ejpmr, 2020,7(11), 301-310

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

COVID19: A PANORAMIC VIEW (PART 2)

[1]Dr. Sunanda Gaddalay, *[2]Dr. Ruchi Rathi, [3]Dr. Anita Kale, [4]Dr. Revtee Birajdar, [5]Dr. Ramchandra Kabir and [6]Dr. Amol Badgire

[1]Professor and HOD, Dept. of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur.
[2,4]PG Student, Dept. of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur.
[3,5]Professor, Dept. of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur.
[6]Reader, Dept. of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur.

***Corresponding Author: Dr. Ruchi Rathi**

PG Student, Dept. of Conservative Dentistry and Endodontics, MIDSR Dental College and Hospital, Latur.

Article Received on 10/09/2020 **Article Revised on 30/09/2020** **Article Accepted on 20/10/2020**

ABSTRACT

COVID-19 has led to more than 3.39 million confirmed cases accross the country and is known to be caused by a highly pathogenic strain of human corona virus (HCoV) - SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus 2). The pandemic began in December 2019, when a cluster of 41 cases with severe pneumonia of unknown origin emerged from Wuhan, China. Extensive research and scientific response has helped to fight the pandemic by availing more information about the disease. The surface of the virus displays prominent club shaped projections, composed of its spike protein, under the electron microscope; which imparts the virus its pathogenicity. virus being a potent inducer of inflammatory cytokines; activates immune cells thereby resulting in profound cytokine storm and ARDS. Knowledge of incubation period, basic reproduction number, pathogenesis, viral structure and viral life cycle will help to provide better treatment to those infected.

KEYWORDS: SARS-CoV-2, COVID-19, Incubation period, Basic reproduction number, Spike protein, Cytokine storm, ARDS, Pneumonitis.

INTRODUCTION

The world is currently facing one of the deadliest pandemics resulting from an outbreak of coronavirus disease which has led to 3.39 million confirmed cases accross the country, as of 28th August, 2020.[1] On 30th January 2020, India's first confirmed case of the coronavirus infection was reported in the state of Kerala. The affected person had travel history from Wuhan, China; where the disease is said to have emerged from. COVID-19 (coronavirus disease 2019) is known to be caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus 2); a highly pathogenic strain of human corona virus (HCoV) which is known to cause zoonotic diseases.[2] The Director-General of World Health Organization (WHO); Dr. Tedros Adhanom Ghebreyesus, named the disease caused by SARS-CoV-2 as COVID-19.[3] COVID-19 manifests in the form of severe respiratory tract infections in humans and on 30th January 2020, was declared to be a Public Health Emergency of International Concern (PHEIC).[4] The world has witnessed previous outbreaks of coronavirus (CoV) which were caused by SARS-CoV and MERS-CoV (Middle East Respiratory Syndrome-CoV) in 2002-2003 and September 2012 respectively.[5] A swift scientific response has helped to fight the pandemic by availing more information about the disease. Through this article, we aim to bring together the knowledge of the disease, its clinical features and pathogenesis.

Natural history

In December 2019, a cluster of 41 cases with severe pneumonia of unknown origin emerged from Wuhan, Hubei province in China. These cases were epidemiologically related to a seafood and wet animal market in Wuhan. Clinical features of these patients were described, based on the data prospectively obtained by Huang et al. All 41 patients had pneumonia with abnormal findings on chest CT. 27 (66%) of 41 patients had been exposed to Huanan seafood market. Lower respiratory tract samples were obtained including bronchoalveolar-lavage fluid from these patients and subjected to rigorous sequencing and analysis. Based on the results, 2019-nCoV was implicated in the Wuhan outbreak. The 2019-nCoV antigen was identified in the lung tissue of patients by immunohistochemical analysis, IgM and IgG antiviral antibodies were detected in the serum samples from patients to demonstrate seroconversion, and animal (monkey) experiments revealed pathogenicity of 2019-nCoV (later named COVID-19). There was conclusive evidence of COVID-19 infection in these patients by January 2, 2020.[6,7,8]

Consequently, on 1st January, the Huanan wet seafood market was closed with the view of implementing necessary public health measures to prevent further spread of the virus within China and elsewhere.[9]

Chen et al reported that of 99 patients with SARS-CoV-2 pneumonia, 50 (51%) patients had chronic diseases. The conditions of 17 [17%] patients evolved into acute respiratory distress syndrome. It was observed that elderly men with other underlying diseases often have a higher fatality rate when infected with SARS-CoV-2 than that of elderly women or younger and healthier patients.[10]

Li et al conducted a study on 425 confirmed cases of COVID19 between January 1 and January 22, 2020. More than half of the cases (55%) with onset before January 1, 2020, were linked to the Huanan Seafood Wholesale Market,with clinical features in accordance with Huang et al. The mean incubation period was 5.2 days (95% confidence interval [CI], 4.1 to 7.0), with the 95th percentile of the distribution at 12.5 days.[11]

The findings of these studies were confirmed by two subsequent studies conducted by Song et al[12] and Chen et al[13] in 2020. These studies together indicated that fever, cough and dyspnea were the chief clinical findings of the disease along with fatigue, myalgia, sputum production, headache and hemoptysis. A small fraction of the cases also present with gastrointestinal symptoms.

