Severe acute respiratory syndrome coronavirus 2 for physicians: Molecular characteristics and host immunity (Review)

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Abstract. Recently, severe acute respiratory syndrome (SARS) coronavirus (CoV) 2 (SARS-CoV-2)-causing CoV disease 2019 (COVID-19) emerged in China and has become a global pandemic. SARS-CoV-2 is a novel CoV originating from β-CoVs. Major distinctions in the gene sequences between SARS-CoV and SARS-CoV-2 include the spike gene, open reading frame (ORF) 3b and ORF 8. SARS-CoV-2 infection is initiated when the virus interacts with angiotensin-converting enzyme 2 (ACE2) receptors on host cells. Through this mechanism, the virus infects the alveolar, esophageal epithelial, ileum, colon and other cells on which ACE2 is highly expressed, causing damage to target organs. To date, host innate immunity may be the only identified direct factor associated with viral replication. However, increased ACE2 expression may upregulate the viral load indirectly by increasing the baseline level of infectious virus particles. The peak viral load of SARS-CoV-2 is estimated to occur ~10 days following fever onset, causing patients in the acute stage to be the primary infection source. However, patients in the recovery stage or with occult infections can also be contagious. The host immune response in patients with COVID-19 remains to be elucidated. By studying other SARS and Middle East respiratory syndrome coronaviruses, it is hypothesized that patients with COVID-19 may lack sufficient antiviral T-cell responses, which consequently present with innate immune response disorders. This may to a certain degree explain why this type of CoV triggers severe inflammatory responses and immune damage and its associated complications.

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Contents

1. Introduction

During the Middle of December 2019, a virus-induced pneumonia emerged in Wuhan, China, which ultimately resulted in the current global pandemic (1). In the first few days of its emergence, this disease was simply identified as a virus-related respiratory disease. However, the original pathogen remained unclear (2). In early January 2020, the specific virus was isolated and later identified as a novel coronavirus by sequencing (3). The virus was first officially named the 2019 novel coronavirus (nCoV) by the World Health Organization (WHO) and subsequently termed 'severe acute respiratory syndrome CoV 2 (SARS-CoV-2)' by The International Committee on Nomenclature of Viruses. The virus is highly infectious and has infected >10,000 individuals in China and other countries. Since the virus presented the potential to result in a pandemic, the WHO declared a public health emergency of international concern on this epidemic on the 31st January 2020. The virus primarily infects the respiratory tract, resulting in pneumonia, acute respiratory distress syndrome (ARDS) and other fatal complications, including acute kidney injury, coagulation dysfunction and shock, according to a published report (4). Collectively, all the associated diseases caused by SARS-CoV-2 are termed Coronavirus Disease of 2019 (COVID-19). Therefore, knowledge on the molecular characteristics and host immunity in reaction to the virus would aid physicians to further understand the disease, manage patients and implement the occupational precautions. However, since it

is a novel virus, the detailed underlying mechanisms are yet to be fully elucidated. As an accurate profile of the virus is urgently required, the present article screened the available literature on SARS-CoV-2 and other members of the coronavirus family to perform a literature review.

2. Literature review

The databases of Pubmed (date of access, 20/08/2020; https://pubmed.ncbi.nlm.nih.gov/), Web of Science (date of access, 20/08/2020; https://apps.webofknowledge.com/), Library, Information Science & Technology Abstracts (date of access, 20/08/2020; https://connect.ebsco.com/) and Library of Congress (date of access, 20/08/2020; https://www.loc.gov/) were screened with a date range from 2003 to 2020, using primary key words, including 'coronavirus', 'human coronavirus', 'coronavirus pneumonia', '2019 novel coronavirus', '2019-nCoV', 'severe acute respiratory syndrome coronavirus 2', 'SARS-CoV-2', 'Coronavirus Disease 2019' or 'COVID-19', and secondary key words, including 'molecular characteristics', 'biological characteristics', 'immune response', 'immunity' or 'pathogenesis'. Articles with information on epidemiology, etiology, pathophysiology, clinical characteristics, therapeutic and preventive strategies of COVID-19 were preferred, along with articles concerning severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) or other coronavirus infections in high-quality journals with high impact factors, high index or with reputations in infectious diseases. The selected articles were categorized based on the abstract and those deemed to be pertinent were carefully read by ≥ two authors: LD, JS and/or JW. The major conclusions of the selected articles were then summarized in the current review. Through properly summarized information and scientific speculation, the present review aimed to provide insights regarding the novel coronavirus for clinical physicians.

