Virology, Molecular Pathogenesis and Diagnosis of SARS-CoV-2: A Systematic Review

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Abstract : *After past episodes of Zika, Nipah and Ebola viruses and also previous emergencies due to other viruses like swine flu and bird flu viruses, now in 21st century global disaster caused by nCoV virus. As the virus is still investigated for proper identification, there are very less option in choosing the proper detection method. So we have reviewed various article for giving an idea about pathogenesis and diagnostic methods. We had screened Google Scholar database with the keywords nCoV pathogenesis, molecular immune pathogenesis of COVID-19, virology of SARS-CoV-2, diagnosis of SARS-CoV-2 and advancement of nCoV diagnosis. In the final review we have included a total of 84 articles. As a result we have reviewed the molecular virology along with molecular pathogenesis of COVID-19 in human body. We have found various ongoing researches on detection of nCoV related to nucleic acid amplification test, different types of RT-qPCR, serological assay and different HRCT methods.*

Keywords: Diagnosis, Molecular pathogenesis, SARS-CoV-2, Virology, WHO

1.   INTRODUCTION

From December 2019, numerous cases of pneumonia with unknown etiology had been reported in Wuhan, China from a local Seafood Market.[1] Later the causative agent was identified and named as SARS-CoV-2 or nCoV or 2019 novel coronavirus or COVID-19 by WHO.[2] Within two months, this disease spreads from China to 33 other countries. At the end of February 2020, the total confirmed cases were more than 77658, with 9126 severe cases, 2663 deceased cases in China[3] and 23309 confirmed, 33 deceased cases worldwide.[4]

As the proper therapeutic options are not discovered yet, so the early detection of the disease can lead us to the prevention. This virus is the part of beta coronavirus family. The Nucleic acid detection methods are showing the path for the proper confirmation of COVID-19. The clinical features are similar to SARS-CoV and MERS-CoV. The comparison of SARS-CoV-2, MERS-CoV and SARS-CoV is listed in Table 1. The virus transmitted through droplets and direct or indirect contacts with the infected person. This can cause respiratory damage, neurological damage and hepatic damage.[5] In this study we described the virology, molecular immune pathogenesis and various methods for detecting SARS-CoV and other new researches ongoing related to detection of this.

Table 1: Characteristics of MERS-CoV, SARS-CoV and SARS-CoV-2:[6][7][8]

Characteristic	MERS-CoV	SARS-CoV	SARS-CoV-2
First identified location	Jeddah, Saudi Arabia	Guangdong, China	Wuhan, China
Period	2012–ongoing	2002–2003	2019–present
Host of virus			
Natural host	Bats	Bats	Bats
Intermediate host	Dromedary camels	Civet cat or other animal hosts	Unknown
Mode of transmission	Respiratory droplet, contact	Respiratory droplet, contact	Respiratory droplet, contact
Incubation period	Median 5.2 days (95% CI, 1.9–14.7)	Mean 4.6 days (95% CI, 3.8–5.8)	Median 5.1 days (95% CI, 2.2–11.5)
Case fatality rate	34.4%	9.6%	3.8%

2. MATERIALS AND METHODS

Database screen: We had screened Google Scholar database with the keywords nCoV pathogenesis, molecular immune pathogenesis of COVID-19, virology of SARS-CoV-2, diagnosis of SARS-CoV-2 and advancement of nCoV diagnosis. Then further articles were screened for possible inclusion in the systematic review. Articles that have proper information about diagnosis, pathogenesis and virology were included for further review. The database files were cited using Mendeley application.

3. RESULT AND DISCUSSION
4.

A total of 130 articles were found after preliminary screening of the databases. After title and abstract screening, 15 articles were excluded. Full screening of the remaining 115 articles was done. Among these studies, after full-text screening, a total of 84 articles were included in the final review.

3.1 Virology of SARS-CoV-2

Origin, Family, and Genomic structure

In the end of 2019, nCoV appeared in local hospitals of Wuhan, Hubei, China. Notably, many kinds of live animals including seafood were available for sale in the market of China e.g., Huanan Seafood Market, before it was forced to close on 1st Jan, 2020 by China Center for Disease Control and Prevention. So, the CDC, China suggested the origin of the outbreak is Huanan Seafood Market as the SARS-CoV-2 samples were isolated from this area in the earlier phase. But this decision was disputed as there is no earlier reported case linked to the mention market.[1] In between two months, at least two different strains of nCoV have been discovered.[9] Till date, Chinese health authority conducted many epidemiological and etiological researches to find the origin of nCoV.[10]

