***Review Article***

(all words and passages in green are to be added; all words within green brackets are to be deleted)

A Review of Covid-19 Detailed study of History, Life cycle, Diagnosisand Prevention of Corona virus.

ABSTRACT:

Coronavirus Disease-2019 is a new life-threatening, quickly-spreadable pandemic disease. It is a huge family of viruses known to cause sickness from breathing trouble, fever, fatigue, cough, sore throat, breathlessness, and common cold to the continuation of acute respiratory tract infection and the severity of the infection sometimes visible as pneumonia, acute respiratory syndrome and even death. The disease is commonly known as COVID-19. Since December 2019, Covid-19 emerged in Hunan seafood market at Wuhan in South China and rapidly spreading throughout the world, the virus epidemic has been proclaimed a public health emergency of International concern by World Health Organization (WHO). An exceedinglyinfectious potential for spreading resulted in the universal coronavirus disease 2019 (COVID-19) pandemic in 2020. Thoughsome specialistscast doubts that the virus is transmitted from animals to humans, there are mixed results on the origin of coronavirus. In this review, we go through the history of covid-19, virus life cycle, diagnosis,and preventionwith reference to the historical coronavirus pandemic in 2002.

Keywords: COVID-19, SARS, Symptoms, Respiratory syndrome, Treatment.

## 1. INTRODUCTION:

Up until the SARS outbreak (2002), coronavirusesonly displayed their possibility for epidemic spread and significant pathogenicity in humans, and were mainly known as the cause of mild respiratory syndrome and gastrointestinal disease [1]. Over the pasttwo decades three novel Beta coronavirusesdrastically altered such a perception, these are theSevere Acute Respiratory Syndrome (SARS-CoV), Middle East Respiratory Syndrome (MERS)-CoV and SARS-CoV2, in which the viruses crossed the species barrier and caused significant epidemic characterized by increased case-fatality rates in humans [2–4]. The smallre-emergence of SARS in late 2003 after the restarting of the wildlife market in southern China and the current discovery of a very similar virus in horseshoe bats,the bat SARS-Coronavirus, suggested that SARS can return if circumstances are fit for the introduction, amplification, mutation, and transmission of this threatening virus [5-8]. Seven coronaviruses can bring about infection among people around the world but people mostly get infected with four human coronaviruses, 229E, NL63, OC43, and HKU1[9].The virus typically spreads rapidly from one person to another via respiratory dropletsand aerosol produced through coughing and sneezing. It's considered most infectious

when people are symptomatic, although transmission may be achievable before symptoms showup in patients. Time from infectionto symptom onset is commonly between 2 and 14 days, with an average of 5 days. General symptoms are fever, cough, sneezing and shortness of breath. Complicationsinclude pneumonia, throat pain and acute respiratory syndrome. Initially, there was no specific treatment or vaccine; efforts consisted of symptom mitigation and supportive therapy. Recommended preventive measures such as washing the hands with soap, covering the mouth when coughing by using face mask or handkerchief, maintaining one-meter gap from other people and monitoring and self-isolation for 14 days for people who suspect they are infected [10].The diseaseis diagnosed by using a standard tool of Reverse Transcription Polymerase Chain Reaction (RT-PCR) from a throat swab or nasopharyngeal swab [11]. Diagnostic approaches to COVID-19 can be divided into two broad categories,clinical diagnostics and in vitro diagnostics [12–14]. Clinical diagnostics for COVID-19 begins with assessment of possibly COVID-19 related symptoms and recent contact and exposure. These should be considered in the context of the SARS-CoV-2 incubation period, which is evaluated to be up to 14 days from exposure, with a median of 4 to 5 days [15–17]. Invitro diagnostics for SARS-CoV-2 infection is confirmed by observation of SARS-CoV-2 RNA using NAAT(Nucleic Acid Amplification Test) [18].It usually takes 3.5–4 hours and needs three steps,namely RNA extraction, cDNA synthesis, and amplification and detection of the target nucleic acid, as well as specialized lab equipment [19]. Clinical management of SARSrelies on supportive care. Broad-spectrum antimicrobial coverage for community obtained pneumonia should be given while virological confirmation is pending. Such antibiotics should be discontinued once the diagnosis of SARS-CoV-2 is confirmed, but nosocomial infections as an outcome of extant intubation and useof corticosteroids should be appropriately managed. The correlation between viral loads and clinical result suggests that suppression of viral replication by effective antiviral agents should be the key to fend off morbidity and mortality [20-22].

## 2. HISTORY AND LIFE CYCLE OF VIRUS:

### 2.1 History

The first sufferer of coronaviruses in human present in 1965 by Tyrrell and Bynoe. They noticed that they could isolate a virus named B814. Itwas noticeable in human embryonic tracheal cell culturesof specimens obtained from the respiratory tract of an adult with common cold symptom. The first sufferers of the present pandemic were seen in Wuhan City of Hubei region China in December 2019, and have been associated to the Huanan Seafood Market at South China, and the infection has spread to manycountries around the world [23].

SARS is the first known major pandemic disease caused by a coronavirus. During the outbreak in 2003 in which 8,096 cases with 774 deaths had occurred in over 30 countries in 5 continents [24- 39]. The disease emerged in late 2002, when anepidemic of acute community acquired atypical pneumonia syndrome was first observed in the Guangdong region. Retrospective surveillance tracedseveral cases of the disease in five cities around Guangzhou over a period of 2 months [40]. The index case was announced in Foshan, a city 24 km away from Guangzhou. The

second sufferer involved a chef from Heyuan who worked ata restaurant in Shenzhen. The patient had continuous contact with wild game food animals. His wife, two sisters, and seven hospital staff who had contact with him were also affected. From 16 November 2002 to 9 February 2003,atotal of 305 cases were reported in mainland China, including 105 (of those sufferers involving) health care workers. Next, a devastating outbreak started in Hong Kong Special Administrative Region (HKSAR), when a professor of nephrology from a teaching hospital in Guangzhou, who had contracted the disease from his patients, came to HKSAR on 21 February 2003. On this day, he transmitted the viral infection to 16 other people in the hotel where he resided. His brother-in-law, one of the secondary sufferers, underwent an open lung biopsy from which the etiological agent was found out and first isolated [32]. It is a novel coronavirus, named SARS-CoV.

## 2.2 Life cycle with special reference to themolecular mechanism of SARS-CoV-2 infection at the cellular level.

### 2.2.1 Overview of the basic coronavirus component

The coronavirus basically has three components comprising four major proteins and some other accessory proteins with the viral genome at the center. The outermost zone is formed by the club-shaped S (spike) protein jutting out from the virus membrane, and has an appearance reminiscent of the sun's corona, hence the name of the virus. Then the membrane of the virus carries two membrane proteins, M (for membrane protein) and E (for envelope protein).Thirdly, within the membrane-envelope is the nucleocapsid.The N (for nucleocapsid protein) is the protein component of the viral nucleocapsid and binds to the viral genomic RNAin a "beads-on-a-string" manner. Besides being a structural protein, N protein also contributes to RNA replication and in some cases nuclear localization.The coronavirus RNA is among the largest, if not the largest, RNA in existence. Much of it is taken up for the translation of replicase, a large enzyme complex which expedites the virus genomic replication once it breaks free into the cytoplasm of the host cells. From the remaining genome, the four major structural proteins, S, E, M, N, and, in the particular case of SARS-CoV-2, some eight accessory proteins mostly of uncertain functions.One of these accessory proteins is $3CL^{pro}$(3-chymotrypsin-like protease, corresponding to nsp5/non-structural protein 5) which self-cleaves the coronavirus' polyprotein at 11 sites and plays an essential role in the replication of the virus. It is the target of the anti-viral drug nirmatrelvir.