3. Etiology

Coronavirus. Coronaviruses are RNA viruses that are distributed broadly worldwide. This family of viruses has a large genetic diversity and high prevalence of genome recombination (5). In taxology, these viruses belong to the Nidovirales (Order), Coronaviridae (Family), *Coronavirus* (Genus) and *Orthocoronavirus* (Sub-genus), which consist of four species (α, β, γ and δ) (6). Coronaviruses can only infect vertebrates. The viral particles are spherically and irregularly shaped with a diameter of 60-220 nm. Under an electron microscope, the core particle is wrapped in an envelope with 'spines', giving it a crown-like appearance, and was therefore termed 'coronavirus'. Within the envelope, there are three glycoproteins: i) The spike protein (S protein), which functions as the main antigen and contains receptor-binding sites to mediate cytolytic actions; ii) the envelope protein (E protein), which is a small glycoprotein forming the envelope; and iii) the membrane protein (M protein), which is a transmembrane transportation protein with functions in nutrition intake, maturation of the viral envelope, packaging and budding of progeny viruses (5). The spines observed using electron microscopy are comprised of S proteins. As S protein works as the main epitope for host immune recognition, the spines on different coronaviruses exhibit significant heterogeneity (7).

Over the previous decades, six coronavirus species that are able to cause human diseases have been identified: 229E, OC43, NL63, HKU1, SARS-CoV and MERS-CoV (6). Outbreaks of SARS-CoV and MERS-CoV occurred in 2003 and 2012, respectively, resulting in severe respiratory syndrome and fatal illness (8,9).

Novel coronavirus. SARS-CoV-2. The first reported clinical case of the novel virus can be traced back to the middle of December 2019 (1). Following this, the novel coronavirus was isolated and an outbreak followed in January 2020 (10).

SARS-CoV-2 belongs to the β-coronavirus (β-CoV) subfamily. Similar to its family members, the diameter of SARS-CoV-2 is 60-140 nm. The viral particles exhibit distinctive spikes, which are generally spherical and exhibit certain pleomorphisms (1). According to the RNA sequences of SARS-CoV-2 isolated from three patients with unknown pneumonia in the early stage of the outbreak, the SARS-CoV-2 genome has a typical β-coronavirus composition, with >85% nucleotide sequence similarity to that of the bat SARS-like CoV (bat-SL-CoVZC45; MG772933.1) genome (3). A few days later, the sequence was confirmed further in another study by sequencing the results from nine inpatients, which demonstrated that SARS-CoV-2 is closely related to bat-SL-CoVZC21 (3). Although there is a potential evolutionary link between SARS-CoV and SARS-CoV-2, SARS-CoV-2 is in a different clade from SARS-CoV and MERS-CoV. Genome sequencing revealed that SARS-CoV-2 only shares 79.0% nucleotide homology with SARS-CoV and 51.8% with MERS-CoV (11,12). In addition, the conserved replicase domain (ORF 1ab) of SARS-CoV-2 is <90% homologous to other β-CoVs (3). Therefore, the SARS-CoV-2 is a novel CoV originating from β-CoVs and may have distinct pathogenic features.

Genomic structure of SARS-CoV-2. SARS-CoV-2 and SARS-CoV share a similar genomic structure with other members of the β-CoV family (3). β-CoVs have a typical genome structure with a 5'-untranslated region (UTR), ORF 1ab, S gene, E gene, M gene, nucleocapsid gene (N gene), 3'-UTR and several unidentified nonstructural ORFs (Fig. 1) (13). ORF 1 ab in particular is also known as the pol gene and comprises ~60% of the genome, which encodes RNA-dependent RNA polymerase (RdRp), proteinase and other undefined proteins. Genome sequencing results revealed major distinctions between SARS-CoV and SARS-CoV-2 in the S gene, ORF 3b and ORF 8 (14).

