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SARS-CoV-2 and Coronavirus Ancestors under a Molecular Scope

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Abstract

The Pandemic of COVID-19 has been thoroughly followed and discussed on many levels due to the high level of attention that it has brought by its effect on the world. While this disease might seem like to arise out of the blue, we will shed light on COVID-19 disease which is caused by the virus SARS-CoV2 and belong to family of coronaviruses. We will discuss current knowledge about SARS-CoV2 emergence, diagnosis, its mode of action, and genomic information, For an antiviral treatment to be used, it should be preceded by a foundation of information about the virus genome and its family as discussed in this review.

Keywords: Covid-19, SARS-COV-2, genome, evolution, immunopathology, phylogenetic

1. Introduction

The Coronaviridae have a wide variety of host species, which infect many mammalian and avian species and result in high respiratory, hepatic, and central nervous system diseases. Coronaviruses in humans and fowl mainly cause infections in the upper respiratory tract and enteric infections are caused by pig and bovine Coronavirus [1]. Coronaviruses CoVs are divided into four genera and in 1937 the first coronavirus was identified [2, 3]. Coronaviruses are a family of helical nucleocapsid and extremely large genomes enveloped positive-stranded RNA viruses. Coronaviruses are composed of: 1) Nucleocapsid Protein (N): helical nucleocapsid protein component and is supposed to bind genomic RNA in a bead-on-string mode. 2) spike protein (S): Viral envelope component that mediates binding to the receptor and merging of cell membranes if the virus and host. 3) Membrane Protein (M): the most present component and gives its form to the virion envelope. 4) Envelope Protein (E): A small, only minor component of virions and a small polypeptide between 8.4 and 12 kDa (76–109 amino acids). 5) Accessory Proteins: “Extra” genes may be interspersed with a group of canonical genes, replicase, S, E, M, and N with additional ORFs, or embedded in a separate ORF or heavily overlapped with another gene [4]. (**Figure 1**) Coronaviruses are also one of the few genomically proof-reading RNA viruses that avoid the virus developing mutations that could weaken it. Such capacity may have contributed to the failure of specific antivirals like ribavirin to subdue SARS-CoV-2 meanwhile, can thwart viruses like hepatitis C. Drugs kill viruses by mutations. However, the proofreader can eliminate these changes in coronaviruses. Coronaviruses have a special trick that is fatal: they often recombine,

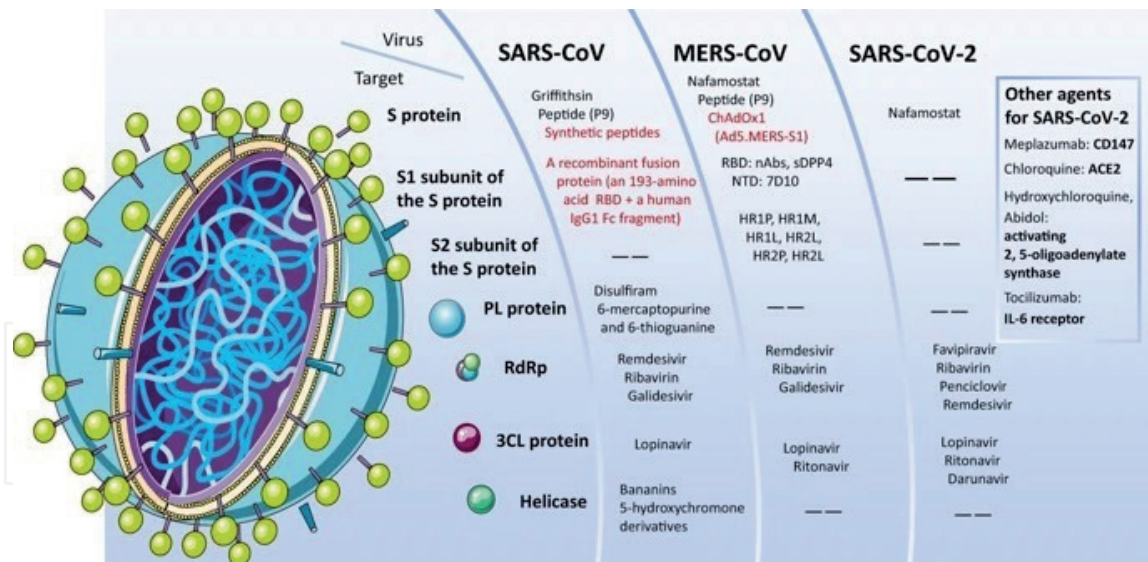


Figure 1. Viral structure diagram showing the envelope, Centre and structure of the nucleoprotein. S, the spike protein and different drug candidates against the three coronaviruses [5].

exchange pieces of RNA and other coronaviruses. This is usually an insignificant trade between viruses like parts [6]. Both mammals are affected by alphacoronaviruses and beta-coronaviruses. Alpha-Coronaviruses and beta-Coronaviruses typically cause human breathing diseases and animal gastroenteritis. Gamma and delta coronaviruses infect birds, but some can infect mammals as well. The SARS-CoV, MERS-CoV viruses, and the other four human coronaviruses (HCoV-NL-63, HCoV-229E, HCoV-OC43 and HKU1) are responsible for severe respiratory syndromes in people with mild conditions in immunocompetent hosts, although some infections are severe in infants and elderly people [7]. Coronavirus transcription is characterized by the development of several mRNAs containing the sequences corresponding to the two ends of the genome. The production of subgenomic mRNA requires discontinuous transcripts. Transcription is known as the process by which subgenomic mRNAs are generated, and replications are the process by which genomic-sized RNA, which also acts as mRNA, is generated [8]. Human coronaviruses (HCoV) were first detected and developed in the nasal cavities of common cold patients in the 1960s. Two human coronaviruses-OC43 and 229E are responsible for about 30% of common colds [9, 10]. Middle East Coronavirus Respiratory Syndrome (MERS-CoV) has also been a global health concern. The initial report for MERS-CoV was in 2012. More than 2000 civilians have been infected in 27 countries in the Middle East and 4 subcontinents. During the SARS outbreak in 26 countries, more than 8000 cases were recorded in 2003 [11]. The ongoing coronavirus disease outbreak (COVID-19), first reported in December 2019 in Wuhan, China. As of 5th of April 2020, the world health organization (WHO) announced this disease as a global public health emergency to extend to 206 countries and territories across the world, with two international correspondence performed on 3,090,445 confirmed cases reported cases, including 217,769 deaths [12]. SARS-CoV-2 virus, the cause of COVID-19 disease that lead to an emergency outbreak that has been going for several months, now it may as well continue to its spread until the finding of new treatments along with the implementation of effective countermeasures. The newly evolving coronavirus (SARS-CoV 2) is becoming increasingly largescale. In the last few weeks, complete genomic sequences were released in order to understand the development and molecular characteristics of the virus by the global scientific community. In this review we will discuss the genomic structure of the virus, the

possible relations between several viruses of the same family and the suspected origins and spill over that might have led to such epidemic and molecular diagnostics used to detect.

2. Emergence of a new virus

The life of people over the centuries has been influenced by Zoonotic diseases. Many of these situations are especially variable in complexity, dynamics and shifts over time, as they emerge, and reappear. Transmission of the pathogen from an animal to human, also known as zoonotic spillovers, is a global public health issue and remains an ambiguous phenomenon, while associated with multiple outbreaks [13]. A mixture of many factors is needed to fulfill a zoonotic spillover, including ecological, epidemiological and behavioral determinants of pathogen transmission and inherent human factors influencing susceptible infection, as well as dietary and societal factors linked with foodborne zoonotic spillover [14]. A new virus is a virus that mutated and went through an evolution process to adapt to new kinds of hosts by a process called spillover. Spillover can happen in wild animals' market as a virus can mutate and go on infecting a new host where it further mutates within new host until it adapts to this new host and become infectious [15]. Over the past two decades, many outbreaks of Zoonotic diseases such as SARS, the Hendra virus and the Nipah virus have been related to the bat-borne viruses. The most definitive proof was included from the separation of the CoV from bats in China, there was over 98% similarity in the genome sequence to SARS-CoV, and can use SARS-CoV-receptor ACE2 on cells of the human race. It is hard to evaluate the possibility for spillover of several similar SARS-CoV Bat CoVs as a result of infringing isolation of viruses, but it should be noted that a "consensus" virus developed through reverse genetics has high evidence of human infection it is clear that bats are the most likely original cause of the current 2019 CoV outbreak in Wuhan, China, which started in December 2019, continuing to spread to many city and province areas in China from a "wet market." The probability of food transmission of derived animal products was also suggested, as it has recently been pointed out to affect the present epidemic as well as the chance of common near contact with animals (a not unusual scenario in these types of markets). Their possible adaptations may lead to new and stable reservoirs, such as human hosts. Those are ideas and problems arisen from the emerged SARS-CoV2, that immediately compares SARS-CoV and MERS-CoV with other beta-coronaviruses with similar natural, intermediate animal hosts with also the possibility of human-to-human transmission in comparison [13, 16].