### 2.2.2 Outline of the virus cycle in the cell from entry through replication to exit.

Havingentered the newly infected human body, the SARS-CoV-2 virus begins its active life cycle by gaining attachment and entry into the host cell. First, its S protein binds to its specific receptor ACE II (angiotensin conversion enzyme II) expressed on many cells in various organs. Conformational changes then occur in the viral S protein resulting in the emergence of FP (fusion peptide) from the virus membrane. FP is thrusted into the host membrane to create a fusion pore. The viral genome then sheds it's coating and enters the host cell cytoplasm. This is followed by translation of viral components, using the host endoplasmic reticulum.Assembly of structural viral proteins, S, E, M, take place in the endoplasmic reticulum-Golgi intermediate

compartment (ERGIC), while nucleocapsids are formed from the encapsidationof the replication products of viral genome organized by viral replicase and the N protein. All these elements coalesce to form new virons, which are transported to the host cell membrane in Golgi sacs or similar vesicles to exit from the cell and infect other cells. Sometimes, a fraction of S protein might not be assembled into virons but reach the cell surface individually, behaving as membrane protein of the host cell with the potential of binding to the ACE II on another cell, mediating direct cell-to-cell fusion, infection, and multinucleated giant cell formation. The following sections further illustrate the complex and often confusing steps involved.

### 2.2.3 The S (spike) protein and binding of corona virus to its receptor ACE II.

The S protein is a trimeric protein, also known as peplomers. It is a class I fusion protein formedfrom an amino-terminal S1 anda carboxyl-terminal S2 subunits connected by a fusion peptide. (In)The two subunits are indispensable for receptor binding and membrane fusion (in) respectively. S1 is responsible for binding to the ACE II. The receptor binding domain(RBD) of S1 has been mapped to residues 318 to 510 [41- 42].Conceivably, mutations in this domain will lead to new subtypes of SARS CoV-2 with different infectivity, pathogenicity, resistance to treatment and evasion of host immunity. S1's binding to the cellular receptorACE II triggers conformational changes, which re-locate the fusion peptide(FP)upstream of the 2 heptad repeats(HR) of S2 in a transmembrane domain and finally resultingin its insertion into the membrane of the target cell creating a fusion porebetween the viral and host cellular lipid membrane barriers.Through the fusion pore the virus genome sheds its coating and enters the cytoplasm of the target cell.

### 2.2.4 Factors facilitating virus entry into host cells.

In order to present the fusion peptide from its original location embedded in the membranous part of the S2 protein two steps are needed. First thesubunit S1, after achieving its mission of gaining attachment to its receptor ACE II,has to be cleaved from S2. Next a further part of S2 has to be cleaved to expedite molecular conformational change and bring out the fusion peptide.Subsequent membrane fusion with the target cell could be facilitated by additional proteolytic activity and lower pH (higher acidity).

Much as the key receptor attached by S, the angiotensin-converting enzyme II(ACE II), is also a metalloprotease, it could not effectively cleave S1 from S2 much less further cleaving S2 at its S2' site.The multiple steps of protein cleavage would still require additional reinforcement. The commonestroute of virus entry is direct membrane fusion on the cell surface.The infected cell membrane that is associated with proteases, like factor Xa, or more commonly TMPRSS-2 (transmembrane protease serine type 2), can contribute to S proteincleaving processes especiallyat S2' to bring out the fusion protein. This route via the cell membrane is shorter and more direct, and is also the preferred route of membrane fusion by the virus.

If a cell's membrane has insufficientTMPRSS-2, the virus' S2 remains intact and it has to settle for a second route of cell entry. Stuckat the cell membrane, it is subjected to endocytosis and then transported into the cytoplasm within the confinement of an endosome i.e. still functionally

separated from the cytoplasm. However,within the endosome, the virus' S2 could be cleaved by cathepsin L under a low pH (acidic) environment.

With either route the ultimate result is to expose the fusion peptide from the membrane remnant of S2 to have it insertedinto the cell membrane or the endosome lipid membraneto create a fusion pore and expedite membrane fusion, allowing the viral genome to enter the host cell cytoplasm.

2.2.5 Other miscellaneous facts and findings related to viral protein cleavage and cell receptors.

Studies showed that the proteolytic cleavage of SARS CoV can be carried out by the protease Factor Xa and specifically inhibited by a protease inhibitor such as Ben-HCl[43].

2.2.5.b Depletion of ACE II and excess angiotensin II might contribute to organ damage.

ACE II is expressed in the cells of the lung, intestine, liver, vascular endothelium, heart, testis, and kidney [44]. It appears to protect against acute lung injury in a mouse model.The binding of S protein to host cells' ACEIIresults in the down-regulation of thisenzyme, leaving an excess of its substrate, angiotensin II,unmodified and free to bind to the angiotensin Ia receptor (AT1aR) to aggravate damage in the respective organs. This mechanism may contribute to the severity of lung damage in SARS [45] and COVID-19.

2.2.5.c The role of Lectin receptors in coronavirus cell entry.

Although the respiratory tract is the major portal of coronavirus entry, yet the well-established virus receptor ACE II is paradoxically under-expressed there. In such situation, the C-type lectin receptors might serve to enhance the ACE II-mediated infection. C-type lectinreceptorsconsist of several types,DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin protein), L-SIGN (liver/lymph-node specific intercellular adhesion molecule-3-grabbing non-integrin protein), and LSECtin (liver sinusoidal endothelial cell lectin). Theyare able to function as receptor facilitators, having been shown to augment the cellular entry of pseudo type virus expressing S in the scanty presence of ACE II [46-49].

2.2.5.d The role of dendritic cell (DC)

Cells expressing the lectins and with low level or absence of ACE II, like dendritic cells,are able to promote the cell-mediated transfer of SARS-CoV to susceptible cells [46]. Lectins like DC-SIGN may enhance the activity of the scarce available ACE II or simple acts as an alternate receptor. Once infected the dendritic cell may travel to lymph follicles and pass the virus to other cells.

2.2.5.e Endosome route of virus entry, role of cathepsin L and pH, and implication on treatment.

Apart from the virus-preferred route of entry through the cell membrane, the alternative route is by endocytosis.Within the internalized endosome, the proteolytic enzyme cathepsin L and alow pH environmentare required for the proteolyticcleavage of S2 protein tobring out the fusion peptide and expeditevirus-to-cell fusion. [50-51]This process of viral entry via endosomal endocytosis and membrane fusion within the cytoplasm for the release of viral RNA for translation and replication remains of secondary importance, and attempts to treat COVID-19

with cathepsin L inhibitors, chloroquine and hydroxychloroquine, have generally proved futile. From theoretical consideration such failures are expected as the preferred pathway of membrane fusion by the virus is not via endosomal endocytosis but by direct fusion with the cell membrane aided by the membrane protease TMPRSS-2 (transmembrane serine protease 2).

2.2.6Translation and replication of coronavirus.