The S protein functions as an epitope of host immune recognition (15). During the SARS epidemic, development of SARS vaccines and several preventive strategies were generally focused on the S protein (16). High variations in the S protein influences viral antigenicity and caused challenges for vaccine development to date. However, there is a conserved region located within the encoding gene of the S protein. The gene sequence of the S2 subunit of the S glycoprotein is highly conserved and shares 99% homology with other SARS-like CoVs and SARS-CoV, which may serve as a potential therapeutic target (3,11). Notably, the receptor-binding domain (RBD) of S1 subunits, which is responsible for interaction with host receptors, is similar (73-76% in homology) between SARS-CoV-2 and SARS-CoV (17,18). However, the RBD is

Figure 1. Schematic of the SARS-CoV-2 RNA genome. SARS, severe acute respiratory syndrome; CoV, coronavirus; UTR, untranslated region; ORF, open reading frame; S, spike; E, envelope; M, membrane; N, nucleocapsid.

different between SARS-CoV-2 and bat SARSr-nCoV. The S protein was reported to interact with the host receptor and angiotensin-converting enzyme 2 (ACE2), and regulate cross-species and human-to-human transmission (19). According to sequence analysis, SARS-CoV-2 also binds with greater affinity to ACE2 compared with SARS-CoV (17). This may partly explain why SARS-CoV-2 exhibits cross-species transmission similar to SARS-nCoV, whilst bat SARSr-nCoV does not.

A novel short putative protein within ORF 3b has been detected in SARS-CoV-2 (20). A deletion in ORF 3b was found to inhibit viral replication by promoting type I interferon (IFN) signaling during SARS-CoV pathogenesis (21,22). The function of this shortened protein within ORF 3b of SARS-CoV-2 warrants further investigation. Furthermore, ORF 8 from SARS-CoV-2 is highly variable from that of SARS-CoV, encoding a novel protein that is distinct from the conserved ORF 8-derived protein (20,23). Theoretically, it may encode serine/threonine kinases with unknown function (24).

Although SARS-CoV-2 has a distinct genome sequence, detection of SARS-CoV-2 is not challenging. Reverse transcription-quantitative PCR is effective in identifying SARS-CoV-2 infection (25,26). Furthermore, the E and RdRp genes are notably different between SARS-CoV-2 and other types of coronaviruses, such that the PCR detection of the E and RdRp genes is also highly sensitive (27). Therefore, E and RdRp genes may also serve to be potential therapeutic targets and promising vaccine candidates, similar to the S gene. The E gene is involved in the viral cycle of CoVs, whilst the RdRp protein is essential for the progression of viral replication and transcription (27). The highly conserved RdRp gene sequence suggests that potent agents developed for SARS-CoV RdRp may also exhibit equal potency and efficacy for SARS-CoV-2 RdRp. For instance, aurintricarboxylic acid, an anionic polymer that has been proven to bind to viral proteins, including RdRp of SARS-CoV and gp120 of human immunodeficiency virus, prevents SARS-CoV replication by targeting RdRp (28). However, the efficacy of targeting the RdRp of SARS-CoV-2 requires further investigation (28).

Infectivity of SARS-CoV-2. SARS-CoV, MERS-CoV and SARS-CoV-2 are the three most notable coronaviruses known to infect humans (2,29). Although they all primarily infect the respiratory tract, clinical differences exist in their transmissibility, incubation period and severity of symptoms (30). Among them, the infectious stages serve a major role in the magnitude of clinical and societal consequences (30). A recent review reported that SARS-CoV-2 had comparable or slightly higher transmissibility (average R0, 2.5) compared with SARS-CoV (average R0, 2.4), with the highest estimate of SARS-CoV-2 R0 being >3.5 compared with 2.5 for SARS-CoV (31). Moreover, the incubation time appears to be longer during illness from SARS-CoV-2 (4-12 days) compared with SARS-CoV (2-7 days). The most notable difference is that the interval between symptom onset and maximum infectiousness for SARS-CoV-2 is 0 days (5-7 days in SARS-CoV), suggesting that the illness can be readily transmitted by patients who are not exhibiting symptoms (31). Therefore, unsurprisingly, SARS-CoV-2 has a higher R0 due to infectiousness in asymptomatic patients and a longer incubation period. As for MERS-CoV, R0 was reported to be 0.9, consistent with the fact that this illness has only resulted in regional outbreaks to date (31). The earliest case of MERS was identified in a hospital-associated cluster, where the disease appeared to be infrequent outside health care settings, though several household clusters have been documented (32). Symptomatic MERS usually presents following an incubation period of 2-14 days (33). Once MERS is suspected, respiratory specimens obtained within 7 days of symptom onset typically have the best diagnostic sensitivity, indicating that the peak in viral load and infectiveness may present during the incubation time of MERS (34). Although several regional studies from South Korea and Middle Eastern areas between 2012 and 2017 have reported different incubation periods (6-7.8 and 4.5-5.5 days, respectively), no difference in incubation times were found (35). Furthermore, the range of incubation time for MERS-CoV (2-14 days) is relatively longer compared with that of SARS-CoV-2 (4-12 days) (36).

