested to administer IVIG for parallel management of the conditions in BP-COVID-19 cases (Drenovska et al., 2021). In addition, doxycycline together with dapsone was effective in both COVID-19 and BP (Farouk and Salman, 2020).

Myasthenia Gravis

Myasthenia gravis (MG) is one of the most common autoimmune disorders induced by autoantibody production against nicotinic

acetylcholine receptors (AchRs) at the neuromuscular junction (Meriggioli and Sanders, 2009; Hübers et al., 2020). It has been proposed that an inflammatory reaction to a virus, as an external agent, can induce antibody production which also induces a triggered immune response. Cross-reaction of this immune response with the AchRs can happen due to molecular mimicry, which may lead to damage. It has been revealed that SARS-CoV-2 has affinity to ACE2 receptors, resulting in the autoantibody formation and an inflammatory cascade (Baig et al., 2020). These receptors are expressed in many organs, such as the kidneys, liver, and lungs. This will cause chemokine and proinflammatory cytokine production along with T and B cell depletion accompanied by high levels of TNF-α and interleukins which are associated with disease severity (Baig et al., 2020). Sriwastava et al. reported a positive case of COVID-19 who presented with left eye diplopia and fatigable ptosis. A combination of laboratory investigations, findings from history, and electrodiagnostic testing confirmed the diagnosis of MG. She received a course treatment of 60 mg of pyrodostigmine every 6 hours, which was followed by subjective improvement of her ptosis and diplopia (Sriwastava et al., 2021a).

Hashimoto's Thyroiditis

Hashimoto's thyroiditis, characterized by thyroid-specific autoantibodies, is considered as one of the most common autoimmune disorders (Ralli et al., 2020). Tee et al. reported a case of COVID-19, without any personal or family history of thyroid or autoimmune disease, who complained of muscle weakness and severe acute-onset generalized fatigue after resolution of the respiratory symptoms. Laboratory findings confirmed the diagnosis of Hashimoto's thyroiditis. After receiving levothyroxine 25 mcg/day for 5 weeks, his symptoms have been resolved (Tee et al., 2020).

Systemic Lupus Erythematosus (SLE)

SLE is a member of autoimmune diseases associated with production of pathogenic autoantibodies and involvement of multiple organs (Zamani et al., 2021).

Several studies reported SLE following SARS-CoV-2 infection. For example, Zamani et al. reported a case of a patient who developed SLE, 2 months after SARS-CoV-2 infection (Zamani et al., 2021). Therapy in the patient was with methylprednisolone (1,000 mg for three consecutive days), hydroxychloroquine and prednisolone (30 mg daily), IV cyclophosphamide (1,000 mg monthly), gabapentin, and vitamin B (300 mg daily), which significantly improved the health status of the patient. At first, treatment with methylprednisolone pulse (1,000 mg) was performed for three consecutive days, and then hydroxychloroquine and prednisolone (30 mg per day) were prescribed to the patient. As a result of this treatment, platelets were reduced to 100,000/mm3 and hemoglobin to 11 g/dl, but paresthesia, proteinuria, and edema persisted. The patient also received monthly doses of 1,000 mg of intravenous cyclophosphamide. The patient also received monthly doses of 1,000 mg of intravenous cyclophosphamide. The patient was discharged but was nevertheless receiving hydroxychloroquine, prednisolone (10 mg dai-ly), cyclophosphamide, gabapentin, and B vitamins (300 mg daily). The patient was followed up with after 6 months. The results were as follows: paresthesia was enhanced. Laboratory tests (CBC, ESR, CRP, T3, and T4) were normal, and urine protein was 230 mg/day. The double-stranded anti-DNA antibody was decreased to the normal range (<35 IU/ml) (Zamani et al., 2021). Also, Bonometti et al. reported a case with SLE following SARS-CoV-2 infection in which treatment with piperacilline/tazobactam, steroid therapy, hydroxychloroquine, and oxygen supplementation was successfully achieved (Bonometti et al., 2020).

One possible explanation for this condition is that infection with SARS-CoV-2 causes severe immune activation, cytokine storm (upregulation in tumor necrosis factor, interferon gamma, IL-2, and other cytokines), thus indicating a form of MAS. Moreover, patients with SLE can develop cytokine storm (elevation of cytokines including TNF-α, IFN-γ, IL-1, IL-6, and IL-18) and MAS more easily (Spihlman et al., 2020).

Systemic Lupus Erythematosus and Varicella-Like Rash

Slimani et al. reported a case with no previous medical history, who was infected with SARS-CoV-2 and was diagnosed with SLE and APS and developed a COVID-19–related varicella-like rash on the trunk. The treatment included steroid therapy with methylprednisolone and a single dose of chloroquine (Slimani et al., 2021). It is hypothesized that SARS-CoV-2 infection can cause autoimmunity. Previous studies reported that viruses could cause autoimmunity by mechanisms such as molecular mimicry, epitope spreading, immortalization in infected B cells, and bystander activation. Furthermore, SARS-CoV-2 infection increases the release of various cytokines, causing a disorder in acquired and innate immune response, which might contribute to the condition. Also, it has been reported that skin symptoms are secondary immune responses to nucleotides of the virus (Slimani et al., 2021).

Graves' Disease

Grave's disease is an organ-specific autoimmune disorder in which the binding of the autoantibodies to the thyroid-stimulating hormone receptor (TSHR) increases thyroid function leading to hyperthyroidism (Davies et al., 2020).

Salat et al. reported two cases of patients who developed Grave's disease following SARS-CoV-2 infection. One of the patients had a previous history of Graves' disease and has been in remission during the past 35 years, and the other had no history of thyroid disease. Both patients showed suppressed levels of TSH and were positive for TSH receptor, thyroperoxidase, and thyroglobulin antibodies. Treatment was started with thiamazole and propranolol in both patients and resulted in improvement of thyroid function and complications (Mateu-Salat et al., 2020).

It has been suggested that the hyper-inflammation caused by severe SARS-CoV-2 infection can trigger the development of Grave's disease. SARS-CoV-2 infection leads to increased IL-6 and Th1 cytokines, whereas autoimmune response in Graves'

disease is mainly associated with Th2 cells. However, IL-6 upregulation is also seen in Grave's disease. IL-6 can exert various complex functions by interacting with cellular receptors (Jiménez-Blanco et al., 2021). Also, IL-6 can inhibit Th1 polarization and promote the Th2 response through stimulation of IL-4 secretion and inhibition of IFN-γ secretion by CD4 T cells (Velazquez-Salinas et al., 2019).

Generalized Pustular Psoriasis

Generalized pustular psoriasis is a scarce demonstration of psoriasis (chronic inflammatory disease) which could be provoked by drugs, viral infections, pregnancy, and variety of medications. Dadras et al. reported a patient with history of psoriasis in childhood who developed generalized pustular psoriasis after COVID-19 infection (Shahidi Dadras et al., 2021).

A hyperinflamatory state in SARS-CoV-2 infection can cause psoriasis manifestation. Also, hydroxychloroquine, a member of important drugs for COVID-19 therapy, can cause psoriasis or lead to lesion recurrence or exacerbation. The condition occurs because hydroxychloroquine influences cholesterol metabolism, which is essential for the skin to function as a barrier, thus leading to weakness of the surface layer of the skin and abnormal keratinocyte proliferation. Treatment for psoriasis in the patient was with systemic retinoids (Shahidi Dadras et al., 2021).

Guillain-Barre Syndrome (GBS)

GBS is a multi-form immune-mediated polyradiculoneuropathy recognized by both sensory and motor symptoms depending on the disease subtype. The most common subtype of GBS, acute inflammatory demyelinating polyradiculoneuropathy (AIDP), is typically characterized by progressive muscle weakness leading to paralysis and sensory deficits (Van den Berg et al., 2014; Willison et al., 2016). The majority of GBS incidences are due to a pre-existing infection such as cytomegalovirus (CMV), Epstein–Barr virus (EBV), Zika virus, influenza virus, and *Campylobacter* jejuni (Jacobs et al., 1998; Cao-Lormeau et al., 2016). The process of post-infection GBS remains to be fully understood; however, in the case of *Campylobacter* jejuni, it is established that the cross-reaction of host antibodies (produced against bacterial antigen) with human peripheral nerve results in neural damage (Yuki and Hartung, 2012). It is possible that this event might be true for other GBS-related pathogens in which there is a structural resemblance between foreign antigen and host nerve glycolipids since anti-ganglioside antibodies are detected in almost 60% of GBS patients (Kaida et al., 2009). In January 2020, the very first report of a patient with co-existing COVID infection and GBS was published. A 61-year-old female was admitted to the hospital with acute muscle weakness and laboratory results confirming GBS. Later, she developed COVID-19 symptoms on day 8 of GBS. Given her history of visiting Wuhan a week prior to admission and primary laboratory results (lymphocytopenia and thrombocytopenia), an asymptomatic COVID-19 infection on admission was assumed (Zhao et al., 2020). This was followed by the various case-series reports indicating a presumable relation between COVID-19 infection and GBS (Caress et al., 2020; Sriwastava et al., 2021b). To date, there is no absolute explanation for this possible link, while some theories have been proposed. The same resemblance theory is suggested; however, no homology between peripheral nerve tissue and SARS-CoV-2 has been discovered yet. Also, the detection of anti-ganglioside antibodies was uncommon in the majority of reports on COVID-associated GBS (Caress et al., 2020; Sriwastava et al., 2021b). Alternatively, some authors speculated that nerve damage may also set in due to T cell activation and cytokines released from macrophages in response to SARS-CoV-2 (Hartung and Toyka, 1990). Nevertheless, the population-based data in the United Kingdom failed to show a temporal relationship between GBS and COVID. Keddie et al. (Keddie et al., 2021) hypothesized that the lockdown policy during the pandemic and more cautious behavior may play a role in reducing the transmission of other GBS-related pathogens. In this scenario, the increase of GBS incidence in COVID-19 patients is the indirect result of a decrease in GBS cases caused by other pathogens.

Autoimmune Disease Following Vaccination

Autoimmune Hepatitis

Autoimmune hepatitis is known as a form of chronic hepatitis with an unknown cause (Krawitt, 2006). Clayton-Chubb et al. reported a case of COVID-19 vaccine–related liver injury. The patient presented with autoimmune hepatitis 26 days after the first dose of the vaccine (Oxford-AstraZeneca) injection. He received a treatment course of prednisolone 60 mg/day. After a few weeks, his general condition improved and the dosage was tapered to 20 mg/day (Bril et al., 2021). This was the first case report of autoimmune hepatitis following COVID-19 vaccination. Further investigation is still required to determine whether there is a causal relationship. Also, other factors such as drugs or toxins may contribute to the condition and their role should be considered in future studies.

Long COVID

Some COVID-19 patients, from mild to severe forms of the disease, may present with debilitating and variable symptoms for several months after the initial diagnosis of COVID-19. This condition, which is called "Long COVID," typically refers to the symptoms lasting for 2 months or longer subsequent to infection (Ortona et al., 2021). The virus may activate an excessive inflammatory response resulting in damage of organs. In addition to this mechanism, an autoimmune reaction which is unmasked by SARS-CoV-2 may have role in Long COVID's symptoms. The higher incidence of long COVID in females can be justified by the autoimmune hypothesis. In order to identify specific and personalized treatments for this syndrome, it is important to study the appearance and the characterization of autoantibodies in the serum of patients (Ortona et al., 2021).

CONCLUSION

As investigated in this study, there could be an association between COVID-19 disease and autoimmune diseases as well as a

multisystem inflammatory syndrome. There are similarities in the immune responses to both diseases, and it should be stated that the damage in both diseases occur to a large extent due to the malfunction of the immune system. Although the main target of SARS-CoV-2 is the lungs, it should be noted that it can affect the function of other organs. Although the exact mechanism of post-COVID-19 autoimmune disease development is unclear, some factors such as pro-inflammatory cytokines and chemokines, damage-associated molecular patterns (DAMPs), molecular mimicry, cross-reactive antibodies, and auto-antibodies were hypothesized to attribute to the diseases. Reports indicated that the spectra of autoimmune and autoinflammatory conditions in SARS-CoV-2–infected populations are mostly responsive to IVIG therapy. Early diagnosis of COVID-19–linked autoimmune and autoinflammatory diseases and prompt initiation of therapy are crucial for successful recovery and preventing end-organ damage and fatality. MIS has also followed the footsteps of the COVID-19 and has been presented as a rare, but life-threatening, complication of the disease, especially in children. Efforts to minimize the risk of exposure to COVID-19 in children, especially those from socioeconomically disadvantaged populations, and prompt recognition of the syndrome, are keys to limit the incidence of this febrile syndrome.

AUTHOR CONTRIBUTIONS

ND designed, supervised, and critically revised the manuscript. MF, PH, GE, AF, SZ, MoP, SE, AA and MaP drafted the manuscript. MF and PH did the data collection.

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REVIEW
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Molecular and Clinical Investigation of COVID-19: From Pathogenesis and Immune Responses to Novel Diagnosis and Treatment

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The coronavirus-related severe acute respiratory syndrome (SARS-CoV) in 2002/2003, the Middle East respiratory syndrome (MERS-CoV) in 2012/2013, and especially the current 2019/2021 severe acute respiratory syndrome-2 (SARS-CoV-2) negatively affected the national health systems worldwide. Different SARS-CoV-2 variants, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and recently Omicron (B.1.1.529), have emerged resulting from the high rate of genetic recombination and S1-RBD/S2 mutation/deletion in the spike protein that has an impact on the virus activity. Furthermore, genetic variability in certain genes involved in the immune system might impact the level of SARS-CoV-2 recognition and immune response against the virus among different populations. Understanding the molecular mechanism and function of SARS-CoV-2 variants and their different epidemiological outcomes is a key step for effective COVID-19 treatment strategies, including antiviral drug development and vaccine designs, which can immunize people with genetic variabilities against various strains of SARS-CoV-2. In this review, we center our focus on the recent and up-to-date knowledge on SARS-CoV-2 (Alpha to Omicron) origin and evolution, structure, genetic diversity, route of transmission, pathogenesis, new diagnostic, and treatment strategies, as well as the psychological and economic impact of COVID-19 pandemic on individuals and their lives around the world.