Epidemology

Incubation Period (IP): Incubation period denotes the time period between exposure to the virus and symptom onset. The range of this period is important as it may influence the monitoring, surveillance, control and other public health activities for infectious diseases.[14] In the current scenario, the role of IP is crucial to determine the effectiveness of screening, transmission potential, suitable duration for quarantine, contact tracing and for estimating the size of epidemic. Backer JA et al[15] estimated the mean incubation period of 2019-nCoV using the travel history and symptom onset data of 88 confirmed cases of COVID-19. The estimated mean incubation period was 6.4 days (95% confidence interval (CI) = 5.6 – 7.7 days) ranging from 2.1 to 11.1 days (2.5th to 97.5th percentile).[15]

According to Linton et al, the mean IP was estimated at 5.0 days (95% CI: 4.2 - 6.0) when excluding Wuhan residents (n=52) and 5.6 days (95% CI: 5.0 - 6.3) when including Wuhan residents (n=158).[16] Li Q et al, in agreement with the above data; estimated the mean IP to be 5.2 days (95% CI = 4.1 to 7.0), with the 95th percentile of the distribution at 12.5 days.[11]

According to the situation report published by WHO in April, 2020; the IP for COVID19 is 5-6 days on an average and may extend up to 14 days. It can also last longer than 2 weeks and a very long IP could be suggestive of double exposure. Subsequently, Lauer SA et al estimated the length of IP to determine its public health implications. The median IP was estimated to be 5.1 days (95% CI = 4.5 to 5.8 days). Also, among those infected, 97.5% will develop symptoms within 11.5 days (CI = 8.2 to 15.6 days).[14] A familial cluster of 5 patients was observed by Bai et al where an IP of 19 days was reported for one person.[17] These estimates of the incubation period of SARS CoV-2 are also in accordance with those of other known human coronaviruses, including SARS (mean = 5 days; range - 2 to 14 days), MERS (mean = 5 to 7 days; range = 2 to 14 days), and non-SARS human coronavirus (mean = 3 days; range = 2 to 5 days).[14] A rapid systematic review and meta-analysis of estimates of IP from observational research was conducted by McAloon C et al. The collective parameters estimated (95% CIs) the median IP of 5.1 (95% CI = 4.5 to 5.8) days, whereas the 95th percentile was 11.7 (95% CI = 9.7 to 14.2) days.[18]

Table I – Summary of Mean IP reported by various authors.

DISEASE	AUTHOR	INCUBATION PERIOD
SARS-CoV-2	Backer JA et al[15]	6.4 Days
	WHO[20]	5-6 Days
	Lauer SA et al[14]	5.1 days
	McAloon C et al[18]	5.1 Days
SARS-CoV	Lauer SA et al[14]	5 Days
MERS-CoV	Lauer SA et al[14]	5-7 Days

Basic reproduction number (R_0): The basic reproduction number (R_0) is also called the basic reproduction ratio or rate or the basic reproductive rate. The concept of R_0 has been adopted from the field of demography, where this metric was first used to count offspring. When used by epidemiologists, it serves as a fundamental metric used to describe the contagiousness or transmissibility of infectious agents and the dynamics of the infectious disease. The magnitude of R_0 aids in predicting the potential size of an outbreak or epidemic and can also be used to estimate the proportion of the population that must be vaccinated to eliminate an infection from that population. Dietz defines R0 as "the number of secondary cases one case would produce in a completely susceptible population"; whereas Diekmann and colleagues have described it as "the expected number of secondary cases".[19]

According to WHO, the range of R0 is estimated to be 1.4 – 2.5 for COVID-19.[20] Jonathan Read and colleagues from Lancaster University determined the R_0 to be around 3.11 (95%CI = 2.39 – 4.13) using a deterministic Susceptible-Exposed-Infected-Recovered (SEIR) metapopulation transmission model of infection.[21] According to Zhou T et al, R_0 ranges between 2.8 and 3.9 when including the number of infected cases from international colleagues in the prediction.[22] In the preliminary investigation of early phase data; Zhao S et al estimated the mean R_0 to be in

the range of 2.24 (95%CI = 1.96–2.55) to 3.58 (95%CI = 2.89–4.39).[23] Li and colleagues analyzed the data from 425 confirm cases and found the value of R0 to be 2.2 but the model was not specified.[11] A review published by Liu Y et al, found the estimated mean R0 for COVID-19 to be around 3.28, with a median of 2.79 which is considerably higher than the WHO estimate.[24]

The R_0 of SARS was 2 and that for pandemic flu H1N1 2009 was 1.3 according to Singhal ; and according to Liu Y et al, the R_0 for SARS was in the range of 2-5, which is expected owing to the similarity of pathogen.[24, 25] The above data interprets as likelihood of epidemic spread. For $R_0>1$, the number infected is likely to increase and for $R_0<1$, transmission is likely to fade out.[24] Based on this information it is apparent that an outbreak of COVID-19 was predicted.

Table II - Summary of R_0 values as reported by various authors.

DISEASE	AUTHOR	R_0
SARS-CoV-2	WHO[20]	1.4 – 2.5
	Read JM[21]	3.11
	Zhou T et al[22]	2.8 – 3.9
	Zhao S et al[23]	2.24-3.58
	Li Q et al[11]	2.2
	Liu Y et al[24]	3.28
SARS	Liu Y et al[24]	2-5
	Singhal[25]	2
H1N1	Singhal[25]	1.3

Phylogenetic analysis

Coronavirus (CoV): Coronaviruses were first identified in the mid-1960s and are the largest known RNA viruses with a diameter of 50-200nm approximately.[10] They belong to the coronaviridae family of Nidovirales order and are single-stranded, positive-strand RNA viruses.[2] They derive their name from the crown-like spikes on their surface. There are six other members of the coronavirus family that are known to infect humans namely; 229E, NL63, OC43, HKU1, MERS-CoV, SARS-CoV and SARS-CoV-2 being the seventh. Of these, 229E, NL63, OC43 and HKU1 are known to commonly infect upper respiratory tracts of people around the world and they are classified as mildly pathogenic HCoVs.[2,26] The MERS-CoV, SARS-CoV and SARS-CoV-2 infect the lower respiratory tracts and are classified as highly pathogenic HCoVs.[2]