3. Genomic characteristics

During infection, the genome has many roles. It first functions as mRNA that is translated into a huge polyprotein called replicase that involves a ribosomal frame-shifting event for complete synthesis. The replicase is the only genome-derived translation product; all downstream ORFs are expressed by Subgenomic RNAs. Next, the genome is the replication and transcription template. Finally, the genome is involved in assembly, as progeny genomes are found in progeny viruses [4]. The genomic RNA for coronavirus of about 30 000 nucleotides encodes structural virus proteins, non-structural proteins with a key part in viral RNA synthesis (which is understood to be replicase transcriptase proteins) and non-structural proteins that are not necessary for viral replication in cell culture but which in vivo tend

to be a selective advantage (which is referred to *in vivo*) [8]. Cis-acting sequence and structural elements involved in the replication, transcription, translation, and packaging are incorporated within RNA virus genomes. Some of these signals are intended to enable the interaction of selective viral RNAs with RNA synthesis machines while some allow or modify events that happen meanwhile the synthesis or assembly of viral protein [17, 18]. Coronaviruses contain the hugest genomes of any RNA virus, and this has hindered the production of full-length coronavirus cDNAs along with the discovery that certain cDNAs originating in the replicase areas of genes are unstable in bacteria. However, the assembly of long-lasting cDNAs in porcine coronavirus transmissible gastroenteritis viral genomic RNA (TGEV) has reportedly been identified with two methods. First, a TGEV full-length cDNA was installed on a bacterial artificial chromosome (BAC). Second, the TGEV total cDNA was installed *in-vitro* using a series of adjacent cDNAs within it engineered unique restriction sites, cDNA of the RNA transcripts derived from bacteriophage T7-RNA polymerase have been then used for infectious virus production [19]. SARS-CoV-2 (**Figure 2**) has a long genome with ORF1ab polyprotein, along with four main structural proteins, involving Spike surface glycoprotein, small envelope protein, matrix protein and nucleocapsid protein, which is also the case in other beta-coronaviruses (**Figure 3; Table 1**). In the ORF1ab polyprotein there were two deletions (three nucleotides and 24 nucleotides) and also one at the 3' end of the genome (ten nucleotides) [21] (**Figure 4**).

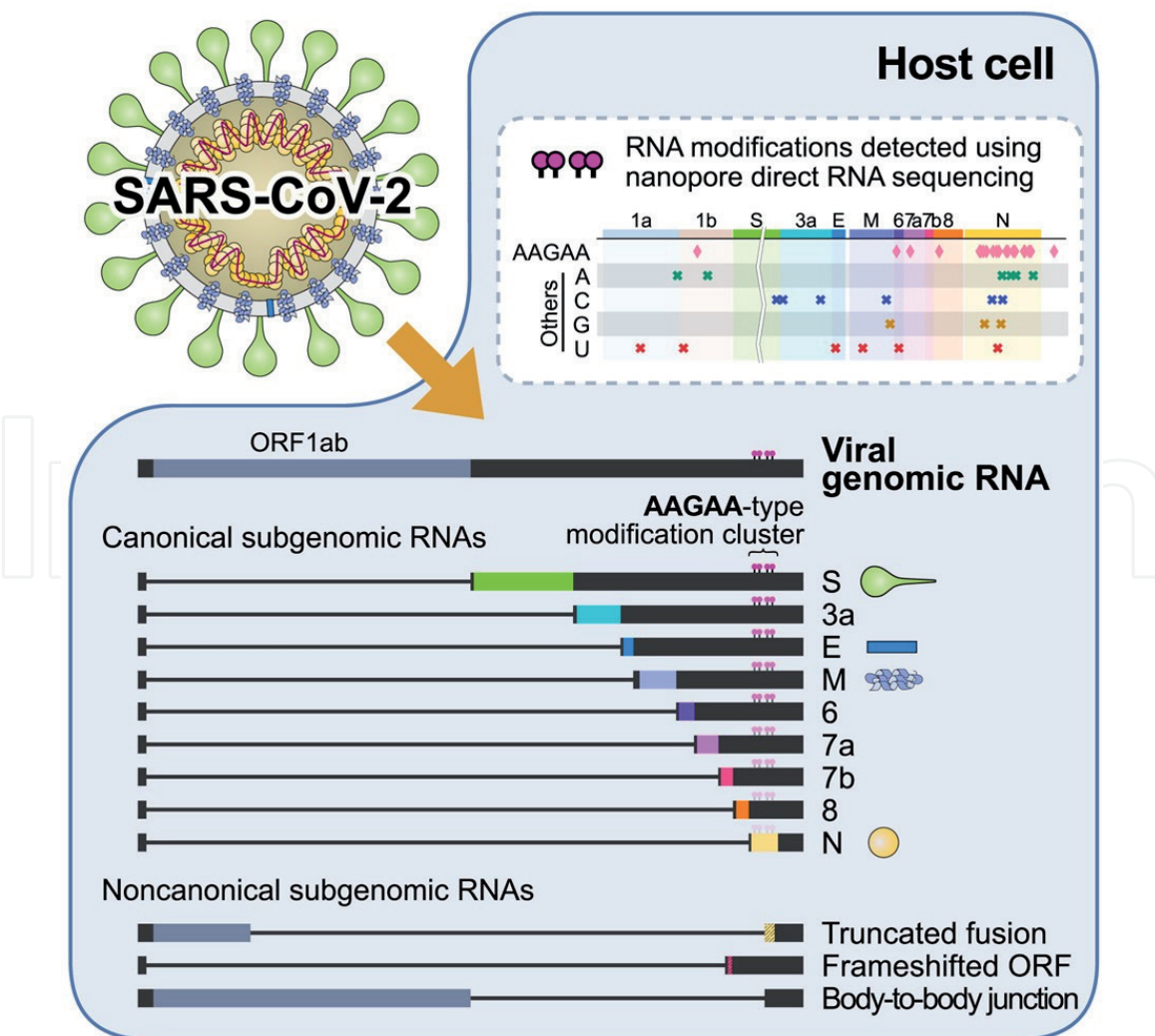


Figure 2.
The structure of SARS-CoV-2 transcriptome [20].

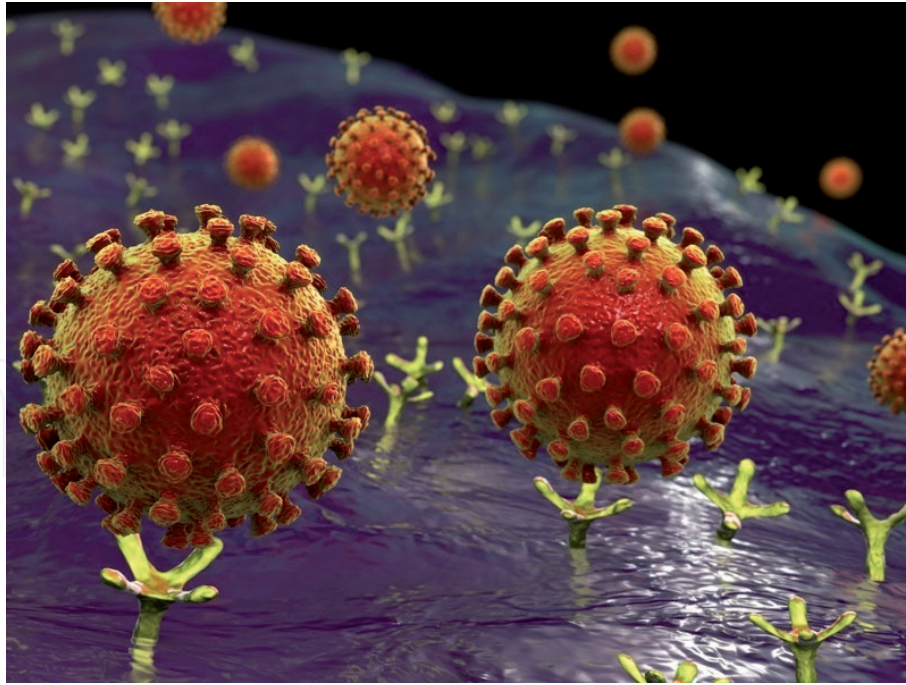


Figure 3.
SARS-CoV-2 binding by its spike protein to ACE2 receptor [12].

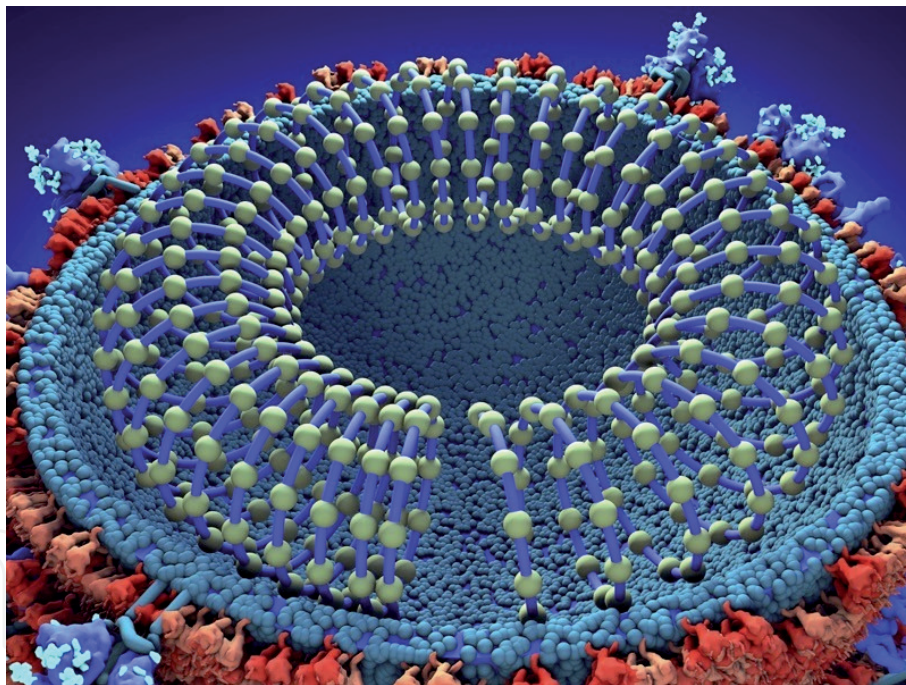


Figure 4.
SARS-CoV-2 inner proteins illustration [12].