From bronchoalveolar lavage of three patients from Wuhan Jinyiantan Hospital on 30th December, 2019, first nCoV was isolated.[11] After the analysis it was considered to be a member of beta-CoVs,[11][12] family *Coronaviridae* of Nidovirales order.[13] There are four subfamilies of CoVs according to serologically and genotypically: alpha, beta, gamma and delta CoVs. In between these groups the humans are affected (respiratory, hepatic, neurologic and enteric diseases) by alpha and beta CoVs.[14][15] According to the analysis, SARS-CoV-2 matches phylogenetically 79.5% with SARS-CoV and 50% with MERS-CoV[11][12][16] and the percent of sequence match is less than 90 between SARS-CoV-2 and other beta-corona viruses[11] which suggested the belonging of novel coronavirus is to lineage B (Sarbecovirus) of beta-coronavirus.[17]

SARS-CoV-2 contain positive single stranded RNA genome of size approx 29.8 kbp with a gene order of 5'-replicase open reading frame 1ab-S-E-M-N- 3'.[13] The nucleocapsid (N) is covered with bilayers of phospholipids and two types of spike proteins: spike glycoprotein trimmer (S) and hemagglutinin-esterase (HE). The envelope contains some structural protein along with S proteins which are membrane protein (M) and envelope protein (E).[17] The open reading frames (ORF), 1a and 1b are responsible for production of polyprotein 1a (pp1a) and polyprotein 1ab (pp1ab), which translates non-structural proteins (nsps).[13] The scientists have predicted the lengths of M, S, N, E and ORF3a genes of nCoV are 669, 3822, 1260, 228 and 828 nt respectively. The prediction also gives an idea of ORF8 gene length of 366 nt present in-between M and N ORF gene of SARS-CoV-2 which is similar to SARS-CoV.[17]

Another study observed that, nCoV protein sequences matches with Bat virus MG772934.1 (91.1%), Bat virus DQ022305.2 (79.7%) and SARS proteome (77.1%). Due to the mutation of ORF8, two variants of 2019-nCoV were observed: ORF8-5 and ORF8-L which changes the structural proteins.[18]

Properties of SARS-CoV-2

The physiochemical properties of nCoV from SARS-CoV or MERS-CoV as it resembles most of the same characters. nCoV is round or oval shaped with 60-100 nm diameter, inactivated by heating upto 56˚C for 30 minutes or ultraviolet rays. Substances like disinfectants e.g., diethyl ether, chlorine, 75%

ethanol, peracetic acid, chloroform is very active against nCoV.[19] According to various studies, on stainless steel, plastic the SARS-CoV-2 is more stable than cardboard and copper. The half-life of SARS-CoV-2 was higher in comparison with SARS-CoV.[20]

Host Cell Entry

To enter into the cell, SARS-CoV-2 takeover the angiotensin converting enzyme-2 (ACE2) as a functional receptor.[21][22] Angiotensin converting enzyme-2, a type I membrane protein, presented in, heart, lung, intestine and kidney which majorly connected with heart diseases. N terminal peptide domain and C terminal collectrin like domain are present on ACE-2. ACE-2 gives a direct bonding location for S proteins of coronaviruses, to break angiotensin-1, for producing other angiotensins (1 to 9).[23] The process of viral membrane structural arrangement to fuse with cell membrane of host is initiated by S1 subunit. The viral membrane binds with host receptor cell through hinge like movements of receptor binding domain (RBD).[15][24] Many research evidences suggest that the binding affinity of SARS-CoV-2 with human ACE-2 is 10-20-fold higher than SARS-CoV. To observe the potential infection effect of SARS-CoV-2, S protein RBD which is in connection with ACE-2 was analysed.[25] According to another research ACE-2-B0AT1 complex may be bind simultaneously to two S proteins.[26]

Ecology of SARS-CoV-2

All coronaviruses which have effects on human are zoonotic in origin and the natural hosts for any coronavirus it is most likely the bat.[27] Chinese horseshoe bats, Rhinolophdae family in Yunnan, China found to be very close regarding SARS-CoV.[28][29] Some bat CoVs like BatCoV RaTG13 shows similar sequence up to 96% nt with SARS-CoV-2.[29]

Naturally BatCoVs cannot affects humans unless until it undergoes through mutation and recombination in any host animal.[30] Different studies shows that SARS-CoV-2 may be originated from pangolin as the sequence of nCoV and pangolin CoVs matches 99%.[31] Till now, different researches are ongoing to track the SARS-CoV-2 animal host.