Keywords: coronavirus, immune system, vaccine, antiviral, respiratory syndrome

Abbreviations: ARDS, acute respiratory distress syndrome; APCs, antigen-presenting cells; CFR, case fatality rate; CRRT, renal replacement therapy; DCs, dendritic cells; CoVs, coronaviruses; ER, estrogens receptors; hACE2, human angiotensin-converting enzyme 2; HLA, human leukocyte antigen; ICTV, International Committee on Taxonomy of Viruses; IFNs, interferons; LUS, lung ultrasound; LncRNAs, long non-coding RNAs; MERS-CoV, Middle East respiratory syndrome coronavirus; RBD, receptor-binding domain; SARS-CoV, severe acute respiratory syndrome coronavirus; SNPs, single nucleotide polymorphisms; WHO, World Health Organization.

HIGHLIGHTS

- The emergence of novel types of COVID-19 can be a serious health threat for humans.
- Genetic variability in certain genes involved in the immune system, encoding human leukocyte antigen (HLA) A, B, and C, may affect susceptibility to and severity of SARS-CoV-2 infection.
- One vaccine or treatment option alone cannot immunize people with genetic variabilities against various strains of SARS-CoV-2 in different areas of the world.
- Among different virus vectors, the adeno-associated virus can be used to deliver the CRISPR/Cas13d system to infected lung cells in SARS-CoV-2 patients.

INTRODUCTION

From the outset of the twenty-first century, three zoonotic β-coronaviruses (CoVs) have crossed the inter-species barriers, infected humans, and caused severe fatal pneumonia. Severe acute respiratory syndrome coronavirus (SARS-CoV) first appeared in 2003 (Poutanen et al., 2003; Zhong, 2004), Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 (Lu L. et al., 2013) and a novel β-coronavirus appeared in Hubei province, China, in December 2019 (Wu and McGoogan, 2020; Zhu et al., 2020). The novel β-coronavirus was named 2019-novel coronavirus (2019-nCoV), and the infection it causes was called COVID-19 disease by the World Health Organization (WHO) on 12 January 2020 (Du Toit, 2020; Gralinski and Menachery, 2020). Afterward, on 11 February 2020, the International Committee on Taxonomy of Viruses (ICTV) study group renamed it severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Gorbalenya et al., 2020). The WHO revised the state of Public Health International Emergency (30 January 2020) to a pandemic (11 March 2020) (Du Toit, 2020). Despite global efforts to control this serious pandemic, it rapidly spread worldwide and continued to rise. Two years after the COVID-19 appearance, the number of confirmed infections reported to WHO has exceeded 435 million, with a death toll of 5,952,215 until March 2022 (World Health Organization, 2022a). COVID-19 can be asymptomatic, mild, or severe or lead to death. Most of the patients infected by COVID-19 clinically manifest with mild symptoms, such as dry cough, breathing difficulty, fever, and nausea (Huang C. et al., 2020). However, others suffer from acute and severe pneumonia, which can develop with serious complications such as septic shock, pulmonary non-cardiogenic edema, acute respiratory distress syndrome (ARDS), organ failure, and damage to the lung parenchyma (Chan W. et al., 2020; Huang C. et al., 2020; Lu H. et al., 2020). The incubation period for COVID-19 is usually <14 days. Advanced age is also associated with increased mortality. Patients having any medical comorbidities (obesity, diabetes, tumor, or heart, lung, and/or kidney diseases) have a greater risk of developing severe COVID-19 and higher mortality rates (World Health Organization, 2022b). Although, no specific animal has been identified to date as an early natural source of novel COVID-19 disease, it is suggested that several animal species or mammalians or birds, including bats, snakes, pangolins, poultry, marmots, and turtles sold at Seafood Wholesale Market in Wuhan's Huanan, were probably associated with SAR-CoV-2 (Chung et al., 2020; Huang P. et al., 2020; Yang Y. et al., 2020). Recent comparative genomic analyses combined with evolutionary tree synthesis assumed that bat coronaviruses such as Bat-CoV-RaTG13a are closest relatives to SARS-CoV-2 with 96% similarity at the genomic level and phylogenetic homology and transmitted to humans through some unknown potential intermediated hosts. The case fatality rate (CFR) of the novel SARS-CoV-2 (2%–4%) is relatively lower than that of SARS-CoV and MERS-CoV (Li W. et al., 2005a; Cui et al., 2019; Li and Shi, 2019; Zhou P. et al., 2020). However, genetics and clinical evidence suggests structural, genetic, and epidemiological similarity of novel COVID-19 to those of SARS and MERS, making the previous findings applicable to COVID-19. Thus far, various SARS-CoV-2 strains (from Alpha to Omicron) with high transmissibility, infectivity, and mortality rates have emerged secondary to a high rate of recombination, S1/S2/RBD mutation, deletion, and substitutions during convergent evolution (Andreata-Santos et al., 2022). This review aims to provide the reader with the most recent information on SARS-CoV-2 special features, such as diverse strains, the host-pathogen interaction, virus pathogenicity, treatment strategies, the mechanisms by which virus evades immune system, transmission route, genetic variability in human immunity-related genes, and finally, different host immune responses. These data may serve as a reference for more precise and comprehensive studies: firstly, to psychologically minimize the potential negative effects of some misinformation about this not-fully-understood virus on people on a worldwide scale and, secondly, to build up a base knowledge as a first step to design an efficient vaccine for COVID-19 (Cui et al., 2019).

ORIGIN AND EVOLUTION OF SARS-COV-2

A profound knowledge of how an animal coronavirus jumped across inter-species boundaries to infect humans will considerably help prevent the following zoonotic outbreaks. At present, the source and intermediate host of SARS-CoV-2 are unknown, and no compelling evidence currently shows that any domestic animal can transmit SARS-CoV-2 to other animals, including humans. Sequencing 2019-nCoV in different patients showed almost 99.9% sequence identity, suggesting that novel SARS-CoV-2 emanated from one source within a very limited period of time (Zhang T. et al., 2020; Zhou P. et al., 2020). Comparisons of this SARS-CoV-2 with other coronaviruses in origin and evolution might help us find its initial reservoirs. In this regard, investigating the evolutionary relationship of the receptor-binding domain (RBD) sequence in spike protein, the cleavage site of S-protein in SARS-CoV-2, and identification of the early sequence, intermediate host its receptor would be of great help in understanding the origin of the virus. The SARS-phylogenetic analysis demonstrated that novel SARS-Cov-2 is closely related to the bat SARS-like CoVs such as bat-SL-

CoVZC45 and bat-SL-CoVZXC21 (Lu R. et al., 2020; Zhou P. et al., 2020). Phylogenetic tree and whole-genome analysis also showed that SARS-CoV-2 shares above 85% nucleotide sequence homology with previous SARS-CoVs, including SARS/MERS CoVs (Dhama et al., 2020; Li W. et al., 2005a). Furthermore, the phylogenetic tree based on S-proteins and the RBD of SARS-CoV-2 showed 74% similarity at the RBD level and 76% S-protein similarity to these SARS-CoVs, respectively (Jaimes et al., 2020; Walls et al., 2020). Because of the high degree of similarity of RBD in spike protein, both novel SARS-CoV-2 and previous SARS-CoVs are likely to use the same receptor for angiotensin-converting enzyme 2 (ACE2) to enter host cells (Jaimes et al., 2020). In addition, the following cell culture studies illustrated that the spike protein of the new SARS-CoV-2 employs the human angiotensin-converting enzyme 2 (ACE2), the same receptor as what SARS-CoV uses for entry (Letko et., 2020). However, the most notable difference between 2019-nCoV S and SARS-CoVS is an insertion in the S1/S2 protease cleavage site that prompts an "RRAR" fur in the recognition site in SARS-CoV-2 compared to the single arginine in SARS-CoV [28,29]. On the contrary, 96% nucleotide homology has been demonstrated between SARS-CoV-2 and Bat-CoV-RaTG13 in a study conducted in Yunnan, China [18, 30]. It has also been shown that SARS-CoV-2 shares 97.43% identity in the spike protein and 89.57% identity in the amino acid sequence of RBD with Bat RaTG13 [18–19,31] as well as the highest similarity in ORF1ab (98.55) and nucleocapsid protein (N) (99.05), respectively (Li Q. et al., 2020). Such a high degree of sequence similarity suggests that the SARS-CoV-2 is more closely related to the Bat-CoVRaTG13 than the other SARS-CoVs (Li C. et al., 2020). These findings also suggest that bats can still be considered a potential source as they host the closest relative of SARS-CoV-2, similar to the case for SARS-CoV and MERS-CoV (Huang J.-M. et al., 2020). Moreover, other animal species might serve as intermediate hosts between bats and humans. SARS-CoV-2 was first reported on 31 December 2019, when most bat species were hibernating in Wuhan. Besides, a variety of other animals but not bats were vended at the Huanan seafood market. Additionally, SARS-CoV-2 has less than 90% sequence identity with its nearest relatives, bat-SL-CoVZC45 and bat-SL-CoVZXC21, which reveals that these Bat-like CoVs are not direct ancestors of 2019-nCoV. What is more, bats have been recognized as natural sources of SARS-CoV and MERS-CoV pathogens, which have been passed on to humans *via* some intermediate hosts such as palms or civets (Y. Guan et al., 2003) or dromedary camels (Azhar et al., 2014), respectively. Therefore, given that the first group of patients infected with COVID-19 were in contact with wild animals sold at a Chinese seafood market, it is suggested that bats are the initial hosts of SARS-CoV-2, which in turn has been transmitted to humans by an unknown wild animal host(s) (Lau et al., 2005; Chan J. F.-W. et al., 2020). Previous studies on the possible intermediate hosts, with regard to viral receptor-binding domains (RBD) and host receptors, have suggested that snakes, pangolins, and turtles may also serve as potential intermediate hosts in transmitting the virus to humans (Liu Z. et al., 2020). With 93.2% nucleotide and 94.1% amino acid identity to SARS-CoV-2, pangolin CoV has been suggested as the most closely related to SARS-CoV-2. Moreover, Pangolin-CoV shows 92.8% nucleotide and 93.5% amino acid identity to Bat RaTG13 (Lam et al., 2020; Zhang T. et al., 2020). However, some Pangolin-CoV genes, including orf1b, the spike (S) protein, orf7a, and orf10, share higher amino acid sequence homology with SARS-CoV-2 than RaTG13 genes. Comparative analysis of SARS-CoV-2, Bat RaTG13, and pangolin CoV in RBD and five essential amino-acid residues engaged with human ACE2 revealed that SARS-CoV-2 has 96.68% RBD identity with pangolin CoV and 89% RBD similarity with Bat RaTG13 (Zhang T. et al., 2020). Furthermore, Pangolin CoV has only 85% RBD similarity with the Bat RaTG13. These findings indicate that Pangolin-CoV is highly similar to SARS-CoV-2 compared to RaTG13. Interestingly, these five key amino-acid residues have a major role in human-to-human and cross-species transmission. However, only one amino acid is different between Pangolin-CoV and SARS-CoV-2, which does not belong to the five cardinal residues engaged in the interaction with human ACE2. Contrarily, RaTG13 accommodates 17 amino acid residues different from SARS-CoV-2, of which four belong to the key amino acid residues (Zhang T. et al., 2020). These findings also provide more evidence to support the hypothesis that chances are higher for pangolin CoV to endure the host defenses and infect humans than Bat RaTG13 (Zhang T. et al., 2020). Besides, the nucleocapsid protein (N-protein) is the most plenteous and conserved protein in coronaviruses, including SARS-CoV-2, Pangolin-CoV, and RaTG13. Phylogenetic analysis showed that the nucleocapsid protein (N-protein) of SARS-CoV-2 and RaTG13 contains four dissimilar amino acids (37S/P, 215G/S, 243G/S, and 267A/Q), while their S-proteins differ by as many as 33 amino acids. It has been shown that the SARS-CoV-2 virus has a very distinctive peptide (PRRA) insertion located at position 680 of the S-proteins, which may be associated with the cellular proteases and proteolytic cleavage, and affects the host's transmissibility. Bat RaTG13 does not have this insertion in its S-protein (Wong S. K. et al., 2004; Li X. et al., 2020c; Ji W. et al., 2020). These findings further support the hypothesis that Pangolin-CoV is a highly possible intermediate host involved in cross-species spread and transmission to humans compared with bat RaTG13 or other SARS-CoVs. For cross-species spread and transmission to humans, SARS-CoV-2 must acquire a cleavage site or undergo some mutations, insertions, and deletions occurring at its spike protein, near the S1–S2 junction, allowing for optimal and improved binding to human-like ACE2 (Ji W. et al., 2020). The interaction of five key amino acid residues of S-protein with the angiotensin-converting enzyme-2 (ACE2) receptor is thought to be critical for human-to-human and cross-species transmission of SARS-CoV-2. It is also possible for the SARS-CoV-2 to jump into humans through an animal host with an ACE2-encoding gene similar to the human orthologous (allowing natural selection to proceed efficiently) (Andersen et al., 2020). Similarity plot analysis of bat, pangolin, and SARS-CoV-2 nucleotide sequence also indicated possible recombination in S-protein of SARS-CoV-2. Both Pangolin-CoV and RaTG13 do not have the fur in the recognition sequence motif at the S1/S2 cleavage site of the S-protein as observed in SARS-CoV-2 (Andersen et al., 2020).