3.1 Transfection

The unusual variations in host diversity and tissue tropism between coronaviruses are primarily due to differences in the spike glycoprotein. The S protein is a broad, glycoprotein type I membrane containing disruptive functional fields near the amino (S1) and carboxy (S2) Termini. Via their receptor specificity and probably by their membrane fusion activities in the cell entry of viral tropism, these spikes can be identified [1]. ACE2 is a primary determinant for the SARS-CoV Host range [23, 24]. The life cycle of COVID-19 starts with the binding of its Angiotensin Converting Enzyme (ACE2) receptor expressed in various

| | |
|--|-----------|
| <i>Group 1</i> | |
| Human coronavirus 229E | HCoV-229E |
| Porcine enteric (transmissible gastroenteritis virus, TGEV; and porcine epidemic diarrhea virus, PEDV) and respiratory (PRCoV) coronavirus | PCoV |
| Canine coronavirus | CCoV |
| Feline coronavirus, including feline infectious peritonitis virus (FIPV) | FCoV |
| <i>Group 2</i> | |
| Human coronavirus OC43 | HCoV-OC43 |
| Bovine coronavirus | BCoV |
| Turkey coronavirus BCoV related | TCoV-B |
| Murine coronaviruses including mouse hepatitis virus (MHV) | MCoV |
| Porcine hemagglutinating encephalomyelitis virus | HEV |
| Rat coronavirus including sialodacryoadenitis virus (SDAV) | RtCoV |
| <i>Group 3</i> | |
| Avian coronavirus including infectious bronchitis virus (IBV) | ACoV |
| Turkey coronavirus IBV related | TCoV-I |
| Unclassified coronavirus | |
| Rabbit coronavirus | RbCoV |

Table 1.
Some of coronaviridae family members [22].

cell types in the body and other susceptible cells throughout the body. (ACE2), the membrane-associated enzyme Carboxypeptidase, is a crucial regulator for cardiac function. Now, recognized and characterized with a sudden second role for ACE2 in mediating viral entry and cell fusion in the form of SARS-CoV spike glycoprotein partner. The coronaviridae family includes this zoonotic virus. The virus has a healthy ssRNA genome and little structural and non-structural protein. Different points of view have been identified with great similarity to SARS-CoV. The Approach of the virus is through S1 protein, which then integrates to the virus membrane with endosomal membranes, possibly by S2 mediation. Then the viral genome is released into the cytoplasm of the cell [25–30]. S-protein has two sub-units with one sub-unit directly binding to the receptor enabling the entrance of the virus into cells. The S-protein RNA binding domain in COVID-19 has a more advanced SARS-CoV homology. Although some of the residues essential to binding are not alike, the structural conformation was not changed in general by the non-identical residues [31]. CoV spike (S) is a key goal for vaccines, antibodies and diagnosis. A 3.5 angle-resolution cryo-electron microscopy structure for the SARS-CoV-2 S was developed cutting conformation in order to promote medical response. The prominent trimer ‘s state possesses rotation in a receptor-accessible conformation in one of the three receptor binding domains (RBDs). Biophysical and structural verification is also given that the SARS-CoV-2 S protein has more affinity than severe acute respiratory (SARS)-CoV S-binding enzyme 2, (ACE2) [32] (Figure 5).

3.2 Replication

Untranslated regions of RNA (UTRs) have 5’ and 3’ viruses that carry RNA-specific signals. The 5’ capped coronavirus genome compromise a 3’ UTR

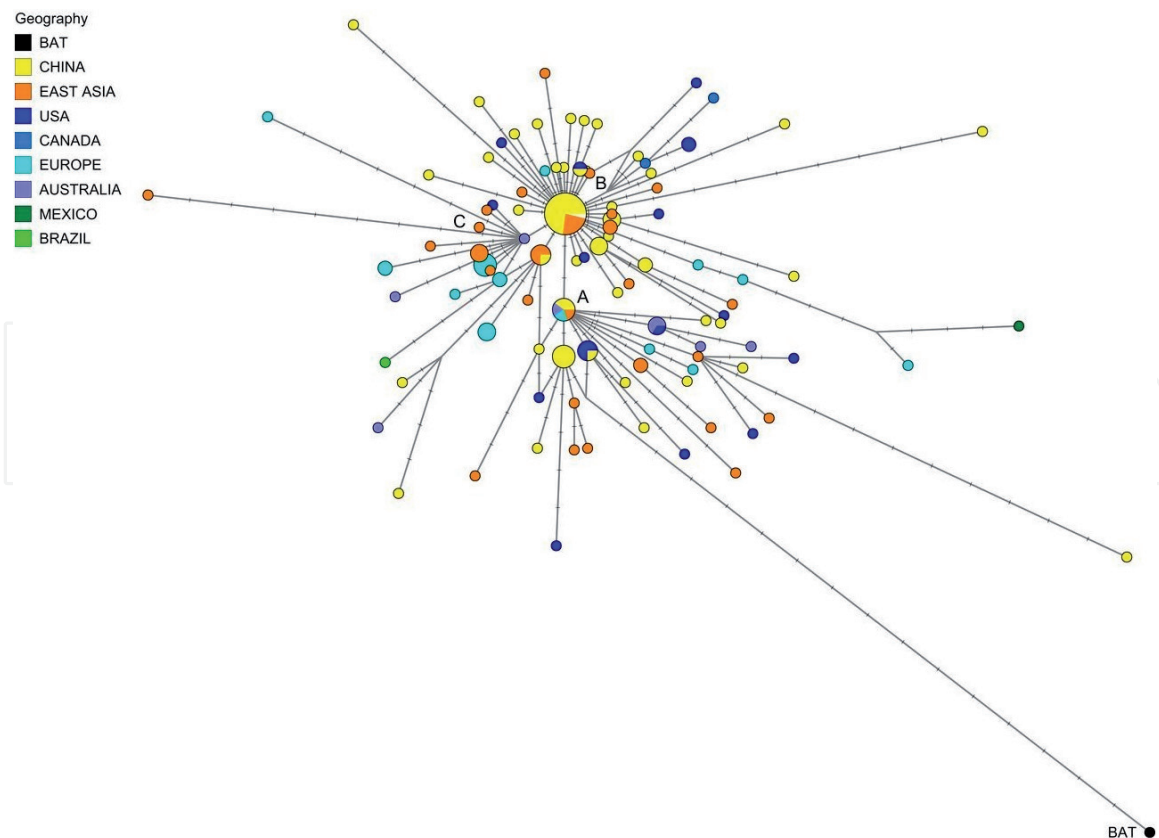


Figure 5.
Phylogenetic tree of 160 SARS-CoV-2 genomes [33].

consisting of 300 to 500 nucleotides) in addition to a poly(A) tail. Host-related factors involving two class-II viruses bovine coronavirus (BCV) and Mouse Hepatitis Coronavirus (MHV) were studied, in order to better understand coronavirus replication. Using gel mobility shift assays unique host protein interactions were identified with BCV 3' UTR [287 nt plus poly(A) tail]. The MHV 3' -UTR [301 nt in addition to poly(A) tail] rivalry indicates that interactivity for the two viruses are preserved. UV cross-linking studies observed proteins with molecular masses of 99, 95 and 73 kDa. The ranges 40- to 50 and 30 kDa even contained less heavily labeled proteins. For binding the 73-kDa protein a poly(A) tail was needed. The 73 kDa proteins have been identified as cytoplasmic poly(A)-binding protein (PABP) by an Immuno-precipitation of UV-cross-linked proteins. To define the significance of the poly(A) tail, the replication of the impaired genomes BCV Drep and MHV MIDI-C was used alongside with several mutants. After transfection to the supporting virus-infected cells, the defect genomes with shortened, 5- or 10-A poly(A) tails have been replicated. BCV Drep RNA lacking a poly(A) tail did not replicate while MHV MIDI-C RNA replication was detected with a deleted tail after multiple mutations of the virus. The kinetics of replication is delayed in both mutants. Noticeable extension or addition of the poly(A) tail in mutants in the replication assay associated with the presence of these RNAs. RNAs exhibit less *in vitro* PABP binding in shorted Poly(A) tails, indicating decreased RNA replication interactions with protein. The data show strongly that the poly(A) tail is a significant indication for the replication of coronavirus [34]. The virus initiates replication and assembly of protein that is followed by the release of new infectious particles into novel target cells. These events are followed by proinflammation chemokines and cytokines producing and triggering which lead to significant pulmonary damage-causing atypical pneumonia with quick abnormalities and failure [35, 36].