Variation of Genome

The earlier genomics were obtained from nine patients of COVID-19 which was 99.98% identical match.[16] Other scientists, on analysing 103 genomes have found two type 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 *S* type has weaker selective pressure so it may persist slowly.[32] These extracted RNA are very unstable, so strong surveillance is required to control SARS-CoV-2.[10]

3.2. 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 gives an idea of nCoV mechanism as it resembles almost similar symptoms and gene sequence.[33]

Entry and replication of Coronavirus

Protein S of CoVs is main responsible for entering into the virus cells.[34] The spike glycoprotein present on the envelope of SARS-CoV-2 makes a bond with ACE-2 of the host cell.[22] This host receptor may be different for SARS-CoV and MERS-CoV i.e. CD209L[35] and DPP4[36] respectively. Through fusion of the membranes between plasma membrane and virus, which occurred at the S2′ position of S protein leads to various proteolytic cleavage, as a result the invasion is completed.[37] After entering the host cell, structural proteins and polyproteins formation starts when the viral RNA comes into cytoplasm and replication of genome started.[38] The glycoprotein forming by the viral genome enters into endoplasmic reticulum membrane and Golgi membrane. The combination of viral RNA and nucleocapsid protein will generate a nucleocapsid. Then, the endoplasmic reticulum Golgi

intermediate compartment (ERGIC) will be the germination hub for viral particles and when the released virus particles containing vesicles will bind with the plasma membrane it releases the virus in the host body.[34] A group of scientists have found a molecule, N-(2-aminoethyl)-1 azirdine-ethanamine as an inhibitor of angiotensin converting enzyme-2 which block the fusion of SARS-CoV RBD with the host cell.[39]

Antigenic action in coronavirus infection

Anti-viral immunity of the body will represent the antigen presenting cells to the viral antigen and the major histocompatibility complex or human leukocyte antigen will present the antigenic peptides which will be recognized by the virus specific cytotoxic T-cells. So, we can understand the pathogenesis of SARS-CoV-2 through the antigen presenting cells but there is not much reports of COVID-19. We only get the research papers from MERS-CoV and SARS-CoV.[40] According to researches the MHC-1 is the main for SARS-CoV presentation.[41] Various researches shows that a number of polymorphisms of human leukocyte antigen e.g., HLA-B*0703, HLA-DRB1*1202, HLA-B*4601,[42] HLA-Cw*0801,[43] can access the SARS-CoV susceptibility. But another research shows that polymorphism like HLA-A*0201, HLA-Cw1502 and HLA-DR0301 may protect from infection like SARS.[44] Apart from polymorphisms, mannose binding lectin is also an antigen presenting cell related to infection of SARS-CoV.[45]

Host cell immunity

The virus-specific T and B cells stimulate the cellular and humoral immunity by the antigen presenting cells which activates the production of IgG and IgM. IgG antibody, specific for S and N can protect the body for long time where IgM only last for 12 weeks.[34][46] Recent researches Shows that the activation of CD8+ and CD4+ is higher but the count in peripheral blood is significantly low for SARS-CoV-2 patients.[47] Different researches on SARS-CoV patients shows that memory T-cells can recognise S-peptide up to four and six years after recovery.[48][49] These data may help in nCoV vaccine designing.

Cytokine storm

Recently published papers shows that SARS-CoV-2 induces the shedding of angiotensin converting enzyme-2 which results in a high activation of inflammatory factors like interferons, interleukins and chemokines.[50] In the earlier stage the viral replication triggers the chemokines and cytokines by causing damage to endothelial, epithelial cell and vascular leakage.[51] As a result of low ACE-2 levels, renin angiotensin system will be effected which will triggers more inflammation causing vascular permeability, ultimately leads to organ failure, acute respiratory distress syndrome etc.[10] Another study suggested that, viral cellular uptake can be improve by antibody dependent enhancement (ADE) through interaction of virus antibody complex and Fc receptor or different receptors, resulting enhancement of target cells.[52] The action between Fc receptor and the virus anti-S protein neutralizing antibodies complex may give an improvement in inflammation and replication of the virus in the lungs.[50]

Immune evasion

SARS-CoV and MERS-CoV have different strategies to dodge immune reactions in host cell. Pattern recognition receptors can identify the pathogen associated molecular pattern. But producing a double membrane vesicles with low PRRs, MERS-CoV and SARS-CoV can avoid host detection.[53] Against MERS-CoV and SARS-CoV infection, IFN alpha and beta can be helpful.[54] But the induction of IFN gets blocked by 4a-protein in MERS-CoV.[55] Alongside there are many proteins (ORF4b, ORF4a, ORF5 etc) which block the IFN regulatory factor-3 and activation of IFN beta promoter in MERS-CoV.[56] If we can closely monitor these processes and reverse the mechanism we may find a treatment strategy.

3.3. Diagnosis of 2019-nCoV

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).[57] The lab techniques commonly used for coronaviruses detection are listed in Table 2.[58]

Table 2: Laboratory techniques for detection of coronaviruses:[58]

Method	Characteristics	Test time	Reference
Antigen EIA	Rapid, poor sensitivity, some are CLIA-waived	<30 min	[59][60]
Antigen IFA	Good sensitivity and specificity, subjective interpretation	1–4 h	[61][62]
Cell culture	Gold standard, pure culture for further research and development, time consuming	1–7 days	[63][64]
Serology	Retrospective, cross-reaction	2–8 h	[63][22]
NAAT, monoplex, pan-HCoV	High sensitivity with universal coverage of all species of HCoV	1–8 h	[65][66]
NAAT, monoplex, specific-HCoV	High sensitivity and specificity for special species, potential quantification	1–8 h	[67][68]
NAAT, multiplex	High sensitivity and specificity, covering other pathogens, FilmArray RP EZ is CLIA-waived	1–8 h	[69][70]
NAAT, POCT	Rapid and safe, good sensitivity and specificity, some are CLIA-waived	15– 30 min	[71][72]