4. Viral distribution and dynamics

Target cells and distribution of SARS-CoV-2 in the host. Coronaviruses primarily infect the respiratory and digestive systems (37). Reported symptoms range from mild and self-limiting illnesses, including common colds, fever or diarrhea, to severe symptoms, including severe pneumonia with renal damage or cardiac involvement and even death (37,38). The global mortality rates of SARS-CoV and MERS-CoV were reported to be 9 and 26.6-59.4%, respectively (39,40).

The initial step of viral infection is entry into host cells (5). A number of studies have demonstrated that the sequences of SARS-CoV-2 and SARS-CoV appear to be homologous (41,42), with similar S proteins that have strong binding affinities to the human cell receptor ACE2 (41). Therefore, ACE2-expressing cells, including pulmonary type II alveolar (AT2) cells, may serve as the primary target cells that are susceptible to SARS-CoV-2 infection (11). Distribution of ACE2 receptor expression is crucial in identifying target cells to provide evidence for the distribution of SARS-CoV-2 infection throughout the body.

A previous article indicated that ACE2 was highly expressed in alveolar, esophageal epithelial, ileum and colon cells (43). Following this study, researchers used the latest single-cell RNA sequencing data to analyze the expression of ACE2 in the respiratory, cardiovascular, digestive and urinary systems. The results demonstrated that cells in the heart, esophagus, kidneys, bladder and ileum exhibited similar or higher expression of ACE2 compared with that in AT2 cells, suggesting that these organs may be at risk of SARS-CoV-2 infection (44).

The lungs are the primary target of SARS-CoV-2 and other SARS-CoV-like coronaviruses, and they exhibit more severe clinical symptoms compared with other organs (4,45). Additionally, the lower respiratory system suffers more prominent damage than the upper system, where certain patients with severe infection rapidly develop ARDS (45,46). On January 24 2020, an article concerning patients with early SARS-CoV-2 first reported that the virus was detected in the bronchoalveolar lavage fluid of three patients (1). A number of studies published in succession indicated that patients with SARS-CoV-2 infection presented with pneumonia and various clinical symptoms, including fever, cough and fatigue (45,47,48). However, symptoms of the upper respiratory system, such as nasal congestion and rhinorrhea, were not obvious. Therefore, recommended specimens of the upper respiratory system for diagnostic tests include nasopharyngeal and oropharyngeal swabs (49). According to a study in Hong Kong, the initial saliva samples of 11/12 patients tested positive following admission (50). In six patients who were continuously monitored, the viral load in the saliva exhibited a declining trend (50). Furthermore, the saliva culture of three patients in this study indicated the presence of the live virus (50).

Aside from pulmonary manifestations, a previous study reported that the levels of liver enzymes in 43 patients were higher compared with those in the normal range, where one patient presented with liver function impairment (48). Moreover, another previous study indicated that the virus may infect cholangiocytes, which also express ACE2, but not hepatocytes, which do not express ACE2 (51,52). It is possible that the abnormal liver function was the result of biliary cell dysfunction or other causes, such as medication, instead of hepatocyte damage (52). Therefore, patients with SARS-CoV-2 should receive specialized tests and medical care to ensure that their liver function recovers during hospitalization.

Patients with coronavirus infection may also present with diarrhea and gastrointestinal-associated symptoms (53). In patients with MERS-CoV, sub-genomic virus RNA, which was indicative of viral replication, was observed in stool specimens (53). Additionally, certain patients with COVID-19 were reported to present with atypical symptoms, including diarrhea, similar to those with SARS-CoV and MERS-CoV infections (48). Concurrently, recent epidemiologic studies have indicated that the nucleic acid test in stool samples for SARS-CoV-2 could return positive (48,54). However, it is unclear whether SARS-CoV-2, an envelope virus, can remain infectious in the presence of bile and proteolytic enzymes in the digestive system.

Although certain patients presented with heart and kidney ailments, there were insufficient studies that have confirmed the presence of the virus in these organs. A previous study indicated that SARS-CoV-2 may bind the ACE2 receptors of tubular cells, causing cytotoxicity and abnormal renal function (55,56). Additionally, the researchers reported high expression of ACE2 in testicular, spermatogenic tube and interstitial cells (56).