3.3 Transcription

For differing coronaviruses, the number of mRNAs varies. The number of mRNAs in the coronavirus species is the number of functional genes. A few hours after infection with the virus in most viral cell systems, coronavirus mRNA synthesis can be identified and proceeded until cells have become invasive. Subgenomic mRNAs are found to be heterogeneous (M. [37]). A two-component support based on expression system was developed and individual genomes were created by selective recombination or by using infectious cDNA clones. Transcription sequences have mainly been characterized by helper-dependent expression systems and can now be validated via single genomes. The coronavirus genome was created through modification of infectious cDNA, resulting in efficient expression of the foreign gene (20 g ml⁻¹) and stable (20 passages) [22, 38]. In a Study, The creation of the full-length infectious cDNA clone and a functional duplicate of the Urbani strain as a bacterial artificial chromosome (BAC) of the extreme acute respiratory syndrome (SARS-CoV). Through this method, the viral RNA was expressed in the cytomegalovirus promoter's cell nucleus and further multiplied by viral replicas in the cytoplasm. The *Escherichia coli* infectious clone and duplicate have been completely stable. The use of the SARS-CoV replica has been shown to be important in efficient coronavirus-RNA synthesis for the recent identification of RNA-processes enzyme exoribonuclease, endo-ribonucleases and 2-line-O-ribose that are found to be essential [39]. The RNA-dependent RNA synthesis is used for coronaviral transcription. The result is that a nested range of 6 to 8 mRNAs of different sizes is produced, depending on the strain of the coronavirus. The mRNAs are five prime and three prime genome -co- terminals. The most significant mRNA is the genomic RNA (gRNA) for both rep1a and rep1b genes. A discontinuous transcription process fuses a lead sequence of 93 nucleotides) (originating from the 5 prime at the end of a genome to 5 prime of the mRNA coding sequence (body) [40]. The RNA virus genomes are comprised of a series of cis-acting and structural elements involved in viral replication. A bulky secondary loop structure was previously established at the upstream end of the 3-way untranslated region (3 tablets of UTR) of the Mouse Hepatitis Virus (MHV) coronavirus genome. This element has proved to be important for viral replication, beginning immediately downstream of the nucleocapsid gene stop codon. A 3 UTR pseudoknot of the corresponding downstream closely related to the bovine coronavirus BCoV. It is an essential pseudoknot for replication and has a preserved counterpart for each coronavirus in groups 1 and 2 [17]. More than one ORF is comprised of 5 'unique regions within multiple mRNA s. For example, mRNA 5 of MHV, which has two ORFs in the coding region which can encode two p 1 3 and p10 proteins, respectively. A negative-stranded RNA template that is represented in an only very small percentage (1–2%) of the intracellular virus-specific RNAs is clearly mediated for Coronavirus RNA synthesis. This negative strand was synthesized by the virus-encoded RNA from the inbound virion. This is likely because the positive-sequenced RNA exceeds the negative-stranded RNA for several rounds of mRNA synthesis. Thus, negative-stranded RNA has more stability. This stability is attributed to the presence in the coronavirus-infected cells of all of the negatively-stranded RNA as a double-stranded RNA [41–43]. The transcriptome Structure was unknown despite the SARS-CoV-2 genome being recorded recently. a high-resolution map was presented of the SARS-CoV-2 transcriptome and epitranscriptome using two complementary sequencing techniques. DNA nanoball sequencing reveals that due to discontinuous transcription occurrences the transcriptome is highly complex SARS-CoV-2 yields transcripts that code unknown ORFs with fusion, deletion and/or frameshift in addition to the canonical genomic and 9 subgenomic RNAs. 41 sites for RNA modification on

viral transcripts were also found with the most common motif being AAGAA with nanopore direct RNA sequencing [20].

3.4 Morphogenesis

Expression studies showed that coronavirus envelope protein E and the more present membrane glycoprotein M were required and adequate to assemble virus particles into cells. Clustered charged-to-alanine Mutagenesis of the gene E was carried, which integrated mutations in mouse hepatitis virus E (MHV) E protein, as a step forward in our understanding of the role of the mouse hepatitis virus E (MHV) E protein. One was apparently lethal and one was a wild-type phenotype of four probable clustered charged-to-alanine E gene mutants. The other two mutants were partly affected by temperature, developing tiny plaques at a nonpermissive temperature. Reverting analyses of these two mutants showed that each mutation was the reason for the temperature-sensitive phenotype and promoted probable interactions among E protein monomers. In permissive temperature, both temperature-sensitive mutants have been substantially thermolabile, indicating that their assembly fails. In the case of the electron microscopy, virions of one of the mutants were discovered to have remarkably aberrant morphology when compared with the wild type: most mutant virions had pinched and extended forms that were seen seldom in the wild [44–46]. Specific recombination of RNA was utilized to create mutants containing chimeric nucleocapsid (N) protein genes in mouse hepatitis virus (MHV) that replace bovine coronavirus N gene segments in place of the correct MHV sequences. This described portions of the two N proteins which were functionally equivalent, given evolutionary divergences. These regions included mostly the RNA binding domain centrally located and two putative spacers connecting the three N protein domains. On the other hand, a bovine coronavirus cannot be transferred from the amino terminus N, the acidic carboxy-terminal region and the central domain serine and arginine-rich section, probably because these parts of a molecule are engaged in protein–protein interactions that are unique to each virus (or possibly each host). The results show that the recombination of the coronavirus genome can be used to produce extensive substitutions and recombinants that cannot otherwise be produced between two viruses separated by species barrier [47].

4. Mutations

RNA viruses must establish an equilibrium between the adaptability to new environmental circumstances or the necessity to preserve the intact and replicative genome to ensure survival and propagation for the host cells. Various virus families with the biggest and most complex replicating RNA genomes identified, up to 32 kb of positive RNA, such as coronaviruses, can achieve these objectives. CoVs, including (MHV) and SARS-CoV, express 3 to 5' of exoribonuclease (ExoN) activity in nsp14. The exoN genetic inactivation of alanine replacement with retained active DE-D Residues in Engineered SARS-CoV and MHV Genomes leads to viable mutants, which display 15 to 20 times higher mutation rates and up to 18 times higher than those endured for other RNA fidelity mutants. Nsp14-ExoN, therefore, is important for the fidelity of the replication and possibly acts as a direct mediator or regulator for a more complex RNA proof-reader, an exceptional process in RNA virus biology. The removal of nsp14-mediated proofreading mechanisms will have significant consequences for our interpretation of RNA virus evolution and will also provide a robust model to research the correlation between fidelity,

| Genomic region | No. nt mutations | Missense mutation | SARV-CoV-2 strain |
|--------------------|--|-------------------|---|
| 5' UTR | 8 | N/A | |
| ORF1ab polyprotein | 48 | | |
| | | A (117) → T | USA/CA3/2020/EPI_ISL_408008 USA/CA4/2020/EPI_ISL_408009 |
| | | P (309) → S | France/IDF0515/2020/EPI_ISL_408430 |
| | | S (428) → N | USA/CA1/2020/EPI_ISL_406034 |
| | | T (609) → I | USA/CA5/2020/EPI_ISL_408010 |
| | | A (1176) → V | Japan/TY-WK-012/2020/EPI_ISL_408665 |
| | | L (1599) → F | Korea/KCDC03/2020/EPI_ISL_407193 |
| | | I (1607) → V | USA/CA3/2020/EPI_ISL_408008 USA/CA4/2020/EPI_ISL_408009 |
| | | M (2194) → T | Shenzhen/SZTH-004/2020/EPI_ISL_406595 |
| | | L (2235) → I | Wuhan/WH01/2019/EPI_ISL_406798 |
| | | I (2244) → T | Wuhan/IPBCAMS-WH-03/2019/ EPI_ISL_403930 |
| | | G (2251) → S | Wuhan/WIV05/2019/EPI_ISL_402128 |
| | | A (2345) → V | Shandong/IVDC-SD-001/2020/EPI_ISL_408482 |
| | | G (2534) → V | Wuhan/IPBCAMS-WH-05/2020/ EPI_ISL_403928 |
| | | D (2579) → A | Wuhan/WIV07/2019/EPI_ISL_402130 |
| | | N (2708) → S | Wuhan/IPBCAMS-WH-01/2019/ EPI_ISL_402123 |
| | | F (2908) → I | Wuhan/IPBCAMS-WH-01/2019/ EPI_ISL_402123 |
| | | T (3058) → I | France/IDF0515/2020/EPI_ISL_408430 |
| | | S (3099) → L | Shenzhen/HKU-SZ-005/2020/EPI_ISL_405839 |
| | | L (3606) → F | Yunnan/IVDC-YN-003/2020/EPI_ISL_408480 Shandong/IVDC-SD-001/2020/EPI_ISL_408482 Chongqing/IVDC-CQ-001/2020/ EPI_ISL_408481 Singapore/3/2020/EPI_ISL_407988 France/IDF0515/2020/EPI_ISL_408430 USA/AZ1/2020/EPI_ISL_406223 |
| | | E (3764) → D | Japan/KY-V-029/2020/EPI_ISL_408669 |
| | | N (3833) → K | Wuhan/WH01/2019/EPI_ISL_406798 |
| | | W (5308) → C | Taiwan/2/2020/EPI_ISL_406031 |
| | | T (5579) → I | USA/CA2/2020/EPI_ISL_406036 |
| I (6075) → T | England/02/2020/EPI_ISL_407073 England/01/2020/EPI_ISL_407071 | | |
| P (6083) → L | Japan/AI/I-004/2020/EPI_ISL_407084 | | |
| F (6309) → Y | Sichuan/IVDC-SC-001/2020/EPI_ISL_408484 | | |
| E (6565) → D | Shenzhen/SZTH-004/2020/EPI_ISL_406595 | | |
| K (6958) → R | Wuhan/WIV05/2019/EPI_ISL_402128 | | |

| Genomic region | No. nt mutations | Missense mutation | SARV-CoV-2 strain | |
|----------------------|------------------|-------------------|---------------------------------|---|
| | | D (7018) → N | Wuhan/WIV02/2019/EPI_ISL_402127 | |
| Spike polyprotein | 14 | 8 | | |
| | | | F (32) → I | Wuhan/HBCDC-HB-01/2019/EPI_ISL_402132 |
| | | | H (49) → Y | Guangdong/20SF174/2020/EPI_ISL_406531 Guangdong/20SF040/2020/EPI_ISL_403937 Guangdong/20SF028/2020/EPI_ISL_403936 |
| | | | S (247) → R | Australia/VIC01/2020/EPI_ISL_406844 |
| | | | N (354) → D | Shenzhen/SZTH-004/2020/EPI_ISL_406595 |
| | | | D (364) → Y | Shenzhen/SZTH-004/2020/EPI_ISL_406595 |
| | | | V (367) → F | France/IDF0372/2020/EPI_ISL_406596 France/IDF0373/2020/EPI_ISL_406597 |
| | | | D (614) → G | Germany/BavPat1/2020/EPI_ISL_406862 |
| | | | P (1143) → L | Australia/QLD02/2020/EPI_ISL_407896 |
| | | Intergenic region | 5 | N/A |
| Envelope protein | 0 | 0 | | |
| Matrix protein | 2 | 1 | | |
| | | | D (209) → H | Singapore/2/2020/EPI_ISL_407987 |
| Intergenic region | 6 | N/A | | |
| Nucleocapsid protein | 7 | 4 | | |
| | | | T (148) → I | Shenzhen/SZTH-004/2020/EPI_ISL_406595 |
| | | | S (194) → L | Shenzhen/SZTH-003/2020/EPI_ISL_406594 Foshan/20SF207/2020/EPI_ISL_406534 USA/CA3/2020/EPI_ISL_408008 USA/CA4/2020/EPI_ISL_408009 |
| | | | S (202) → N | Australia/QLD02/2020/EPI_ISL_407896 |
| | | | P (344) → S | Guangzhou/20SF206/2020/EPI_ISL_406533 |
| | | | | |
| 3'UTR | 3 | N/A | | |
| Complete genome | 93 | 42 | | |