Once within the host cytoplasm, the viral genome assumes its role as a mRNA and translates new viral proteins. Translation begins with the large replicase and two large polyproteins,(since) Orf1a and Orf1ab, that are post-translationally cleaved by the two viral proteases including nsp1 and nsp16. (In)These cleavage products form the replication-transcription complex, (in) which replicates the viral genome and transcribes a 3'-coterminal nested set of 8 sub-genomic RNAs.Therefore, it is conceivable that infected cells contain an increasing number of transcripts-containinggenes towards the 3'coterminus of the viral genome. On this basis, Reverse Transcriptase Polymer Chain Reaction (RT-PCR) using the N gene may have a better sensitivity than using the other genes. And other coronaviruses, SARS-CoV may attach by the hydrophobic domains of their replication equipment to the restrict membrane of autophagosomes and form double-membrane vesicles. Once adequate viral genomic RNA and structural proteins are accumulated, viral assembly by budding of the helical nucleocapsid into theERGIC(endoplasmic reticulum-Golgi intermediate compartment) takes place. At this time, the triplemembrane-spanning M protein interacts with N protein and viral RNA to create the basic structure. It (is) interacts with E and S proteins to induce viral budding and release. Unlike other coronaviruses,(and) the M protein of SARS-CoV also implicates another triple-membrane-spanning protein of Orf3a into the virion [54]. The N protein is the most copiously expressed viral protein in infected cells in which the mRNA levels were amplified 3 to 10 times, increasing at 12 h post-infectionmorethan other structural genes [55] and therefore a main target for immunohistochemistry and antigen detection in the clinical specimens. (Such as )Various diagnostic tests, antiviral drugs, and vaccines are designed on the basis of our comprehension of the structure and function of the several viral proteins involved in the life cycle of this virus.

3.DIAGNOSIS:

RT-PCR assays are conventional or automated type of assay, withalternative terminologies such as rRT-PCR or RT-qPCR.

NAAT (Nucleic Acid Amplification Test)isused to detect the presence of viral RNA [56]. Purified RNA from clinical specimens is reverse transcribed into complementary DNA (or) cDNA, next added to a master mix containing target primers and fluorophore-quencher probe. The RT-PCR process is carried through in a thermal cycler. The fluorophore-quencher probe is cleaved and creating a fluorescent signal that corresponds to the amplified outcome [57-58]. While conventional NAAT start from manual RNA preparation, followed by rRT-PCR, automated systems unsegregate(d) RNA extraction, purification, amplification, detection and outcome in rapid, high-throughput results and decreased contamination [59–62],pre-heating specimens to omit RNA extraction [63–66]. Accuracy with alternative, reduced-invasive

specimens (e.g., Saliva) in comparison with standard NP specimenshas been studied[67–70]. Subsequent respiratory specimens may provide information on progress later in the disease course [71], while non-respiratory specimens may correlate with local symptoms (e.g., stool) or clinical seriousness (e.g., blood) [72-74]. Swab pooling mayincrease testing capacity [75]. Various PCR target regions may act on sensitivity [76–79].Other aspects to consider are: Monitoring effect of SARS-CoV-2 genome mutations on RT-PCR showing [80- 81];First-step (consolidated RT and PCR) versus Second-step (separate RT and PCR) assays;(and)Uniplex versus Multiplex RT-PCR [82- 83, 58]; Sub-genomic RNA or Ct value as the surrogate for infectivityoflive virus [84].

3.1 FUTURE MANAGEMENT:

Availability of the diagnostic technologies has enabled researchers to quickly adapt them to COVID-19 [58]. Lessons since the 2002 SARS epidemic have guided development of COVID-19 detection strategies. Only three weeks elapsed since visualization of the virus by using transmission electron microscopy to the elucidation of SARS-CoV-2 genetic sequence, whileforSARS-CoV ittook five months (to be grant)[58, 85]. Control of outbreak requires extensive, ongoing (on) surveillance, and quick sharing of epidemiological data [86]. Smartphones are used (in) which increased exponentially (and) including in sub-Saharan Africa, and can be leveraged for this motive as they possess link, computational power, and hardware to ease electronic reporting, epidemiological data basing and sharp end-of-care testing [58, 87]. Combining diagnostics tools with smartphone combination could support better management, curb transmission of infection and decrease mortality [58]. Safety of laboratory employee who conduct COVID-19 testing is also paramount. Concern for laboratory-associated infection is of specific concern in the setting of individual or personal protective equipment (PPE) shortages, inappropriate microbiological techniques, insufficiency of training, and inadequate detoxification protocols or biosafety measure [88],all of which are more expected to occur when systems are overwhelmed. Optimization of mechanisms to safe-guard laboratory employees should occur in parallel with optimization of COVID-19 diagnostics.

4. PREVENTION:

To diminished COVID-19 transmission from potentially asymptomatic or pre-symptomatic people, the ECDC (European Centre for Disease prevention and Control) recommends (to) the use of face masks [89],(While included) social distancing, travel restrictions on visitors arriving from increasingly high-risk provinces, quarantine for nationals returning from increasingly risk locations, and closure of schools, colleges and certain types of workplaces,the full or partial closure of educational institutions and certain workplaces, restrict the number of visitors and restrict the contact between the residents of confined settings, including prolonged-term care facilities and prisons, cancellation, barring and reduction of mass gatherings and smaller meetings, obligatory quarantine of buildings or residential areas, internal or external boundary closures, and stay-at-home restrictions for whole regions or countries. All ministriesshouldpublish(ed) common instructions on COVID-19 prevention and control measures in their organizations [90]. In April 13, approximately 40,000 tests have been reached

per day with a total of 73 authorized laboratories, and the number of daily tests is step by step increasing.

(5. TREATMENT:This section is totally outdated and should be deleted

5.1 Antimalarial Drugs against SARS-CoV-2

Hydroxy Chloroquine:

Chloroquine is a phosphate and sulphate derivative drug it can be medically used the drug administered as antimalarials, and hydroxychloroquine is an immunomodulatory agent in systemic lupus erythematosus. Chloroquine present antiviral activity against Influenza, seasonal CoVs, Chikungunya virus and SARS [91-94]. Chloroquine derivatives against SARS-CoV2 was identified in vitro early on [95].The drug was quickly introduced into clinical use, and preliminary reports suggested enhanced viral clearance and clinical result in COVID-19 patients receiving a 10 days course of Hydroxychloroquine [96]. And randomizing 36 patients with COVID-19 suggested accelerated viral clearance in patients treated with a combination of hydroxychloroquine and azithromycin [97]. However, others have challenged outcome and found no pros in either disease results or viral clearance [98]. Disappointingly, the largest retrospective study to date assessing Hydroxychloroquine on its own or in combination with azithromycin found no pros, but indeed an enhance mortality risk among patients receiving hydroxychloroquine [99]. A study exploring chloroquine diphosphate in two dosing regimens was forced to terminate early for concerns over in fact mortality in the high dose arm. The authors conclude that treatment with high dose chloroquine for 10 days is not sufficiently safe and should no longer used in severe SARS-CoV2 patients [100].

Azithromycin produced synergistic effects while azithromycin and hydroxychloroquine against SARS-CoV2 have been observed in vitro, which seem to translate into clinical practice [97,101-102].

Interestingly, azithromycin is a weak base, and it’s accumulates in endosomes, with an alkalinizing effect at least parallel to Hydroxychloroquine. Inclusion to its antimicrobial properties, azithromycin is sometimes used for its immunomodulatory properties, mainly in patients with chronic pulmonary disorders. Azithromycin polarizes macrophages towards an anti-inflammatory M2 phenotype, and prevent pro-inflammatory STAT1 and NFκB signaling pathways [103,104]. In the context of anti-inflammatory effects, in particular interest that azithromycin is used for patients requiring intensive care for nonCOVID-19 related ARDS and is associated with a significant reduction in mortality and low time to extubation [105–107].