CoVs have been classified into four categories by The International Committee on Taxonomy of Viruses (ICTV): α, β, γ, and δ. The SARS-CoV-2 is a beta CoV and is postulated to have mutated from bat coronaviruses. Through genetic sequencing of the COVID-19, more than 80% identity to SARS-CoV and 50% to the MERS CoV was found; and phylogenetic analysis revealed that both SARS-CoV and MERS-CoV originate in bats.[5] A high degree of homology has been reported of the ACE2 receptor from a diversity of animal species indicating bats to be the intermediate host. The single intact open reading frame on gene 8 further affirms the assumption of bat-origins.[2] Also, a high level of genetic similarity (96.3%) is shared between SARS-CoV-2 and the bat coronavirus RaTG13, which was obtained from bats in Yunnan in 2013.[1, 2]

Viral structure and genome

The surface of the virus displays prominent club shaped projections, composed of its spike protein, under the electron microscope. The viral genome consisting of 26,000 to 32,000 bases is wrapped in the nucleocapsid and located inside the virus particle. The positive strand viral RNA consists of a cap structure at 5’ end and multiple poly(A) tails at the 3’ end. This permits the translation of replicase/transcriptase and viral proteins thereby serving as the messenger RNA (mRNA).

Approximately 2/3rd of the 5’-end RNA sequence is occupied by replicase/transcriptase genes. It is composed of 2 open reading frames (ORFs): ORF1a and ORF1b. About 16 nonstructural proteins are encoded by the ORFs. Remaining 1/3rd RNA sequence encodes for the four classical viral structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein and nucleocapsid (N) protein. Among these, the envelope plays a crucial role in pathogenicity of the virus.[2,3]

Pathogenesis

Research indicates that SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as a receptor to enter human cells.[27,28,29,30,31] ACE2 converts Ang II to Ang I and is expressed by several tissues like renal, cardiovascular and gastrointestinal; in addition to lung alveolar epithelial cells, enterocytes of the small intestine, arterial and venous endothelial cells and arterial smooth muscle cells.[32] Therefore these organs are potential sites of viral replication. The common transmission routes of novel coronavirus include direct transmission (cough, sneeze, and droplet inhalation transmission) and contact transmission (contact with oral, nasal and eye mucous membranes.[33] Once gaining entry, the virus begins its replication cycle.

Viral Life cycle: The primary determinant for a coronavirus to infect a host species is the S-protein–receptor interaction. Interactions between the S protein and its receptor initiate the initial attachment of the virion to the host cell.[34,35] Depending on the virus, the sites of receptor binding domains (RBD) vary within the S1 region of a coronavirus S protein; some having the RBD at the N-terminus of S1 (murine hepatitis virus, MHV), while others (SARS-CoV) having the RBD at the C-terminus of S1. SARS-CoV and HCoV-NL63 utilize angiotensin converting enzyme as their cellular receptor.[36, 37]

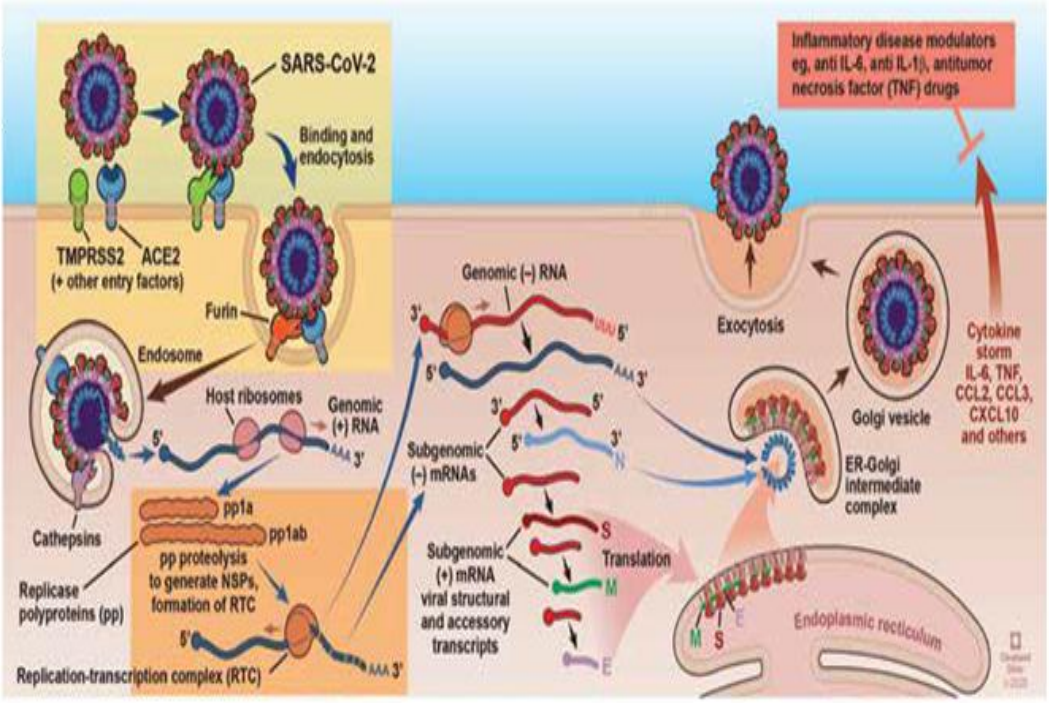
Following receptor binding, the virus needs access to the cytosol of the host cell. Generally, this is accomplished by proteolytic cleavage of S protein in an acid dependent manner by a cathepsin, transmembrane protease serine

S2 receptor (TMPRSS2) or another protease (like furin) and subsequently, fusion of the viral and cellular membranes occurs. The pH dependent fusion of viral and cellular membranes allows the release of viral genome into the cytoplasm.[38]