The first pathological report on patients who succumbed to COVID-19 only obtained specimens of the lungs, liver and heart by puncture, rather than performing a complete autopsy (57,58). Therefore, data that could be used to support the hypothesis that SARS-CoV-2 directly damages the heart and liver was not obtained (57). Further pathological evidence is urgently required to study COVID-19 in this regard.

Viral load at different clinical stages. Since the viral load is proportional to the transmission capacity of the virus, clarifying the viral dynamic patterns of SARS-CoV-2 is crucial (59). During SARS-CoV infection, viral concentration in the respiratory tract is the highest during the febrile period (60). As SARS-CoV-2 is a relative of SARS-CoV, it can be hypothesized that respiratory secretions are highly contagious during the acute febrile phase of SARS-CoV-2 infection. Previous publications on SARS-CoV reported that certain patients presented with fever, myalgia and other symptoms, which were resolved within a few days. During this phase, the viral load increased continuously, peaking on day 10. As the disease progressed, IgG seroconversion occurred and the viral load began to decrease between days 10-15 (59). Lung damage during this phase may be caused by immunopathological damage as a result of an hyperactive host response (59). Other studies have demonstrated a slightly different peak in viral load on days 10-15 following fever onset (61-63). However, patients in these studies were treated with hydrocortisone, which may have resulted in a delayed peaking of viral load (61-63). Furthermore, Lo *et al* (64) previously reported that SARS-CoV was detected in plasma on days 3 and 4 following fever onset, where peak concentration was identified during the first week, followed by a rapid decline in the second week. This indicated that the SARS-CoV-2 may reach peak viral load ~10 days following fever onset like its relatives.

Apart from the febrile period, the virus replicates from initial infection up until elimination (59). Early in the latent period, the virus endows patient infectivity (65). Furthermore, the presence of the virus can be detected during the recovery phase, indicating that recovering patients may also be contagious (33). Moreover, according to several case reports, individuals with occult infections of SARS-CoV-2 can also be contagious, although this has not been previously reported in patients with SARS-CoV (43,44). Two scenarios may occur in patients during the asymptomatic phase: Either the patient is in the early stage of disease or the patient is an asymptomatic carrier. In the former scenario, previous studies have reported a relatively high viral load of coronavirus in the epithelial cells of the upper respiratory tract in the first few days following infection compared with the latter days (64,66). As death of these epithelial cells occurs in the early stage of disease, the virus released by those cells primarily account for the infectiousness (64). In the latter scenario, since ACE2-induced angiotensin II degradation is essential for the pathogenesis of coronaviruses (67), disease severity depends on the maturity and binding capacity of ACE2. Therefore, asymptomatic

carriers may exhibit lower expression of ACE2, causing an impaired binding capacity with SARS-CoV-2 in certain patients. Notably, during the outbreak of SARS in 2003, reports of asymptomatic patients were rare (68). However, asymptomatic SARS-CoV carriers were reported in a retrospective study in Guangzhou (China), following a community outbreak in 2004 (69). Similarly, in the early phase of the MERS outbreak, asymptomatic carriers were also rare (70). Nonetheless, a recent review in 2019 reported an increased asymptomatic rate of ≤28.6% among all MERS-CoV infections (71). It is possible that the increased rate of asymptomatic carriers was associated with advanced surveillance and completion of more diagnostic tests. When COVID-19 emerged in China, healthcare workers were prepared due to experience with surveillance and had access to abundant facilities for testing. Consequently, asymptomatic carriers were identified rapidly following the outbreak in Wuhan (China). Additionally, cases of transmission were confirmed. Viral load served an important role in viral transmission (72,73). The viral load in an asymptomatic carrier of SARS-COV-2 was similar to that in symptomatic patients, suggesting that the transmission capacity of asymptomatic carriers was equal to that in clinically diagnosed patients (73).

These aforementioned publications indicate that, in addition to patients in the acute stage, those at different clinical stages or those with asymptomatic infection also require proper evaluation of their contagious capacities and potentially isolation for disease management.

Factors influencing viral replication of SARS-CoV-2. The natural course of viral infection is intricate and variable, making it challenging to identify the influential factors underlying viral replication. Currently, the full-length genome sequencing results of different virus samples obtained from patients with COVID-19 were almost identical with a few site mutations (74), indicating that this virus has yet to undergo significant mutations.

