Review Article

Pathogenesis, immune response and laboratory diagnosis of severe acute respiratory syndrome associated Coronavirus 2
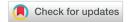
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Abstract

The pandemic unleashed by Severe Acute Respiratory Syndrome Associated Coronavirus 2 (SARS-CoV-2) has crippled the social, health and economic affairs of the world. This is the third stint with a highly contagious virus being introduced into the human population after the Severe Acute Respiratory Syndrome Associated Coronavirus (SARS-CoV) and Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) incidence in this twenty first century. This mini review provides a brief overview on the basic structure, pathogenesis, immune response and laboratory diagnosis of SARS-CoV-2 for better understanding of the disease process in order to induce novel insights to further intensify the prevention, containment and identification of CoVID-19.

Abbreviations

SARS-CoV, MERS-CoV, CoVID-19, CCR, INF, ORF, Nsp, MHC, IL

Introduction

The CoVID-19 caused by a novel strain of coronavirus, known as Severe Acute Respiratory Syndrome Associated Coronavirus 2 (SARS-CoV-2), has spread worldwide with about 15 million confirmed cases and more than half a million deaths as of now according to Center for Disease Control and Prevention (CDC). The first incidence of the disease as clustered outbreak, reported in the Wuhan city of China during early December 2019 originated in the Huanan Seafood Market. The SARS-CoV-2 genome sequenced in January 2020 revealed the novel coronavirus have greater similarity to (96%) bat coronavirus compared with (79%) Severe Acute Respiratory Syndrome Associated Coronavirus (SARS-CoV) and (50%) Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV) that had caused an earlier outbreaks in China (2002) and

Middle East (2012) respectively [1]. The intermediate host for SARS-CoV and MERS-CoV was implicated to be civet cats and camel respectively [2]. The SARS-CoV-2 debuted into human host causing severe respiratory disorders and the intermediate host is unclear, probably bat [3] snake or pangolin [2]. However SARS CoV-2 is more contagious than the SARS-CoV and MERS-CoV [4].

Taxonomy

The coronavirus belong to coronavirinae subfamily coming under the coronaviridae family both of which belong to the Nidoviales order or superfamily [5]. The occurrence of a set of multiple 3′-nested (Nido in latin means nest) subgenomic RNAs after transcription, the uniquely large replicase complex system translated by ribosomal frame shifting mechanism and a multi-spanning integral membrane protein are salient features of the Nidoviales superfamily [6]. The coronavirinae subfamily has 4 genera on the basis of their phylogenetic relationships and genomic structures - alphacoronavirus, betacoronavirus, gamacoronavirus (mainly infecting viruses

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of whales and birds) and deltacoronavirus (virus isolated from pigs and birds) [7]. The alphacoronavirus consists of many animal virus and human virus (HCoV-229E and HCoV-NL63). The betacoronavirus genus includes prototypes of mouse Hepatitis Virus (HMV) and the human viruses such as the HCoV-OC43, SARS-HCoV, HCoV-HKU1, MERS-CoV, SARS-CoV including the SARS-CoV-2 capable of causing mild to severe respiratory infections. The *Coronaviridae* Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) proposes to use the convention SARS-CoV-2/host/location/isolate/date to name a particular isolate (for example SARS-CoV-2/human/Wuhan/X1/2019) with various characteristics like pathogenic potential in human and other hosts. In publications it would be further extended with a sequence database ID linking it to the specific genome sequence of the virus in the public database such as GenBank enabling further epidemiological and other studies [5].

Genome and proteins

The virus belonging to coronavirus subfamily, have the largest RNA genome of 28 to 32 kb. The enveloped single stranded positive RNA virus contain the replicase locus encoded at its 5' end and various structural genes arranged in the order Heamaggluttin esterase or HE (not seen in SARS-CoV 2), spike or S, small membrane or envelope or E, membrane or M and nucleocapsid or N at its 3'end [8]. Inside the envelope, the helical capsid structure is the RNA complexed with N whereas the S trimer, embedded in envelop, gives the virus a crown like morphology and hence giving the name 'corona"[9].

The Spike (S) glycoprotein of the SARS-CoV-2 is cleaved by a trypsin-like host serine protease (TMPRSS) into S1 and S2 domains to mediate viral entry into the host cell through the angiotensin-converting enzyme 2 (ACE2) receptors. The S2 domain is conserved in coronavirus forms the highly coiled stalk portion while the divergent S1 domain forms the bulb of the spike. The binding of S1 domain to the ACE2 receptors results in conformational changes in S2 domain leading to the fusion of virion and host cell membrane [6]. The interaction between S and corresponding receptors on the host cell determines the species range and tissue tropism of the virus. However S protein in SARS-CoV 19 have a peculiar furin-like cleavage site missing in the MERS-CoV and SASR-CoV [10]. Further, the virus is internalized by receptor mediated endocytosis [11].

