UPDATES ON COVID-19 PATHOGENESIS & DIAGNOSIS

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I. INTRODUCTION

Numerous instances of pneumonia with uncertain cause were reported in December 2019 from small seafood market in Wuhan, China [1]. The World Health Organization's Institution (WHOI) then gave this unique virus, which is a member of the beta coronavirus family, the names severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and new coronavirus (nCoV) [2]. This disease spreads from China to 33 other nations within two months. At the end of February 2020, there were around 77658 confirmed cases, including 9126 severe cases and 2663 deaths in China and 23309 confirmed cases and 33 deaths abroad [3]. As the correct therapeutic alternatives have not yet been developed, early disease detection remains the only method for disease prevention. Methods for detecting nucleic acids point the way for a proper confirmation of COVID-19. Clinical signs displayed by

patients infected with this virus are comparable to those seen in SARS-CoV and MERS-CoV cases. Both direct and indirect contact with an infected person as well as droplet transmission of the virus are possible. This method of transmission has the potential to harm the liver, nervous, and respiratory systems [4]. This comprehensive study gives an overview of the virology, molecular immunological pathogenesis, and established detection techniques for SARS-CoV2.

II. VIROLOGY OF SARS-CoV-2

1. **Origin, Family, and Genomic Structure:** Towards the end of 2019, hospitals in Wuhan, Hubei, China, experienced the emergence of a novel coronavirus (nCoV). It is important to remember that Chinese markets, especially the Huanan Seafood Market, offered a wide range of live animals for sale, including seafood. The China Centre for Disease Control did, however, issue a directive for the market's closure on January 1st, 2020. The Human Seafood Market, where the first SARS-CoV-2 samples were obtained, was named as the outbreak's likely source by the Chinese CDC. In between two months, at least two different strains of nCoV have been discovered [5]. The Chinese health authority has conducted numerous epidemiological and etiological studies to determine the origin of nCoV. On December 30, 2019, three patients who had been admitted to Wuhan Jinyuantan Hospital had their bronchoalveolar lavage samples used to isolate the first nCoV. These patients were affected by alpha and beta CoV (causing respiratory, hepatic, neurologic, and enteric diseases) [6]. Following the investigation, it was discovered that SARS-CoV-2 and SARS-CoV share a phylogenetic similarity of 79.5 and 50%, respectively, and the percent of sequence match about 90% between SARS-CoV-2 and other beta-corona viruses [7] The envelope contains some structural proteins along with spike proteins, which are membrane protein (M) and envelope protein (E). The production of polyprotein la (ppla) and polyprotein lab (pplab), responsible for translating non-structural proteins (NSPs), is attributed to the open reading frames (ORF) known as la and 1b [8]. Various study has predicted the lengths of the M. S. N, E, and ORF3a genes of CoV are 669, 3822, 1260, 228, and 828 nt respectively. The length of the ORF8 gene, which is predicted to be 366 m and is located between the M and N ORF genes of SARS-CoV-2, is also included. This length matches that of SARS-CoV exactly [9]. According to another study, the protein sequences of nCoV share similarities with the SARS proteome (77.1%), Bat situs DQ022305.2 (79.7%), and but virus MG772934.1 (91.1%). Two variants of 2019-nCoV with changed structural proteins, ORFS-5 and ORFS-L, have been discovered as a result of the ORFS mutation [10]. Table 1 provides a complete comparison of SARS-CoV-2, MERS- Coy, and SARS-CoV.