Table 2.
 Mutations of SARS-CoV-2 strains found throughout the whole genome. The number in the parentheses shows where amino acid is found in its protein [21].

diversity and pathogenesis [48–52]. COVID-19 is very related to SARS-CoV Middle East Respiratory Syndrome (MERS). Yet another human attack by coronaviruses. A research attempted to explore potential changes/developments in the ‘spike protein’ element that enables the virus to bind to cell receptor(s) and in the silicon design and discovery of B epitopes in which antibody synthesis is used to neutralize and block this connection. The findings show that this protein varies constantly between

| Accession | Location-date | Nucleotide variation | Gene | Amino acid change | Mutation type |
|-----------|----------------------------|----------------------|--------|-------------------|---------------------|
| MT240479 | 04-03-2020/Pakistan Gilgit | 1 1497G > A | Orf1ab | | Synonymous mutation |
| MN996527 | 30/Dec/2019-China Wuhan | 21316G > A | Orf1ab | D7018N | Missense |
| MN996527 | 30/Dec/2019-China Wuhan | 24292A > G | S | | Synonymous mutation |
| LC528232 | 10/Feb/2020-Japan | 11083 T > G | Orf1ab | L3606F | Missense |
| LC528232 | 10/Feb/2020-Japan | 29642C > T | ORF10 | | Synonymous mutation |
| LR757995 | 05/Jan/2020-China Wuhan | 28144 T > C | ORF8 | L84S | Missense |
| LR757998 | 12/26/2019-China Wuhan | 6968C > A | Orf1ab | L2235I | Missense |
| LR757998 | 12/26/2019-China Wuhan | 11749 T > A | Orf1ab | | Synonymous mutation |
| MN938384 | 1/10/2020-China Shenzhen | 8782C > T | Orf1ab | | Synonymous mutation |
| MN938384 | 1/10/2020-China Shenzhen | 28144 T > C | ORF8 | L84S | Missense |
| MN938384 | 1/10/2020-China Shenzhen | 29095C > T | N | | Synonymous mutation |
| MN975262 | 11/Jan/2020-China | 8782C > T | Orf1ab | | Synonymous mutation |
| MN975262 | 11/Jan/2020-China | 9534C > T | Orf1ab | T3090I | Missense |
| MN975262 | 11/Jan/2020-China | 29095C > T | N | | Synonymous mutation |
| MN975262 | 11/Jan/2020-China | 28144 T > C | ORF8 | L84S | Missense |
| MN975262 | 11/Jan/2020-China | 8782C > T | Orf1ab | | Synonymous mutation |
| MN985325 | 19/Jan/2020-USA WA | 28144 T > C | ORF8 | L84S | Missense |
| MN994467 | 23/Jan/2020-USA CA | 1548G > A | Orf1ab | S428N | Missense |
| MN994467 | 23/Jan/2020-USA CA | 8782C > T | Orf1ab | | Synonymous mutation |
| MN994467 | 23/Jan/2020-USA CA | 26729 T > C | M | | Synonymous mutation |
| MN994467 | 23/Jan/2020-USA CA | 28077G > C | ORF8 | V62L | Missense |
| MN994467 | 23/Jan/2020-USA CA | 28144 T > C | ORF8 | L84S | Missense |
| MN994467 | 23/Jan/2020-USA CA | 28792A > C | N | | Synonymous mutation |
| MN994467 | 23/Jan/2020-USA CA | 1912C > T | Orf1ab | | Synonymous mutation |

| Accession | Location-date | Nucleotide variation | Gene | Amino acid change | Mutation type |
|------------------|-------------------------|----------------------|--------|-------------------|---------------------|
| GWHABKF000000001 | 23/Dec/2019-China Wuhan | 3778A > G | Orf1ab | | Synonymous mutation |
| GWHABKF000000001 | 23/Dec/2019-China Wuhan | 8388A > G | Orf1ab | N2708S | Missense |
| GWHABKF000000001 | 23/Dec/2019-China Wuhan | 8987 T > A | Orf1ab | F2908I | Missense |
| GWHABKK000000001 | 30/Dec/2019-China Wuhan | 24325A > G | S | | Synonymous mutation |
| GWHABKK000000001 | 30/Dec/2019-China Wuhan | 21316G > A | Orf1ab | D7018N | Missense |
| GWHABKH000000001 | 30/Dec/2019-China Wuhan | 6996 T > C | Orf1ab | I2244T | Missense |
| GWHABKJ000000001 | 01/Jan/2019-China Wuhan | 7866G > T | Orf1ab | G2534V | Missense |
| GWHABKM000000001 | 30/Dec/2019-China Wuhan | 21137A > G | Orf1ab | K6958R | Missense |
| GWHABKM000000001 | 30/Dec/2019-China Wuhan | 7016G > A | Orf1ab | G2251S | Missense |
| GWHABKO000000001 | 30/Dec/2019-China Wuhan | 8001A > C | Orf1ab | D2579A | Missense |
| GWHABKO000000001 | 30/Dec/2019-China Wuhan | 9534C > T | Orf1ab | T3090I | Missense |
| MT188341 | 05/Mar/2020-USA MN | 6035A > G | Orf1ab | | Synonymous mutation |
| MT188341 | 05/Mar/2020-USA MN | 8782C > T | Orf1ab | | Synonymous mutation |
| MT188341 | 05/Mar/2020-USA MN | 16467A > G | Orf1ab | | Synonymous mutation |
| MT188341 | 05/Mar/2020-USA MN | 18060C > T | Orf1ab | | Synonymous mutation |
| MT188341 | 05/Mar/2020-USA MN | 21386insT | Orf1ab | | Insertion |
| MT188341 | 05/Mar/2020-USA MN | 21388-21390insTT | Orf1ab | | Insertion |
| MT188341 | 05/Mar/2020-USA MN | 23185C > T | S | | Synonymous mutation |
| MT188341 | 05/Mar/2020-USA MN | 28144 T > C | ORF8 | L84S | Missense |
| MT188339 | 09/Mar/2020-USA MN | 8782C > T | Orf1ab | | Synonymous mutation |
| MT188339 | 09/Mar/2020-USA MN | 17423A > G | Orf1ab | Y5720C | Missense |
| MT188339 | 09/Mar/2020-USA MN | 18060C > T | Orf1ab | | Synonymous mutation |
| MT188339 | 09/Mar/2020-USA MN | 21386C > T | Orf1ab | | Synonymous mutation |

| Accession | Location-date | Nucleotide variation | Gene | Amino acid change | Mutation type |
|-----------|-----------------------------|----------------------|--------|-------------------|---------------------|
| MT188339 | 09/Mar/2020-USA MN | 22432C > T | S | | Synonymous mutation |
| MT188339 | 09/Mar/2020-USA MN | 28144 T > C | ORF8 | L84S | Missense |
| MT121215 | 02/Feb/2020-China Shanghai | 6031C > T | Orf1ab | | Synonymous mutation |
| MT123290 | 05/Feb/2020-China Guangzhou | 15597 T > C | Orf1ab | | Synonymous mutation |
| MT123290 | 05/Feb/2020-China Guangzhou | 29095C > T | N | | Synonymous mutation |
| MT126808 | 2/28/2020-Brazil | 26144G > T | ORF3a | G251V | Missense |
| MT066175 | 31/Jan/2020-Taiwan | 8782C > T | Orf1ab | | Synonymous mutation |
| MT066175 | 31/Jan/2020-Taiwan | 28144 T > C | ORF8 | L84S | Missense |
| MT093571 | 07/Feb/2020-Sweden | 13225C > G | Orf1ab | | Synonymous mutation |
| MT093571 | 07/Feb/2020-Sweden | 13226 T > C | Orf1ab | | Synonymous mutation |
| MT093571 | 07/Feb/2020-Sweden | 17423A > G | Orf1ab | Y5720C | Missense |
| MT093571 | 07/Feb/2020-Sweden | 23952 T > G | S | | Synonymous mutation |
| MT066156 | 30/Jan/2020-Italy | 11083 T > G | Orf1ab | L3606F | Missense |
| MT066156 | 30/Jan/2020-Italy | 26144G > T | ORF3a | G251V | Missense |
| LC522975 | 20/JAN/2020-JAPAN | 8782C > T | Orf1ab | | Synonymous mutation |
| LC522975 | 20/JAN/2020-JAPAN | 29095C > T | N | | Synonymous mutation |
| LC522975 | 20/JAN/2020-JAPAN | 28144 T > C | ORF8 | L84S | Missense |
| LC522975 | 20/JAN/2020-JAPAN | 2662C > T | ORF1ab | | Synonymous mutation |
| LC522974 | 20/JAN/2020-JAPAN | 8782C > T | ORF1ab | | Synonymous mutation |
| LC522974 | 20/JAN/2020-JAPAN | 29095C > T | N | | Synonymous mutation |
| LC522974 | 20/JAN/2020-JAPAN | 28144 T > C | ORF8 | L84S | Missense |
| LC522974 | 20/JAN/2020-JAPAN | 2662C > T | ORF1ab | | Synonymous mutation |
| LC522973 | 20/JAN/2020-JAPAN | 8782C > T | ORF1ab | | Synonymous mutation |
| LC522973 | 20/JAN/2020-JAPAN | 29095C > T | N | | Synonymous mutation |
| LC522973 | 20/JAN/2020-JAPAN | 3792C > T | ORF1ab | A1176V | Missense |
| LC522973 | 20/JAN/2020-JAPAN | 29095C > T | N | | Synonymous mutation |
| LC522973 | 20/JAN/2020-JAPAN | 2662C > T | ORF1ab | | Synonymous mutation |
| LC522973 | 20/JAN/2020-JAPAN | 28144 T > C | ORF8 | L84S | Missense |