These findings suggest that SARS-CoV-2 is a recombinant evolved virus of Bat-CoV and Pangolin-CoV with some genetic mutations and recombination in the spike protein gene as a result of natural selection. In fact, some homologous recombination has happened between bat and pangolin CoVs, triggering cross-species transmission of SARS-CoV-2, leading to the evolution that increases its adaptability during the outbreak (Huang J.-M. et al., 2020). This indicates that SARS-CoV-2 might gain optimized ACE2 proteins from an intermediate host such as a bat to facilitate its entry into host cells and suggests that the SARS-CoV-2 S-protein RBD–ACE2 host receptor interaction mediates infection in humans and other animals (Zhou P. et al., 2020). In SARS-CoV-2, some mutations have also been detected in five genes of S, N, ORF8, ORF3a, and ORF1ab, of which about 42% are non-synonymous mutations (Tang et al., 2020). Because of the global spread of SARS-CoV-2, its amino acid sequence has also changed significantly, resulting in increased viral diversity in some SARS-CoV-2 infected patients. This explains the probable cross-species transmission, adaptation of viruses to the human body, human-to-human transmission, and the viral genome evolution in the human population (Shen et al., 2020). Structural studies and biochemical experiments have demonstrated a high affinity for SARS-CoV-2 RBD toward human ACE2 and other species with high receptor homology. Notably, the high-affinity binding of the SARS-CoV-2 spike protein to human ACE2 is most likely the result of natural selection on a human-like ACE2. There is a strong body of evidence in the literature that SARS-CoV-2 might not be a purposefully manipulated laboratory-based virus (Wan et al., 2020).

Coronavirus: Genome Structure and Classification

Coronaviruses are enveloped, non-segmented positive-sense RNA viruses containing a very large RNA (~26–32 kb) surrounded by a symmetrical nucleocapsid (Song et al., 2019). They are the largest group of viruses belonging to the Riboviria realm, the idovirales order, including the Coronaviridae, Arteriviridae, and Roniviridae families. The Arteriviridae family are phylogenetically classified into four genera: the Alpha, Beta, Gamma, and Delta coronaviruses (α, β, γ, and δ) (Chandra and Awasthi, 2020). Each genus is further subdivided into linage subgroups of A, B, C, and D. Human CoVs consist of HCoV-229E and NL63, MERS-CoV, SARS-CoV, HCoV-OC43, and HCoV-HKU1 (Chandra and Awasthi, 2020). Four α-coronaviruses of HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 are associated with mild symptoms in humans, while two human β-coronaviruses of SARS-CoV (Peiris et al., 2003) and MERS-CoV cause severe disease (Xu et al., 2004). The newly emerged SARS-CoV-2 is the seventh human coronavirus, belonging to β-coronaviruses and lineage B subgenus. β-Coronaviruses (including SARS-CoV-2) are classified by the club-like spikes (S-proteins) that project from their outer surface, their large RNA genome, and their replication strategy. Human SARS-CoV-2 genome consisted of 29,903 nucleotide-based RNA molecules (Papanikolaou et al., 2022) with at least ten open reading frames (ORFs), encoding 27 proteins (including 15 nonstructural proteins, 4 structural proteins, and 8 auxiliary proteins). The first ORF (ORF1a/b) forms about two-thirds of viral RNA and encodes two nonstructural polyproteins involved in the formation of viral replicas transcriptase complex. Other ORFs on the remaining one-third of the genome encode four main structural proteins of spike surface glycoprotein (S), envelope (E), nucleocapsid (N), and membrane or matrix (M) proteins. They also encode for sixteen non-structural proteins (NSP1–NSP16) virus' critical molecules such as helicase and RNA-directed RNA polymerase, which participate in viral replication and translation and facilitate virus entry into the cells (Papanikolaou et al., 2022). The most important feature of coronaviruses is the heavily glycosylated spike glycoprotein (S) (~150 kDa), located on the surface of CoVs to help them enter target cells. Spike mediates viral entry into host cells through homodimers protruding from the viral surface (Tortorici and Veesler, 2019; Walls et al., 2020). Spike S encompasses two subunits of S1 (N-terminal) and S2 (C-terminal), which create a unique crown-like formation (corona) on virion's surface. S1 subunit acts as the main receptor-binding domain (RBD) and recognizes and binds to the host cell surface receptor, whereas the S2 domain is involved in the fusion mechanism between cell membrane and virus (Li F. et al., 2005; Ge et al., 2013; Lu G. et al., 2013; Raj et al., 2013; Gui et al., 2017; Yuan et al., 2017; Letko et al., 2020; Walls et al., 2020). These two subunits (S1 and S2) have a major role in viral infection and pathogenesis and are critical targets for antiviral neutralizing antibodies. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a receptor to enter target cells (Hoffmann et al., 2020a; Yao H.-P. et al., 2020). ACE2 is expressed on human nasal epithelial cells, lung, spermatogonia, Sertoli, gastric, duodenal, and rectal epithelial cells (Song et al., 2018). ACE2 is a substrate for membranous attachment, activating the S1 and S2 subunits. Then, ACE2–SARS-CoV-2 cell complex triggers intracellular signaling transduction affecting hypoxia regulatory molecules (Tsiambas et al., 2020). Specific proteases, such as furin, trypsin, cathepsin, or serino-protease (transmembrane serine protease 2-TMPRSS2), are involved in the virus entry into the cell, leading to the intracellular infection signal (Coutard et al., 2020; Lukassen et al., 2020). As a host cell protease, furin cleaves the S-protein into two separate polypeptides: the S1 and S2 subunits. The S1 subunit contains the receptor-binding domain (RBD) (with 193 amino acid residues), which contains two subdomains of the core and external portions (Wong S. K. et al., 2004). The RBD core subdomain is responsible for the formation of S trimer particles (Bosch et al., 2003), and the external subdomain with two exposed loops on the surface binds to the ACE2 receptor (Raj et al., 2013). Six RBD amino acids of SARS-CoV-2, including L455, F486, Q493, S494, N501, and Y505, are essential for binding to ACE2 receptors and determining the host range of SARS-CoV-like viruses. Spike RBD has all the structural information needed for virus attachment to the host receptor ACE2 (Andersen et al., 2020). The receptor-binding domain (RBD) in the spike protein is the most variable part of the coronavirus genome (Andersen et al.,

2020), which might be influenced by positive selection. Five of these six residues differ between SARS-CoV-2 and SARS-CoV (Raj et al., 2013).

Different Variants of Novel COVID-19

Like other viruses, SARS-CoV-2 evolves over time. SARS-COV-2 has undergone many mutations since its earlier detection in Wuhan at the end of 2019. Different SARS-COV-2 variants such as Alpha (α), Beta (β), Gamma (γ), Delta (δ), and recently Omicron variant (B.1.1.529) have appeared by a broad spectrum of recombinations, point mutations, deletions, and amino acid substitutions, particularly in spike RBD, and RBM of novel SARS-CoV-2. Beta, Gamma, and Delta variants have been associated with disease severity and higher virulence but lower pathogenicity. WHO classified SARS-CoV-2 variants into three main categories: variants of concern (VOCs), variants of interest (VOIs), and variants under monitoring (VUMs) (Andreata-Santos et al., 2022). The four previously reported VOCs are Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) variants, whereas the recent variant, Omicron (B.1.1.529), was firstly recognized in South Africa on 26 November 2021, designated as the fifth VOC (He X et al., 2021). It seems that the SARS-CoV-2 virus tries to survive through some variants with//ORF deletions with a low level of activity. Furthermore, under the pressure of natural selection and evolution, point deletions sometimes lessen the viral spread (Akkiz, 2021). However, some of the mutations enhance the ability of the virus to spread and transmit across species and infect multiple cell types leading to a variety of human diseases. Typically, various point mutations (substitutions) and specific deletions have occurred in the S1/S2 domains and RBD areas of RNA genomic sequences in SARS-CoV-2, resulting in the emergence of a new set of SARS-CoV-2 variants. Major (non) synonymous mutations affecting the RBD region in novel SARS-CoV-2 include N501Y, E484K, L452R, and K417N/T (Papanikolaou et al., 2022; Rahimi et al., 2021; Winger and Caspari, 2021). P681H/R substitution has been recognized in S1/S2 furin cleavage site, D614G and G142D in S1 and V1176F, A701V, and T20N on the S2 region. Interestingly, critical deletions (ΔH69/ΔV70/Δ156/Δ157/ΔY144/ΔL242/ΔA243/ΔL244) influencing the non-RBD S1 region increase the transmission ability and infectivity of the virus and negatively affect the potency of immunity that neutralizing antibodies provide (decreased serum neutralization titles) (Papanikolaou et al., 2022). However, frequent deletions observed in other regions, including open reading frames (ORF) 7 and 8, surprisingly lead to a low replication load and strong response to mAbs. Various mutations of D614G, N501Y, E484K-Q, K417N/T, and L452R are associated with a significantly strong hACE2 binding affinity, elevated viral load production, increased human-to-human transmissibility and infectivity, enhanced disease severity, and immune escape against vaccine and antibody therapeutic strategies due to low immune response rates (Daniloski et al., 2021; Gobeil et al., 2021; Nelson et al., 2021). All of these mutations were predominantly found in Alpha, Beta, Gamma, Delta, Theta, and Omicron variants. Amino acid D614G substitution (glycine for aspartic acid) in spike occurred in all of the five VOCs. D614G mutation demonstrates a higher load of infectious virus in the upper respiratory tract and an increased replication and transmissibility in SARS-COV-2 variants. The combination of deletions of D614G and E484Q only in the Delta variant granted the virus a significantly increased infectivity and transmissibility and empowered the virus as the response rate to mAbs and targeted vaccines reduced substantially. The Omicron SARS-CoV-2 variant also shares N501Y with the Alpha, Beta, and Gamma variants. This mutation is believed to enhance the binding affinity between spike and angiotensin-converting enzyme 2 (ACE2) and enhance transmissibility (Papanikolaou et al., 2022; Kumar et al., 2021). Recent evidence indicates that the Omicron variant is probably more infectious than the Delta and Beta variants (Pulliam et al., 2021; Lippi et al., 2022)). It has been reported that the Omicron variant has at least 35 mutations in its spike (S) protein compared with early SARS-CoV-2. Generally, 15 of the 29 substitutions located in the receptor-binding domain (RBD) are the early target for neutralizing the monoclonal antibody. Moreover, 10 of those on the receptor-biding motif (RBM), involved in recognizing the human angiotensin-converting enzyme 2 (ACE2) receptors, are suggested to increase the spread and virulence of the Omicron variant. These findings suggest that the monoclonal antibodies approved by the Food and Drug Administration (FDA) may be less effective against the Omicron variant. Although the impact of Omicron on morbidity and mortality is still unknown, the number and combination of mutations/deletions/insertions in this recent variant is impressively associated with high transmissibility, infectivity, and possibly increased re-infection rates (Andreata Santos et al., 2022).

L and S Types of SARS-CoV-2 (SNP)

Population-based genetic analyses of SARS-CoV-2 genomes indicated that this virus has evolved into two major types, L and S, by means of two different SNPs that show nearly complete linkage across the viral strains' sequence. Although the L type (~70%) is more prevalent than the S type (~30%), the S type was the ancestral strain. Although the L type was more prevalent in the early stages of the SARS-CoV-2 outbreak in Wuhan, the frequency of the L type decreased after early January 2020. It is likely that human intervention may have exerted more severe selective pressure on the L type, leading it to be more aggressive, and spread more quickly. On the contrary, the frequency of the S type, which is evolutionarily older and less aggressive, might have relatively increased due to the lower selective pressure imposed on it. A combination of these findings strongly supports an urgent need for further immediate and comprehensive studies on genomic data, epidemiological data, and clinical symptoms of COVID-19 patients, facilitating the development of effective drugs and vaccines against the virus and aiding us in predicting when and where potential epidemics may occur and preventing their tolls (Huang J.-M. et al., 2020; Wang W. et al., 2020).