5.2 Antiviral Drugs against SARS-CoV-2

The antiviral drugs are especially used in case of HIV/AIDS, such as Lopinavir and Ritonavir. Other drugs and nucleoside analogs such as Favipiravir, Ribavirin, Remdesivir, and Galidesivir have been tested for present activity in the prevention of viral RNA synthesis [108]. Among these drugs, Lopinavir, Ritonavir, and Remdesivir are listed in the Solidarity trial by the World Health Organization (WHO).

(5.3 Plasma from recuperating patients:

Recuperating plasma, the plasma since individuals following COVID19 resolution and affluent in immunoglobulins directed against SARSCoV2, is being entertained as believable treatment option [109-110]. Unscientific use in SARS, MERS, Ebola and Influenza patients supports its use of neutralizing or immunomodulatory agent [111-112].But, a larger randomized controlled estimate of hyperimmune intravenous immunoglobulin use for severe influenza [113-114] and Ebola [115] manifest this intervention without superior to placebo. Similarly, rigorously estimated data used in coronaviral infections is lacking but not only for its use in SARS-CoV2 [116], and a possible study exploring use in MERS found that in numerous survivors, antibody titres were not high enough, so further restrict the donor pool [117]. Different dosing, issues surrounding donor recruitment in times of quickly increasing patient numbers, and drawbacks concerning safety of widespread use of human blood products all limit availability and its benefit widely available treatment option. Eventually, the viruses that are subject to ADE (such as SEARS-CoV2) by non-neutralizing antibodies, the choice of plasma therapy also holds significant risks. This complication has lately exemplified by anti-Zika virus antibodies increasing Dengue virus infection [118].so, the administration of hyperimmune/convalescent plasma may carry the threat of significant sickness upon future exposure to related or yet-to-emerge coronaviruses.)

## 5. CONCLUSION

In this review, we conclude that the disease description of COVID-19 is dynamic and continues to quickly evolve. (As more and more suspected cases of COVID 19 infection arises, crisis prospect of RT-PCR kits may also be enhanced.)(This has led to)Chest CT has been utilized to aid diagnosis in the absence or doubt of RT-PCR, (as demonstrated in a recent case reported since China and all over world.) The development of the lung changes of COVID-19 on CT imaging is also similar to SARS, with the ground-glass and consolidation fare worse or better over several days. This would be expected, as the 2 infectious agents are part of the coronavirus family. We are only beginning to understand host factors, such as various expression of cell surface proteins that may determine infection risk, disease presentation and results. Unveiling tissue and stage-specific factors contributing to pathology will result in new, effective and disease-specific(stage particular) therapeutic approaches thatwill control virus replication while restricting inflammatory damage.(until vaccinations become available.)

REFERENCES:


1. Z. Song, Y. Xu, L. Bao, L. Zhang, P. Yu, Y. Qu, et al., From SARS to MERS, thrusting coronaviruses into the spotlight, Viruses 11 (1) (2019).
2. C. Drosten, S. Gunther, W. Preiser, S. van der Werf, H.R. Brodt, S. Becker, et al., Identification of a novel coronavirus in patients with severe acute respiratory syndrome, N. Engl. J. Med. 348 (20) (2003) 1967–1976.

3. A.M. Zaki, S. van Boheemen, T.M. Bestebroer, A.D. Osterhaus, R.A. Fouchier, Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia, N. Engl. J. Med. 367 (19) (2012) 1814–1820.
4. F. Wu, S. Zhao, B. Yu, Y.M. Chen, W. Wang, Z.G. Song, et al., A new coronavirus associated with human respiratory disease in China, Nature. 579 (7798) (2020) 265–269.
5. Che, X. Y., B. Di, G. P. Zhao, Y. D. Wang, L. W. Qiu, W. Hao, M. Wang, P. Z. Qin, Y. F. Liu, K. H. Chan, V. C. Cheng, and K. Y. Yuen. 2006. A patient with asymptomatic severe acute respiratory syndrome (SARS) and antigenemia from the 2003–2004 community outbreak of SARS in Guangzhou, China. Clin. Infect. Dis. 43:e1–e5.
6. Lau, S. K., P. C. Woo, K. S. Li, Y. Huang, H. W. Tsoi, B. H. Wong, S. S. Wong, S. Y. Leung, K. H. Chan, and K. Y. Yuen. 2005. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc. Natl. Acad. Sci. USA 102:14040–14045.
7. Li, W., Z. Shi, M. Yu, W. Ren, C. Smith, J. H. Epstein, H. Wang, G. Crameri, Z. Hu, H. Zhang, J. Zhang, J. McEachern, H. Field, P. Daszak, B. T. Eaton, S. Zhang, and L. F. Wang. 2005. Bats are natural reservoirs of SARS-like coronaviruses. Science 310:676–679.
8. Wang, M., M. Yan, H. Xu, W. Liang, B. Kan, B. Zheng, H. Chen, H. Zheng, Y. Xu, E. Zhang, H. Wang, J. Ye, G. Li, M. Li, Z. Cui, Y. F. Liu, R. T. Guo, X. N. Liu, L. H. Zhan, D. H. Zhou, A. Zhao, R. Hai, D. Yu, Y. Guan, and J. Xu. 2005. SARS-CoV infection in a restaurant from palm civet. Emerg. Infect. Dis. 11:1860–1865.
9. WHO Q&A on coronaviruses (COVID-19) March 2020, https://www.who.int/news-room/q-a-detail/q-a-coronaviruses.
10. Center for disease controle and prevention, coronavirus disease 2019 (COVID-19). https://www.cdc.gov/ coronavirus/2019-ncov/symptoms-testing/symptoms.html.
11. Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health. 2020; 25(3):278–280. doi:10.1111/tmi.13383 https:// pubmed.ncbi.nlm.nih.gov/32052514/. [CrossRef]
12. Carter LJ, Garner L V, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay techniques and test development for COVID-19 diagnosis. ACS Cent Sci. (2020) 6:591–605. doi: 10.1021/acscentsci.0c00501
13. Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, et al. Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2: a narrative review. Ann Inter Med. (2020) 172:726–34. doi: 10.7326/M20-1301