| Accession | Location-date | Nucleotide variation | Gene | Amino acid change | Mutation type |
|-----------------|---------------------------------|----------------------|--------|-------------------|---------------------|
| LC522972 | 20/JAN/2020-JAPAN | 29303C > T | N | P344S | Missense |
| LC522972 | 20/JAN/2020-JAPAN | 25810C > G | ORF3a | L140V | Missense |
| LC522972 | 20/JAN/2020-JAPAN | 11557G > T | ORF1ab | E3764D | Missense |
| LC522972 | 20/JAN/2020-JAPAN | 15324C > T | ORF1ab | | Synonymous mutation |
| LC521925 | 21/JAN/2020-JAPAN | 1912C > T | ORF1ab | | Synonymous mutation |
| LC521925 | 21/JAN/2020-JAPAN | 18512C > T | ORF1ab | P6083L | Missense |
| LC521925 | 21/JAN/2020-JAPAN | 359_382del | ORF1ab | G32_L39del | Deletion |
| MN988713 | 21/JAN/2020-USA Chicago | 24034C > T | S | | Synonymous mutation |
| MN988713 | 21/JAN/2020-USA Chicago | 26729 T > C | M | | Synonymous mutation |
| MN988713 | 21/JAN/2020-USA Chicago | 8782C > T | ORF1ab | | Synonymous mutation |
| MN988713 | 21/JAN/2020-USA Chicago | 490 T > A | ORF1ab | D75E | Missense |
| MN988713 | 21/JAN/2020-USA Chicago | 3177C > T | ORF1ab | P971L | Missense |
| MN988713 | 21/JAN/2020-USA Chicago | 28854C > T | N | S194L | Missense |
| MN988713 | 21/JAN/2020-USA Chicago | 28077G > C | ORF8 | V62L | Missense |
| MN988713 | 21/JAN/2020-USA Chicago | 28144 T > C | ORF8 | L84S | Missense |
| MN997409 | 21/JAN/2020-USA Arizona | 8782C > T | ORF1ab | | Synonymous mutation |
| MN997409 | 21/JAN/2020-USA Arizona | 29095C > T | N | | Synonymous mutation |
| MN997409 | 21/JAN/2020-USA Arizona | 11083G > T | ORF1ab | L3606F | Missense |
| MN997409 | 21/JAN/2020-USA Arizona | 28144 T > C | ORF8 | L84S | Missense |
| MT072688 | 26/JAN/2020-USA: Massachussetts | 24034C > T | S | | Synonymous mutation |
| NMDC60013002-09 | 01/JAN/2019-China Wuhan | 27493C > T | ORF7a | P34S | Missense |
| NMDC60013002-09 | 01/JAN/2019-China Wuhan | 28253C > T | ORF8 | | Synonymous mutation |
| NMDC60013002-10 | 30/Dec/2019-China Wuhan | 20679G > A | ORF1ab | | Synonymous mutation |
| NMDC60013002-01 | 30/Dec/2019-China Wuhan | 11764 T > A | ORF1ab | N3833K | Missense |
| NMDC60013002-06 | 30/Dec/2019-China Wuhan | 24325A > G | S | | Synonymous mutation |
| NMDC60013002-04 | 05/Dec/2019-China Wuhan | 28144 T > C | ORF8 | L84S | Missense |

Table 3.
 Coding mutation list detected in SARS-CoV-2 genomes [57].

| Accession | Location-date | Nucleotide variation | UTR type |
|-----------------|-----------------------------------|----------------------|----------|
| MT240479 | 04-03-2020/Pakistan Gilgit | 241C > T | 5 UTR |
| MT123290 | 05/Feb/2020-China Guangzhou | 4A > T | 5 UTR |
| MT007544 | 25/Jan/2020-Australia Victoria | 29749-29759del | 3 UTR |
| NMDC60013002-07 | 07/JAN/2019-China Wuhan | 29869del | 3 UTR |
| NMDC60013002-04 | 05/Dec/2019-China Wuhan | 29856 T > A | 3 UTR |
| NMDC60013002-04 | 05/Dec/2019-China Wuhan | 29854C > T | 3 UTR |
| NMDC60013002-04 | 05/Dec/2019-China Wuhan | 16C > T | 5 UTR |
| MT049951 | 17/Jan/2019-China Yunnan | 75C > A | 5 UTR |
| LC522975 | 20/JAN/2020-JAPAN | 29705G > T | 3 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 124G > A | 5 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 120 T > C | 5 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 119C > G | 5 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 112 T > G | 5 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 111 T > C | 5 UTR |
| GWHABKG00000001 | 30/Dec/2019-China Wuhan | 104 T > A | 5 UTR |

Table 4.
Non-coding mutation list detected in SARS-CoV-2 genomes [57].

the sequences of proteins obtained worldwide. Some B epitopes (part of an antigen molecule to which an antibody attaches itself), 177-MDLEGKQGNFKNL-189-555-SNKKFLPF-562-656 -VNSYECDIPI-666, 1035- GQSKRVDFC-1043, from the Cons sequence constructed from global protein sequences released between 11 Feb and 06 April, have been found to meet most of the criteria required for real wet application [53]. SARS-CoV is well suited to cultural development and does not seem to be selected in humans. It was also assessed that, in late October 2002, the alleged root of the SARS outbreak was consistent with a previous report of case use from China. The higher structural and antigenic sequence divergence and significant deletions within 3' – of much of the viral genome indicate that some selection pressures conflict along with the functional structure of these confirmed and suspected ORFs [54]. In three regions the SARS and SARSr of bats-CoVs are largely different: S, ORF8 and ORF3. SARSr-CoVs bats share high sequence with the SARS- COV in the S2 but are highly different in the S1 region. However, bat MERSr-CoVs bats and human and camel MERS-CoVs share similar genomics but are significantly different from their genomic sequences [7]. Comparison of COVID-19, SARS-CoV and MERS-CoV genome sequence showed that COVID-19 has better sequence similarity

with SARS-CoV compared to MERS CoV. Nevertheless, the COVID-19 amino acid sequence differed from the other coronavirus in specific areas of 1ab polyprotein and surface glycoprotein or S-protein [31]. Considering the high rate of mutation that characterizes RNA viruses, it is clear that several more mutations will emerge in the viral genome to monitor the spread of SARS-CoV-2 knowing that also their mutations rate are lower than other RNA viruses due to their proofreading activity described above [55, 56] (**Tables 2–4**).

5. Evolution and origin

Most SARS-CoV strains are derived from bats. SARS-CoV bat is a probable progenitor for SARS – CoV that is contagious to humans and civets, and thus it is important to study ACE2 receptor for monitoring origins of SARS-CoV and avoiding and controlling the outbreak. Though palm civets were involved in SARS emergence, most early MERS index cases had contact with dromedary camels. Indeed, the MERS-CoV strains separated from camels were nearly matching to those from humans [7]. The virus shares 96% of its genetic material with a virus detected from a bat found in a cave in Yunnan in China. A persuasive argument that it comes from bats but there is a critical alteration. The coronaviral spike proteins have a unit called a receptor-binding domain that is essential to the successful entry of human cells. Especially powerful is the SARS-CoV-2 binding domain and it varies from the bat virus Yunnan which appears to not affect human. Another Complicating matter, a scaly anteater called the pangolin with a coronavirus which was almost similar to the human version with a receptor-binding domain. However, the majority of the coronavirus was genetically identical just 90%, and some researchers do not believe that pangolin was the intermediary. It is difficult to draw a family tree since both mutations and recombinations are involved [58–60]. An article identifies and uses a machine learning-based alignment-free approach to identify a COVID-19 intrinsic genomic signature for an ultra-fast, scalable, and extremely precise classification of all COVID-19 virus genomes. The technique presented incorporates supervised machine learning with MLDSF for genome analysis, improved by a machine learning component decision tree approach and a Spearman-leading correlation coefficient analysis of tests. These methods are used to examine a broad collection of more than 61.8 million bp, including the 29 COVID-19 virus sequences on 27 January 2020, with over 5,000 unique viral genomic sequences. The findings endorse a bat hypothesis and the COVID-19 virus is classed under Betacoronavirus as the Sarbecovirus. Without any advanced biological expertise, training or genome annotations, our method achieves a 100% precise classification of the COVID-19 virus sequences, and determines the most important relationships between more than 5000 genomes in minutes, from the beginning on, with the sole use of raw DNA sequence details [61]. In a recent research, they have developed a phylogenetic tree, including other members of coronaviridae including Bat coronavirus (BCoV) and extreme acute respiratory 2019 disease, taking advantage of all of the available genomic knowledge. The closest BCoV sequence, with a 96,2% sequence 2019 SARS-CoV2 identity, confirm that all available genomes of the sequence are of zoonotic origin. We have confirmed the high sequence similarity (> 99%) among all available genomes. Given the low 2019 SARS-CoV2 heterogeneity, at least two genomic hyper various hotspots were identified, including one of the Serine/Leucine variations in viral ORF8 Protein encoded, can be detected [62]. (**Figures 6 and 7**) In the study a Malayan pangolin-isolated coronavirus showed 100%, 98.6%, 97.8% and 90.7% SARS-CoV-2 amino acid identity in genes E, M, N, and S respectively. Particularly in the S protein of