BASIC VIROLOGY AND PATHOGENESIS OF SARS-COV-2

Coronavirus encompasses a large family of viruses that may cause severe respiratory disease. The early site of SARS-CoV-2 infection

and its pathogenesis is still unknown. For betacoronaviruses such as SARS-CoV-2, cell entry is the mainstay of cross-species transmission. SARS-CoV-2 binds to ACE2 receptors on alveolar epithelial cells, infects the lower respiratory tract, and causes lethal and severe pneumonia in humans (Fan et al., 2020). The process of SARS-CoV-2 infection consists of virus attachment to the cell surface, receptor involvement, protease cleavage, and membrane fusion. Cell entry depends on the binding of the viral spike S glycoprotein (subunit S1) to the host receptor ACE2 in SARS-CoV-2 [61] and DPP4 in MERS-CoV (Millet and Whittaker, 2014; Ou et al., 2016). In fact, the S-protein-receptor interaction is the first determinant for coronavirus to infect host species. After binding to the host receptor, the virus must access the host cell cytosol. This is generally done through a two-step process of acid-dependent cleavage of S-protein by the host serine protease of TMPRSS2 or other proteases. This cleavage will activate S-protein by its conformational changes, leading to the fusion of the virus to the cellular membranes. The cleavage of the SARS-CoV-2 S-protein occurs at two sites: 1) at the junction site of S1 and S2, a.k.a. S-protein priming, which is important for separating the RBD and the fusion domains of the S-protein; 2) at the S2 site, immediately upstream of the fusion peptide in the S2 subunit, releasing the fusion peptide and causing the virus-membrane fusion (Fehr and Perlman, 2015; Walls et al., 2020). These processes produce two subunits, including an N-terminal S1 that recognizes the cell surface receptor and a C-terminal S1 that promotes the fusion of the viral envelope with the cellular membrane. Notably, the cleavage site sequence can determine the zoonotic potential of coronaviruses. The S-protein interacts with the host receptor ACE2, mediating the receptor-binding domain (RBD) region of the S-protein through a conformational rearrangement (Millet and Whittaker, 2014; Ou et al., 2016). The site of RBD within coronavirus S-protein varies according to the type of the virus. Some viruses (e.g., MHV) have the RBD at the N-terminus of their S1 region, while in others (e.g., SARS-CoV), the RBD is located at the C-terminus of their S1. The fusion of the virus S-protein and host receptor ACE2, which occurs within acidified endosomes (plasma membrane in MHV), is of pivotal importance for the entry of the virus into cells and for inducing the humoral immune response during infection (Fehr and Perlman, 2015; Kirchdoerfer et al., 2016; Yang X. et al., 2020). Cleavage at S2 exposes a fusion peptide, which is inserted into the membrane. Then, the joining of two heptad repeats in S2 forms an antiparallel six-helix bundle (Li F. et al., 2005), which causes the fusion of virus and cellular membranes, resulting in the viral genome release into the cytoplasm. Lack of an adequate and timely immune response against the infection or immunosuppression in infected patients enables rapid viral replication and spread, which initiates critical and deadly stages of the disease, and severe pneumonia, lung damage, and subsequent respiratory failure will ensue (Zheng, 2020). The high binding affinity between ACE2 and the S-protein in SARS-CoV-2 (~15 nM and 10–20-fold higher than SARS-CoV S) is thought to play a significant role in the human-to-human transmission of SARS-CoV-2 and predicts disease severity in humans (Kuba et al., 2005; Li W. et al., 2005b). Additionally, high expression and activation of serine protease TMPRSS2 at S1/S2 subunits probably is an important determinant of the virus's tendency to enter the infected cells, viral pathogenesis, transmissibility, and spread among humans (Tortorici et al., 2019). Notably, the ACE2 expression protects against lung injury and is downregulated by SARS-CoV-2; it would thus be interesting to find out whether SARS-CoV-2 interferes with the ACE2 expression (Li, 2008). Moreover, the capability of the virus to engage with ACE2 from different animal species appears to reflect host susceptibility to SARS-CoV infection and explains zoonotic spillover and numerous SARS-CoV-2 human-to-human transmission events reported to date (Muus et al., 2020).

Sex–Age-Based Differences in Susceptibility to SARS-CoV-2 Infection Sex Difference

A higher mortality rate among older males (≥65 years) suggests a gender and age-dependent difference in susceptibility to SARS-CoV-2 infection and disease outcomes. Epidemiological data from previous COVs and the recent COVID-19 pandemic revealed that male patients demonstrate a more severe disease and increased mortality than females on a global scale (Karlberg et al., 2004). Furthermore, immunological differences suggest that females have a rapid and aggressive innate and adaptive immune response to combat the invading virus, while the reduced antiviral response in males may lead to more susceptibility to severe diseases. The enhanced antiviral response in females results in a reduced viral RNA copy number and reduced expression of viral antigens in a sex hormone-dependent manner. Thus, it is assumed that sex differences in COVID-19 are represented in the early viral infections and hormonal signaling pathways. In SARS-CoV-2, more infection severity in men is associated with increased plasma cytokine levels of the innate immune system, such as IL-8 and IL-18. In contrast, less infection severity in females corresponds with higher T-cell activation. Furthermore, increases in TNF-α and IL-6 immunological activation are significant and independent predictors of severity and mortality in COVID-19 (Viveiros et al., 2021). Typically, genetic, epigenetic, and hormonal factors and the immune system activity are major factors explaining the gender and age differences in SARS-CoV-2 infection. Two major factors in sex-specific immune responses to COVID-19 infection are different expression levels of immune-related genes such as ACE2 located on the X chromosome, and the sex-specific steroid hormones, androgen, and estrogen, which regulate different immune responses in men and women (Markle and Fish, 2014; Asselta et al., 2020). The SARS-CoV-2 virus uses angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) to facilitate infection. A meta-analysis study indicated that increased expression levels of ACE2, TMPRSS2, and CTSL in specific cell types are correlated with advanced age and male gender. It has been suggested that the cell-type-specific expression or co-expression of ACE2 and proteases, as the mediators of SARS-CoV-2 viral entry, may affect susceptibility, severity, and transmissibility of COVID-19, as well as certain aspects of the

epidemiology, and clinical course of the disease (Channappanavar et al., 2017; Muus et al., 2021). Further investigations are needed to understand whether gender, age, and comorbidity are risk factors for COVID-19 infection and death (Liu et al., 2010; Wei et al., 2020).

X Chromosome

A recent paper highlighted an association between specific ACE1 I/D genotypes and differences in clinical characteristics of COVID-19 patients. This can be due to the hACE2 gene located on chromosome X (band Xp22.2), which is a crucial molecule involved in the immune response. Aggressive clinic-pathological phenotypes of male patients can be explained by chromosome X-linked genes modifications (Tsiambas et al., 2020). It is suggested that the different expression levels of the genes on the X chromosome may affect the susceptibility to and the severity of SARS-CoV-2 infection. Certain genes on the X chromosome regulate immune responses by encoding proteins such as human leukocyte antigens (HLA), ACE2 receptor, Toll-like receptors (TLR7, TLR8), cytokine receptors (IL2RG and IL13RA2), and a transcription factor for regulatory T cells (FOXP3) (Libert et al., 2010; Case et al., 2012; Conti and Younes, 2020). The Y chromosome also possesses various gene regulatory properties and polymorphisms that influence sex-based susceptibility to viral infection (Yang et al., 2010). As a host cell receptor for SARS-CoV-2, ACE2 is one of the major sex-based genes on the X chromosome. The expression level of ACE2 is strongly upregulated by estrogen and androgen and repressed by inflammatory cytokines and T2D (which increases with age and chronic diseases). There is a strong association between ACE2 expression and COVID-19 infection, susceptibility, severity, and fatality. Higher expression of ACE2 in males compared to females is one of the factors related to the severe symptoms and even death of COVID-19 infection (Liu et al., 2010; Fischer et al., 2015). In females, the X-inactivation mechanism (XCI) is the main factor for the sex-dependent expression of ACE2. Females have two X chromosomes, whereas males carry one X and one Y chromosome. Many immune-associated genes are X-linked, and females have two copies of these genes. Females benefit from a large reserve of proteins provided by the two X chromosomes. The X-inactivation mechanism in females balances the double allelic dosage of genes on the X chromosome by the epigenetic silencing of one of the X chromosomes in the early development process. The ACE2 gene is an X-linked gene that resides in the Xp22.2 region of the X chromosome and escapes X chromosome inactivation, which may confer a "double-dosage" of ACE2 mRNA. This mechanism leads to cellular mosaicism in women. ACE2 expression is dependent on sex hormones. In addition, certain mutations in X chromosome genes such as ACE2 in COVID-19 patients have been detected in some lung cells in females, whereas all the cells in males will exhibit the risky allelic variant (Liu et al., 2010; Bianchi et al., 2012; Case et al., 2012; Markle and Fish, 2014). Like the ACE2 receptor, the protease TMPRSS2 is crucial for SARS-CoV-2 entry into the host cells (Zhou P. et al., 2020). Both genes mediate COVID-19 infection through their sex-dependent expression in the lung cell. Higher expression of TMPRSS2 in male lung cells promotes SARS-CoV-2 entry to cells *via* membrane fusion; additionally, the TMPRSS2 expression is induced by androgen/estrogen stimulation (Souyris et al., 2018). Furthermore, some genetic variants in the 3' region of the gene TMPRSS2 identified in lung cells might have a significant impact on the TMPRSS2 expression and its catalytic activity, leading to more severe disease (Jin et al., 2020). The Toll-like receptor 7 (TLR7) gene, encoded on the X chromosome, is another gene that may escape X inactivation, resulting in a higher expression level of TLR7 in female immune cells, which causes more cytokine production against viral infection. Stronger Th1 immune responses in females also cause a lower susceptibility to the COVID-19 infectious pathogen than in males (Pinheiro et al., 2011; Markle and Fish, 2014). However, sex hormones may modulate ACE2 expression.

MicroRNAs and Long Non-Coding RNAs

MicroRNAs (miRNAs) are small non-coding RNAs with 18–24 nucleotides, which play an important role in many biological processes, including regulating gene expression. Long noncoding RNAs (lncRNAs) are RNA molecules larger than 200 nucleotides, which modulate gene expression at transcriptional and translational levels. lncRNAs are RNAs encoded by the human genome that are not translated into proteins (Ratti et al., 2020). The X chromosome contains 10% of all the miRNAs in the human genome, whereas the Y chromosome only contains two miRNAs. The X-linked ACE2 gene is regulated downstream by micro RNAs (miRNAs) and proteolytic cleavage. However, the TMPRSS2 gene is positively regulated by androgens in the prostate and, thus, may demonstrate male-biased expression. The X chromosome encodes major microRNAs such as miRNA-18 and miRNA-19, which play a role in sex differences in immune responses to coronaviruses diseases, including SARS-CoV-2 infection. Other X-linked microRNAs are miR-233 (regulating neutrophil differentiation), miR-106A, miR-424, miR542, and miR-503 (negative regulation of monocyte differentiation), expression of which can be under sex hormone control (Dai et al., 2013; Channappanavar et al., 2017). The high expression of miRNA genes on the X chromosome in females is a result of incomplete X inactivation, which furthers the sex differences in susceptibility to COVID-19 diseases. lncRNAs also play a crucial role in the regulation of innate and adaptive immune responses as catalysts of X inactivation, which leads to sex-differential (Channappanavar et al., 2017).

Genetic Polymorphisms

Polymorphism in autosomal genes on sex chromosomes encoding immunological proteins can have sex-differential effects on immune responses. It is proposed that epigenetic and hormone-dependent mechanisms might affect sex-based differences in gene variants (SNP) expression, but this needs stronger evidence support (Souyris et al., 2018).

Sex Hormones

Sex steroids such as estrogen and androgen, as regulators of immune responses, exert their function by binding to estrogen receptors (ER), androgen receptors (AR), and progesterone

receptors (PR). These receptors are expressed by immune cells such as B- and T-lymphocytes, macrophages, monocytes, natural killer cells, dendritic cells, and myeloid cells. It has been suggested that androgen receptors may be one of the important factors responsible for gender differences in COVID-19 presentation. As an anti-inflammatory hormone, androgen has a significantly higher expression in men than in women (Chien et al., 2006; Malkin et al., 2004; vom Steeg and Klein., 2016). Androgen in men suppresses pro-inflammatory responses by reducing the secretion of pro-inflammatory cytokines and increasing the production of anti-inflammatory cytokines. An investigation found a higher expression of IL-16, IL-7, ILCs, and IL-18 in males with COVID-19 than in females, which may promote COVID-19-related cytokine storms. Most patients with severe COVID-19 infection have experienced "cytokine storms" that can trigger the immune system to attack the body violently, resulting in an acute respiratory distress syndrome and multiple organ failure. A previous study demonstrated a positive correlation between the severity of pneumonia and the cytokine storm and inflammatory response caused by COVID-19 infection. These findings may hold answers to the question of why the risk of severe COVID-19 and case fatality rate (CFR) in males is higher than in females (Chien et al., 2006; Garanina et al., 2019; Guan et al., 2020b).

Age

Epidemiologic studies have revealed that individuals older than 60 and those with chronic or preexisting diseases (such as diabetes and hypertension) are more likely to present with severe or lethal forms of COVID-19 infection than younger cases without underlying diseases. This difference is possibly associated with systemic inflammation or cytokine storm (Abdulamir and Hafidh, 2020), considering suboptimal innate and adaptive responses to viral infection in older patients with underlying diseases. It is suggested that differences in immunity response, gene expression level, or even genetic background might explain the variations in susceptibility to SARS-CoV-2 infection, its severity, and mortality rate. Without an adequate immune response, the virus begins to replicate more aggressively, and the deadly critical phase of the disease occurs more frequently and in a shorter time. Severe pneumonia and respiratory failure in COVID-19 infection appear to be the result of an exaggerated immune response and severe inflammation rather than a direct harmful effect of the virus (Yao H.-P. et al., 2020). Another study demonstrated that patients over 60 years have a lower T-cell count, indicating that TNF-α might be directly involved in these patients, leading to an insufficient and weak immune response against viral infection. Children are not immune to COVID-19 and are vulnerable to most of the circulating common coronaviruses. Given that the adults are exposed to many respiratory infections, numerous memory cells are found in adult hosts (Yao H.-P. et al., 2020). However, the reason why COVID-19 does not aggressively attack children as it does for elderly adults is yet unknown. Besides, COVID-19-infected children younger than age 9 show mild signs and symptoms compared to older patients. It is probably due to insufficient memory cells specific to SARS-CoV-2 antigens, leading to a much milder cell-mediated immune response and milder inflammation than what happens in adults. Studies on S-protein RBD and ACE2 demonstrated that a higher binding affinity of RBD to ACE2 leads to higher virus infectivity and pathogenicity. The fact that the RBD of 2019-nCoV exhibits a much stronger affinity to ACE2 in elderly individuals and patients with a weaker immune system or accompanying underlying diseases might be related to a lower level of ACE2 expression in those people, resulting in a high virulence potential for 2019-nCoV (Yao H.-P. et al., 2020).