14. Vashist SK. In vitro diagnostic assays for COVID-19: recent advances and emerging trends. Diagnostics. (2020) 10:202. doi: 10.3390/diagnostics10040202.
15. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. (2020) 382:1708–20. Doi: 10.1056/NEJMoa2002032.
16. 18. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Inter Med. (2020). 172:577–82. Doi: 10.7326/M20-0504.
17. 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. N Engl J Med. (2020) 382:1199–207. Doi: 10.1056/NEJMoa2001316.
18. Alsuliman T, Sulaiman R, Ismail S, Srour M, Alrstom A. COVID-19 paraclinical diagnostic tools: updates and future trends. Curr Res Transl Med. (2020) 68:83–91. Doi: 10.1016/j.retram.2020.06.001.
19. Weissleder R, Lee H, KO J, Pittet MJ. COVID-19 diagnostics in context. Sci Transl Med. (2020) 12:eabc1931. Doi: 10.1126/scitranslmed.abc1931.
20. Cinatl, J., B. Morgenstern, G. Bauer, P. Chandra, H. Rabenau, and H. W. Doerr. 2003. Treatment of SARS with human interferons. Lancet 362:293– 294.
21. Hensley, L. E., L. E. Fritz, P. B. Jahrling, C. L. Karp, J. W. Huggins, and T. W. Geisbert. 2004. Interferon-beta 1a and SARS coronavirus replication. Emerg. Infect. Dis. 10:317–319.
22. Tan, E. L., E. E. Ooi, C. Y. Lin, H. C. Tan, A. E. Ling, B. Lim, and L. W. Stanton. 2004. Inhibition of SARS coronavirus infection in vitro with clinically approved antiviral drugs. Emerg. Infect. Dis. 10:581–586.
23. Murphy A, and Bell D.J, et al. COVID-19, Radiopedia. https:// radiopaedia.org/articles/covid-19-2?lang=us.
24. Drosten, C., S. Gunther, W. Preiser, S. van der Werf, H. R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R. A. Fouchier, A. Berger, A. M. Burguiere, J. Cinatl, M. Eickmann, N. Escriou, K. Grywna, S. Kramme, J. C. Manuguerra, S. Muller, V. Rickerts, M. Sturmer, S. Vieth, H. D. Klenk, A. D. Osterhaus, H. Schmitz, and H. W. Doerr. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. 348:1967–1976.
25. Guan, Y., B. J. Zheng, Y. Q. He, X. L. Liu, Z. X. Zhuang, C. L. Cheung, S. W. Luo, P. H. Li, L. J. Zhang, Y. J. Guan, K. M. Butt, K.

L. Wong, K. W. Chan, W. Lim, K. F. Shortridge, K. Y. Yuen, J. S. Peiris, and L. L. Poon. 2003. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302:276–278.

26. Hon, K. L., C. W. Leung, W. T. Cheng, P. K. Chan, W. C. Chu, Y. W. Kwan, A. M. Li, N. C. Fong, P. C. Ng, M. C. Chiu, C. K. Li, J. S. Tam, and T. F. Fok. 2003. Clinical presentations and outcome of severe acute respiratory syndrome in children. Lancet 361:1701–1703.
27. Ksiazek, T. G., D. Erdman, C. S. Goldsmith, S. R. Zaki, T. Peret, S. Emery, S. Tong, C. Urbani, J. A. Comer, W. Lim, P. E. Rollin, S. F. Dowell, A. E. Ling, C. D. Humphrey, W. J. Shieh, J. Guarner, C. D. Paddock, P. Rota, B. Fields, J. DeRisi, J. Y. Yang, N. Cox, J. M. Hughes, J. W. LeDuc, W. J. Bellini, and L. J. Anderson. 2003. A novel coronavirus associated with severe acute respiratory syndrome. N. Engl. J. Med. 348:1953–1966.
28. Kuiken, T., R. A. Fouchier, M. Schutten, G. F. Rimmelzwaan, G. van Amerongen, D. van Riel, J. D. Laman, T. de Jong, G. van Doornum, W. Lim, A. E. Ling, P. K. Chan, J. S. Tam, M. C. Zambon, R. Gopal, C. Drosten, S. van der Werf, N. Escriou, J. C. Manuguerra, K. Stohr, J. S. Peiris, and A. D. Osterhaus. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 362:263–270.
29. Lee, N., D. Hui, A. Wu, P. Chan, P. Cameron, G. M. Joynt, A. Ahuja, M. Y. Yung, C. B. Leung, K. F. To, S. F. Lui, C. C. Szeto, S. Chung, and J. J. Sung. 2003. A major outbreak of severe acute respiratory syndrome in Hong Kong. N. Engl. J. Med. 348:1986–1994.
30. Marra, M. A., S. J. Jones, C. R. Astell, R. A. Holt, A. Brooks-Wilson, Y. S. Butterfield, J. Khattra, J. K. Asano, S. A. Barber, S. Y. Chan, A. Cloutier, S. M. Coughlin, D. Freeman, N. Girn, O. L. Griffith, S. R. Leach, M. Mayo, H. McDonald, S. B. Montgomery, P. K. Pandoh, A. S. Petrescu, A. G. Robertson, J. E. Schein, A. Siddiqui, D. E. Smailus, J. M. Stott, G. S. Yang, F. Plummer, A. Andonov, H. Artsob, N. Bastien, K. Bernard, T. F. Booth, D. Bowness, M. Czub, M. Drebot, L. Fernando, R. Flick, M. Garbutt, M. Gray, A. Grolla, S. Jones, H. Feldmann, A. Meyers, A. Kabani, Y. Li, S. Normand, U. Stroher, G. A. Tipples, S. Tyler, R. Vogrig, D. Ward, B. Watson, R. C. Brunham, M. Krajden, M. Petric, D. M. Skowronski, C. Upton, and R. L. Roper. 2003. The genome sequence of the SARS-associated coronavirus. Science 300:1399–1404.
31. Nicholls, J. M., L. L. Poon, K. C. Lee, W. F. Ng, S. T. Lai, C. Y. Leung, C. M. Chu, P. K. Hui, K. L. Mak, W. Lim, K. W. Yan, K. H.

Chan, N. C. Tsang, Y. Guan, K. Y. Yuen, and J. S. Peiris. 2003. Lung pathology of fatal severe acute respiratory syndrome. Lancet 361:1773–1778.

32. Peiris, J. S., S. T. Lai, L. L. Poon, Y. Guan, L. Y. Yam, W. Lim, J. Nicholls, W. K. Yee, W. W. Yan, M. T. Cheung, V. C. Cheng, K. H. Chan, D. N. Tsang, R. W. Yung, T. K. Ng, and K. Y. Yuen. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361:1319–1325.
33. Peiris, J. S., K. Y. Yuen, A. D. Osterhaus, and K. Stohr. 2003. The severe acute respiratory syndrome. N. Engl. J. Med. 349:2431–2441.
34. Poutanen, S. M., D. E. Low, B. Henry, S. Finkelstein, D. Rose, K. Green, R. Tellier, R. Draker, D. Adachi, M. Ayers, A. K. Chan, D. M. Skowronski, I. Salit, A. E. Simor, A. S. Slutsky, P. W. Doyle, M. Krajden, M. Petric, R. C. Brunham, and A. J. McGeer. 2003. Identification of severe acute respiratory syndrome in Canada. N. Engl. J. Med. 348:1995–2005.
35. Rota, P. A., M. S. Oberste, S. S. Monroe, W. A. Nix, R. Campagnoli, J. P. Icenogle, S. Penaranda, B. Bankamp, K. Maher, M. H. Chen, S. Tong, A. Tamin, L. Lowe, M. Frace, J. L. DeRisi, Q. Chen, D. Wang, D. D. Erdman, T. C. Peret, C. Burns, T. G. Ksiazek, P. E. Rollin, A. Sanchez, S. Liffick, B. Holloway, J. Limor, K. McCaustland, M. Olsen-Rasmussen, R. Fouchier, S. Gunther, A. D. Osterhaus, C. Drosten, M. A. Pallansch, L. J. Anderson, and W. J. Bellini. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300:1394–1399.
36. Ruan, Y. J., C. L. Wei, A. L. Ee, V. B. Vega, H. Thoreau, S. T. Su, J. M. Chia, P. Ng, K. P. Chiu, L. Lim, T. Zhang, C. K. Peng, E. O. Lin, N. M. Lee, S. L. Yee, L. F. Ng, R. E. Chee, L. W. Stanton, P. M. Long, and E. T. Liu. 2003. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 361:1779–1785.
37. So, L. K., A. C. Lau, L. Y. Yam, T. M. Cheung, E. Poon, R. W. Yung, and K. Y. Yuen. 2003. Development of a standard treatment protocol for severe acute respiratory syndrome. Lancet 361:1615–1617.
38. Tsang, K. W., P. L. Ho, G. C. Ooi, W. K. Yee, T. Wang, M. Chan-Yeung, W. K. Lam, W. H. Seto, L. Y. Yam, T. M. Cheung, P. C. Wong, B. Lam, M. S. Ip, J. Chan, K. Y. Yuen, and K. N. Lai. 2003. A cluster of cases of severe acute respiratory syndrome in Hong Kong. N. Engl. J. Med. 348:1977–1985.
39. World Health Organization. 2003. WHO issues consensus document on the epidemiology of SARS. Wkly. Epidemiol. Rec. 78:373–375.