Table 1: Characteristics of MERS-CoV, SARS-CoV and SARS-CoV-2

Features	SARS-CoV2	SARS-CoV	MERS-CoV
First case observed at	Wuhan, China	Guangdong, China	Jeddah, Saudi Arabia
Period	2019–present	2002–2003	2012-2013
Primary transmitting host	*Chiroptera* (Bats)	*Chiroptera* (Bats)	*Chiroptera* (Bats)
Intermedial	Unknown	Asian palm civet and other	Dromedary camels

host		zoonotic hosts	
Transmission modality	Pneumatic aerosols	Pneumatic aerosols	Pneumatic aerosols
Period of latency	Median 5.1 days (95% CI, 2.2–11.5)	Mean 4.6 days (95% CI, 3.8–5.8)	Median 5.2 days (95% CI, 1.9–14.7)
Mortality rate	3.8%	9.6%	34.4%

2. **Properties of SARS-CoV-2:** Given that it has many similarities to both SARS-CoV and MERS-CoV, nCoV displays physiochemical traits that are analogous to both viruses. It has an oval or circular form, and its diameter ranges from 60 to 100 nm. nCoV can become inactive by being heated to 56°C for 30 minutes or by being exposed to UV light. Numerous chemicals have shown high efficiency against nCoV, including diethyl ether, chlorine, 75% ethanol, acetic acid, and chloroform [11]. According to several studies, SARS-CoV-2 is more stable on plastic and stainless-steel surfaces than it is on cardboard and copper. Additionally, SARS-CoV-2 has a longer half-life than SARS-CoV [12].

3. **Host Cell Entry:** To gain entry into the cell, SARS-CoV-2 utilizes the angiotensin-converting enzyme-2 (ACE2) as its functional receptor. ACE2, classified as a type I membrane protein, is found in vital organs such as the heart, lung, gut, and kidney, which are closely associated with cardiovascular disease. It consists of N-terminal peptide domains and a C-terminal collect in-like domain. ACE2 serves as a direct binding site for the S proteins of coronaviruses, enabling the degradation of angiotensin-1 and the production of angiotensin (1-9) [13]. The S1 subunit initiates the process of viral membrane structural arrangement to merge with the cell membrane of the host. By means of a hinge-like movement exhibited by the receptor binding domain (RBD), the viral membrane establishes a connection with the host receptor cell [14]. Extensive scientific evidence suggests that the binding affinity between SARS-CoV-2 and human ACE-2 is 10-20 times stronger than that observed with SARS-CoV. In order to assess the potential infectious impact of SARS-CoV-2, the RBD of the S protein, which interacts with ACE-2, was thoroughly examined [15]. Other research indicates that the ACE-2-BOAT1 complex may simultaneously bind to two S proteins [16]

4. **Ecology of SARS-CoV-2:** It is important to note that all coronaviruses that impact humans originate from zoonotic sources, with bats being the probable natural hosts [17]. In the case of SARS-CoV, Chinese horseshoe bats from the Rhinolophus family in Yunnan, China, were identified as having close genetic resemblance [18] Some bat CoV. like RaTG13, show similar sequences up to 96% nt with SARS-CoV-2 [19]. Naturally, Bat COV cannot affect humans unless it undergoes mutation and recombination in any host animal [20]. Different studies show that SARS-CoV-2 may have originated from pangolin, as the sequences of nCoV and pangolin CoV match 99% [21].

5. **Variation of Genome:** The earlier genomics were obtained from nine patients with COVID-19, which was a 99.98% identical match [22]. Other scientists, after analyzing 103 genomes, have found two types of major evolution of SARS-CoV-2: S and L. The L type is more aggressive and spreads rapidly as it has severe selective pressure, but the S type has weaker selective pressure, so it may persist slowly. These extracted RNAs are very unstable, so strong surveillance is required to control SARS-CoV-2 [23]