Clinical Symptoms and Immunopathology Features

COVID-19 infection can cause mild, moderate, severe, and/or life-threatening pneumonia. The most common symptoms of COVID-19 are fever, cough, running nose, and body ache. These symptoms may develop in different SARS-CoV-2 variants, including Alpha, Mega, Delta, and recently Omicron. In severe cases of COVID-19, loss of smell and taste sensation and difficulty in breathing have been observed, while Omicron-positive people did not have breathing issues to date (Ettaboina et al., 2021). The incubation period of the SARS-CoV-2 infection ranges from 2 to 14 days (Chan J. F.-W. et al., 2020; Guan et al., 2020b; Jiang et al., 2020) compared to the Delta variant of SARS-CoV-2 with a 4-day incubation period. COVID-19 infection may culminate in progressive respiratory failure due to alveolar damage (Ye et al., 2020). The most common manifestation on chest CT scans of the infected patients is peripheral and bilateral ground-glass opacities or consolidative lesions, in which their density and extension have been correlated with disease severity and mortality (Guan et al., 2020a). Patients with mild COVID-19 present with fever, cough, sore throat, fatigue, headache, dyspnea, or myalgia, have normal or decreased leukocyte counts (Peiris et al., 2004), and might show increased blood levels of ALT, AST, LDH, CK-MB, CRP, and ESR (Chan J. F.-W. et al., 2020). Patients with moderate infection often experience dyspnea after 1 week. Patients with severe COVID-19 rapidly progress to critical conditions, including acute respiratory distress syndrome (ARDS), severe pulmonary infection, acute respiratory failure, severe metabolic acidosis and coagulation disorders, hypoxemia unresponsive to conventional oxygen therapy, and septic shock, which can result in multi-organ failure (e.g., acutely altered function in liver and kidneys) and even death. Pathologically, inflammation and immune cell infiltration, necrosis, and hyperplasia are typically inspected in infected tissues and secretions (Guo et al., 2019; Jiang et al., 2020). Damage to the pulmonary interstitial arteriolar walls indicates that inflammatory response is the main determinant of the course of the disease, despite the pathogenic effect of CoVs (Wong C. et al., 2004). Notably, patients with severe forms of infection also had more prominent laboratory abnormalities, including lymphocytopenia, thrombocytopenia, and leukopenia (Guo et al., 2019) (**Figure 1**). Patients who require intensive care in the course of hospitalization have high levels of pro-inflammatory cytokines including IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1A, and TNF-α circulating in their bloodstream, which leads to cytokine storm and is highly relevant to disease

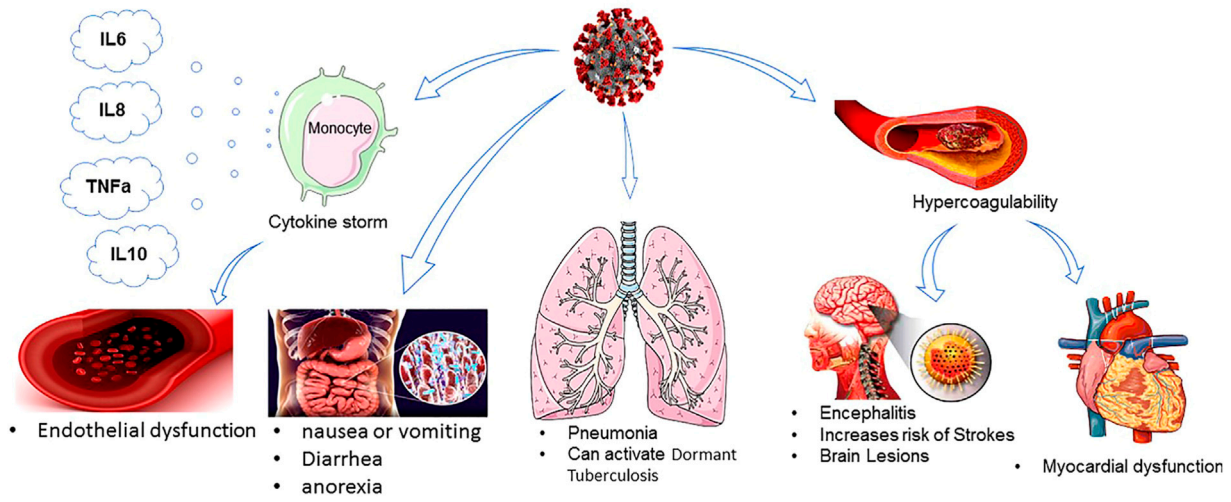

FIGURE 1 | COVID-19 effect on different organisms.

severity and final clinical outcomes (Huang C. et al., 2020). An early surge in the serum level of pro-inflammatory cytokines can lead to potentially severe disease, similar to what precedes a cytokine storm (Prompetchara et al., 2020; Wu F. et al., 2020). In COVID-19 patients with a higher level of IL-6 and IL-10; increased CRP; elevated levels of ALT, AST, and creatine kinase; increased neutrophils; and decreased lymphocytes, the clinical coarse of disease is more severe and the mortality rate is considerably higher (Huang C. et al., 2020; Wu and Yang, 2020).In contrast, a high level of IL1B, IFNγ, IP10, and MCP1 probably leads to the activation of the T-helper-1 (Th1) cell responses and increases the secretion of T-helper-2 (Th2) cytokines (IL4 and IL10) that suppress inflammation (Mescher et al., 2006). The majority of COVID-19 infected patients had a lower absolute count of lymphocytes. This finding suggests that 2019-nCoV might mainly act on lymphocytes, especially T lymphocytes, as does SARS-CoV. Viral particles cross the respiratory mucosa and infect other cells, induce a cytokine storm in the body, and generate a series of immune responses that, in turn, cause alterations in the number and functionality/activity of peripheral white blood cells such as lymphocytes. Some studies suggest that a substantial decrease in the total number of lymphocytes implies that coronavirus consumes many immune cells and inhibits the cellular immune function of the host. Damage to the T lymphocytes might be an important factor in exacerbating the condition and leading to a rapid clinical deterioration in COVID-19 patients (Chen N. et al., 2020). Moreover, the total count of other immune cells, including CD4+T, CD8+T, dendritic cells (DCs), macrophages, and natural killer cells (NKCs), significantly decreases. What is more, according to previous studies, CD4+T cells, but not CD8+T cells, are important for harnessing the SARS-CoV infection (Bai et al., 2020; Zhu et al., 2010). Nearly 85% of COVID-19 patients are asymptomatic or present with mild symptoms, around 10% may develop with severe symptoms such as the acute respiratory distress syndrome (ARDS), and at least 5% are critical cases who need treatment in the intensive care unit (ICU), of which roughly half die, with an average morality rate of approximately 2.4% (Dandekar and Perlman, 2005; Wu F. et al., 2020). Notably, children and teenagers with COVID-19 infections have milder disease than adults, and fatal cases are more common in elderly patients with chronic and multiple comorbidities, such as hypertension, heart disease, diabetes, endocrine system pathology, digestive system disorders, malignancy, nervous system disease, and respiratory disease, due to weakened immune function (Cai, 2020; Wu C. et al., 2020a; Zhou F. et al., 2020). In an investigation by Guo et al., most of the patients were men, with an average age of 55.5 years. In total, 41.9% of the patients were female, and 0.9% of the patients were younger than 15 years (Guo et al., 2019). Despite the phylogenetic homogeneity between SARS-CoV-2 and SARS-CoVs, it seems that the severity and fatality rate of COVID-19 are lower than those of SARS and MERS (Guo et al., 2019; Li X. et al., 2020a).

DIAGNOSIS OF COVID-19

The detection of the disease at an early stage is essential to isolate COVID-19 patients from healthy people. According to the China guideline of Diagnosis and Treatment of Pneumonitis Caused by SARS-CoV-2 (Corman et al., 2020), available and widely accepted clinical diagnostic tools for COVID-19 include molecular methods of full genome sequencing and real-time quantitative polymerase chain reaction (RT-qPCR)), lung CT scan, serological evaluation of anti-viral immunoglobulin M (IgM), G (IgG) antibodies, and viral culture (Ettaboina et al., 2021). In RT-PCR, respiratory specimens are collected by nasal swab or swab from the throat of an infected patient. This technique is performed by targeting the specific primers in the ORF1ab and N gene regions of SARS-CoV-2. Genome sequencing is almost not applicable given its high cost. Early laboratory assessments mostly

consist of complete blood count, blood chemical analysis, coagulation testing, liver function tests, blood urea nitrogen and creatinine, CRP, LDH, ESR, procalcitonin, creatine kinase, and electrolytes (Adams et al., 2020). The low blood count of lymphocytes has also been proposed as a paraclinical indicator to diagnose the COVID-19 infection (Channappanavar et al., 2017). Besides, RT-qPCR also has some limitations, including sample collection and transportation, sample safety, kit performance, and a low positive rate of RT-PCR for throat swab samples (30%–60%) at initial presentation, its false-negative rate, its lack of sensitivity, insufficient stability, and its relatively long processing time (Ai et al., 2020; Pan et al., 2020; Xie et al., 2020). The number of mutations involving epitopes of the SARS-CoV-2 variants (particularly Omicron) has made several single-target molecular tests ineffective, raising the false-negative rate results in patients infected by this VOC. Virus antigens or serological antibody testing kits can be implemented for diagnosis (Channappanavar et al., 2017). Although antigen tests rely mainly on nucleocapsid proteins, they can also detect the proteins of the SARS-CoV-2 Omicron variant but maybe with a lower sensitivity (Tiecco et al., 2022; Ettaboina et al., 2021). The antigen tests are generally less sensitive to very early infections compared with molecular tests. Following the FDA's long-standing rapid test recommendations, a negative antigenic test in a symptomatic patient or patients with a high likelihood of infection inevitably requires follow-up molecular testing (Tiecco et al., 2022). Over the last few decades, lung ultrasound (LUS) has been a useful, bedside, safe, and non-ionizing imaging modality for diagnosing a variety of acute respiratory diseases, including COVID-19 pneumonia, and it has been superior to chest x-ray and clinical examination (Copetti, 2016; Lichtenstein and Malbrain, 2017). It can be a beneficial diagnostic/triaging tool in emergency departments given resource-limited settings (Lichtenstein and Meziere, 2008). LUS has several advantages over RT-PCR (real-time polymerase chain reaction) as it can precede RT-PCR test positivity (Kalafat et al., 2020), and it is consistent with CT imaging. Moreover, LUS is more sensitive than chest x-ray (plain chest radiography) in both children and adults and in pregnant women without radiation exposure and with ease of sterilization (Pagano et al., 2015; Mayo et al., 2019; Denina et al., 2020; Feng et al., 2020; Huang Y. et al., 2020; Kalafat et al., 2020; Poggiali et al., 2020). Chest CT imaging is a non-invasive, easy-to-perform, and fast method of diagnosis with high diagnostic accuracy and timelier COVID-19 diagnosis compared to RT-PCR, with the only drawback of radiation. It has been reported that almost all COVID-19 patients demonstrate peripherally distributed radiographic features of ground-glass opacity, multifocal organizing pneumonia, and interstitial changes on their CT scan (Ai et al., 2020; Huang P. et al., 2020). Like LUS, chest CT may show pulmonary abnormalities in COVID-19 infected patients with initial negative RT-PCR results (Chung et al., 2020). Individuals who have negative RT-PCR results may benefit greatly from a combination of repeated RT-qPCR tests and chest CT scans (Yang Y. et al., 2020). A low-dose chest CT protocol (mainly based on reducing CT tube current from 100 to 150 mAs in the standard CT protocol to 50 mAs) has been introduced and implemented to overcome the main chest CT scan downside, which does not decrease diagnostic accuracy of CT images but reduces radiation dose considerably (up to 89%) in comparison to the standard-dose protocol (Azadbakht et al., 2021). Regarding some limitations of RT-PCR and CT scans to diagnose COVID-19, immunological detection kits are used to target viral antigens or antibodies in clinical laboratories. SARS-CoV-2 IgG/IgM Antibody Test Kit and ELISA kits for SARS-CoV-2 can detect and quantify SARS-CoV-2 IgM and IgG and are highly sensitive for IgG identification from 10 days following symptoms onset (Adams et al., 2020).

CRISPR Based Methods and Their Usage in COVID-19 Detection and Treatment

These methods are alternative RNA-based antiviral strategies acting against infections caused by RNA viruses, with the capability of targeting highly different conserved regions by multiple crRNAs, further reducing the chances of viral escape from inhibition through mutation and drug reissuance. Another advantage of this novel strategy is that it targets not only viruses infecting humans but also those that are currently found in animal reservoirs and might transfer to humans unexpectedly. This approach relies on a CRISPR-based system for recognizing and degrading the virus genome or mRNAs. The Cas13a RNA-targeting technique of SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking) as a new efficient CRISPR-based diagnostic test has been developed to rapidly detect all types of RNA viruses. This approach consists of two guide RNAs (gRNAs), which combine with a Cas13 protein and form a SHERLOCK system to detect SARS-CoV-2 RNA in human lung epithelial cells. The gRNAs are designed based on certain specific regions of the S gene and Orf1ab gene in SARS-CoV-2, which can bind to their complementary sequences in the SARS-CoV-2 RNA and recognize the viral RNA. In this system, a paper strip is used for visual readout, similar to a pregnancy test. After dipping a paper strip into a prepared sample, if a new line appears on the strip, it indicates virus existence in the sample. As it takes only 45 min to detect viral RNA in the sample, the new test works faster than widely used RT-PCR techniques, taking about 4 hours to reveal the result in a respiratory sample (Curti et al., 2020). DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) is another low-cost and accurate CRISPR-Cas12-based approach developed for SARS-CoV-2 detection in respiratory samples in approximately 30 min. Cas12 gRNAs are designed to specifically detect the N gene in SARS-CoV-2 and the E (envelope) and the N (nucleoprotein) genes in other viral strains (Freije et al., 2019; Mustafa and Makhawi, 2020). Moreover, two novel CRISPR-Cas13 techniques, CARVER (Cas13-assisted restriction of viral expression and readout) and PAC-MAN (Prophylactic Antiviral CRISPR in Human Cells), have been developed for therapeutic purposes, which target the SARS-CoV-2 virus and restrict its replication (**Figure 2**).