In the host, the innate immune response determines the level of the coronavirus replication (50). Previously published studies reported that patients with cancer may have an increased risk of contracting SARS-COV-2 and present with associated poor prognosis due to immunosuppression (37,75,76). Additionally, age was revealed to be an independent risk factor for incidence of severe events, including intensive care unit (ICU) admission, invasive ventilation and death in patients with tumors (75,77). Therefore, factors associated with impaired immunity, including old age and the presence of tumors, may downregulate the innate immune response of the host and accelerate viral replication, resulting in disease deterioration (76,77).

To date, host innate immunity may be the only direct factor identified to be associated with viral replication (78). However, increased ACE2 expression may also upregulate the viral load indirectly by increasing the baseline level of infectious viruses (79). A study previously analyzed large-scale datasets of four lung tissues from patients with lung cancer to investigate differences in ACE2 gene expression by ethnicity, age, sex and smoking status. The results demonstrated that the expression of ACE2 was higher in individuals who smoked compared to non-smoking controls (80). Therefore, smoking may upregulate the expression of cellular receptors, indirectly increasing viral replication. Nonetheless, direct evidence of smoking being associated with viral replication remains to be elucidated.

5. Immune response and pathogenesis

Innate immune response to coronavirus infection. As mentioned previously, the replication of human coronaviruses (hCoVs) is regulated by various host factors and immune interaction (81). The first interaction occurs through innate immunity (82). Although antiviral T-cells and antibodies are essential for virus clearance, the effectiveness of the innate immune response serves a decisive role in viral replication and sequential clearance from the host (81). Innate immunity is initiated by pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns or virus-associated molecules (83,84). The virus activates PRRs on cells and triggers the production and release of type I and III IFNs or other proinflammatory mediators. These mediators then initiate the sequential immune response or extensive immune damage (13,85). During SARS-CoV infection, this specialized type I IFN response pattern is essential for controlling potentially fatal viral infections (86). Researchers have previously demonstrated that plasma cell-like dendritic cells inhibited SARS-CoV replication through the immediate production of type I IFN, via the toll-like receptor 7-mediated recognition of viruses (87). Considering the potential antiviral efficacy of type I IFN in eliminating coronaviruses, aerosol inhalation of IFN was recommended in China for patients with COVID-19, to inhibit viral replication in the respiratory tract (88).

Besides activation, hCoVs can also inhibit the innate immune response by shielding viral RNA from host cell sensors to inhibit IFN induction (89). MERS-CoV encodes several structural and nonstructural proteins to negate the innate antiviral immune response (90,91). The structural proteins M and N and the auxiliary proteins derived from the ORF 3 and ORF 4 in MERS-CoV have been demonstrated to downregulate IFN signaling (90,91). Therefore, conserved structural proteins M, N and ORF 3 in SARS-CoV-2 may also downregulate endogenous IFN signaling via this mechanism, resulting in delayed or even failed clearance of the virus in certain patients.

Furthermore, coronaviruses induce the production of chemokines and cytokines other than IFN in the innate immune system. SARS-CoV infection can result in the moderate upregulation of inflammatory cytokine such as tumor necrosis factor (TNF) and interleukin (IL)-6 and significant upregulation of the chemokine ligand (CCL) 3, CCL5, CCL2 and chemokine (C-X-C motif) ligand (CXCL) 10 in macrophages and dendritic cells (92,93). When this response is overactivated, inflammatory damage occurs. For example, higher serum levels of proinflammatory cytokines (IFN-γ, IL-1, IL-6, IL-12 and transforming growth factor β) and chemokines (CCL2, CXCL10, CXCL9 and IL-8) were observed in patients with severe SARS-CoV infection, compared with those with milder symptoms (94,95). During MERS-CoV infection, chemokines and proinflammatory cytokines, including CCL2, CCL3, CCL5, IL-2 and IL-8, were found to be elevated in monocyte-derived macrophages and dendritic

cells, albeit in a delayed pattern compared with SARS-CoV infection (96-98). Consistently, serum proinflammatory cytokines (IL-6 and IFN-α) and chemokines (IL-8, CXCL10 and CCL5) were found to be elevated in patients with severe MERS compared with those in patients with mild or moderate conditions (99,100). By analyzing the plasma of patients with COVID-19 from Wuhan, a previous study observed that the baseline levels of IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, fibroblast growth factor, granulocyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, IFNγ, interferon γ-induced protein 10 kDa (IP10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP) 1A, MIP1B, platelet-derived growth factor, TNFα and vascular endothelial growth factor concentrations in serum were higher in both patients in ICU and non-ICU patients compared with those in healthy individuals (45). By contrast, serum levels of IL-5, IL-12p70, IL-15, eotaxin and CCL5 were similar between patients with COVID-19 and healthy adult individuals (45). Further comparison between patients in the ICU and non-ICU patients demonstrated higher serum levels of IL-2, IL-7, IL-10, G-CSF, IP10, MCP1, MIP1A and TNFα in patients in the ICU (45). These observations suggest that cytokine and chemokine dysregulation and/or overreaction caused by viral infection may serve an important role in the pathogenesis of coronavirus infection.