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

Nucleic acid detection method

There are two methods for nCoV detection: high-throughput sequencing and real-time quantitative polymerase chain reaction – RT-qPCR.[12] The high-throughput sequencing is not preferred due to its high cost and equipment dependency. Whereas RT-qPCR is an effective and direct technique for identify viruses from blood and secretions from respiratory track.[73] Different studies trying to isolate live viral genome of nCoV from stool [74] and tear fluid.[75]

Scientists are conducting different type of RT-PCR and RT-LAMP for better and rapid identification like using iCycler thermocycler- IQSYBR Green SuperMix.[76] RT-LAMP isothermal amplification is also a rapid and very sensitive method in detection of cDNA or RNA of SARS-CoV-2 and the detection can be seen under natural light by the presence turbidity in the kit.[77][78] There are some guidelines for handling Nucleic Acid Amplification Test in different countries which are considered as standard operating procedure by WHO and the mechanism is according to their guidelines given in Table 3.[79]

Table 3: Current following protocol for Nucleic Acid Amplification Testing:[79]

Institute/Country	Gene targets
China CDC, China	ORF 1ab and N
Charitè, Germany	RdRp, E, N

HKU, Hong Kong SAR	ORF 1b-nsp14, N
National Institute of Infectious Diseases, Department of Virology, Japan	Pancorona and multiple targets, spike protein
National Institute of Health, Thailand	N
US CDC, USA	Three targets in N gene
Pasteur Institute, Paris, France	Two targets in RdRp gene
As listed in WHO	

The primers and probes used for RT-PCR for COVID-19 is listed in Table 4.[73]

Table 4: Primers and probes for real-time RT-PCR for COVID-19:[73]

Assay/use	Oligonucleotide	Sequence (a)
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG

(a)- W is A/T; R is G/A; M is A/C; S is G/C. FAM - 6-carboxyfluorescein; BBQ - blackberry quencher.

The comparison of real-time reverse transcription polymerase chain reaction between World Health Organisation and Centre for Disease Control and Prevention for diagnosis purpose of SARS-CoV-2 are given in Table 5.[80] The comparison of PCR and LAMP is given in Table 6.

Table 5: Comparison of rRT-PCR of WHO and CDC for diagnosis of SARS-CoV-2:[80]

Test	Molecular targets	Scope	Limit of blank	Specimens	Storage conditions
WHO	E gene	First-line screening	3.9 copies × reaction	Nasopharyngeal AND oropharyngeal swab or wash in ambulatory patients, lower respiratory specimens (sputum and/or endotracheal aspirate or bronchoalveolar lavage)	≤5 days: 2–8 °C
	RdRp gene	Confirmatory testing	3.6 copies × reaction		>5 days: ≤70 °C
	N gene	Additional confirmatory testing	N/A		(Dry-ice)
CDC	N1/2/3 gene	Combined assay	1.0–3.2 copies/µL	Nasopharyngeal AND oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage and nasopharyngeal wash/aspirate or nasal aspirate	≤4 days: 4 °C
	RNase P gene	Control assay	N/A		> 4 days: ≤70 °C

E gene - envelop gene; N gene- nucleocapside gene; RdRp gene- RNA-dependent RNA polymerase gene; RNase P gene- human RNase P gene.

Table 6: Comparison between PCR and LAMP:[83][84]

LAMP	PCR
Isothermal and continuous amplification (Smaller, simpler, portable).	Thermal cycling (Multiple heating and cooling cycle; bulky and cumbersome).
Always requires sample concentration and Preparation (Time-consuming).	For virus detection, for example, influenza or human norovirus, LAMP assay offers one-step detection. Sample preparation steps are simplified.
Single protocol (Faster).	Multiple protocols (Complicated and requires a skilled technician).
Tolerate inhibitors and more stable.	Inhibitors hinder the reaction.
Diagnostic sensitivity > 95%.	Diagnostic sensitivity (95%) is currently reported lower than LAMP
Still exploring.	Established technique.