40. Zhong, N. S., B. J. Zheng, Y. M. Li, Poon, Z. H. Xie, K. H. Chan, P. H. Li, S. Y. Tan, Q. Chang, J. P. Xie, X. Q. Liu, J. Xu, D. X. Li, K. Y. Yuen, J. S. Peiris, and Y. Guan. 2003. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. Lancet 362:1353–1358.
41. Babcock, G. J., D. J. Esshaki, W. D. Thomas, Jr., and D. M. Ambrosino. 2004. Amino acids 270 to 510 of the severe acute respiratory syndrome coronavirus spike protein are required for interaction with receptor. J. Virol. 78:4552–4560.
42. Wong, S. K., W. Li, M. J. Moore, H. Choe, and M. Farzan. 2004. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J. Biol. Chem. 279:3197–3201.
43. Du, L., R. Y. Kao, Y. Zhou, Y. He, G. Zhao, C. Wong, S. Jiang, K. Y. Yuen, D. Y. Jin, and B. J. Zheng. 2007. Cleavage of spike protein of SARS coronavirus by protease factor Xa is associated with viral infectivity. Biochem. Biophys. Res. Commun. 359:174–179.
44. Hamming, I., W. Timens, M. L. Bulthuis, A. T. Lely, G. J. Navis, and H. van Goor. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J. Pathol. 203:631–637.
45. Kuba, K., Y. Imai, S. Rao, H. Gao, F. Guo, B. Guan, Y. Huan, P. Yang, Y. Zhang, W. Deng, L. Bao, B. Zhang, G. Liu, Z. Wang, M. Chappell, Y. Liu, D. Zheng, A. Leibbrandt, T. Wada, A. S. Slutsky, D. Liu, C. Qin, C. Jiang, and J. M. Penninger. 2005. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat. Med. 11:875–879.
46. Chan, V. S., K. Y. Chan, Y. Chen, L. L. Poon, A. N. Cheung, B. Zheng, K. H. Chan, W. Mak, H. Y. Ngan, X. Xu, G. Screaton, P. K. Tam, J. M. Austyn, L. C. Chan, S. P. Yip, M. Peiris, U. S. Khoo, and C. L. Lin. 2006. Homozygous L-SIGN (CLEC4M) plays a protective role in SARS coronavirus infection. Nat. Genet. 38:38–46.
47. Gramberg, T., H. Hofmann, P. Moller, P. F. Lalor, A. Marzi, M. Geier, M. Krumbiegel, T. Winkler, F. Kirchhoff, D. H. Adams, S. Becker, J. Munch, and S. Pohlmann. 2005. LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. Virology 340:224–236.
48. Jeffers, S. A., S. M. Tusell, L. Gillim-Ross, E. M. Hemmila, J. E. Achenbach, G. J. Babcock, W. D. Thomas, Jr., L. B. Thackray, M. D. Young, R. J. Mason, D. M. Ambrosino, D. E. Wentworth, J. C. Demartini, and K. V. Holmes. 2004. CD209L (L-SIGN) is a receptor for severe acute respiratory syndrome coronavirus. Proc. Natl. Acad. Sci. USA 101:15748–15753.

49. Yang, Z. Y., Y. Huang, L. Ganesh, K. Leung, W. P. Kong, O. Schwartz, K. Subbarao, and G. J. Nabel. 2004. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J. Virol. 78:5642– 5650.
50. Huang, I. C., B. J. Bosch, F. Li, W. Li, K. H. Lee, S. Ghiran, N. Vasilieva, T. S. Dermody, S. C. Harrison, P. R. Dormitzer, M. Farzan, P. J. Rottier, and H. Choe. 2006. SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. J. Biol. Chem. 281: 3198–3203.
51. Simmons, G., D. N. Gosalia, A. J. Rennekamp, J. D. Reeves, S. L. Diamond, and P. Bates. 2005. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. Proc. Natl. Acad. Sci. USA 102:11876– 11881.
52. Keyaerts, E., L. Vijgen, P. Maes, J. Neyts, and M. Van Ranst. 2004. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. Biochem. Biophys. Res. Commun. 323:264–268.
53. Vincent, M. J., E. Bergeron, S. Benjannet, B. R. Erickson, P. E. Rollin, T. G. Ksiazek, N. G. Seidah, and S. T. Nichol. 2005. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol. J. 2:69.
54. Ito, N., E. C. Mossel, K. Narayanan, V. L. Popov, C. Huang, T. Inoue, C. J. Peters, and S. Makino. 2005. Severe acute respiratory syndrome coronavirus 3a protein is a viral structural protein. J. Virol. 79:3182–3186.
55. Hiscox, J. A., D. Cavanagh, and P. Britton. 1995. Quantification of individual subgenomic mRNA species during replication of the coronavirus transmissible gastroenteritis virus. Virus Res. 36:119–130.
56. Alsuliman T, Sulaiman R, Ismail S, Srour M, Alrstom A. COVID-19 paraclinical diagnostic tools: updates and future trends. Curr Res Transl Med. (2020) 68:83–91. doi: 10.1016/j.retram.2020.06.001
57. Premraj A, Aleyas AG, Nautiyal B, Rasool TJ. Nucleic acid and immunological diagnostics for SARS-CoV-2: processes, platforms and pitfalls. Diagnostics. (2020) 10:866. doi: 10.20944/preprints202009.0526.v1
58. Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, Li VYC, et al. Diagnosing COVID-19: the disease and tools for detection. ACS Nano. (2020) 14:3822–35. doi: 10.1021/acsnano.0c02624
59. Pfefferle S, Reucher S, Nörz D, Lütgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV2 using a high throughput system. Euro