Adaptive immune response to coronavirus infection. Previous reports have demonstrated that the acute phase in patients with SARS-CoV infection is associated with leukopenia and severe lymphopenia in >80% patients, involving a marked loss of 90-100 and 80-90% in $CD4^+$ T and $CD8^+$ T-cells, respectively (101-103). Additionally, leukopenia and associated lymphopenia were observed in patients with MERS, but to a lesser degree compared with patients with SARS (30,104). A previous clinical study reported that 14% patients with MERS had leukopenia, whilst 34% presented with lymphopenia (104). Furthermore, early in the epidemic, leukopenia and associated lymphopenia were included into the diagnostic criteria of COVID-19 (105). A study of 41 patients with COVID-19 revealed that 63% patients had lymphopenia (45). Another previous analysis of 1,099 patients with COVID-19 reported lymphopenia in 82.1% of the samples obtained (4).

Two Chinese studies reported impaired $CD4^+$ and $CD8^+$ T-cell activation in patients with SARS-CoV (106,107). The suppressed activation of T lymphocytes indicated reduced cellular immunity in patients with SARS-CoV, particularly those with severe conditions (107). Due to the lack of optimal animal models of SARS, a number of laboratories developed an adaptive strain of SARS-CoV, termed MA15 and established an *in vivo* mouse model (108-110). The results demonstrated that following SARS-CoV-MA15 infection in elderly mice (age, 6 months), proinflammatory cytokines (TNF-α, IL-6, IL-8, IP-10 and MCP-1) and chemokines (CXCL1, CXCL2, CCL3 and CCL5) were upregulated, but the levels of virus-specific $CD8^+$ T-cells were significantly reduced compared to young mice (age, 6 weeks). Furthermore, in older mice (age, 12 and 22 months), the levels of cytokines and virus-specific $CD8^+$ T-cells were not significantly different compared with the elderly mice (age, 6 months) (108,111). Although decreases in the levels and activation of T-cells were closely associated the severity of SARS in the acute phase (102,112), the effect of T-cells in patients with MERS remains unclear. Considering T-cells are essential for the control of the innate immune response, lack of an effective antiviral T-cell response may result in disorders of the innate immune response and pathological deterioration (113). Additionally, reported data indicated that older patients (≥65 years old) with COVID-19 were more commonly admitted into the ICU (4).

Several studies have previously identified virus-specific memory $CD4^+$ and $CD8^+$ T-cells in patients who recovered from SARS (114-116). Virus-specific memory $CD8^+$ T-cells targeting major histocompatibility complex class $IA^*02:01$ restricting the epitopes on the S protein (SSp-1, S978 and S1202) were observed >1 year post-infection (117). These cells produced high levels of antiviral cytokines (IFN-γ and TNF-α) and cytotoxic molecules (perforin and granzyme B) following peptide stimulation *in vitro (*117). However, whether virus-specific memory T-cells are present in patients who recovered from COVID-19 remains controversial. This information is important for estimating the impact of future COVID-19 epidemics.

Immune damage caused by coronavirus infection. A recent meta-analysis compiled >600 studies to report a full clinical profile of COVID-19 (118). Among these patients, fever was the leading symptom [88.7%; 95% confidence interval (CI), 84.5-92.9%], followed by cough (57.6%; 95% CI, 40.8-74.4%) and dyspnea (45.6%; 95% CI, 10.9-80.4%). In hospitalized patients, 32.8% presented with ARDS (95% CI, 13.7-51.8), 6.2% with shock (95% CI, 3.1-9.3) and 13.9% with fatal outcomes (95% CI, 6.2-21.5) (118).