The next step is translation of the replicase gene from the virion genomic RNA. A frameshift between the two ORFs guides the production of pp1a and pp1ab polypeptides. The precise reason behind the use of frame-shifting to control protein expression is unknown, but it is postulated that: (a) either it helps to control the precise ratio of rep1b and rep1a proteins or (b) To delay the production of rep1b products until a suitable environment for RNA replication is created.[39, 40, 41]

Polyproteins are processed by proteases to yield 16 nonstructural proteins (nsps). Coronaviruses express only one protease - the papain-like proteases (PLpro). The cleavage of the nsps are attributed to the PLpros.[42, 43]

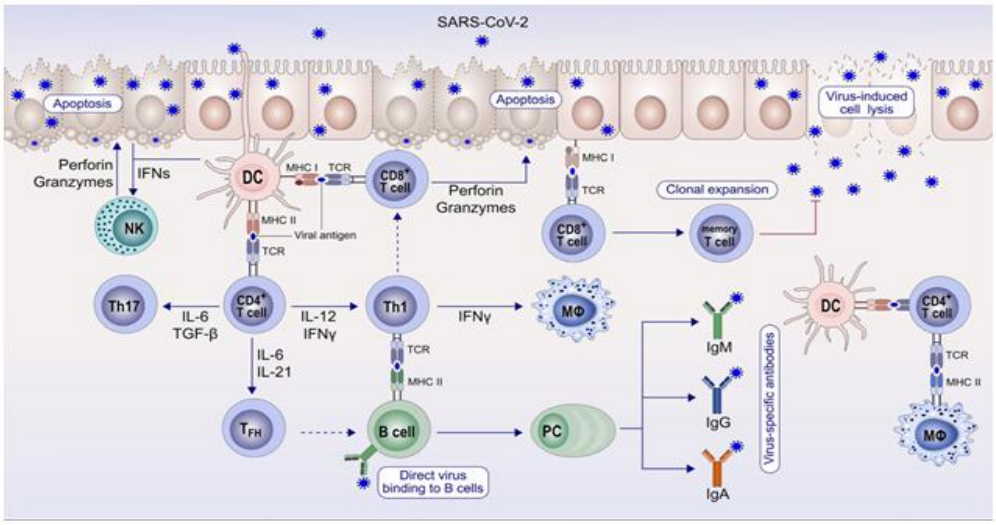
Image 1



RNA synthesis occurs as a result of assembling of the nsps into the replicase–transcriptase complex (RTC) followed by RNA replication and transcription of the sub-genomic RNAs.[44] A series of subgenomic mRNAs formed by discontinuous transcription are translated into relevant viral structural proteins, S, E, and M; following which they are inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum–Golgi intermediate compartment (ERGIC). There, viral genomes are encapsidated by N protein and assembled into membranes of the ERGIC containing viral structural proteins, forming mature virions.[45,46] Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.[35] These events have been summarized in Image 1.[34] As the viral titers increase, innate immunity responds with antiviral mechanisms.[2]

Immunopathogenesis: The SARS-CoV-2 virus is a potent inducer of inflammatory cytokines (Image 2).[47] The virus activates immune cells and induces the production of IFN-I or IFN-a/ß. IFN-I is the principal natural immune defense response in the early stages of viral infection. In mice, the rapid replication of SARS-CoV induces the delayed release of IFNs, which is accompanied by the influx of many pathogenic inflammatory mononuclear macrophages which interferes with the body's antiviral response.[48, 49] Owing to the activating signals received by the IFN receptors on the surface, the accumulated mononuclear macrophages produce more monocyte chemo-attractants (such as CCL2, CCL7, and CCL12); causing further accumulation of mononuclear macrophages. These macrophages produce elevated levels of proinflammatory cytokines (TNF, IL-6, IL1- ß, and inducible nitric oxide synthase), thereby increasing the severity of the disease.[50]

Image 2



High levels of expression of IL-1B, IFN-γ, IP-10, and monocyte chemo-attractant protein 1 (MCP-1) have been detected in patients with COVID-19. These inflammatory cytokines may activate the T-helper type 1 (Th1) cell response; which signifies activation of specific immunity.[7,51] These patients also have elevated levels of Th2 cell-secreted cytokines (such as IL-4 and IL-10), which inhibit the inflammatory response. Serum levels of IL-2R and IL-6 in patients with COVID-19 are positively correlated with the severity of the disease (i.e., critically ill patients>severely ill patients>ordinary patients).[13]

The pro-inflammatory cytokines induce T cell apoptosis, which further prevents viral clearance.[2] The rapid viral replication and vigorous pro-inflammatory cytokine/chemokine response induces apoptosis in lung epithelial and endothelial cells. Alternatively, IFNs induce inflammatory cell infiltration through mechanisms involving Fas–Fas ligand (FasL) or TRAIL–death receptor 5 (DR5) and cause the apoptosis of airway and alveolar epithelial cells.

Apoptosis damages the pulmonary microvascular and alveolar epithelial cell barriers and causes vascular leakage and alveolar edema, eventually leading to hypoxia, resulting in ARDS.[52,53,54] It is now known that several proinflammatory cytokines (IL-6, IL-8, IL-1 ß, granulocyte-macrophage colony-stimulating factor, and reactive oxygen species) and chemokines (such as CCL2, CCL-5, IFN-induced protein 10 (IP-10), and CCL3) all contribute to the occurrence of acute respiratory distress syndrome (ARDS).[55, 56, 57]

In COVID-19, the inflammatory cytokine storm is closely related to the development and progression of ARDS. The serum levels of cytokines are significantly increased in patients with ARDS, and the degree of increase is positively correlated with mortality rate.[58] The cytokine storm is also an important factor in determining the clinical course of extra-pulmonary multi-organ failure. This partially explains the signs of extra-pulmonary organ failure (such as elevated liver enzymes and creatinine) seen in some COVID-19 patients without respiratory failure, suggesting that the damage to extra-pulmonary tissues and organs is caused by the cytokine cascade.[59] The immunologic changes are summarized in Image 3.[47]