CRISP-FDS is a sensitive assay for SARS-CoV-2 detection with very less equipment and low cost. There are researches going on placing a microfluidic chip of RT-PRA based CRISPR-FDS which can be usable by any smart phone.[81] RT-RAA kit is also a promising tool for nCoV detection.[82]

Radio imaging and other diagnostic techniques

Although the RT-PCR is a specific test for detection of SARS-CoV-2 but still most physician suggested CT-imaging as they believe to be more sensitive. 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 multilobar 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, lymph adenopathy, 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.[85] 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.[86]

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.[87] Infact, enzyme-linked immunosorbent assay using chemiluminescence (CLEIA) has also emerged as a newer technique to detect the viral nucleocapsid (N) antigen in nasopharyngeal aspirate (NPA). Similar studies have found, rS- and rN- based ELISAs may give us a specific confirmatory reaction for COVID-19.[88] Salivary detection of secretory immunoglobulin A specific for COVID-19 can be a beneficial research as animal model of this was successful for SARS-CoV.[89] Some researchers said that, IL-6 and D-dimer levels can give an idea about the severity of SARS-CoV-2 infection.[90]

4. CONCLUSION

The issue under consideration in COVID 19 pandemic is the asymptomatic infected cases or the very mild cases who are considerably a large number in the population. Testing them for viral RNA is a real impractical situation; therefore, development of a specific IgG kit is the need of an hour. With this development large scale sero-diagnosis will be possible and true rate of infection in the population will also be known making us to understand the disease better and human to human transmission will also be easily traced. Hence, a rapid-performing serologic assay is keenly required for the fute and current viral needed for the current and future irruption. Again, as a preventive measure, strict vigilance of viral changes in different hosts is very important.

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Conflicts of Interest

There are no conflicts of interest.

Summary of work done by the contributors

VS, KJ and NP planned the concept and design. VS, KJ, PPK and AS carried out the Literature search and drafting the manuscript. PPK, AS and NP contributed in Manuscript editing and interpretation of the results. All the authors listed in manuscript have contributed substantially to the writing and revising of the manuscript.

Abbreviations

SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; MERS-CoV: Middle East respiratory Syndrome Coronavirus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; nCoV: novel Corona-virus; COVID-19: 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

REFERENCE

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.
2. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. Journal of Medical Virology. 2020; 92: 418–23.
3. NHC. National Health Commission of the People's Republic of China main [Internet]. 2019 [cited 2020 Jun 21]. Available from: http://en.nhc.gov.cn/
4. CDC Weekly C. The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) — China, 2020. China CDC Wkly [Internet]. 2020;2(8):113–22.
5. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology. 2019;17: 181–92.
6. Park SE. Epidemiology, virology, and clinical features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; coronavirus disease-19). Pediatr Infect Vaccine. 2020;27(1):1–10.
7. Chen J. Pathogenicity and transmissibility of 2019-nCoV—A quick overview and comparison with other emerging viruses. Microbes Infect. 2020;22(2):69–71.
8. Hui DS. Epidemic and Emerging Coronaviruses (Severe Acute Respiratory Syndrome and Middle East Respiratory Syndrome). Vol. 38, Clinics in Chest Medicine. 2017;71–86.
9. Xiong C, Jiang L, Chen Y, Jiang Q. Evolution and variation of 2019-novel coronavirus. bioRxiv [Internet]. 2020;2020.01.30.926477. Available from: https://www.biorxiv.org/content/10.1101/2020.01.30.926477v1
10. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, et al. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses [Internet]. 2020;12(4):372. Available from:

https://www.mdpi.com/1999-4915/12/4/372

11. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–33.

12. Zhou P, Yang X Lou, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–3.

13. Guo Y-R, Cao Q-D, Hong Z-S, Tan Y-Y, Chen S-D, Jin H-J, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Mil Med Res [Internet]. 2020 Dec 13;7(1):11. Available from: https://mmrjournal.biomedcentral.com/articles/10.1186/s40779-020-00240-0

14. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. In: Advances in Virus Research. 2011:85–164.

15. M.J. de WAHSEJKM and van H. Host Factors in Coronavirus Replication. In: Current Topics in Microbiology and Immunology [Internet]. 2017; 1–42. Available from: http://link.springer.com/10.1007/82_2017_25

16. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet [Internet]. 202;395(10224):565–74.

17. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265–9.

18. Ceraolo C, Giorgi FM. Genomic variance of the 2019-nCoV coronavirus. J Med Virol. 2020;92(5):522–8.

19. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). Chin Med J (Engl) [Internet]. 2020;133(9):1087–95. Available from: http://journals.lww.com/10.1097/CM9.0000000000000819

20. Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. New England Journal of Medicine. 2020: 382:564–7.

21. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. New England Journal of Medicine. 2020;382: 1199–207.

22. Li W, Moore MJ, Vasllieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–4.

23. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res [Internet]. 2000;87(5):E1-9. Available from: https://www.ahajournals.org/doi/10.1161/01.RES.87.5.e1

24. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 2016;3(1):237–61.

25. Loganathan SK, Schleicher K, Malik A, Quevedo R, Langille E, Teng K, et al. Rare driver mutations in head and neck squamous cell carcinomas converge on NOTCH signaling. Science. 2020;367(6483):1264–9.