Surveill. (2020) 25:2000152. doi: 10.2807/1560-7917.ES.2020.25.9.2000152
60. Craney AR, Velu PD, Satlin MJ, Fauntleroy KA, Callan K, Robertson A, et al. Comparison of two high-throughput reverse transcription-PCR systems for the detection of severe acute respiratory syndrome coronavirus 2. J ClinMicrobiol. (2020) 58:e00890–20. doi: 10.1128/JCM.00890-20
61. U. S. Food and Drug Administration. In Vitro Diagnostics EUAs - Molecular Diagnostic Tests for SARS-CoV-2. (2021). Available online at: https://www. fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergencyuse-authorizations-medical-devices/in-vitro-diagnostics-euas-moleculardiagnostic-tests-sars-cov-2
62. Poljak M, Korva M, Knap Gašper N, FujsKomloš K, Sagadin M, Uršic T, ˇ et al. Clinical evaluation of the cobas SARS-CoV-2 test and a diagnostic platform switch during 48 hours in the midst of the COVID-19 pandemic. J ClinMicrobiol. (2020) 58:e00599–20. doi: 10.1128/JCM.00599-20
63. Alcoba-Florez J, González-Montelongo R, Íñigo-Campos A, de Artola DGM, Gil-Campesino H, The Microbiology Technical Support Team, et al. Fast SARS-CoV-2 detection by RT-qPCR in preheated nasopharyngeal swab samples. Int J Infect Dis. (2020) 97:66–8. doi: 10.1016/j.ijid.2020.05.099
64. da Silva SJR, Silva CTA da, Guarines KM, Mendes RPG, Pardee K, Kohl A, et al. Clinical and laboratory diagnosis of SARS-CoV2, the virus causing COVID-19. ACS Infect Dis. (2020) 6:2319–36. doi: 10.1021/acsinfecdis.0c00274
65. Fomsgaard AS, Rosenstierne MW. An alternative workflow for molecular detection of SARS-CoV-2 - escape from the NA extraction kit-shortage, Copenhagen, Denmark, March 2020. Eurosurveillance. (2020) 25:2000398. doi: 10.2807/1560-7917.ES.2020.25.14.2000398
66. Lübke N, Senff T, Scherger S, Hauka S, Andrée M, Adams O, et al. Extraction-free SARS-CoV-2 detection by rapid RT-qPCR universal for all primary respiratory materials. J ClinVirol. (2020) 132:104579. doi: 10.1016/j.jcv.2020.104579
67. Péré H, Podglajen I, Wack M, Flamarion E, Mirault T, Goudot G, et al. Nasal Swab sampling for SARS-CoV-2: a convenient alternative in times of nasopharyngeal swab shortage. J ClinMicrobiol. (2020) 58:e00721–20. doi: 10.1128/JCM.00721-20
68. McCulloch DJ, Kim AE, Wilcox NC, Logue JK, Greninger AL, Englund JA, et al. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for

detection of SARS-CoV-2 infection. JAMA Netw Open. (2020) 3:e2016382. doi: 10.1001/jamanetworkopen.2020.16382 .
69. To KKW, Tsang OTY, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV2: an observational cohort study. Lancet Infect Dis. (2020) 20:565–74. doi: 10.1016/S1473-3099(20)30196-1
70. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, et al. Saliva is a reliable tool to detect SARS-CoV-2. J Infect. (2020) 81:e45–50. doi: 10.1016/j.jinf.2020.04.005
71. Martinez RM. Clinical samples for SARS-CoV-2 detection: review of the early literature. ClinMicrobiolNewslett. (2020) 42:121–7. doi: 10.1016/j.clinmicnews.2020.07.001
72. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARSCoV-2 in different types of clinical specimens. JAMA. (2020) 323:1843–4. doi: 10.1001/jama.2020.3786
73. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe. (2021) 2:e13–22. doi: 10.1016/S2666-5247(20)30172-5
74. Khoury NC, Russi TJ. A case of gastrointestinal-predominant COVID19 demonstrates value of stool PCR test. J MedVirol. (2021) 93:662–3. doi: 10.1002/jmv.26448
75. Abdalhamid B, Bilder CR, Mccutchen EL, Hinrichs SH, Koepsell SA, Iwen PC. Assessment of specimen pooling to conserve SARS CoV-2 testing resources. Am J ClinPathol. (2020) 153:715–8. doi: 10.1101/2020.04.03.20050195
76. Matsumura Y, Shimizu T, Noguchi T, Nakano S, Yamamoto M, Nagao M. Comparison of 12 molecular detection assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J Mol Diagn. (2021) 23:164–70. doi: 10.1016/j.jmoldx.2020.11.007
77. Vogels CBF, Brito AF, Wyllie AL, Fauver JR, Ott IM, Kalinich CC, et al. Analytical sensitivity and efficiency comparisons of SARS-CoV2 RT–qPCR primer–probe sets. Nat Microbiol. (2020) 5:1299–305. doi: 10.1038/s41564-020-0761-6.
78. Nalla AK, Casto AM, Huang M-LW, Perchetti GA, Sampoleo R, Shrestha L, et al. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. J ClinMicrobiol. (2020) 58:e00557–20. doi: 10.1128/JCM.00557-20.
79. van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, et al. Comparison of seven commercial

RT-PCR diagnostic kits for COVID-19. J ClinVirol. (2020) 128:104412. doi: 10.1016/j.jcv.2020.104412.
80. Artesi M, Bontems S, Göbbels P, Franckh M, Maes P, Boreux R, et al. A recurrent mutation at position 26340 of SARS-CoV-2 is associated with failure of the E gene quantitative reverse transcription-PCR utilized in a commercial dual-target diagnostic assay. J ClinMicrobiol. (2020) 58:e01598– 20. doi: 10.1128/JCM.01598-20.
81. Galloway SE, Paul P, MacCannell DR, Johansson MA, Brooks JT, MacNeil A, et al. Emergence of SARS-CoV-2 b. 1.1. 7 lineage—United States, December 29, 2020–January 12, 2021. Morb Mortal Wkly Rep. (2021) 70:95. doi: 10.15585/mmwr.mm7003e2.
82. Premraj A, Aleyas AG, Nautiyal B, Rasool TJ. Nucleic acid and immunological diagnostics for SARS-CoV-2: processes, platforms and pitfalls. Diagnostics. (2020) 10:866. doi: 10.20944/preprints202009.0526.v1.
83. Afzal A. Molecular diagnostic technologies for COVID-19: limitations and challenges. J Adv Res. (2020) 26:149–59. doi: 10.1016/j.jare.2020. 08.002.
84. Bullard J, Dust K, Funk D, Strong JE, Garnett L, Boodman C, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis. (2020) 71:2663–6. doi: 10.1093/cid/ciaa638.
85. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. JAMA. (2020) 323:1239–42. doi: 10.1001/jama.20 20.2648
86. Smith RD. Responding to global infectious disease outbreaks: lessons from SARS on the role of risk perception, communication and management. Soc Sci Med. (2006) 63:3113–23. doi: 10.1016/j.socscimed.2006.08.004
87. Wood CS, Thomas MR, Budd J, Mashamba-Thompson TP, Herbst K, Pillay D, et al. Taking connected mobile-health diagnostics of infectious diseases to the field. Nature. (2019) 566:467–74. doi: 10.1038/s41586-019-0956-2
88. Karthik K, Aravindh Babu RP, Dhama K, Chitra MA, Kalaiselvi G, AlagesanSenthilkumar TM, et al. Biosafety Concerns during the collection, transportation, and processing of COVID-19 samples for diagnosis. Arch Med Res. (2020) 51:623–30. doi: 10.1016/j.arcmed.2020. 08.007
89. European Centers for Disease Control (ECDC) (2020). Using face masks in the community reducing COVID-19 transmission from potentially asymptomatic or presymptomatic people through the use