Image 3

Immunologic changes	COVID-19
T-cell responses	Lymphopenia in severe cases (<20%). Initial lymphopenia is predictive of severe disease.
$CD8^+$ T cells	Severe lymphopenia (<5%) is observed in $CD8^+$ T cells and can be a predictor of severe disease.
Th1-Th2 responses	Normal antiviral immunity requires a CD4 and CD8 Th1 response. Severe disease shows a systemic severe inflammatory response with a cytokine storm. Cytokine storm response is mainly Th1 and inflammatory. It can also have a major role in inflammasome activation.
Eosinophils	Decreased circulating eosinophil numbers in 50%-80% of the hospitalized patients.
Specific antibody levels	In the acute phase, virus-specific IgM increases followed by virus-specific IgG during convalescence.
Cytokine storm	Innate and adaptive cytokines are released in high amounts linked to severe disease.
Acute-phase reactants	High in severe cases. Initially high values are predictive of severe disease.

Signs and symptoms

There are similarities in the symptoms between COVID-19 and previously known beta coronaviruses. Most common symptoms at onset of COVID-19 illness are fever, cough and fatigue; while other symptoms include hyposmia, sputum production, headache, haemoptysis, diarrhoea, dyspnoea and lymphopenia.[7, 60, 61, 62]

COVID-19 showed some unique clinical features like the targeting of the lower airway as evident by upper respiratory tract symptoms like rhinorrhoea, sneezing and sore throat. Additionally, based on results from chest radiographs upon admission, some of the cases show an infiltrate in the upper lobe of the lung that is associated with increasing dyspnea with hypoxemia.[63] A small proportion of cases also presented with gastro-intestinal symptoms like diarrhoea.

These events have been summarized in the images 4 and 5.[64]

Image 4

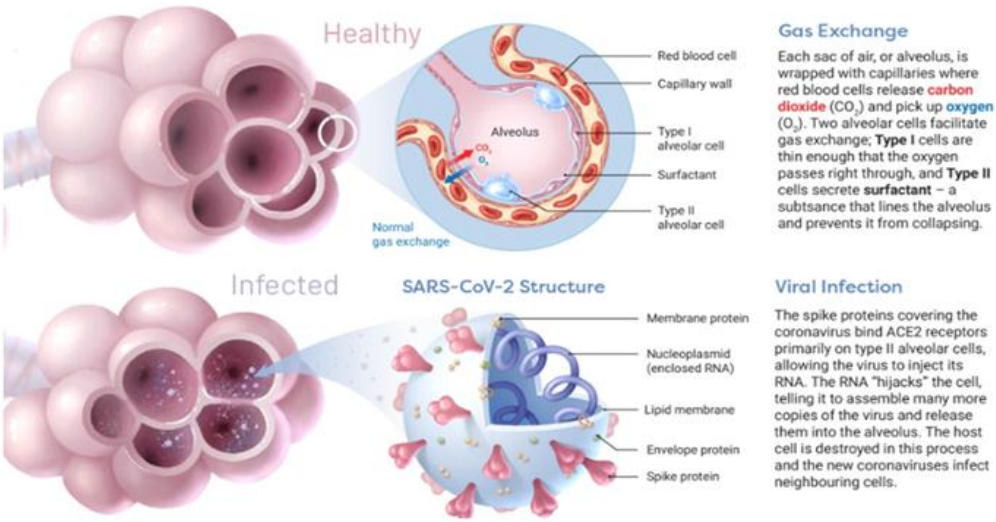
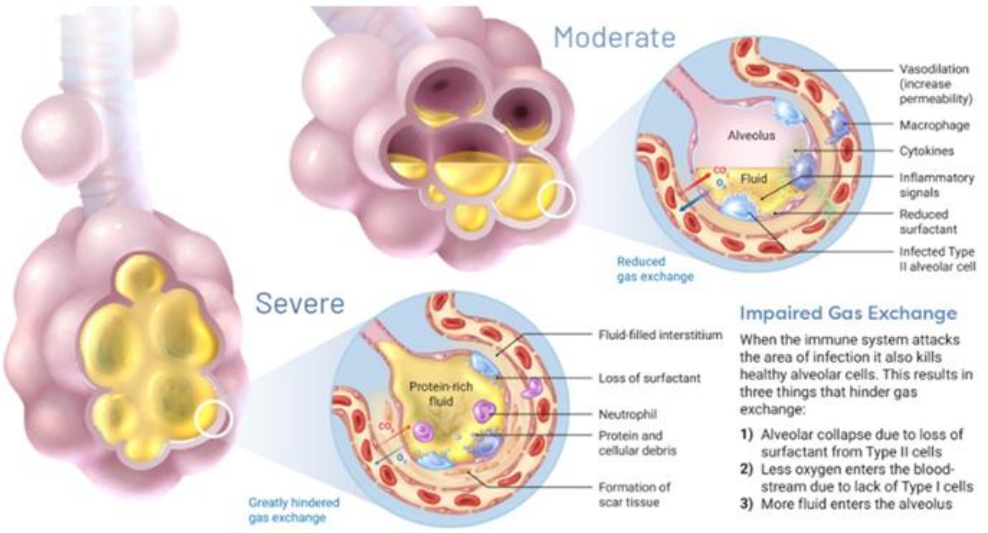