The Membrane (M) glycoprotein, a membrane protein, contributing to virion shape is the most abundant constituent of the virus [6]. The glycosylated ectodomain of M molecule, is the least conserved part and is highly sensitive to proteolysis. The endodomain of M molecule, closely connected to the membrane surface, is compact and somewhat resistant to proteolysis. The transmembrane domain of M, which aids in anchoring and inserting the protein in its native orientation in the membrane is the central organizer of the viral assembly process [12].

The small membrane or envelope (E) protein, a minor constituent of virion has a short hydrophilic and long hydrophobic segment. The palindromic hairpin configuration of the transmembrane segment in the E protein is unique to SARS CoV [13]. The E protein have significant role in viral assembly, budding and spread. The N (Nucleocapsid) protein bind to the genomic RNA like beads on strings and interacts with M protein for viral assembly [14].

The coronavirus family has the largest genome among all RNA viruses enabling expansive coding capacity and gene expression strategies. The extremely large genome of coronaviruses are similar to eukaryotic mRNA with a 5'caps and 3' poly (A) tails at either ends. The gene coding for replicase is towards the 5' end whereas those coding for the structural genes like S, M and E are clustered at 3'end [15]. Another salient feature of the corona virus is the presence of multiple Open Reading Frames (ORFs). Several accessory proteins of SARS-CoV is supposed to have evolved from mutation through duplication and scavenging of ORFs and code for accessory proteins as well as junk genes. The SARS-CoV isolated from 2002 found to have 29 nucleotide missing from the previous reported animal isolates probably due to jump of virus from animal to human and fusion of ORFs 8a and 8b into a single ORF 8 [16].

Translation and replication

After viral entry into host cell the nucleocapsid and viral genome are disassembled. The ORFs 1a and 1b at the 5' end of the genome RNA are translated into pp1a and pp1ab via a frameshift mechanism and co-translational proteolytic processing results in production of 16 nonstructural proteins (designated as nsp 1 to nsp 16) including various accessory proteins [17]. The ORF1a encodes proteases (nsp1 to nsp 11) which function to process pp1a and pp1ab into the mature replicase proteins, assembling machinery for RNA synthesis and prime the host cell for infection whereas the ORF1b encodes (nsp 1 to nsp 10 and nsp11 to nsp 12) multiple enzymes promoting the replicase complex in viral metabolism associated with catalyzing RNA replication, transcription and also interfere with host cell process [11].

The nsp1 induces cell cycle arrest and inhibits interferon synthesis by infection cells preparing a favorable environment for viral replication. It co-localizes with replicase complex during early stage of infection and later it is seen colocalized with the M protein at the virus assembly and binding site. The deletion of nsp2 region is found to delay viral growth and kinetics. A unique domain is contained in nsp3 with encodes papain like protease. Along with nsp 4 and nsp 6, nsp 3 anchors replicase complex to intracellular membrane and remodel into double membrane dedicated for viral progeny RNA synthesis. Nsp 5 encodes the Mpro, 3C like protease. Nsp 7 to nsp 10 are localized into the auto-phagosomal RNA synthases compartment in the infected cells along with nsp 2. Nsp 8 is a primase that partner with nsp9 which have nonspecific RNA binding activity. The nsp 7 to nsp 10 is clustered at the carboxy terminal of PPla. Nsp 12 to 16 encodes enzymes for RNA replication and transcription. Nsp 12 is RNA dependent RNA polymerase. Nsp 4 (with exonuclease activity) and nsp 15 (NendoU with endoribonuclease activity) have the ribonucleolytic function in the replicase complex. Nsp 16 which has 20-O-methyltransferase activity is involved with capping and protection [11].

The progeny viral assembly occur in the endoplasmic reticulum Golgi intermediate compartment complex. The process of formation of nucleocapsid by cooperative binding of N protein to viral RNA (or encapsidation) results in the production of both positive and negative stranded RNAs. During genome packaging the corona viruses selectively incorporate positive stranded RNA into virions from a variety of newly synthesized negative and positively RNAs during the infection time [6].