6. **Pathogenesis of SARS-CoV-2:** The knowledge about COVID-19 pathogenesis is poorly understood as it is a new strain, but from the previous studies of MERS-CoV and SARS-CoV, one can get an idea of the CoV mechanism as it resembles almost identical symptoms and gene sequences [24]. The penetration of the host cell by the coronavirus is facilitated by the entry and duplication of the CoV's protein S [25]. The envelope of SARS-CoV-2 contains spike glycoproteins that establish a connection with the ACE-2 receptors on the host cell surface [26]. This host receptor may be different for SARS-CoV and MERS-CoV, i.c.. CD2091 and DPP4 respectively [27]. Through fusion of the membranes between the plasma membrane and the virus, which occurred at the $2 position of S protein. various proteolytic cleavages occur, and as a result, the invasion is completed [28]. Upon infiltration of the host cell, the initiation of structural protein synthesis and polyprotein formation commences as the viral RNA enters the cytoplasm, triggering genome replication [29]. The glycoprotein synthesized from the viral genetic material infiltrates the membranes of the endoplasmic reticulum and Golgi apparatus. The combination of viral RNA and nucleocapsid protein leads to the formation of a nucleocapsid. Subsequently, the endoplasmic reticulum Golgi intermediate compartment (ERGIC) acts as the focal point for viral particle assembly. Once the newly formed virus particles are enclosed within vesicles, they bind with the plasma membrane, ultimately releasing the virus into the host organism [30]. A team of researchers has identified a molecule called N-(2-aminoethyl)-1 azirdineethanamine as an inhibitor of angiotensin converting enzyme-2. This molecule effectively hinders the fusion between the SARS-CoV receptor-binding domain (RBD) and the host cell [31].

7. **Antigenic Action in Coronavirus Infection:** The antiviral defence system of the body recruit's antigen-presenting cells to display viral antigens. Antigenic peptides presented by the major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules are identified by virus-specific cytotoxic T-cells. Understanding the pathogenesis of SARS-CoV-2 is aided by the involvement of antigen-presenting cells; nevertheless, there is limited documentation regarding COVID-19 in this context. We only receive research articles pertaining to MERS-CoV and SARS [32] According to research. MHC-1 is the principal determinant of SARS-CoV presentation. There are many polymorphisms of the human leukocyte antigen, such as HLA-B*0703, HLA-DRB1*1202. HLAB*4601, and HLA-Cw*0801 have been shown to influence SARS-CoV susceptibility. Other research, however, demonstrates that polymorphisms such as HLA- A*0201, HLA-Cw1502, and HLA-DR0301 may provide protection against viruses such as SARS. In addition to polymorphisms, mannose-binding lectin is an antigen-presenting cell associated with SARS-CoV infection [33].

8. **Host Cell Immunity:** The virus-specific T and B cells stimulate cellular and humoral immunity by the antigen- presenting cells, which activate the production of IgG and IgM. IgG antibodies, specific for S and N, can protect the body for a long time, whereas IgM antibodies only last for 12 weeks [34]. Recent research shows that the activation of CD8+ and CD4+ is higher, but the count in peripheral blood is significantly lower for SARS-CoV-2 patients [35]. Different research on SARS-CoV patients shows that memory T cells can recognize the S-peptide for up to four to six years after recovery. These findings could aid in the development of an nCoV vaccine [36].

9. **Cytokine Storm:** Recently published papers shows that the SARS-CoV-2 induces shedding of angiotensin-converting enzyme type 2 which results in a high activation of

inflammatory factors like interferons, interleukins and chemokines [37]. In the earlier stage the viral replication triggers the chemokines and cytokines by causing damage to endothelial, epithelial cell and vascular leakage. As a result of low ACE-2 levels, renin angiotensin system will be affected which will triggers more inflammation causing vascular permeability, ultimately leads to organ failure, acute respiratory distress syndrome etc [38]. Another study suggested that, viral cellular uptake can be improved by antibody dependent enhancement (ADE) through interaction of virus antibody complex and Fe receptor de different receptors, resulting enhancement of target cells. The interaction between the Fc receptor and the complex formed by neutralising antibodies against the virus's S protein may enhance the reduction of pulmonary inflammation and viral replication [39].

10. **Evading the Immune System:** SARS-CoV and MERS-CoV have distinct methods for evading immune responses in host cells. Pattern recognition receptors can identify the molecular pattern associated with pathogens. MERS-CoV and SARS-CoV can elude host detection if they produce double membrane vesicles with low PRRs. [53] IFN alpha and beta may be useful against MERS-CoV and SARS-CoV infections, but MERS-4a-protein inhibits the induction of IFN [40]. In addition, several proteins (ORF4b, ORF4a, ORF5, etc.) inhibit IFN regulatory factor-3 and IFN beta promoter activation in MERS CoV. If we can carefully monitor these processes and reverse the underlying mechanism, we may be able to develop a therapy method [41] .