In order to clear the virus, these techniques contain guide RNAs (gRNAs) designed to organize the viral RNA by specifically binding to complementary sequences in the viral RNA genome and effectively cut it using an RNA-targeting Cas13 nuclease

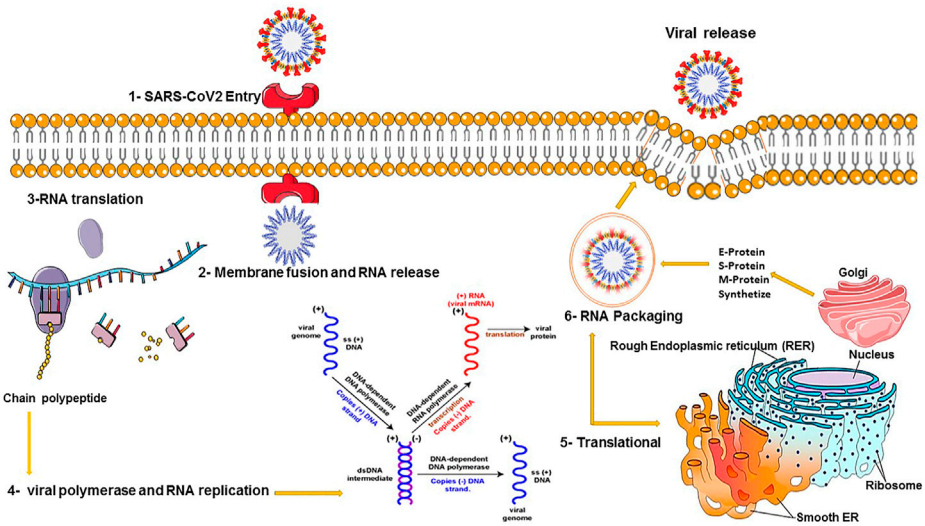

FIGURE 2 | COVID-19 viral replication and translation.

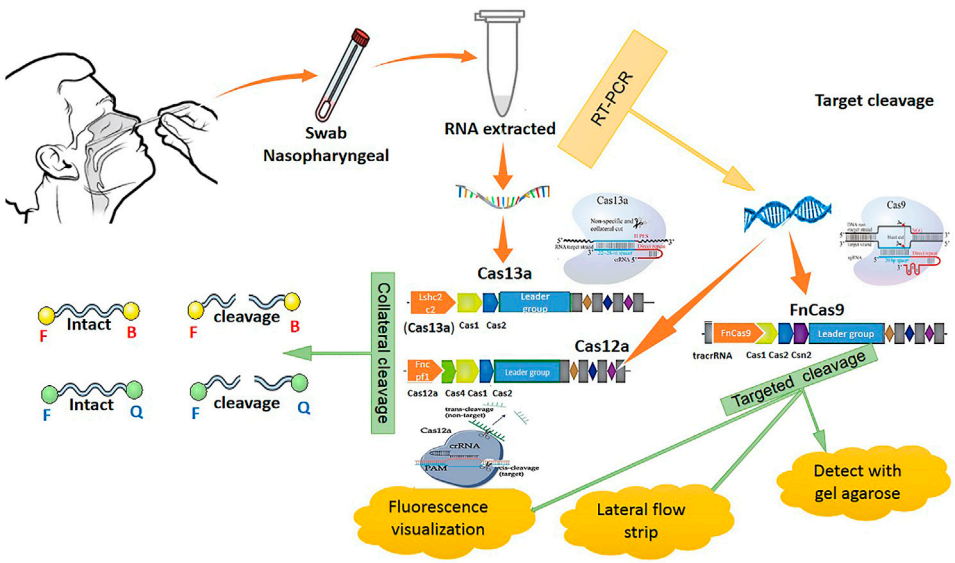

FIGURE 3 | CRISPR methods for COVID-19 detection.

(Konermann et al., 2018). Guide RNAs have been designed based on highly conserved regions of the viral genome encoding the major structural proteins of SARS-CoV-2, including the orf1ab, S (spike), M (membrane), N (nucleocapsid), and E (envelope) genes. Targeted inhibition of these proteins can lead to disabled viral replication and function. A total of 10,333 guide RNAs have been designed to specifically target 10 peptide-coding sites on the RNA genome of SARS-CoV-2. This novel approach is applicable to defend against the virus variants that evolve and may escape traditional drugs and vaccines. It particularly targets the different regions of the same virus or different SARS-CoV-2 strains (L and S strains) by the crRNAs pool (Konermann et al., 2018). Cas13d and its components can be delivered within polymers or lipid nanoparticles with chemical alterations to increase stability. Besides, a recently developed DNA-based liposomal delivery strategy (i.e., the HEDGES platform) is also optimal in this regard (Konermann et al., 2018). If we find this therapeutic strategy secure and effective with a safe delivery method, it could be a good alternative to traditional vaccines in which viruses can escape from inhibition. Among different virus vectors, the adeno-associated virus (AAV) can be used to deliver the CRISPR/Cas13d system to infected lung cells in SARS-CoV-2 patients (Hoffmann et al., 2020b). Before *in vivo* therapeutic application of the CRISPR/Cas13d system on

patients, it is necessary to determine the safety and efficacy of this system in clearing 2019-nCov and other viruses in animals (Broughton et al., 2020; Cui et al., 2019) (**Figure 3**).

Innate Immune Responses Mechanism to SARS-CoV-2 Infection

The epithelium of the lungs and the respiratory tract are exposed to viruses existing in the inhaled air. SARS-CoV-2 entry into the respiratory tract cells depends on its attachment to the ACE2 receptor on the surface of the host's lung cells by the viral spike (S) protein, leading to antigen presentation *via* APCs. Antigen detection by the innate immune sensors in the respiratory tract stimulates humoral and cellular immunity, mediated by virus-specific B and T cells. Indeed, the appearance of viral dsRNA in the cytoplasm and its detection by pattern recognition receptors (PRRs) [including the Toll-like receptors (TLR3 and TLR7), cytotoxic T lymphocytes (CTLs), cytosolic RNA sensor, and RIG-I/MDA5], triggers the downstream signaling cascade of NF-κB and IRF3 against RNA viruses in the lungs (Totura et al., 2015; Zhou et al., 2014). Alveolar lymphocytes and macrophages, dendritic cells (DCs), airway epithelial cells, and innate lymphoid cells as innate immune contributors induce the secretion of large amounts of cytokines and chemokines, establishing an antiviral state in the lung cells (Taniguchi and Takaoka, 2001). This antiviral state stimulates the production of type I and type III interferons (IFNs), genes (ISGs), and other proinflammatory cytokines (TNF-α and IL-6), which control viral replication by inducing an effective adaptive immune response. Furthermore, type I IFN simulates the release of antiviral proteins to protect neighboring uninfected cells (Trottein and Paget, 2018; Zhou et al., 2018). In general, T helper cells, especially Th17 cells, produce the proinflammatory cytokine IL17 *via* the STAT3 and NF-κB signaling pathways and assist cytotoxic T cells and B cells to clear the virus. Natural killer T cells, mucosal-associated invariant T cells, and neutrophils also effectively mediate the innate and adaptive immune responses, clearing viruses from the respiratory system (Martin and Frevert, 2005; Gabay, 2006). All the aforementioned immune responses can prompt an uncontrolled immune-inflammatory response called a "cytokine storm." The cytokine storm denotes highly increased levels of pro-inflammatory cytokines (IFN-α, IFN-γ, G-CSF, IL-1b, IL-2, IL-6, IL-7, IL-10, IL-12, IL-18, IL-33, TNF-α, TGFb, MCP-1, MIP-1A, etc.) and chemokines (CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, etc.) in severe SARS-CoV-2 infection (Kenway-Lynch et al., 2014; Jones and Jenkins, 2018). The serum levels of TNF-α, IL-6, and IL-10 in SARS-CoV-2-infected patients are negatively correlated to the total number of T cells, CD4+T, and CD8+T cells, suggesting that cytokine storm might reduce T-cell count *via* apoptosis or necrosis (Williams and Chambers, 2014). Moreover, high initial viral loads, and an increased number of IMMs and neutrophils in the lungs, accompanied by elevated pro-inflammatory cytokine/chemokine levels, cause lung damage in SARS patients (Xu Z. et al., 2020). Acute respiratory distress syndrome (ARDS) and multiple organ failure, which are the main death causes in COVID-19 infections, are results of the cytokine storm following severe pulmonary inflammation (Cameron et al., 2008; Sui et al., 2008). Humoral immunity (B cells), as the adaptive immune response, defends only against viruses outside the cell (i.e., extracellular viral particles). In fact, neutralizing antibodies bind to the crown-like spikes of these extracellular viruses and prevent them from interacting with host cells and even prevent re-infection in the future. Incomplete, delayed, or even strongly induced host immune response can result in pulmonary tissue damage (Ter Meulen et al., 2006; Kikkert, 2020). The adaptive immune evasion mechanism is a primary viral reaction to escape host immune detection and suppress innate immune responses aimed at lengthening the virus's survival, boosting its replication rate, and promoting the host's cell infection (Prompetchara et al., 2020). For this purpose, SARS-CoV-2 can downregulate MHC class I, MHC class II, and CD80/86 in antigen-presenting cells (APCs) by infecting macrophages or dendritic cells, which inhibit T-cell activation. SARS-CoV-2 also modifies intracellular membranes to form double-membrane vesicles that lack PRRs, thereby replicating in these vesicles and avoiding the host detecting their dsRNA (Lessler et al., 2009; Liu et al., 2018; Snijder et al., 2006). This is probably associated with a longer incubation period of 2–14 days in SARS-CoV-2 compared to other CoVs (Martin and Frevert, 2005). Besides, some accessory proteins of SARS-CoV-2 can attach to the dsRNA during replication to block the TLR-3 activation and evade the immune response. Another viral strategy for suppressing the host immune response is encoding viral proteases for cleaving innate immune factors (Martin and Frevert, 2005). This interaction between the cellular immune responses and viral evasion mechanisms identifies infection outcomes (Gralinski and Baric, 2015; Farrag and Almajhdi, 2016). Moreover, delayed innate immune response that is secondary to temporary suppression when an innate immune response has been evaded contributes to an overreaction and exacerbated immune response, which leads to cytokine storms, damaging inflammation, and other severe complications (Røsjø et al., 2011; Farrag and Almajhdi, 2016). However, in-depth knowledge of this virus–host interaction proposes important opportunities for designing and creating novel antiviral strategies. Because each virus uses various strategies for suppressing the host immune response *via* evolved multifunctional proteins, mostly it is difficult to determine the immune evasive pattern to offer novel antiviral treatment strategies based on it. Notably, long-lasting immune protection after SARS-CoVs infection has not yet been proved, and indeed, CoVs can re-infect individuals sometime after earlier infection. It might be associated with the virus's high genetic variation and genetic drift and shift, causing new strains that are not efficiently recognized by the innate immunity system. This is the reason why individuals experience various CoV infections in the course of their lives (Gralinski and Baric, 2015).

Genetic Variability in Immune System and Susceptibility to COVID-19

Recently, a study investigated the probable relationship between the global distributions of three immune system genes, called human leukocyte antigen (HLA) A, B, and C genes, involved in recognizing pathogens, with potential epidemiological outcomes of the current pandemic. This investigation revealed that the

effect of genetic variation in the human immune system genes (HLA), as significant segments of the viral antigen presentation pathway, may affect an individual's susceptibility to COVID-19 and the severity of infection. It has been suggested that genotype variability in HLA genes causes differences in the capability of recognizing a pathogen and affects the capacity of the immune response against COVID-19 infection. Thus, certain alleles might be responsible for more susceptibility and more severe infection in some individuals. Because of poor recognition of SARS-CoV-2 by this haplotype, some patients may be more vulnerable to the virus. It has been demonstrated that individuals with the HLA-B*46:01 allele and the fewest binding peptides for SARS-CoV-2 show a particular vulnerability to COVID-19, as previously shown for SARS. Using this knowledge, individuals with high-risk HLA types could be prioritized in vaccine design against SARS-CoV-2 (Cui et al., 2019).