In addition to virological tests, CT scans served vital roles for early detection and disease evaluation (119,120). Peripherally distributed multifocal ground-glass opacities with patchy consolidations and/or posterior or lower lobe involvement predilection were the typical findings in lungs of patients with COVID-19, which warrant further evaluation (119). In the majority of cases, pulmonary architecture was compromised by the host immune response rather than hCoV replication (85). This is consistent with the fact that the immune response to respiratory viral infection, instead of direct injury from the virus, accounted for the pathological damage of cells in the respiratory system (85). Furthermore, an increase in neutrophils and monocyte numbers in the peripheral blood was reported to correlate with a reduction in $CD4^+$ and $CD8^+$ T cells in patients with severe SARS (103,121). Histological evaluation of pulmonary samples from patients who succumbed to SARS revealed extensive infiltration of neutrophils and macrophages in the lung interstitial and alveolar cells (122,123). Similarly, in patients with MERS, the severity of lung lesions was associated with extensive infiltration of neutrophils and macrophages in the lungs and peripheral blood (124). Additionally, inflammatory mediators serve a key role in the pathogenesis of ARDS (125). Several proinflammatory cytokines (IL-6, IL-8, IL-1β and GM-CSF) and chemokines (CCL2, CCL5, IP10 and CCL3) contribute to the incidence of ARDS, ultimately making ARDS the leading cause of death in patients with SARS or MERS (126-128).

Previous histological reports of patients who succumbed to COVID-19 revealed extensive infiltration of immune cells,

including $CD4^+$ helper T lymphocytes and $CD163^+$ M2 macrophages (129,130). In accordance with other previous reports concerning coronavirus infection, it can be hypothesized that the pathogenesis induced by the novel coronavirus is characterized by the continuously hyperactive innate immune response, induced by the delayed development of the adaptive immune response and prolonged virus clearance (131). Rapid virus replication and excessive pro-inflammatory cytokine/chemokine production then induce infiltration of inflammatory cells, resulting in airway and alveolar epithelial cell apoptosis (132). Moreover, the strong inflammatory response further reduces T-cell response by TNF-mediated T-cell apoptosis, aggravating tissue damage (133).

Immune damage results in thromboembolism in the lungs, heart and brain (134,135). Microorganisms and their components activate the expression of multiple cytokines after binding to the pathogenic PPRs on immune cells (136). Host proinflammatory cytokines exhibit pleiotropic effects on the activation of coagulation (135). In patients with COVID-19, abnormal coagulation test results were observed in the early stages of disease though clinical bleeding did not occur (137). According to the present data, the SARS-CoV-2 virus did not present with an intrinsic procoagulant effect (137). Therefore, development of coagulopathy was likely a result of the profound inflammatory response. In the 99 patients first reported in Wuhan, >1/3 presented with abnormal coagulation parameters (48).

Activation of coagulation and thrombin generation results from the interaction between pathogens and host innate immunity (138). Thromboinflammation or immunothrombosis refers to the humoral and cellular pathways of signaling amplification (138). Invasive pathogen-associated components activate platelets, mast cells and factor XII in the contact pathway and serve further downstream roles (135). Circulating serine protease inhibitors, including antithrombin, are largely decreased during severe inflammatory responses (139). This may explain why the representative coagulopathy indicator, D-dimer, is associated with the severity of inflammatory parameters, such as IL-6, in patients with COVID-19 in the ICU (45).

Besides thromboinflammation, endotheliopathy also contributes to coagulation disorders in patients with COVID-19 (140). ACE2 on endothelial cells mediate viral adhesion and invasion (141). Sequential viral replication induces the infiltration of inflammatory cells and apoptosis of endothelial cells, leading to microvascular prothrombotic effects (141). Along with microcirculatory clot formation, microvascular endothelial injury was reported to facilitate thrombotic microangiopathy, including cerebrovascular complications or myocardial ischemia (137).

6. Conclusions

In conclusion, the current review compiled available data concerning COVID-19, which were then compared with SARS and MERS to profile the molecular characteristics of SARS-CoV-2 and the immune response against the virus. As a novel CoV originating from β-CoVs, SARS-CoV-2 infects alveolar cells and other cells in which ACE2 is highly expressed, causing damage to target organs. Host immunity is crucial for viral replication and sequential pathogenesis. However, the comprehensive underlying mechanisms are yet to be fully elucidated. Based on the current data, it can be hypothesized that patients with SARS-CoV-2 infection may have insufficient antiviral T-cell responses, resulting in disorders of the innate immune response. To a certain degree, this may explain why this type of CoV triggers excessive inflammatory responses and immune damage due to COVID-19 and its associated complications.

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Availability of data and materials

Not applicable.

Authors' contributions

HT and LB conceptualized and designed the current work. LD, JS, NH, DL and JW researched and evaluated the literature obtained from the database. LD and JS drafted the manuscript. LD, LB, HT and HY revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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