III. DIAGNOSES OF NCOV-2019

1. **Rapid to Molecular Diagnosis of nCoV-2019:** Several examinations are involved for confirming a COVID-19 case firstly patient's travel history clinical appearances and then lab investigation and radiological imaging (CT). Lab investigation involves blood culture, nucleic acid detection (NAAT, RT-PCR, LAMP). serological investigation, immune identification techniques (POCT, IIFT. ELISA) [42]. The lab techniques commonly used for coronaviruses detection are listed in Table 2.

Table 2: Comparison of Different Diagnostic Approach ofnCoV-2019

Approach	Scope	Turnaround Time
Enzyme immunoassay antigen technique	Spontaneous, feebly sensitive, some are CLIA-waived	1–4 h
Immunofluorescence assay antigen technique	Good sensitivity and accuracy, subjective explanation	1–4 h
Tissue culture	Gold standard, axenic culture for future prospect of R&D, cumbersome	1–7 days
Analytical Serology	Retroactive, cross-reaction	2–8 h
NAAT, Monoplex, pan-HCoV	High sensitivity to all the human CoV species known till date	1–8 h
NAAT, Monoplex, specific-HCoV	High sensitivity and specificity for exclusive species, potentially quantitative	1–8 h

NAAT, multiplex	High sensitivity and specificity, undertaking different pathogens, Film Array RP EZ is CLIA-waived	1–8 h
NAAT, POCT	Spontaneous and secure, good sensitivity and specificity, some are CLIA-waived	15– 30 min

EIA- enzyme immunoassay; IFA- immunofluorescent assay; NAAT- nucleic acid amplification test; CLIA- Clinical Laboratory Improvement Act.

2. **Nucleic Acid Detection Method:** Antiviral immunity of the body presents antigen-presenting cells to the viral antigen, and the major histocompatibility complex or human leukocyte antigen presents antigenic peptides that are recognized by virus-specific cytotoxic T-cells. The antigen-presenting cells allow us to comprehend the pathogenesis of SARS-CoV-2, but there are few reports of COVID-19. We only receive research articles pertaining to MERS-CoV and SARS [32]. Research indicates that MHC-1 is the primary component in SARS-CoV presentation. A number of polymorphisms of the human leukocyte antigen, such as HLA-B*0703, HLA-DRB1*1202, HLAB*4601, and HLA-Cw*0803, have been linked to vulnerability to SARS-CoV. Other research, however, demonstrates that polymorphisms such as HLA-A*0201, HLA-Cw1502, and HLA-DR0301 may provide protection against viruses such as SARS. In addition to polymorphisms, mannose-binding lectin is an antigen-presenting cell associated with SARS-CoV infection [43]. Table 3 represents the Current protocols approved by respective authority for nucleic acid amplification testing for COVID-19.

Table 3: Current Following Protocol for Nucleic Acid Amplification Testing [79]

Institute/Country	Gene Targets	Target Gene Primer
Chinese Centre for Disease Control, China	ORF 1ab F	CCC TGT GGG TTT TAC ACT TAA
	ORF 1ab R	ACG ATT GTG CAT CAG CTG A
	N-F	GGG GAA CTT CTC CTG CTA GAA T
	N-R	CAG ACA TTT TGC TCT CAA GCT G
Charite, Germany	RdRp F	GTG ARA TGG TCA TGT GTG GCG G
	RdRp R	CAR ATG TTA AAS ACA CTA TTA GCA TA
	E-F	ACAGGTACGTTAATAGTTAATAGCGT
	E-R	ATATTGCAGCAGTACGCACACA
	N-F	CACATTGGCACCCGCAATC
	N-R	GAGGAACGAGAAGAGGCTTG
HKU, Hong Kong SAR	ORF 1b-nsp14 F	TGG GGY TTT ACR GGT AAC CT
	ORF 1b-nsp14 R	AAC RCG CTT AAC AAA GCA CTC
	N-F	TAA TCA GAC AAG GAA CTG ATT A
	N-R	CGA AGG TGT GAC TTC CAT G