26. Yan R, Zhang Y, Li Y, Xia L, Zhou Q. Structure of dimeric full-length human ACE2 in complex with B0AT1. bioRxiv [Internet]. 2020;2020.02.17.951848. Available from: http://biorxiv.org/content/early/2020/02/18/2020.02.17.951848.abstract

27. Vijaykrishna D, Smith GJD, Zhang JX, Peiris JSM, Chen H, Guan Y. Evolutionary Insights into the Ecology of Coronaviruses. J Virol. 2007;81(8):4012–20.

28. Ge XY, Li JL, Yang X Lou, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503(7477):535–8.

29. Lau SKP, Woo PCY, Li KSM, Huang Y, Tsoi HW, Wong BHL, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A. 2005;102(39):14040–5.

30. Corman VM, Muth D, Niemeyer D, Drosten C. Hosts and Sources of Endemic Human Coronaviruses. In: Advances in Virus Research [Internet]. 2018. p. 163–88. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0065352718300010

31.   Lam TT-Y, Jia N, Zhang Y-W, Shum MH-H, Jiang J-F, Zhu H-C, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature [Internet]. 2020 Mar 26; Available from: http://www.nature.com/articles/s41586-020-2169-0

32.   Tang X, Wu C, Li X, Song Y, Yao X, Wu X, et al. On the origin and continuing evolution of SARS-CoV-2. Natl Sci Rev [Internet]. 2020;7(6):1012–23. Available from: https://academic.oup.com/nsr/article/7/6/1012/5775463

33.   Peiris JSM, Guan Y, Yuen KY. Severe acute respiratory syndrome. Vol. 10, Nature Medicine. 2004. p. S88–97.

34.   De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: Recent insights into emerging coronaviruses. Nature Reviews Microbiology. 2016;14:523–34.

35.   Jeffers SA, Tusell SM, Gillim-Ross L, Hemmila EM, Achenbach JE, Babcock GJ, et al. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A. 2004;101(44):15748–53.

36.   Raj VS, Mou H, Smits SL, Dekkers DHW, Müller MA, Dijkman R, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. 2013;495(7440):251–4.

37.   Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. Proc Natl Acad Sci U S A. 2004;101(12):4240–5.

38.   Perlman S, Netland J. Coronaviruses post-SARS: Update on replication and pathogenesis. Nature Reviews Microbiology. 2009;7:439–50.

39.   Chen Y, Guo Y, Pan Y, Zhao ZJ. Structure analysis of the receptor binding of 2019-nCoV. Biochem Biophys Res Commun. 2020;525(1):135–40.

40.   Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. Vol. 10, Journal of Pharmaceutical Analysis. 2020; 10:102–8.

41.   Liu J, Wu P, Gao F, Qi J, Kawana-Tachikawa A, Xie J, et al. Novel Immunodominant Peptide Presentation Strategy: a Featured HLA-A*2402-Restricted Cytotoxic T-Lymphocyte Epitope Stabilized by Intrachain Hydrogen Bonds from Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Protein. J Virol. 2010;84(22):11849–57.

42.   Keicho N, Itoyama S, Kashiwase K, Phi NC, Long HT, Ha LD, et al. Association of human leukocyte antigen class II alleles with severe acute respiratory syndrome in the Vietnamese population. Hum Immunol. 2009;70(7):527–31.

43.   Chen YMA, Liang SY, Shih YP, Chen CY, Lee YM, Chang L, et al. Epidemiological and genetic correlates of severe acute respiratory syndrome coronavirus infection in the hospital with the highest nosocomial infection rate in Taiwan in 2003. J Clin Microbiol. 2006;44(2):359–65.

44.   Wang SF, Chen KH, Chen M, Li WY, Chen YJ, Tsao CH, et al. Human-leukocyte antigen class i Cw 1502 and Class II DR 0301 genotypes are associated with resistance to severe acute respiratory syndrome (SARS) infection. Viral Immunol. 2011;24(5):421–6.

45.   Tu X, Chong WP, Zhai Y, Zhang H, Zhang F, Wang S, et al. Functional polymorphisms of the CCL2 and MBL genes cumulatively increase susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect. 2015;71(1):101–9.

46.   Li G, Chen X, Xu A. Profile of Specific Antibodies to the SARS-Associated Coronavirus. N Engl J Med [Internet]. 2003 Jul 31;349(5):508–9. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM200307313490520

47.   Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8(4):420–2.

48.   Fan YY, Huang ZT, Li L, Wu MH, Yu T, Koup RA, et al. Characterization of SARS-CoV-specific memory T cells from recovered individuals 4 years after infection. Arch Virol. 2009;154(7):1093–9.

49.   Tang F, Quan Y, Xin Z-T, Wrammert J, Ma M-J, Lv H, et al. Lack of Peripheral Memory B Cell Responses in Recovered Patients with Severe Acute Respiratory Syndrome: A Six-Year Follow-Up Study. J Immunol. 2011;186(12):7264–8.