Image 5



Pathological findings in other organs: In addition to the lungs, SARS-CoV-2 was detected in several other organs, including the heart, liver, kidneys, gastrointestinal tract, spleen, lymph nodes, skin, and placenta. However, pathological findings in these organs were nonspecific. Epithelial damage and notable inflammatory infiltrates—possibly related to SARS-CoV-2 infection were found in the liver, kidneys, gastrointestinal tract and placenta; suggesting delayed involvement during the later stages of COVID-19. This finding is supported by previous reports of SARS-CoV-2 in urine and feces.[65] Evidence of microvascular damage such as thrombi, endotheliitis, and complement activation was not limited to the lungs, but was also found in the heart, liver, kidneys, gastrointestinal tract, skin, adrenal gland, and prostate, possibly reflecting systemic hyper-inflammation in these cases.[66]

According to a systematic review conducted by Polak SB et al,[66] lab investigations revealed lymphocytopenia (81%), elevated (>10mg/L) CRP levels (100%), elevated (>0.5 µg/ml) D-dimer levels (98%). Majority of the cases

were measured in the first three days of illness and notably, CRP levels were >100mg/L in 50% cases and D-dimer levels were >5.0µg/ml in 29% cases.

The histopathological picture of COVID-19–related pneumonitis appears to encompass epithelial (35%), vascular (4%) and fibrotic (1%) patterns of lung injury. Overlapping patterns of injury were also seen where a maximum number of cases showed a combination of epithelial and vascular pattern of injury (41%). This vascular pattern of COVID-19 lung injury is prominent and is in accordance with clinical studies reporting 49% of cases with thrombotic events. This is in line with the fact that the ACE2 receptor are present on both alveolar epithelium and capillary endothelium.

Radiographic findings: On Chest CT scan, bilateral ground-glass opacities (GGOs) were observed in the lung field. The lesions at the early stage of COVID-19 are relatively localized and mainly manifest as inflammatory infiltration restricted to the subpleural or peribronchovascular regions of one lung or both lungs, exhibiting patchy or segmental pure GGOs with vascular dilation. Very few cases have negative CT findings at the early stage.

In the progressive stage, CT shows mainly an increased range of pure GGOs, the involvement of multiple lobes, consolidations of some lesions and GGOs surrounding consolidated lesions (the characteristic change of the progressive stage). Interlobular septal thickening and an obvious crazy-paving pattern are often present.

At the advanced stage, the CT manifestations of patients are similar to those in other types of pneumonia and mainly include diffuse lesions in both lungs, which are mostly consolidated lesions, and GGOs surrounding consolidated lesions, mostly accompanied by parenchymal bands and occasionally by a small amount of pleural effusion. This CT appearance is called lung whiteout.[67]

Inflammatory biomarkers and risk assessment

The summary of changes in biomarkers seen during a severe COVID-19 infection is illustrated in image6.[68]

Image 6

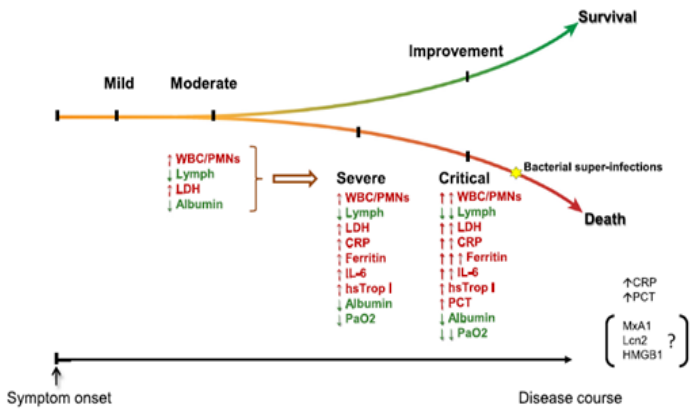
Summary of Changes in Biomarkers Seen in Severe COVID-19 Infection.

Biomarker	Change in severe COVID-19 infection
CRP	Increase
SAA	Increase
IL-6	Increase
LDH	Increase
WCC	NLR increases
	LC decrease
D-dimer	Increase
Platelet count	Decrease
Cardiac troponin	Increase
Renal biomarkers	Urea & creatinine increase

CRP = C-reactive protein; SAA = serum amyloid A; IL-6 = interleukin 6; LDH = lactate dehydrogenase; WCC = White cell count.

The progression of disease is illustrated in image 7 along with the severity of symptoms.[69]

Image 7



Mild: Mild symptoms, no imaging findings of pneumonia.
Moderate: Fever ***OR*** respiratory symptoms.
Severe: Respiratory distress and the respiratory rate >30/min ***OR*** saturation <93% at rest ***OR*** PaO2: FiO2 ratio ≤300mmHg.
Critical: Respiratory failure requiring mechanical ventilation (including ARDS) ***OR*** shock ***OR*** other organ failure requiring ICU.

Different manifestations at different stages are in accordance with pathological mechanism of viral pneumonia which at first is prone to affect the terminal bronchioles and their surrounding pulmonary parenchyma and then develop into infiltration of pulmonary lobules and, lastly, diffuse alveolar damage.

COVID-19 exhibits a range of clinical manifestations, from mild flu-like symptoms to life-threatening conditions. Until a commercial vaccine becomes available, it is important to identify individuals who have been infected with SARS-CoV-2, with or without accompanying symptoms, and who have developed antiviral immunity. This allows for additional analyses of strength and durability of immunity across general populations.[70]