of face masks ECDC Technical Report [online]. Website https://www.ecdc.europa. eu/en/publications-data/using-face-masks-communityreducing-covid-19-transmission [accessed 8 April 2020].
90. T.C. SağlıkBakanlığı (2020). KurumlarİçinAlınanKararlar [online]. Website https://covid19bilgi.saglik.gov.tr/tr/alinankaralar.htm [accessed 13 April 2020].
91. A. Savarino, J.R. Boelaert, A. Cassone, G. Majori, R. Cauda, Effects of chloroquine on viral infections: an old drug against today's diseases? Lancet Infect. Dis. 3 (11) (2003) 722–727.
92. Y. Yan, Z. Zou, Y. Sun, X. Li, K.F. Xu, Y. Wei, et al., Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model, Cell Res. 23 (2) (2013) 300–302.
93. E. Keyaerts, L. Vijgen, P. Maes, J. Neyts, M. Van Ranst, In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine, Biochem. Biophys. Res. Commun. 323 (1) (2004) 264–268.
94. M.J. Vincent, E. Bergeron, S. Benjannet, B.R. Erickson, P.E. Rollin, T.G. Ksiazek, et al., Chloroquine is a potent inhibitor of SARS coronavirus infection and spread, Virol. J. 2 (2005) 69.
95. M. Wang, R. Cao, L. Zhang, X. Yang, J. Liu, M. Xu, et al., Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro, Cell Res. 30 (3) (2020) 269–271.
96. J. Gao, Z. Tian, X. Yang, Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies, Biosci Trends. 14 (1) (2020) 72–73.
97. P. Gautret, J.C. Lagier, P. Parola, V.T. Hoang, L. Meddeb, M. Mailhe, et al., Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial, Int. J. Antimicrob. Agents 105949 (2020).
98. J.M. Molina, C. Delaugerre, J. Le Goff, B. Mela-Lima, D. Ponscarme, L. Goldwirt, et al., No evidence of rapid antiviral clearance or clinical benefit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID19 infection, Med. Mal. Infect. (2020), https://doi.org/10.1016/j.medmal.2020. 03.006 pii: S0399-077X(20)30085-8. [Epub ahead of print].
99. J.N. Magagnoli, F. Pereira, T. Cummings, J.W. Hardin, S.S. Sutton, J. Ambati, Outcomes of Hydroxychloroquine usage in United States veterans hospitalized with COVID-19, medRciv Server (2020), https://doi.org/10.1101/2020.04.16. 20065920.

100. Silva Borba MGFAV, V. Sousa Sampaio, M. Almeida Araújo Alexandre, G. Cardoso Melo, M. Brito, M.P. Gomes Mourão, J.D. Brito-Sousa, D. Baía-da-Silva, Chloroquine diphosphate in two different dosages as adjunctive therapy of hospitalized patients with severe respiratory syndrome in the context of coronavirus (SARS-CoV-2) infection: Preliminary safety results of a randomized, doubleblinded, phase IIb clinical trial (CloroCovid-19 Study), medRxiv Preprint (2020), https://www.medrxiv.org/content/10.1101/2020.04.07.20056424v1.full.pdf.
101. P. Gautret, J.C. Lagier, P. Parola, V.T. Hoang, L. Meddeb, J. Sevestre, et al., Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: A pilot observational study, Travel Med. Infect. Dis. 101663 (2020).
102. J.L.B. Andreania, I. Duflota, P. Jardota, C. Rollanda, M. Boxberger, J.Y. BouKhalila, J.P. Baudouin, N. Wurtza, vitro testing of Hydroxychloroquine and Azithromycin on SARS-CoV-2 shows synergistic effect. Mediterranee Infection, https://www.mediterranee-infection.com/wp-content/uploads/2020/03/LaScola-et-al-V1.pdf, (2020).
103. D. Haydar, T.J. Cory, S.E. Birket, B.S. Murphy, K.R. Pennypacker, A.P. Sinai, et al., Azithromycin Polarizes Macrophages to an M2 Phenotype via Inhibition of the STAT1 and NF-kappaB Signaling Pathways, J. Immunol. 203 (4) (2019) 1021–1030.
104. J.C. Gensel, T.J. Kopper, B. Zhang, M.B. Orr, W.M. Bailey, Predictive screening of M1 and M2 macrophages reveals the immunomodulatory effectiveness of post spinal cord injury azithromycin treatment, Sci. Rep. 7 (2017) 40144.
105. A.J. Walkey, R.S. Wiener, Macrolide antibiotics and survival in patients with acute lung injury, Chest. 141 (5) (2012) 1153–1159.
106. K. Kawamura, K. Ichikado, M. Suga, M. Yoshioka, Efficacy of azithromycin for treatment of acute exacerbation of chronic fibrosing interstitial pneumonia: a prospective, open-label study with historical controls, Respiration. 87 (6) (2014) 478–484.
107. K. Kawamura, K. Ichikado, M. Takaki, Y. Eguchi, K. Anan, M. Suga, Adjunctive therapy with azithromycin for moderate and severe acute respiratory distress syndrome: a retrospective, propensity score-matching analysis of prospectively collected data at a single center, Int. J. Antimicrob. Agents 51 (6) (2018) 918–924.

108. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nat Rev Drug Discov. (2020) 19:149– 50. doi: 10.1038/d41573-020-00016-0.
109. E.M. Bloch, S. Shoham, A. Casadevall, B.S. Sachais, B. Shaz, J.L. Winters, et al., Deployment of convalescent plasma for the prevention and treatment of COVID19, J. Clin. Invest. (2020), https://doi.org/10.1172/JCI138745 pii: 138745. [Epub ahead of print].
110. T.C. Luke, A. Casadevall, S.J. Watowich, S.L. Hoffman, J.H. Beigel, T.H. Burgess, Hark back: passive immunotherapy for influenza and other serious infections, Crit. Care Med. 38 (4 Suppl) (2010) e66–e73.
111. J. Mair-Jenkins, M. Saavedra-Campos, J.K. Baillie, P. Cleary, F.M. Khaw, W.S. Lim, et al., The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis, J. Infect. Dis. 211 (1) (2015) 80–90.
112. A. Casadevall, L.A. Pirofski, The convalescent sera option for containing COVID19, J. Clin. Invest. 130 (4) (2020) 1545–1548.
113. I.F. Hung, To KK, C.K. Lee, K.L. Lee, K. Chan, W.W. Yan, et al., Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection, Clin. Infect. Dis. 52 (4) (2011) 447–456.
114. R.T. Davey Jr., E. Fernandez-Cruz, N. Markowitz, S. Pett, A.G. Babiker, D. Wentworth, et al., Anti-influenza hyperimmune intravenous immunoglobulin for adults with influenza A or B infection (FLU-IVIG): a double-blind, randomised, placebo-controlled trial, Lancet Respir. Med. 7 (11) (2019) 951–963.
115. J. van Griensven, T. Edwards, X. de Lamballerie, M.G. Semple, P. Gallian, S. Baize, et al., Evaluation of Convalescent Plasma for Ebola Virus Disease in Guinea, N. Engl. J. Med. 374 (1) (2016) 33–42.
116. J.H. Beigel, H.H. Nam, P.L. Adams, A. Krafft, W.L. Ince, S.S. El-Kamary, et al., Advances in respiratory virus therapeutics - A meeting report from the 6th isirv Antiviral Group conference, Antivir. Res. 167 (2019) 45–67.
117. Y.M. Arabi, A.H. Hajeer, T. Luke, K. Raviprakash, H. Balkhy, S. Johani, et al., Feasibility of Using Convalescent Plasma Immunotherapy for MERS-CoV Infection, Saudi Arabia. Emerg Infect Dis. 22 (9) (2016) 1554–1561.

118. A.B. Kawiecki, R.C. Christofferson, Zika Virus-Induced Antibody Response Enhances Dengue Virus Serotype 2 Replication In Vitro, J. Infect. Dis. 214 (9) (2016) 1357–1360.