50.   Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virol Sin [Internet]. 2020 Mar 3; Available

from: http://link.springer.com/10.1007/s12250-020-00207-4

51. Yang M. Cell Pyroptosis, a Potential Pathogenic Mechanism of 2019-nCoV Infection. SSRN Electron J [Internet]. 2020; Available from: https://www.ssrn.com/abstract=3527420

52. Takada A, Kawaoka Y. Antibody-dependent enhancement of viral infection: Molecular mechanisms and in vivo implications. Reviews in Medical Virology. 2003;13:387–98.

53. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJM, van der Meulen J, Koerten HK, et al. Ultrastructure and Origin of Membrane Vesicles Associated with the Severe Acute Respiratory Syndrome Coronavirus Replication Complex. J Virol. 2006;80(12):5927–40.

54. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, et al. 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.

55. Niemeyer D, Zillinger T, Muth D, Zielecki F, Horvath G, Suliman T, et al. Middle East Respiratory Syndrome Coronavirus Accessory Protein 4a Is a Type I Interferon Antagonist. J Virol. 2013;87(22):12489–95.

56. Yang Y, Zhang L, Geng H, Deng Y, Huang B, Guo Y, et al. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. Protein Cell. 2013;4(12):951–61.

57. Xie C, Jiang L, Huang G, Pu H, Gong B, Lin H, et al. Comparison of different samples for 2019 novel coronavirus detection by nucleic acid amplification tests. Int J Infect Dis [Internet]. 2020 Apr;93:264–7. Available from: https://doi.org/10.1016/j.ijid.2020.02.050

58. Loeffelholz MJ, Tang YW. Laboratory diagnosis of emerging human coronavirus infections– the state of the art. Emerg Microbes Infect. 2020;9(1):747–56.

59. Lau SKP, Woo PCY, Wong BHL, Tsoi HW, Woo GKS, Poon RWS, et al. Detection of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in SARS patients by enzyme-linked immunosorbent assay. J Clin Microbiol. 2004;42(7):2884–9.

60. Sastre P, Dijkman R, Camuñas A, Ruiz T, Jebbink MF, Van Der Hoek L, et al. Differentiation between human coronaviruses NL63 and 229E using a novel double-antibody sandwich enzyme-linked immunosorbent assay based on specific monoclonal antibodies. Clin Vaccine Immunol. 2011;18(1):113–8.

61. He Q, Manopo I, Lu L, Leung BP, Chng HH, Ling AE, et al. Novel immunofluorescence assay using recombinant nucleocapsid-spike fusion protein as antigen to detect antibodies against severe acute respiratory syndrome coronavirus. Clin Diagn Lab Immunol. 2005;12(2):321–8.

62. Sizun J, Arbour N, Talbot PJ. Comparison of immunofluorescence with monoclonal antibodies and RT-PCR for the detection of human coronaviruses 229E and OC43 in cell culture. J Virol Methods. 1998;72(2):145–52.

63. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003;348(20):1953–66.

64. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814–20.

65. Zlateva KT, Coenjaerts FEJ, Crusio KM, Lammens C, Leus F, Viveen M, et al. No novel coronaviruses identified in a large collection of human nasopharyngeal specimens using family-wide CODEHOP-based primers. Arch Virol. 2013;158(1):251–5.

66. Kuypers J, Martin ET, Heugel J, Wright N, Morrow R, Englund JA. Clinical Disease in Children Associated With Newly Described Coronavirus Subtypes. Pediatrics [Internet]. 2007 Jan 1;119(1):e70–6. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2006-1406

67. Chan JFW, Choi GKY, Tsang AKL, Tee KM, Lam HY, Yip CCY, et al. Development and evaluation of novel real-time reverse transcription-PCR assays with locked nucleic acid probes targeting leader sequences of human-pathogenic coronaviruses. J Clin Microbiol. 2015;53(8):2722–6.

68. Dare RK, Fry AM, Chittaganpitch M, Sawanpanyalert P, Olsen SJ, Erdman DD. Human Coronavirus Infections in Rural Thailand: A Comprehensive Study Using Real-Time Reverse-Transcription Polymerase Chain Reaction Assays. J Infect Dis. 2007;196(9):1321–8.

69.   Gaunt ER, Hardie A, Claas ECJ, Simmonds P, Templeton KE. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. J Clin Microbiol. 2010;48(8):2940–7.

70.   Babady NE, England MR, Jurcic Smith KL, He T, Wijetunge DS, Tang YW, et al. Multicenter Evaluation of the ePlex Respiratory Pathogen Panel for the Detection of Viral and Bacterial Respiratory Tract Pathogens in Nasopharyngeal Swabs. McAdam AJ, editor. J Clin Microbiol [Internet]. 2018 Dec 6;56(2):e01658-17. Available from: https://jcm.asm.org/content/56/2/e01658-17

71.   Kozel TR, Burnham-Marusich AR. Point-of-care testing for infectious diseases: Past, present, and future. Journal of Clinical Microbiology. 2017;55:2313–20.