REFERENCES

1. Zhai P, Ding Y, Wu X, Long J, Zhong Y, Li Y. The epidemiology, diagnosis and treatment of COVID-19. Int J Antimicrob Agents, 2020; 55(5): 105955.
2. Ye Q, Wang B, Mao J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. J Infect, 2020; 80(6): 607-613.
3. Di Gennaro F, Pizzol D, Marotta C, et al. Coronavirus Diseases (COVID-19) Current Status and Future Perspectives: A Narrative Review. Int J Environ Res Public Health, 2020; 17(8): 2690.
4. Jiang F, Deng L, Zhang L, Cai Y, Cheung CW, Xia Z. Review of the Clinical Characteristics of Coronavirus Disease 2019 (COVID-19). J Gen Intern Med, 2020; 35(5): 1545-1549.
5. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun, 2020; 109: 102433.
6. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine, 2020; 24.
7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet, 2020; 395(10223): 497-506.
8. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. Journal of medical virology, 2020; 92(4): 401-2.
9. Wang C, Horby PW, Hayden FG, Gao GF. A novel coronavirus outbreak of global health concern. The Lancet, 2020; 395(10223): 470-3.
10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet, 2020; 395(10223): 507-13.
11. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KS, Lau EH, Wong JY, Xing X. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. New England Journal of Medicine, 2020.
12. Song F, Shi N, Shan F, et al. Emerging Coronavirus 2019-nCoV Pneumonia. Radiology, 2020; 6: 200274.
13. Chen L, Liu HG, Liu W, et al. [Analysis of clinical features of 29 patients with 2019 novel coronavirus pneumonia]. Zhonghua jie he he hu xi za zhi = Zhonghua Jiehe he Huxi Zazhi = Chinese Journal of Tuberculosis and Respiratory Diseases, 2020; 43(0): 005.
14. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Azman AS, Reich NG, Lessler J. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Annals of internal medicine, 2020; 172(9): 577-82.
15. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China. Eurosurveillance, 2020; 25(5): 2000062.
16. Linton NM, Kobayashi T, Yang Y, Hayashi K, Akhmetzhanov AR, Jung SM, Yuan B, Kinoshita R, Nishiura H. Incubation period and other epidemiological characteristics of 2019 novel coronavirus infections with right truncation: a statistical analysis of publicly available case data. J. Clin. Med, 2020; 9(2): 538.
17. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, Wang M. Presumed asymptomatic carrier transmission of COVID-19. Jama, 2020; 323(14): 1406-7.
18. McAloon C, Collins Á, Hunt K, et al. Incubation period of COVID-19: a rapid systematic review and meta-analysis of observational research. BMJ Open, 2020; 10: 039652.
19. Delamater PL, Street EJ, Leslie TF, Yang YT, Jacobsen KH. Complexity of the basic reproduction number (R0). Emerging infectious diseases, 2019; 25(1): 1.
20. WHO. Statement on the meeting of the International Health Regulations Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV), 2020.
21. Read JM, Bridgen JR, Cummings DA, Ho A, Jewell CP. Novel coronavirus 2019-nCoV: early estimation of epidemiological parameters and epidemic predictions. MedRxiv, 2020; 1.
22. Zhou T, Liu Q, Yang Z, Liao J, Yang K, Bai W, Lu X, Zhang W. Preliminary prediction of the basic reproduction number of the Wuhan novel coronavirus 2019-nCoV. Journal of Evidence-Based Medicine, 2020; 13(1): 3-7.
23. Zhao S, Lin Q, Ran J, Musa SS, Yang G, Wang W, Lou Y, Gao D, Yang L, He D, Wang MH. Preliminary estimation of the basic reproduction number of novel coronavirus (2019-nCoV) in China, from A data-driven analysis in the early phase of the outbreak. International journal of infectious diseases, 2020; 92: 214-7.

24. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. Journal of travel medicine, 2020; 13.
25. Singhal T. A review of coronavirus disease-2019 (COVID-19). The Indian Journal of Pediatrics, 2020; 13: 1-6.
26. CDC https://www.cdc.gov/coronavirus/types.html
27. Cao Y, Li L, Feng Z, et al. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov, 2020; 6: 11.
28. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. N Engl J Med, 2020.
29. Chen J, Jiang Q, Xia X, Liu K, Yu Z, Tao W, Gong W, Han JJ. Individual variation of the SARS-CoV2 receptor ACE2 gene expression and regulation, 2020; p. 2020030191.
30. Sommerstein R, Kochen MM, Messerli FH, Gräni C. Coronavirus disease 2019 (COVID-19): do angiotensin-converting enzyme inhibitors/angiotensin receptor blockers have a biphasic effect? J Am Heart Assoc, 2020; 9(7): 016509.
31. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med, 2020; 46(4): 586–90.
32. Li G, He X, Zhang L, Ran Q, Wang J, Xiong A, Wu D, Chen F, Sun J, Chang C. Assessing ACE2 expression patterns in lung tissues in the pathogenesis of COVID-19. Journal of autoimmunity, 2020; 13: 102463.
33. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet (London, England), 2020; 395(10224): 39.
34. Bergmann CC, Silverman RH. COVID-19: Coronavirus replication, pathogenesis, and therapeutic strategies. Cleveland Clinic journal of medicine, 2020; 4.
35. Fehr, A. R. & Perlman, S. Coronaviruses: an overview of their replication and pathogenesis. Methods Mol. Biol, 2015; 1–23.
36. Kubo H, Yamada YK, Taguchi F Localization of neutralizing epitopes and the receptor-binding site within the aminoterminal 330 amino acids of the murine coronavirus spike protein. J Virol, 1994; 68: 5403–5410.
37. Cheng PK, Wong DA, Tong LK et al Viral shedding patterns of coronavirus in patients with probable severe acute respiratory syndrome. Lancet, 2004; 363: 1699–1700.
38. Bosch BJ, van der Zee R, de Haan CA et al The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol, 2003; 77: 8801–8811.
39. Baranov PV, Henderson CM, Anderson CB et al (2005) Programmed ribosomal frameshifting in decoding the SARS-CoV genome. Virology, 332: 498–510.
40. Brierley I, Digard P, Inglis SC Characterization of an effi cient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. Cell, 1989; 57: 537–547.
41. Araki K, Gangappa S, Dillehay DL et al Pathogenic virus-specifi c T cells cause disease during treatment with the calcineurin inhibitor FK506: implications for transplantation. J Exp Med, 2010; 207: 2355–2367.
42. Ziebuhr J, Snijder EJ, Gorbalenya AE Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol, 2000; 81: 853–879.
43. Mielech AM, Chen Y, Mesecar AD, Baker SC. Nidovirus papain-like proteases: multifunctional enzymes with protease, deubiquitinating and deISGylating activities. Virus research, 2014; 194: 184-90.
44. Sethna PB, Hofmann MA, Brian DA Minus-strand copies of replicating coronavirus mRNAs contain antileaders. J Virol, 1991; 65: 320–325.
45. Krijnse-Locker J, Ericsson M, Rottier PJM et al Characterization of the budding compartment of mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. J Cell Biol, 1994; 124: 55–70.
46. Tooze J, Tooze S, Warren G Replication of coronavirus MHV-A59 in saccells: determination of the fi rst site of budding of progeny virions. Eur J Cell Biol, 1984; 33: 281–293.
47. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, O'Mahony L, Gao Y, Nadeau K, Akdis CA. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy, 2020; 75(7): 1564-81.
48. García-Sastre A, Biron CA. Type 1 interferons and the virus-host relationship: a lesson in detente. Science, 2006; 312(5775): 879-82.
49. Channappanavar R, Fehr AR, Zheng J, Wohlford-Lenane C, Abrahante JE, Mack M, Sompallae R, McCray PB, Meyerholz DK, Perlman S. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. The Journal of clinical investigation, 2019; 129(9).
50. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, Perlman S. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell host & microbe, 2016; 19(2): 181-93.
51. Marchingo JM, Sinclair LV, Howden AJ, Cantrell DA. Quantitative analysis of how Myc controls T cell proteomes and metabolic pathways during T cell activation. Elife, 2020; 9: 53725.