Pathogenesis

The spread of SARS-CoV-2 is by close person to person contact, through infectious droplets and fomites. The SARS-CoV-2 infection mainly progress through three phases corresponding to different clinical stages after onset. The 1st asymptomatic stage may last for early 1 to 3 days. Here, the inhaled virus attaches to the respiratory cells and undergo replication but with limited innate immune response. During the next stage the virus propagates along the respiratory tract triggering a more robust immune response in the conducting airways, however majority of the infected patients might show mild to moderate infection in the upper respiratory tract during this phase [18]. Clinical manifestations range from fever, unproductive cough, fatigue, diarrhea, myalgia, sore throat, lymphopenia to severe pneumonia [19]. The third stage progresses to pulmonary destruction with hypoxia and acute respiratory distress syndrome [18]. The infiltrated lung and damaged alveoli shows multinucleated giant cells containing macrophages and cells of epithelial origin along with numerous characteristic syncytium like formation [20].

There is marked lymphopenia, hemophagocytosis and in some cases diarrhea, hepatic involvement, atrophy of white pulp in speen, lymphadenopathy suggesting wide systemic spread dissemination of the virus along with copious viral shedding in respiratory and excretory secretions. Some develop cardiovascular complications, with tachyarrhythmia and thromboembolic events characterized by troponin rise [21]. Clinical investigations showed fevers, lymphopenia, highly elevated C-reactive protein, proinflammatory cytokines, serum ferritin, and D-Dimers. One possible explanation of these proinflammatory cytokine expression is the virus induced endothelial damage, apoptosis, and necrosis during viral replication and dissemination, leading to immune cell recruitment and activation, which in turn exhibited strong uncontrolled hyper-inflammation. Lethal cases of Covid 19 showed increased number of neutrophils and activated monocyte/macrophage in the airways [19].

Upto 80% of the cases remain asymptomatic, about 15% proceeds to severe pneumonia, however approximately 5% develop various complications including ARDS, septic shock and multi-organ failure [22]. Death occurred as early as 4 days to 108 days from the onset and shedding of virus from respiratory tract peaked during 10 days. The autopsy of SARS patients who succumbed to death in 2002 outbreak during early phase of disease showed a primary pathology of hyaline membrane, edema, fibrin exudates, microthrombi, sloughing of pneumocytes and mixed infiltration of polymorphonuclear cells, lymphocytes and macrophages as well as multinucleated giant cells in the lungs. Histology sections obtained during later phase of diseases showed type II pneumocyte hyperplasia, metaplasia, vasculitis, hypercoagulability, bronchiolitis obliterans with severe lung damage [22].

Host immune response

The virus entry into the cells activate both innate and adaptive immunity initially by presentation of viral antigen on type I and II MHC molecules of infected and immune cells like macrophage and dendritic cells as an antiviral response. Toll like receptor (TLR) 1 is modulated to TLR 10 and chemokine receptors like CCR5, CCR3, and CCR1 are unregulated in the host cells [23]. The mechanism by which virus enters the immune cells like macrophage, lymphocytes and monocyte in the lungs, which express ACE2 receptor minimally, is still unclear. Modulation of neutrophil counts and lymphocytopenia, the common hall mark of the disease, correlates directly with disease severity and death. Various downstream transduction pathways such as IRF3 (IFN regulatory factor-3), nuclear factor κB (NF-κB), JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signaling pathways, are activated which are pivotal in host antiviral immune response [24]. Yet the coronavirus uses different strategies to evade the defense mechanism of the host immune system. The replication of corona virus occurs in double membrane vesicle (DMV) shielding it from detection by cytosolic pattern recognition receptors. Coronavirus also suppresses the induction of interferon γ which is crucial for viral clearance. T cell activation is also delayed by the virus through mechanisms that down regulate the class I and II MHC molecules in the cells thereby evading the adaptive immune response [23].

Innate immune response shows a reduction in immune cells such as T cell and NK cells that are also crucial for cell mediated immunity. The absolute count of circulating CD4+ cells, CD8+ cells, B cells monocytes, eosinophils and basophils and natural killers (NK) cells is markedly decreased [24]. A cohort study with 452 severely affected SARS patients in Wuhan reported a decrease in total T cells where the helper, suppressor and regulatory subset of T cells and memory T cells where decreased while the percentage of naïve T cell was increased [25]. This imbalance biased towards the naïve T cell, not favoring the memory and other subset, probably unleash a massive uncoordinated cytokine response, hyper-inflammation and relapse of Covid infection [24].