72.   Beal SG, Posa M, Gaffar M, Reppucci J, Mack JA, Gurka MJ, et al. Performance and Impact of a CLIA, Point-of-care Respiratory PCR Panel in a Pediatric Clinic. Pediatr Infect Dis J [Internet]. 2020;39(3):188–91. Available from: http://journals.lww.com/10.1097/INF.0000000000002544

73.   Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance [Internet]. 2020 Jan 23;25(3). Available from: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045

74.   Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581(7809):465–9.

75.   Xia J, Tong J, Liu M, Shen Y, Guo D. Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. J Med Virol. 2020;92(6):589–94.

76.   Hao W, Li M. Clinical diagnostic value of CT imaging in COVID-19 with multiple negative RT-PCR testing. Travel Med Infect Dis [Internet]. 2020 Mar;34:101627. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1477893920300958

77.   Yu L, Wu S, Hao X, Dong X, Mao L, Pelechano V, et al. Rapid Detection of COVID-19 Coronavirus Using a Reverse Transcriptional Loop-Mediated Isothermal Amplification (RT-LAMP) Diagnostic Platform. Clin Chem [Internet]. 2020 May 12; Available from: https://academic.oup.com/clinchem/advance-article/doi/10.1093/clinchem/hvaa102/5823294

78.   Kitagawa Y, Orihara Y, Kawamura R, Imai K, Sakai J, Tarumoto N, et al. Evaluation of rapid diagnosis of novel coronavirus disease (COVID-19) using loop-mediated isothermal amplification. J Clin Virol [Internet]. 2020;129(May):104446. Available from: https://doi.org/10.1016/j.jcv.2020.104446

79.   Padhi A, Kumar S, Gupta E, Saxena SK. Laboratory Diagnosis of Novel Coronavirus Disease 2019 (COVID-19) Infection. In 2020; 95–107. Available from: http://link.springer.com/10.1007/978-981-15-4814-7_9

80.   Lippi G, Simundic A-M, Plebani M. Potential preanalytical and analytical vulnerabilities in the laboratory diagnosis of coronavirus disease 2019 (COVID-19). Clin Chem Lab Med [Internet]. 2020 Jun 25;58(7):1070–6. Available from: https://www.degruyter.com/view/journals/cclm/58/7/article-p1070.xml

81.   Huang Z, Tian D, Liu Y, Lin Z, Lyon CJ, Lai W, et al. Ultra-sensitive and high-throughput CRISPR-p owered COVID-19 diagnosis. Biosens Bioelectron [Internet]. 2020 Sep;164:112316. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0956566320303110

82.   Wang J, Cai K, He X, Shen X, Wang J, Liu J, et al. Multiple-centre clinical evaluation of an ultrafast single-tube assay for SARS-CoV-2 RNA. Clin Microbiol Infect [Internet]. 2020 May 15;(xxxx). Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X20302846

83.   Nguyen T, Bang DD, Wolff A. 2019 Novel coronavirus disease (COVID-19): Paving the road for rapid detection and point-of-care diagnostics. Micromachines. 2020;11(3):1–7.

84.   Wang X, Seo DJ, Lee MH, Choi C. Comparison of conventional PCR, multiplex PCR, and loop-mediated isothermal amplification assays for rapid detection of Arcobacter species. J Clin Microbiol. 2014;52(2):557–63.

85.   Salehi S, Abedi A, Balakrishnan S, Gholamrezanezhad A. Coronavirus Disease 2019 (COVID-19): A Systematic Review of Imaging Findings in 919 Patients. Am J Roentgenol [Internet]. 2020 Jul;215(1):87–93. Available from:

https://www.ajronline.org/doi/10.2214/AJR.20.23034

86. Li Y, Xia L. Coronavirus disease 2019 (COVID-19): Role of chest CT in diagnosis and management. Am J Roentgenol. 2020;214(6):1280–6.

87. Woo PCY, Lau SKP, Wong BHL, Tsoi HW, Fung AMY, Kao RYT, et al. Differential sensitivities of severe acute respiratory syndrome (SARS) coronavirus spike polypeptide enzyme-linked immunosorbent assay (ELISA) and SARS coronavirus nucleocapsid protein ELISA for serodiagnosis of SARS coronavirus pneumonia. J Clin Microbiol. 2005;43(7):3054–8.

88. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of Nucleocapsid and Spike Protein-Based Enzyme-Linked Immunosorbent Assays for Detecting Antibodies against SARS-CoV-2. J Clin Microbiol. 2020;58(6):1–7.

89. Sabino-Silva R, Jardim ACG, Siqueira WL. Coronavirus COVID-19 impacts to dentistry and potential salivary diagnosis. Clin Oral Investig. 2020;24(4):1619–21.

90. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 2020;92(7):791–6.