Viral Transmission and Spread

SARS-CoV-2 was initially zoonotic in nature and then gained the potential for human-to-human transmission. The source of infection for this novel virus is mainly a virus-contaminated hand touching the mouth and nose (Jiang et al., 2020). The most important route of transmission for SARS-CoV-2 is person-to-person transmission through close contact with infected patients or respiratory droplets, which they spray when coughing, talking loudly, or sneezing (Phan et al., 2020; Rothe et al., 2020). Vertical transmission of the novel coronavirus or transmission through breastfeeding has not yet been proven (Rothe et al., 2020). COVID-19 is most contagious during the asymptomatic incubation period (roughly between 2 and 14 days) (Lu C.-w. et al., 2020). Viral load and the severity of the disease are the main determinants of inter-host transmission. However, there is no direct evidence for the transmissibility of coronaviruses from contaminated surfaces to hands (Cao et al., 2020). Although early reports were supportive of the absence of human-to-human transmission for the virus, it is currently clear that efficient human-to-human transmission is essential for the large-scale spread of 2019-nCoV. The basic reproductive number (R0) of COVID-19, the number of individuals that each case can infect, determines its transmissibility, ranging from 2.2 to 2.6 (Li X. et al., 2020b). Given that 2019-nCoV uses the human angiotensin-converting enzyme 2 (hACE2) as a host receptor, it is likely that 2019-nCoV will further adapt to the human host by increasing its binding affinity to hACE2. Adaptation to the human host depends on some mutations that frequently occur during viral RNA replication errors or through recombination events in SARS-CoV-2, which support their tendency for human infection (Ge et al., 2013; Chen J et al., 2020). The viral mutations mostly occur in the receptor-binding motif (RBM) of the SARS-CoV S-protein and increase the binding affinity of the viral spike protein to the human cell receptor ACE2. These mutations facilitate the virus's replication and assist the virus in adapting to the new conditions in the human host cell. Consequently, the new virus could undergo adaptive evolution, which results in more efficient human-to-human transmission and can increase the virus's virulence. In fact, interactions between the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein and its host receptor, angiotensin-converting enzyme 2 (ACE2), mediate both the cross-species and human-to-human transmissions (Chan J. F.-W. et al., 2020; Liu Z. et al., 2020). Among different SARS-CoV-2 variants, Omicron shows the highest transmissibility. It might be due to multiple mutations in the virus genome that increase the binding affinity to the ACE2 receptor. For instance, N501Y mutation combined with Q498R, E484K, S477N, and H69/V70 deletions might increase the binding affinity to ACE2 receptor by up to 1000-fold and up to the level of low pM in KD value. The existence of H655Y and N679K mutations in the furin cleavage site is supposed to enhance spike cleavage (S1/S2 junction) and lead to more transmission. Moreover, the P681H mutation Alpha variant and P681R in the Delta VOC probably increase the transmission rate through the same mechanism. Other Omicron spike mutations, including K417N, G446S, E484A, Q493R, G496S, and Y505H, might also impact virus transmissibility *via* increasing binding affinity to human ACE2. In contrast, RBD mutations such as E484A, Y145del, and Y505H may result in the complete loss of interactions between an antibody and RBD, raising immune escape and reinfections (Tiecco et al., 2022). In the case of COVID-19, it is suggested that one or more mutations may be selected and sustained during the SARS-CoV-2 outbreak as the virus adapts to human hosts, which possibly reduces the virus's ability to infect cells and might have influenced its transmissibility (Anwar et al., 2013). Moreover, the currently reported 17 non-synonymous mutations that happened in the ORF1ab, ORF7a, ORF8, and spike genes of the SARS-CoV-2 genome (Tortorici et al., 2019) in a family cluster of COVID-19 patients supported the hypothesis that the viral mutations could have occurred during person-to-person transmission. Indeed, due to positive selection pressure, these mutations can enhance the transmission of this novel virus to the new host(s) for its survival (Anwar et al., 2013).

Treatment Strategies Against SARS-CoV-2

Since the beginning of the new pandemic, many clinical trials have been conducted and numerous specific drugs have been utilized to treat COVID-19 infection and prevent mortality. However, several drugs that were documented to be useful in small clinical trials were ineffective in larger studies. Current COVID-19 treatment options, proved or authorized by the FDA, include antiviral drugs (molnupiravir, nirmatrelvir, and remdesivir), anti-SARS-CoV-2 monoclonal antibodies (bamlanivimab-etesevimab, casirivimab-imdevimab, and sotrovimab), anti-inflammatory drugs (dexamethasone), and immunomodulator agents (baricitinib, tocilizumab) (Takashita et al., 2022). Despite the success of some SARS-CoV-2 therapeutic strategies, such as effective vaccines, COVID-19 treatment remains challenging due to the large number of mutations that have continuously emerged in the SARS-CoV-2 variants and may contribute to attenuating the effectiveness of current treatments. Remdesivir was the first FDA-approved drug in COVID-19 treatment. To date, only the antiviral remdesivir has been shown to facilitate significant clinical improvements and has been approved by major drug safety regulators for COVID-19 (Takashita et al., 2022). Supportive treatment consists of oxygen

therapy, invasive mechanical ventilation, membrane oxygenation, prescribing systemic glucocorticoids, and renal replacement therapy (CRRT) (Lu L. et al., 2013). Oxygen therapy is used for patients with a severe acute respiratory infection, respiratory distress, hypoxemia, or shock (Schultz et al., 2017; Bai et al., 2020). For early treatment for fever, antipyretic therapies are administered (e.g., paracetamol), and guaifenesin would be helpful for non-productive cough (Liu K. et al., 2020). Intravenous administration of immunoglobulin and steroids (methylprednisolone) can enhance the anti-infection status in critical patients with ARDS and septic shock, who otherwise may experience multiple organ failures. In the absence of shock, intravenous fluids should be carefully administered (Channappanavar et al., 2017). Renal replacement therapy (RRT) is an option for patients with AKI, and antibiotics are the mainstay of therapy in sepsis (Li and De Clercq, 2020). Antiviral RNA-target therapy, antibody and plasma therapy, immunomodulatory therapy, oligonucleotide-based therapies, peptides and interferon therapies, traditional Chinese therapies, and vaccines are also used to treat 2019-nCoV infection (Coleman et al., 2014; Lu, 2020). SARS-CoV-2 components might serve as potential targets for developing antiviral drugs and vaccines, particularly spike protein (S), serine protease TMPRSS2, polymerases, and ACE2 receptors, which are mainly involved in the virus's entry into the host cell and viral replication (Wu C. et al., 2020b). Because the SARS-CoV-2 spike protein has a higher binding affinity to ACE2 than the spike proteins of other SARS-CoVs, it may be an ideal target for neutralizing antibodies and vaccines designed to combat viral infection. Anti-SARS-CoV antibody functionally intervenes with a special feature of the coronavirus (i.e., viral S-protein-receptor attachment) (Yang et al., 2013). Structural proteins, membrane proteins, and other accessory proteins of SARS-CoV-2 (ORFs, RBD) can also make credible vaccine targets (Li Y. et al., 2020). Specific inhibitors targeting key proteases such as TMPRSS2 (involved in replication and proliferation of the virus) are among the most effective drugs for treating COVID-19 infection (Xu H. et al., 2020). ACE2, as a receptor protein in both SARS-CoV and 2019-nCoV, is highly expressed in the epithelia of the human lung and small intestine, and coronavirus can infect human cells *via* binding to this receptor (Kawase et al., 2012). Thus, ACE2 can be considered an ideal target for 2019-nCoV infection treatment. Camostat mesylate is an approved serine protease TMPRSS2 inhibitor used in Japan for the off-label treatment of SARS-CoV-2 infected patients (Sheahan et al., 2020).

Antiviral Therapy

Antiviral therapy against SARS-CoV-2 consists of many of the medications previously prescribed for SARS/MERS, influenza, and HIV infections, including nucleoside analogs (lopinavir, ribavirin, chloroquine, remdesivir, ritonavir, favipiravir, and galidesivir), neuraminidase inhibitors (oseltamivir), spike protein inhibitors (griffithsin), RNA synthesis inhibitors (TDF, 3TC), anti-inflammatory drugs, fusion peptide (EK1), abidol, and Chinese traditional medicine (ShuFengJieDu capsules and Lianhuaqingwen capsule) (Savarino et al., 2006; Zumla et al., 2016; Ji S. et al., 2020; Wang M. et al., 2020). However, the efficacy and safety of some of these drugs in COVID-19 treatment have not been confirmed. Hydroxychloroquine, which was used to prevent COVID-19, had no effect on preventing illness, hospitalization, or death from the disease and may increase the risk of cardiac arrhythmia, blood and lymph disorders, kidney injury, and liver problems. Based on previous evidence, ribavirin and lopinavir-ritonavir have been effective for HIV, SARS, and MERS infection therapy. The clinical application of two HIV-1 protease inhibitors, namely, lopinavir and ritonavir, appears to be effective in suppressing COVID-19 infection (Wang et al., 2021). Remdesivir, an inhibitor of the viral RNA-dependent RNA polymerase (RdRp), has a similar chemical structure to HIV reverse-transcriptase inhibitors. It targets the RNA-dependent RNA polymerase and inhibits viral RNA synthesis in human coronaviruses. Remdesivir, originally used to treat Ebola, is an effective and nearly safe treatment for COVID-19 infection (Savarino et al., 2006; Richardson et al., 2020). A recent study suggested the various mutations in the RdRp (V557L, V473F, N491S, F480 L/S/C, P323L, and or E802D) are associated with remdesivir resistance in the Omicron SARS-CoV-2 variant (Chen et al., 2022). A combination of lopinavir/ritonavir or ribavirin as nucleoside analogs with IFNs as protease inhibitors can act synergistically upon 2019-nCoV and reduce the mortality rate in critical conditions (Ji S. et al., 2020; Wang M. et al., 2020). The antiviral drug nirmatrelvir (PF-07321332), co-administered with ritonavir, inhibits the SARS-CoV-2 protease involved in viral replication. Molnupiravir, used orally for patients with mild-to-moderate COVID-19, increases the frequency of viral RNA mutations and impairs SARS-CoV-2 replication. Molnupiravir is expected to be effective against all SARS-CoV-2 variants, including the recently discovered Omicron (Jayk Bernal et al., 2022). Several approved antiviral drugs such as nafamostat, camostat, or aprotinin have been effective in SARS-CoV-2 variants, especially Omicron. The Pfizer company confirmed that the nirmatrelvir pill was effective in patients infected with Omicron (Ettaboina et al., 2021).

Antibody and Plasma Therapy

Neutralizing monoclonal antibodies include sotrovimab (GSK4182136 or S309), bebtelovimab (LY-CoV1404), bamlanivimab-etesevimab (LY-CoV016-LY-CoV555), and casirivimab-imdevimab (REGN-CoV2), which prevent infection of human cells by blocking the S-protein–ACE2 attachment and mediate SARS-CoV-2 entry into the human respiratory epithelial cells (Tiecco G et al., 2022). These antibodies target the spike protein (S), act as antigenic proteins, and stimulate the antibody response by interacting with the cell surface receptor angiotensin-converting enzyme 2 (ACE2). Long-term antibody response is affected by antibody concentration and the severity of the infection (Liu and Wang, 2020; Tian et al., 2020). Bamlanivimab-etesevimab (LY-CoV016-LY-CoV555) is a cocktail of two antibodies binding to the RBD of the SARS-CoV-2 S-protein, blocking the virus attachment to the human ACE2 receptor, and thereby increasing the neutralization rate. Casirivimab-imdevimab (REGN-CoV2) also reduces the risk of severe COVID-19 infection by binding to distinct

epitopes of the S-protein. The neutralization potency of both cocktails is still considered a treatment option for Delta VOC but is greatly reduced in the Omicron variant (Quiros-Roldan et al., 2021). Recombinant human monoclonal antibodies (mAbs), including CR3022, m396, and CR3014, can also neutralize viral function *via* binding to the receptor-binding domain (RBD) of SARS-CoV-2 (Gierer et al., 2013; Nguyen et al., 2020). It is notable that the receptor-binding domain (RBD) on the spike protein is a critical target for neutralizing antibodies. In contrast to the Beta or Delta SARS-CoV-2 variants, the Omicron variant has a significant ability to escape humoral immunity and neutralizing antibodies due to 15 mutations (such as E484A, K417N, D614G, N501Y, K417N, and P681H) identified in antigenic sites (RBS-A, RBS-B, RBS-C, the CR302, and S309) on its spike RBD. These mutations result in increased ACE2 binding affinity, increased transmissibility and pathogenicity, potential resistance to monoclonal antibodies targeting these sites, and immune evasion (Yuan et al., 2021). Besides, both Beta and Gamma variants could escape the neutralizing antibodies of LY-CoV555 due to the E484K mutation in spike and LY-CoV016 due to the K417N/T mutation (Starr et al., 2021). Because CoVs are very potent in generating antibody-escape mutations in the RBD, antibodies that target non-RBD epitopes are wiser choices for COVID-19 treatment (Sui et al., 2008; Shimojima et al., 2015).