Blood picture of patients from Wuhan also reported increased IL6, elevated C-Reactive protein (CRP), thrombocytopenia, anemia, hypofibrinogenemia, hypoalbuminemia, increased C-X-C motif chemokine 10 (CXCL10)/Interferon gamma-induced protein 10 (IP-10), chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemo-attractant protein 1 (MCP-1), Macrophage Inflammatory Protein (MIP-)1A/CCL3 and TNF-α, apart from neutrophilia and lymphopenia, suggesting a dysregulated immune response. Elevated levels of proinflammatroy cytokines like IL-6, IL-1β, IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP-10, MCP-1, CCL3 and TNFα, directly correlated with the severity of the disease suggesting

a hyperinflammatory immune response in the patient [26]. The hypercytokinemia or 'cytokine storm' triggers various pathological events such as vascular permeability, plasma leakage, disseminated vascular coagulation accounting life threatening respiratory symptoms and multi-organ failure. This avalanche of cytokines in SARS-CoV2 infected patients attributes to the progressive tissue injury, ADS and systemic effects involving gastrointestinal tract, neuroendocrine, renal and cardiovascular organs [24].

The B cells involved in humoral immunity through antibody production, acquired changes in B cell receptor. The naïve B cell count decrease but the plasma cells in circulation is increased. As much as 96% patients showed seroconversion of IgG or IgM in some days after symptom onset [27]. Virus specific IgG appeared about 17-19 days in 100% patients while virus specific IgM appeared in about 20-22 days in the 94% patients after symptom onset corresponding to decrease in viral load and suggesting active humoral immunity. Early production of neutralizing antibodies against coronavirus causes accumulation of antigen antibody complex and antibody dependent enhancement (ADE) resulting in immune complex mediated inflammatory response leading to organ damage [27, 28].

Immunity in children with their high plasticity for adaption are effective in clearing the viral load may explain the mild symptoms and development of disease in some of them. Furthermore ACE2 receptors are lesser in children compared to adults for viral entry into the cell important for the mediation of infection [29]. Women also showed less viral load, ACE2 expression and inflammation and higher level of CD4+ T cells and antibody response, TLR8, CD40L and CXCR3 and high antiviral IF-Gama production when compared to men. The reasons for this is the hormonal and genetic difference that lower incidence of comorbidities affecting lungs such as smoking, cardiovascular diseases and X chromosomes that produce associated cytokines [30]. Patient over 60 showed more than 50% mortality. This extreme increase or decrease in age is correlated with an increase in severity and mortality in the disease [21].

Diagnosis

Radiological finding: Typical CT scans of Covid 19 patients show ground glass opacities mostly involving multiple lobes with a peripheral and subpleural distribution in majority of cases and a "white lung appearance" in severe cases. CT can be recommended for long term evaluation of fibrosis in lungs [31]. Chest X Rays, though not as sensitive as CT, can also be helpful in monitoring the rapid progress of infected patients in emergency cases. However the imaging reports are variable, nonspecific, have significant overlap with pneumonia from other etiology and so the standard reference relies mainly on laboratory diagnosis for confirmation of the disease [32].

Specimen collection, biosafety and transport

Specimen collected are nasopharyngeal (OP) and/or oropharyngeal (ON) swabs using dracon or polyester swabs from the Upper Respiratory Tract (URT), sputum or Bronchoalveolar Lavage (BAL) or endotracheal aspirate collected in sterile containers from Lower Respiratory Tract (LRT). During initial stages RNA of SARS Cov-2 is detected more from NP swabs (63%) than in OP swabs (32%) [33]. World Health Organization recommends collecting OP and NP swabs in same the tube to increase the detection. URT can be easily collected compared to sputum and BAL, which increases the exposure and biosafety risk to health workers [34]. Viral RNA peaks in URT during 7 to 10 days after disease onset and declines after that whereas in LRT the viral detection rate are high after 3rd week of illness onset and maintains thus throughout [35]. Other specimens including rectal swabs, stool, urine and blood in appropriate sterile containers as recommended by the WHO. The source of specimen, severity of disease and various underlying factors like diabetes, heart disease determine the shedding of RNA virus and the detection rate in the specimen. Paired sera during acute (1st week of illness) and convalescent phase (2 or 3 weeks later) should be collected for serological assay whenever possible. Specimens in viral transport medium should be immediately shipped after triple packaging maintaining proper cold chain. It can be maintained at refrigerated temperature for up to 72 h, or frozen at –70°C or below in case longer transport is expected [36].