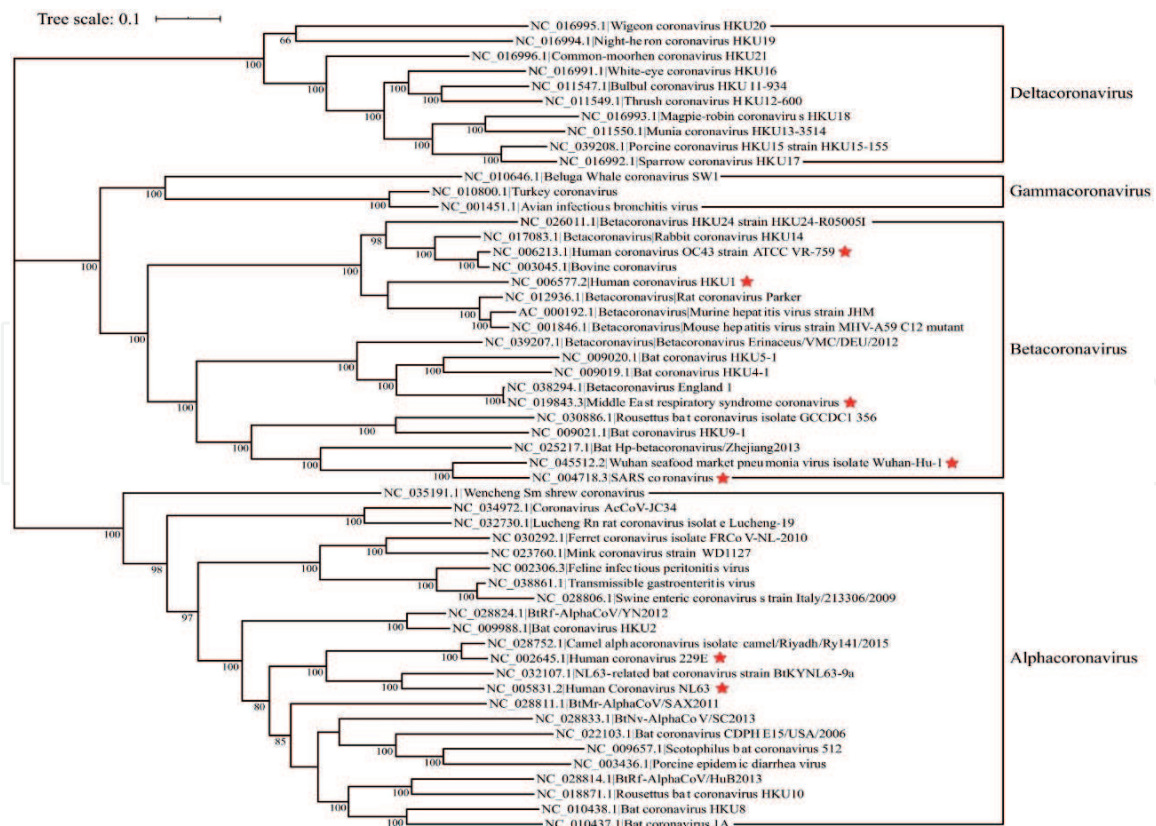


Figure 6.

A coronavirus phylogenetic tree based on full-length genome sequences. Both complete coronavirus genome sequences have been obtained from RefSeq, the NCBI reference sequence database [5].

Pangolin-CoV, the receptor-binding domain is nearly the same as the SARS-CoV-2 with vital one-amino acid alteration. Results of comparative genomic analysis indicate that SARS-CoV-2 may have been the result of a Pangolin-CoV-like virus recombination with a Bat-CoV-RaTG13 virus [63].

6. Immunopathology of SARS-CoV2

Pneumonia, lymphopenia, drained lymphocytes and a cytokine storm are distinguishable symptoms of Extreme Coronavirus Disease 2019 (COVID-19). Major antibody development is detected, but it remains to be determined if this is defensive or pathogenic. Defining the immunopathological changes in COVID-19 patients presents future drug development targets and is critical for clinical management [64]. Asymptomatic condition is found in a large but generally unexplained proportion of the infected people, analogous to many other viral diseases. Usually, a 1-week, self-limiting viral respiratory disease develops in most patients, and ends with the production of neutralizing antiviral T cell and antibody immunity [65]. SARS-CoV-2 has been shown to weaken natural immune responses, resulting in a compromised immune system and an unregulated inflammatory response in extreme and vital COVID-19 patients. These patients display lymphopenia, stimulation and malfunction of lymphocytes, defects of granulocytes and monocytes, elevated levels of cytokines, and higher amounts of immunoglobulin G (IgG) and total antibodies [66] (**Figure 8**). Extreme and fatal COVID-19 is linked with lymphopenia and an elevated amount of blood neutrophils [67]. Lymphocyte counts of 800 cells/ μ l and a decreased probability of recovery are reported in ICU patients suffering from COVID-19. The mechanism of action and causes of

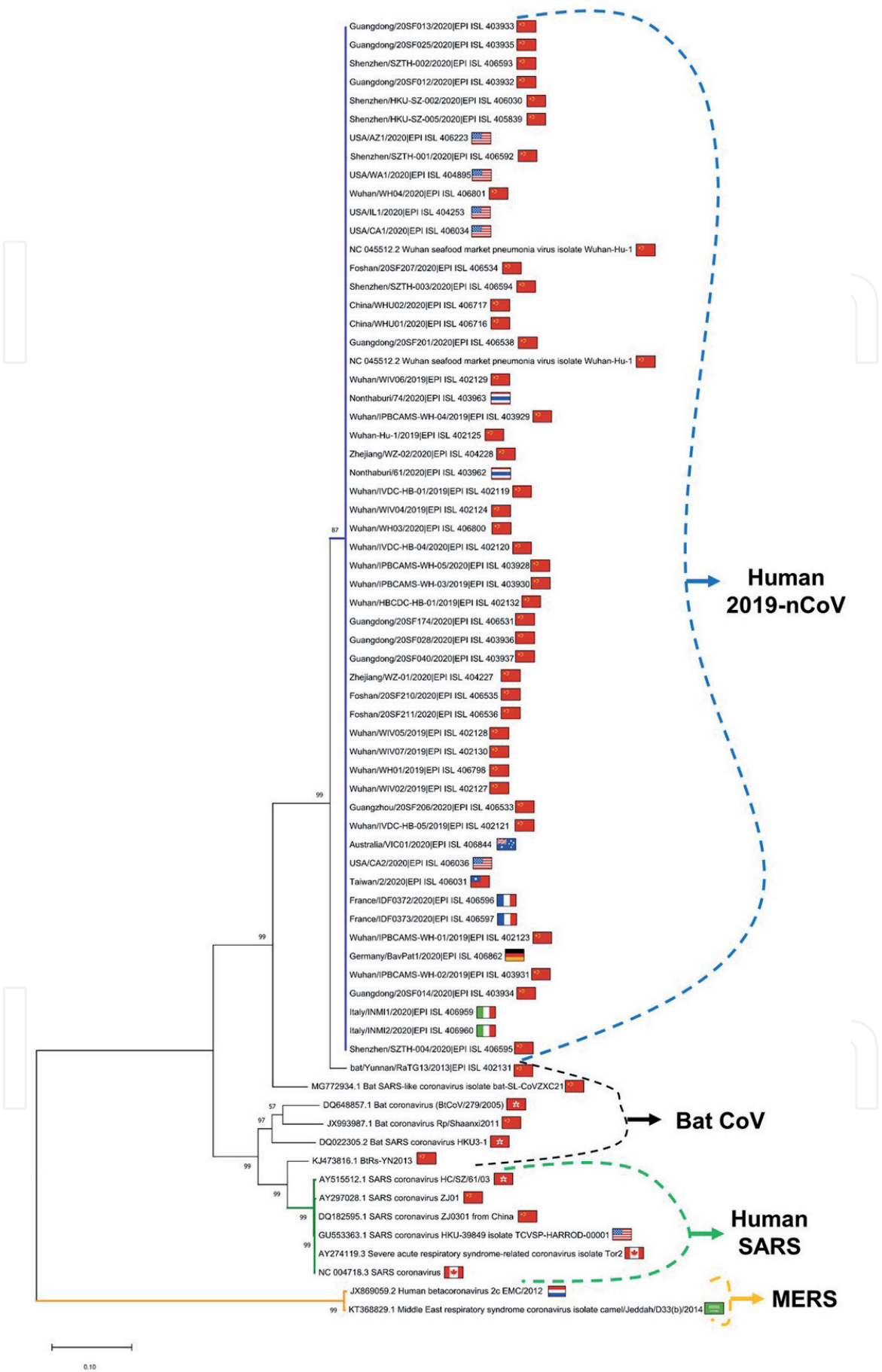


Figure 7.
 A phylogenetic tree with all the sequences of SARS-CoV-2 available from the 02-Feb-2020 sequence in the blue divisions, plus six Bat coronavirus sequences split in multiple taxa, six human SARS sequences (green) and two MERS sequences (orange); the bootstrap percentage of each branch is recorded [62].