52. Herold S, Steinmueller M, von Wulffen W, Cakarova L, Pinto R, Pleschka S, et al. Lung epithelial apoptosis in influenza virus pneumonia: the role of macrophage-expressed TNF-related apoptosis-inducing ligand. J Exper Med, 2008; 205(13): 3065–77.
53. Högner K, WolffT, Pleschka S, Plog S, Gruber AD, Kalinke U, et al. Macrophage–expressed IFN-βcontributes to apoptotic alveolar epithelial cell injury in se- vere influenza virus pneumonia. PLoS Pathogens, 2013; 9(2): 1003188-e.
54. Rodrigue-Gervais IG, LabbéK, Dagenais M, Dupaul-Chicoine J, Champagne C, Morizot A, et al. Cellular inhibitor of apoptosis protein cIAP2 protects against pulmonary tissue necrosis during influenza virus infection to promote host sur- vival. Cell Host Microbe, 2014; 15(1): 23–35.
55. Jiang Y, Xu J, Zhou C, Wu Z , Zhong S, Liu J, et al. Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. Am J Res- pirat Critical Care Med, 2005; 171(8): 850–7.
56. Reghunathan R, Jayapal M, Hsu LY, Chng HH, Tai D, Leung BP, Melendez AJ. Expression profile of immune response genes in patients with severe acute respiratory syndrome. BMC immunology, 2005; 6(1): 2.
57. Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ . Human immunopathogenesis of severe acute respiratory syndrome (SARS). Virus Res, 2008; 133(1): 13–19.
58. Parsons PE, Eisner MD, Thompson BT, Matthay MA, Ancukiewicz M, Bernard GR, Wheeler AP, NHLBI Acute Respiratory Distress Syndrome Clinical Trials Network. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. Critical care medicine, 2005; 33(1): 1-6.
59. Wang H, Ma S. The cytokine storm and factors determining the sequence and severity of organ dysfunction in multiple organ dysfunction syndrome. The American journal of emergency medicine, 2008; 26(6): 711-5.
60. L.L. Ren, Y.M. Wang, Z.Q. Wu, Z.C. Xiang, L. Guo, T. Xu, et al., Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study, Chinese Med J, 2020; 133(9): 1015–1024.
61. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. Journal of medical virology, 2020; 92(4): 441-7.
62. Carlos WG, Dela Cruz CS, Cao B, Pasnick S, Jamil S. Novel Wuhan (2019-nCoV) Coronavirus. Am J Respir Crit Care Med, 2020; 201(4): 7-8.
63. Phan LT, Nguyen TV, Luong QC, et al. Importation and Human-to-Human Transmission of a Novel Coronavirus in Vietnam. N Engl J Med, 2020; 382(9): 872-874.
64. Infographic by Azuravesta Rastan as posted on www.azuravesta.com, https://www.azuravesta.com/covid-19-pandemic
65. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, Tan W. Detection of SARS-CoV-2 in different types of clinical specimens. Jama, 2020; 323(18): 1843-4.
66. Polak SB, Van Gool IC, Cohen D, von der Thüsen JH, van Paassen J. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression [published online ahead of print]. Mod Pathol, 2020; 1-11.
67. Dai WC, Zhang HW, Yu J, et al. CT Imaging and Differential Diagnosis of COVID-19. *Can Assoc Radiol J*, 2020; 71(2): 195-200.
68. Skevaki C, Fragkou PC, Cheng C, Xie M, Renz H. Laboratory characteristics of patients infected with the novel SARS-CoV-2 virus. J Infect, 2020; 21.
69. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19–A systematic review. Life Sci, 2020; 117788.
70. Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, Sasso JM, Gregg AC, Soares DJ, Beskid TR, Jervey SR, Liu C. Assay Techniques and Test Development for COVID-19 Diagnosis. ACS Cent Sci, 2020; 6(5): 591-605. doi: 10.1021/acscentsci.0c00501.