Serological assays

Serological assay including Enzyme-Linked Immunosorbent Assays (ELISA), rapid antibody immunochromatographic tests, point-of-care test (POCT)-fluorescence assays, and chemiluminescence immunoassays (CLIAs) detects viral antigens or antibodies against the viral antigens in the specimens [37]. These are cheaper, require lesser analytical time and have greater productivity as far as automated instruments in hospitals and laboratories are concerned when compared to the molecular tests. Serological tests can be used for screening and tracing contact for serological surveillance and epidemiologic studies at a broader level as well as for understanding the dynamics of immune response to disease severity and to assess cross reactivity leading cross protection and long term immunity from an individual level [34]. The viral antigens mostly used in serological assays are the N protein, S protein and RBD of the S protein. Sensitivity wound be higher if N and S proteins are used together [38]. The serum titer increase in IgM and Ig A is relatively slower in SARS-CoV-2 compared to other virus. However seroconversion after exposure is comparable to other viral infections with IgG concentration peaking as IgM concentration is maintained (plateau) [27]. The median seroconversion time for total antibody against the receptor binding domain of the S protein of SARS-CoV-2 was 11 days while for IgM and IgG were 12 and 14 respectively [39]. Hence, during the initial phase of the disease the serological assays are likely to give false negative as antibodies are detectable between 5 to 14 days after the disease onset. The sensitivity of serology tests for total antibody, IgM and IgG were 100.0%, 94.3% and 79.8%, respectively during l5 to 39 days after onset (later phase) while RNA was detectable only in 45.5% of the samples from 15 to 39 days [40].

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Viral antigen particles directly detected by immunochromatographic assay, uses antibodies against the nucleoprotein of the virus pre-coated in a line over a membrane strip with colloid gold conjugate impregnation. The specimen applied at one end and, flows laterally over the membrane and if there is viral antigens in the sample, a visible band is formed on the test line corresponding to the antibody–antigen–antibody gold conjugate complex deposit. Even though rapid and easy to perform,

immunochromatographic assay can give false negative results depending on variable viral load in different samples. The fully automated CLIA assay limits exposure to samples offering complete traceability as in fluorescence assay where the reading is through a detector system. In both cases the results can be directly fed to the Laboratory Information System enabling easier documentation and access [41].

Molecular methods

The detection of viral RNA using nuclei acid amplification tests such as RT-PCR require approved laboratory facilities and standardizations. The viral load is highest during the initial phase and decreases afterwards in NP swabs requiring lower pre-specified Ct value and fewer replications cycle than when viral load is lower [42]. Primers for gene encoding the structural proteins, ORFs and RNA dependent RNA polymerase are provided by WHO and different countries follow different protocols. Specimens of poor quality with little patient materials, very early or late collection, improper handling during transportation, repeated thawing leading to non-maintenance of cold chain and other technical factors inhibiting RNA amplification or detection can give faulty results [38]. Other molecular methods such as loop-mediated isothermal amplification, multiplex isothermal amplification followed by microarray detection, and CRISPR (clustered regularly interspaced short palindromic repeats)-based assays are also being evaluated [34].

Diagnostic interpretation

A real time instantaneous picture of possible viral infection in acute case can be accomplished from molecular diagnosis whereas serology helps to demonstrate the presence of virus during a wider range of process of infection period. In early phase of infection sensitivity of RT-PCR assay is 66.7% while that of antibody assays is just 38.3%. However from day 8 after symptom onset the sensitivity of antibody assay increased and overtook RT-PCR and even crossed 90% by 12 days of onset. Interpretation of results is subject to accuracy of test, time of specimen collection, type of specimen collected, the type of antibodies or antigen (in case of serology) and the probe (in case of RT-PCR) used in the assay [43]. If two targets sequence (N and S) tests positive then the disease can be confirmed if the Ct value is less than 40 for both. However the tests requires confirmation and is deemed indeterminant if Ct is more than 40 for one target sequence. Low Ct value indicating high viral load is an indication of transmissibility. A positive RT-PCR along with direct antigen test also confirms a current infection, however negative tests has to be carefully interpreted in the current pandemic scenarios. A single negative assay in a symptomatic patient should not be relied upon to exclude the disease. In case of serological tests, the aspect of long term immunity through seroconversion should also be taken into clinical consideration along with critical evaluation of previous infection or exposure as a false positive in such situations could lead to false reassurance and inappropriate behavior encouraging community spread [44].

Conclusion

To control the current outbreak extensive measures that increase person to person transmission should be strictly followed. Susceptible populations such as health care providers should be made aware of the guidelines and protective measures recommended by WHO. Accurate diagnosis of the disease and preventive measures are important to appropriately treat and contain the spread of the disease.

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