Immunomodulatory Therapy With Anti-Inflammatory Drugs

Cytokine storms (CRS) are the result of the high activation of immune cells and inflammatory responses. Many investigations have reported high levels of pro-inflammatory cytokines and chemokines, including IL-2, IL-6, IL-10, IFN-γ, CXCL10, and CCL2 in COVID-19. Interleukin-6 (IL-6) is a strong pro-inflammatory cytokine that plays an important role in the body's fight against viral infection (Hertanto et al., 2021; Villaescusa et al., 2022). IL-6 is produced by several cell types, such as T cells, B cells, monocytes, and fibroblasts. TNF-α and IL-1b activate IL-6 expression, which has a key role in the mechanism of acute inflammation. According to the findings of clinical trials, both immunosuppression (IL-1 and IL-6 inhibitors) and immunomodulation (interferon alpha (IFN-α) and interferon beta (IFN-β)) and convalescent plasma therapy have been proposed as an effective treatment only in severe or critical COVID-19 conditions who require respiratory support and ventilation. Anti-inflammatory drugs that inhibit the IL-6 pathway include sarilumab and tocilizumab, which block the IL-6 receptor, siltuximab and lizumab, which block IL-6 itself. Moreover, IFN-β-1a, IFN-α-2b, chloroquine, lopinavir, ritonavir, atazanavir, and ritonavir prevent mortality by reducing inflammation *via* viral clearance from the upper respiratory tract (Hertanto et al., 2021; Villaescusa et al., 2022). Interleukin-10 plays an immunomodulatory role by affecting T-helper 1 and inhibiting IFN-γ, IL-12, and TNF-α. This might result in a reduction of the damaging effects of inflammatory pneumonia (which is a critical phase in COVID-19 pathophysiology). Interferons (IFNs) are cytokines made and released by host cells in response to viral pathogens. A combination of interferons (IFNs) and ribavirin is more effective against coronaviruses compared to the IFNs alone (Morgenstern et al., 2005; Tian et al., 2020). Furthermore, SARS-CoV-2 can escape innate immunity early during the infection by inhibiting IFN effects in viral replication and spread. Currently, corticosteroids (dexamethasone, hydrocortisone, and prednisone) with potent anti-inflammatory effects have been used to treat only severe or critical COVID-19, according to WHO recommendations. However, the role of immune system modulation in SARS-CoV-2 variants (Alpha to Omicron) remains unknown.

Vaccine

To date, the vaccine design for coronaviruses has faced many challenges. Due to the high rate of genetic recombination and mutation in SARS-CoV-2 variants, developing a suboptimal vaccine may potentially increase the evolution and diversity of the virus in the wild. Despite this, several strategies are being considered for vaccine development to reduce the likelihood of recombination (Prompetchara et al., 2020). In general, it is assumed that live attenuated vaccines must either induce stronger immune responses than the original virus or diminish the disease during secondary mucosal infection. In this regard, for developing vaccines, spike (S) and its receptor-binding domain (RDB) might be considered as an antigen protein, which can stimulate the production of neutralizing antibodies to block the entry of the virus into the host cells (Pardi et al., 2018). According to disruptive vaccine technology, nucleic acid-based vaccines (including RNA or mRNA vaccines) can be designed to provoke an immune response against COVID-19 infection and can be delivered by lipid nanoparticles or an LNP mechanism. Reverse genetic technology can be used to develop new vaccines to combat COVID-19 by utilizing the virus's innate immune evasive function (Maruggi et al., 2019; Ramaiah and Arumugaswami, 2020). The attenuated virus may trigger better innate immune responses due to the lack of one or more of its evasive functions. Experimental SARS vaccines, including recombinant S-protein and inactivated viruses, induce neutralizing antibodies to raise against SARS-CoV and can offer some protection against SARS-CoV-2 infection (Yang et al., 2004; Wang C. et al., 2020). Current COVID-19 vaccines validated for use by WHO include Pfizer–BioNTech-BNT162b2, Moderna COVID-19 vaccine (mRNA 1273), SII/COVISHIELD and AstraZeneca/AZD1222 vaccine, Sinopharm, Sinovac-CoronaVac, Covovax (NVX-CoV2373) vaccine, and Bharat Biotech BBV152. These vaccines are based on different platforms, including DNA plasmid-based, RNA platform, innovative nucleoside-modified viral messenger RNA encapsulated within nanoparticles, specifically lipid ones (LNPs), non-replicating viral vector, inactivated or weakened versions of the virus, and protein subunit platform (Tsiambas et al., 2021). Recent evidence suggests that the current COVID-19 vaccines might be less effective against the Omicron variant in comparison to other SARS-CoV-2 variants. That is why even three doses of mRNA vaccines may not be sufficient to prevent infection and symptomatic disease with this VOC. This is due to

an elevated number of mutations in the Omicron N-terminal domain (NTD) and RDB, significantly making them difficult to be recognized by the NTD/RDB targeting neutralizing antibodies, allowing reinfection, and reducing the efficacy of currently used vaccine (Liu, L et al., 2021). Other observations revealed the importance of variant-specific vaccines based on the mutated spike, especially against the Omicron variant. However, the two-dose Pfizer vaccine had a high level of neutralizing power against other variations, such as Delta, which have been proven to help reduce disease severity, hospitalizations, and death (Ettaboina et al., 2021).

Psychological Impact of COVID-19 Pandemic

Since the emergence of SARS-CoV-2, continuing COVID-19 crises such as experiencing the new waves of infections caused by different SARS-CoV-2 variants (Alpha to Omicron), viral reinfection, vaccination delay and the non-existence of potent pharmacological treatments for COVID-19, and pandemic fatigue eventually have led to psychological exhaustions, which negatively affects the adherence to health protocols. The COVID-19 epidemic has spread fear, anxiety, depression, and even panic in the community, leading to relapses or worsening of the pre-existing psychiatric disorders in patients because of their higher susceptibility to stress in comparison to the general population (Yao H. et al., 2020). Mental disorders are associated with increased susceptibility to infections and can enhance the risk of infections, including pneumonia. The psychiatric illness itself may also result in lower COVID-19 treatment efficacy. Therefore, clear information on the role of mental disorders in COVID-19 transmission, infection, and the mental health status of the community is urgently required (Santos, 2020). Research conducted on previous infections such as SARS/MERS-CoVs and Ebola has revealed their vast psychosocial impacts on individuals, communities, and international levels during infection outbreaks (Yao H. et al., 2020). Although, currently, there is no detailed data on the psychological impact of the COVID-19 pandemic on the public health while it hit its peak, some pieces of evidence show the impact of the COVID-19 pandemic with anxiety disorders, especially cases of obsessive-compulsive disorder (OCD) who suffer from continuous hand washing immediately after coughing, sneezing, or after touching contaminated objects. Long-lasting perceived stress resulting from COVID-19 can lead to severe OCD symptoms for those who are overanxious about the virus (Zhang and Ma, 2020). Research has also reported that vast, negative, and extended psychological effects such as anger, confusion, and post-traumatic stress symptoms may be associated with long-lasting quarantine, fear of infection, frustration, a lack of basic and needed supplies, misinformation, and financial problems (Zhang and Ma, 2020). Many COVID-19 patients with mental disorders have not been able to get regular health services, and many medical appointments have been suspended. This results in an increased risk of psychiatric disorders, creates a new set of challenges in treatment, and can potentially make therapeutic measures less effective (Santos, 2020). Also, healthcare professionals and many medical practitioners, as the first line of defense against COVID-19, experience severe depression, anxiety, emotional distress, burnout, and post-traumatic stress disorder (PTSD) at the onset, during, and after the end of the outbreak of such infections because of the extreme workloads, physical and mental exhaustion, insomnia, anxiety, and the fear of being infected or transmitting the infection to their families while doing their job with little or substandard protective equipment (Zhang and Ma, 2020). However, several psychiatric comorbidities have been observed even among non-infected people during the COVID-19 pandemic, including depression, panic attacks, anxiety, cognitive distress, psychomotor excitement, suicidality, delirium, and post-trauma stress symptoms due to the fear of getting exposed to COVID-19 (Ayittey et al., 2020; Santos, 2020). Symptoms of the viral infection, such as cough and fever, may also be associated with the emergence or worsening of cognitive distress and anxiety. In a survey study, the psychological impact of the COVID-19 outbreak on mental health status was measured using the Depression, Anxiety, and Stress Scale (DASS-21) and IES-R scale. The results revealed that the COVID-19 pandemic could trigger the onset of the mental symptoms of depression, moderate-to-severe anxiety, insomnia, and distress among the physicians and nurses, caretakers, and young or old affected people (Ayittey et al., 2020). In a study investigating the psychological impact of the infection outbreak, a significant correlation was found between the female gender, student status, and specific physical symptoms such as myalgia, dizziness, coryza, and poor health status with higher levels of stress, anxiety, and depression (Ding et al., 2017). The prevalence of moderate or severe psychological impact, as measured by IES-R, was higher than depression, anxiety, and stress, as measured by the DASS-21. Most respondents (>70%) were worried about their family members contracting COVID-19. Sociodemographic data suggest that females are more affected by the psychological impact of the outbreak (i.e., they suffer from higher levels of stress, anxiety, and depression). This finding was in line with previous exhaustive epidemiological studies, which found that women were at a higher risk of mental disorders such as depression (Ding et al., 2017). Furthermore, the uncertainty of the COVID-19 status could have an adverse effect on the mental health of students and their educational status. Some studies revealed that the general population experienced the negative psychological impact of the COVID-19 outbreak to a higher degree. This also correlates to the high rate of older adult suicide deaths reported in Hong Kong among the affected individuals during the SARS and MERS epidemics (Cui et al., 2019). A study conducted on the psychological impact of SARS infection on affected families showed that they were socially isolated even after being treated and disease-free because the public avoided them (Ayittey et al., 2020). However, the clear and up-to-date information on medicines and treatment plans, routes of transmission, health status and number of both infected cases and recovered individuals, and involved families provided for the medical staff and general public is associated with decreased COVID-19 related psychological impact and levels of stress, anxiety, and depression (Ayittey et al., 2020; Zhang and Ma,

2020). Generally, behavioral therapy based on the model of stress adaptation coupled with psychotherapeutic treatments should also be provided for people with signs of mental disorders to reduce the cognitive effects of the pandemic. Using masks and medical gloves, regardless of the presence or absence of infection symptoms, is associated with less degrees of anxiety and depression. Psychological first aid (PFA) or psychosocial support is helpful in decreasing the acute distress and focuses on the mental health of the affected survivors and clinicians to offer them safety, comfort, and practical help and help them meet their needs after traumatic events such as the COVID-19 pandemic (Ayittey et al., 2020).

ECONOMIC IMPACTS OF COVID-19 ON CHINA AND THE WORLD

The impact of the recent COVID-19 outbreak on the world economy has become a matter of increasing concern. Governments are struggling to prevent the spread of the COVID-19 infection, contain the pandemic, and diminish the adverse effects of the pandemic on trade between nations. Uncertainties about the COVID-19 pathophysiology and its causative pathogen have interrupted global trade and supply chains, decreased asset prices, and forced international businesses to make hard decisions (Atkeson, 2020; Fernandes, 2020; McKibbin and Fernando, 2020). China's position as the world's largest manufacturer and importer of crude oil has caused economists to ignore their prediction of yearly global growth, especially in the stock market (Fernandes, 2020). During the SARS-CoV outbreak in China from 2002 to 2003, the global economy nearly lost roughly $40 billion. The current Chinese economy is several times larger than during the SARS epidemic and is even more connected to the whole world. This integration of China, the world's second-largest economy after the United States, with the rest of the world has resulted in a significant impact of COVID-19 on the global economy. As China now contributes to approximately 16.3% of the world's GDP, the country has been the main growth driver worldwide, with IMF estimating that China alone accounted for 39% of the global economic growth in 2019 (McKibbin and Fernando, 2020). This implies that any deceleration in the China economy could likely affect the global economy. Some economists, however, estimate that, in the event of the continuous spread of the Wuhan 2019-nCoV, China is expected to lose up to $62 billion in the first quarter of 2021, while the world is likely to lose over $280 billion in the same period. Nevertheless, some economists believe that it is too early to estimate the total impact of COVID-19 on the world's economy and trade since the infection has not yet reached its peak. According to an estimated model by the same experts, the global GDP is likely to decline by roughly 0.42% in the first quarter of 2021 due to the outbreak. Furthermore, the negative impact of the crisis will be greater on the service-oriented economies, which means more jobs are at risk. Countries whose economies are highly dependent on tourism (more than 15% of GDP), such as Greece, Portugal, and Spain, will be more negatively influenced by this crisis. This current crisis is posing adverse effects on countries that are highly dependent on foreign trade by affecting supply chains. The literature suggests that if the crisis continues, it will cost 2.5%–3% of global GDP per month on average (McKibbin and Fernando, 2020).

CONCLUSION

The continuous emergence of new SARS-CoV-2 variants has made the control of the COVID-19 pandemic more complicated. It is also not clearly known whether new variants evolving after the Omicron variant will bear a higher transmission rate, infection capacity, and immune-escape potential. However, there is no doubt that the Omicron variant will not be the last variant of SARS-CoV-2. Fortunately, we have accumulated a lot of experience and methods to deal with the novel coronavirus, and we know what we need to do to stop the spread of virus variants. With global collaboration and rapid data sharing, human society would ultimately win the war against COVID-19. As the SARS-CoV-2 continues evolving and becoming more transmissible between humans and given that it raises serious health concerns worldwide, it is a matter of intense research nowadays. We still do not know whether different SARS-CoV-2 strains, resulting from genetic mutations or adaptive evolution, have affected the transmissibility, susceptibility, and severity of COVID-19 infection. Thus, a convincing knowledge of the SARS-CoV-2 characteristics, including pathogenic mechanism, viral mutation and genetic recombination, its interaction with the host immunopathological response, evolution and genetic diversity, transmission network, and any specific and effective treatment option, may provide a clearer biological understanding of how the virus infects some patients severely, while the majority of patients are only mildly symptomatic or even asymptomatic. This comprehension can also boost a scientist's ability to successfully design an effective vaccine and take some efficient prophylactic and therapeutic measures, not only for 2019-nCoV but also for future outbreaks of similar coronaviruses.

AUTHOR CONTRIBUTIONS

ES and NK provided direction and guidance throughout the preparation of this manuscript. JA, HE, HH, HR, EA, and HN conducted the literature and drafted the manuscript. All authors reviewed the manuscript, made significant revisions, and approved the final version of the manuscript draft.

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