"National Institute of Infectious Diseases, Department of Virology", Japan	nCOV_N-F		AAA TTT TGG GGA CCA GGA AC
	nCOV_N-R		TGG CAG CTG TGT AGG TCA AC
National Institute of Health, Thailand	NIC N-F		CGT TTG GTG GAC CCT CAG AT
	NIC N-R		CCC CAC TGC GTT CTC CAT T
US CDC, USA	Three targets in N gene	NI-F	GAC CCC AAA ATC AGC GAA AT
		N1-R	TCT GGT TAC TGC CAG TTG AAT CTG
		N2-F	TTA CAA ACA TTG GCC GCA AA
		N2-R	GCG CGA CAT TCC GAA GAA
		N3-F	GGG AGC CTT GAA TAC ACC AAA A
		N3-R	TGT AGC ACG ATT GCA GCA TTG
Pasteur Institute, Paris, France	Two targets in RdRp gene	RdRp_IP2-F	ATGAGCTTAGTCCTGTTG
		RdRp_IP2-R	CTCCCTTTGTTGTGTTGT
		RdRp_IP4-F	GGTAACTGGTATGATTTCG
		RdRp_IP4-R	CTGGTCAAGGTTAATATAGG

3. **Radio Imaging and Other Diagnostic Techniques:** Despite the fact that RT-PCR is a specific diagnostic test for detecting SARS-CoV-2, many physicians prefer CT imaging due to its perceived greater sensitivity. As in many cases it has been seen that the RT-PCR report is negative but according the CT scans the patient is probably affected by nCoV as the image clearly shows the bilateral and multi-lobar GGO which can be distributed peripherally or posteriorly. Some other findings may include septal thickening, pleural thickening, bronchiectasis etc. Some uncommon but considerable findings are pleural effusion, lymphadenopathy, pneumothorax, cavitation etc. Follow-up case findings may be high number of GGO, septal thickening etc. The changes in lungs can occurs within 10 days of symptomatic actions. There are five stages of CT finding: (a) ultra-early, (b) early, (c) rapid progression, (d) consolidation and (e) dissipation stage [44]. But CT findings are still limited as the changes of the lungs can be due to other viral infection like adenovirus, MERS-CoV and SARS-CoV. In addition to nucleic acid detection and CT-imaging many researchers are developing kit for immunological detection and the detection rate of such serological kit (POCT of IgG/IgM, ELISA) is

higher than nucleic acid detection. As an additional method for detecting the viral nucleocapsid (N) antigen in nasopharyngeal aspirate (NPA), a more recent technique known as chemiluminescent enzyme-linked immunosorbent assay (CLEIA) has emerged [45].

Similar studies have found, rS- and N-based ELISAs may give us a specific confirmatory reaction for COVID-19. Salivary detection of secretory immunoglobulin A specific for COVID-19 can be beneficial research as animal model of this was successful for SARS-CoV. Several studies have demonstrated that assessing the levels of IL-6 and D-dimer in SARS-CoV-2-infected individuals can aid in determining the severity of their illness [46].

IV. ABBREVIATIONS

SARS CoV	Severe Acute Respiratory Syndrome Coronavirus
MERS CoV	Middle East respiratory Syndrome Coronavirus
SARS CoV nCoV	novel Corona virus
COVID19	2019 Coronavirus
RT-PCR	Real-Time Polymeric Chain Reaction
RT-LAMP	Reverse Transcription Loop-mediated Isothermal Amplification
HRCT	High Resolution Computerised Tomography
ORF	Open Reading Frame
IIFT	Indirect Immunofluorescence Test
POCT	Point-of-care Testing
ACE-2	Angiotensin Converting Enzyme-2
HLA	Human Leukocyte Antigen
NAAT	Nucleic Acid Amplification Testing
ELISA	Enzyme Linked Immunosorbent Assay
RT-RAA	Reverse-Transcription Recombinase Aided Amplification

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