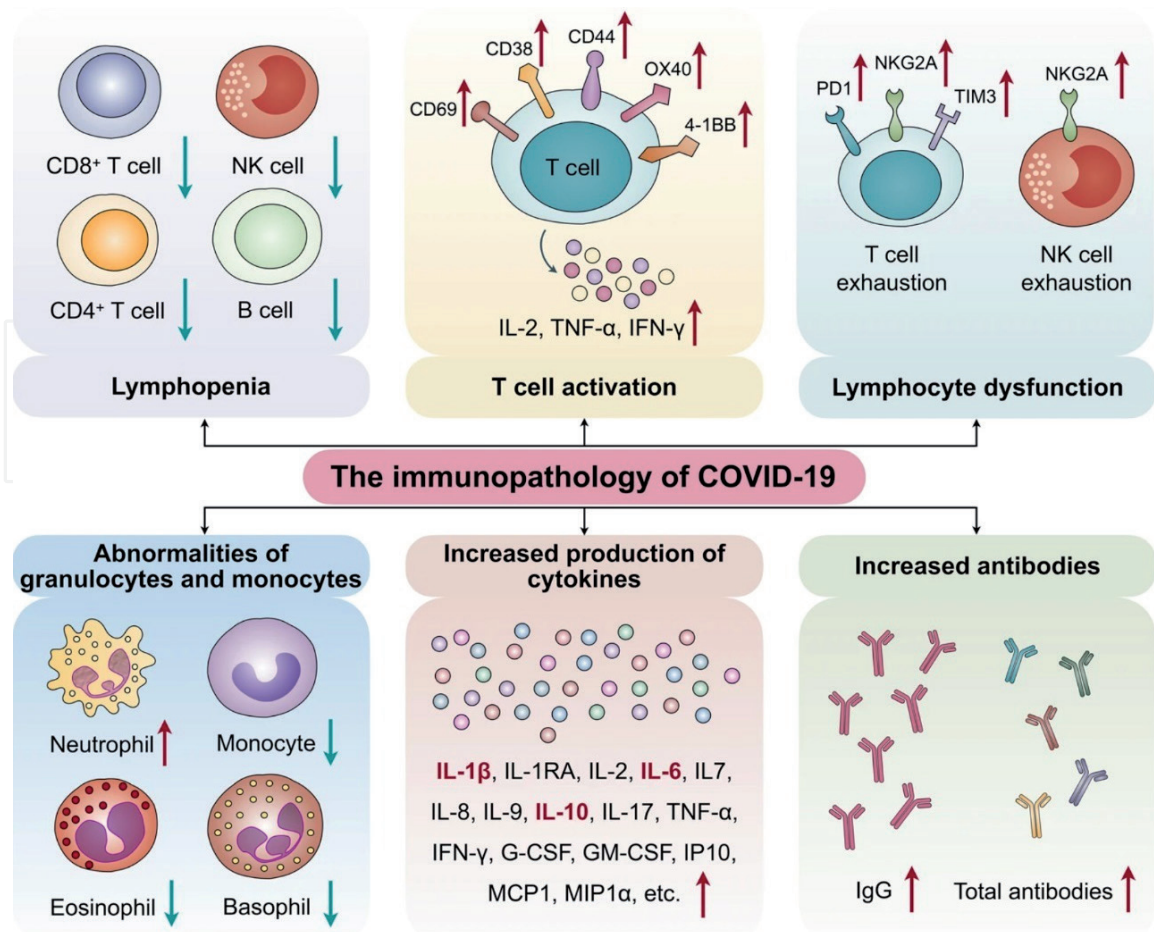


Figure 8.
COVID-19 immunopathology [66].

lymphopenia in patients with COVID-19 are unclear, but SARS-like viral particles and SARS-CoV RNA have been observed in T cells, indicating that the SARS virus may have a detrimental influence on T cells via apoptosis [68]. Accumulating data proves the involvement of T cells in COVID-19 and possibly in the immunological memory that develops after recovering from infection with SARS-CoV-2. Many, but not always, hospitalized patients tend to have both CD8 + and CD4 + T cell responses, and research points to potential T cell responses consistent with extreme disease that are suboptimal, abnormal or otherwise inadequate [5]. In a report, a group of 452 patients with positive test results of COVID-19 in Wuhan, China shows dysregulated immune system. Boosts in NOD-like receptor (NLR) and T lymphopenia, especially a decline in CD4 + T cells, were prominent in COVID-19 patients, and was even more noticeable in extreme cases, but the number of CD8 + cells and B cells did not change significantly. On the basis of these results, it was proposed that COVID-19 may affect lymphocytes, especially T lymphocytes, and that the immune system is disrupted during the infection period [69]. COVID-19 will lead to defects in the routine of peripheral blood parameters. The most noticeable anomalies that are linked to the intensity of the condition and clinical classification are the reduction in lymphocytes and the rise in the NLR ratio. The lower count and delay in eosinophil development can be indicators of weak COVID-19 outcomes. Thus, complex analysis of peripheral blood routine parameters has a significant reference point for COVID-19 progression and prognosis evaluation [70]. Also, In the different stages of COVID-19, multiple cell morphological modifications can be seen. In fact, a strong granulocytic reaction with immaturity, dysmorphism and apoptotic-degenerative morphology was apparent in peripheral blood in the initial stage of symptom aggravation, typically correlating with hospital entry [71].

Cytokine storm plays a crucial role in infected individuals for the pathogenesis of many serious manifestations of the disease. Acute respiratory distress syndrome, thromboembolic disorders such as acute ischaemic strokes caused by myocardial infarction and large vessel occlusion, encephalitis, acute kidney damage, and vasculitis (childhood Kawasaki syndrome and adult renal vasculitis) [72]. Nonetheless, it is uncertain if serious illness is triggered by immune hyperactivity or inability to overcome an inflammatory reaction owing to continuing virus replication or immune dysregulation. However, records of elevated levels of thrombi production and endothelial cell death in patients with COVID-19 suggest disruption to the vascular endothelium and the participation of cytokine elevated activity and immunothrombosis [73]. In response to infection as well as other triggers, cytokine storm is a general term referring to maladaptive cytokine release. The pathogenesis is complicated, but requires the depletion of regulated control at both local and systemic levels of proinflammatory cytokine output. The disease is rapidly progressing, and mortality is elevated. Some data suggests that dysregulated and uncontrolled cytokine release in certain COVID19 patients has been directly correlated with significant deterioration [74].

7. Molecular diagnostics

COVID-19 Test of SARS-CoV-2 is a real-time reverse transcription polymerase chain-reaction (PCR) in upper or lower respiratory samples for the qualitative identification of nucleic acid (such as nasal, nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, nasopharyngeal wash/aspirate or a nasal aspirate) that is individually obtained from those suspected of COVID-19 by their healthcare provider [75]. The latest COVID-19 outbreak can be detected using qPCR, but insufficient possession of reagents and equipment has hindered the identification of diseases. To assist in making COVID-19 more effective in our diagnostics, a new protocol was suggested for the application of the CRISPR-based SHERLOCK technique for detecting COVID-9. COVID-19 objectives were identified between 20 and 200 aM (10–100 copies per input microlitre) with the use of synthetic COVID-19 virus RNA fragments. The test can be performed starting with patient-purified RNA as used in qRT-PCR trials and read in less than an hour with a dipstick, without the need for complex instrumentation [58, 59]. GolayMetaMiner, an in-house software, has identified four different regions over 50 nucleotides for the SARS-CoV-2 genome with 96 SARS-CoV-2 and 104 non-SARS-CoV-2 coronaviral genomes. Primers were made to target the longest and previously not targeted nsp2 region and tailored as a reverse transcription-polymerase chain reaction (RT-PCR) test without a probe. The new COVID-19-nsp2 assay had a detection limit (LOD) of 1.8 TCID₅₀ mL and did not intensify any human coronavirus pathogens and respiratory viruses. The process threshold reproducibility (Cp) values have been adequate and overall imprecision (%CV) values have dropped far below 5%. The latest assay evaluation using 59 clinical samples from 14 reported cases demonstrated a 100% compliance with COVID-19-RdRp/Hel reference assay, which has been previously established. A COVID-19-nsp2, fast sensitive RT-PCR test was developed for SARS-CoV-2 [76].

8. Future perspectives of nucleic acid-based vaccines

Since COVID-19 is new to humanity and the essence of defensive immune responses is incompletely understood, it is unknown which vaccination techniques

are going to be most effective. Therefore, designing diverse vaccine platforms and methods in tandem is crucial. Indeed, researchers worldwide have been racing to produce COVID-19 vaccines since the epidemic started, with at least 166 vaccine candidates now in preclinical and clinical production (Draft landscape of COVID-19 candidate vaccines, 2020). A new pandemic vaccine developing framework has been suggested to address the immediate need for a vaccine, compacting the development period from 10 to 15 years to 1 to 2 years [77]. Recombinant plasmid DNA has been investigated as a vaccine model, although lately, mRNA has appeared as a promising platform. Six mRNA-based COVID-19 vaccines and four DNA-based COVID-19 vaccines are currently in clinical trials, with 27 such vaccines (16 mRNA-based and 11 DNA-based) undergoing preclinical production [78]. (Draft landscape of COVID-19 candidate vaccines, 2020). For protein translation and post-translational modifications, antigen-encoding mRNA encapsulated with a carrier such as lipid nanoparticles can be effectively conveyed *in vivo* into the cytoplasm of host cells, which is a plus over vaccines of the recombinant protein subunit. The mRNA vaccines are non-pathogenic and are synthesized without microbial molecules by *in vitro* transcription [79]. While no mRNA vaccine has been approved for human use yet, recent reports of influenza, rabies and Zika virus infections in animals support its promise in the covid-19 vaccine development race [80]. Plasmid DNA vaccines share many features, such as safety, ease of development and scalability, with mRNA vaccines, but with the differences of having poor immunogenic and having to be administered in several doses coupled with the addition of an adjuvant. This review provides valuable information that can be redirected to the purpose of working on these nucleic acid-based vaccines which provides a new propitious platform of vaccine production.

9. Conclusion

Coronaviruses have proven themselves to be prevailing and a very high threat to our existence by their unique features and ambiguity that caused catastrophic effects in this pandemic. It is obvious that coronaviruses have high spillover abilities and adaptation to new hosts so that enables more appearances in future. For a vaccine to be made or an anti-viral drug to be produced, it is very mandatory that all is known about the virus family, virus genome and its own central dogma, how it differs from its predecessors, their similarities, mutation rates and screening methods. A treatment that will be effective, long-lasting and prepared for any mutation is needed to be able to fight this virus and eradicate this disease and prevent the emergence of a new pandemic by this family of potential and active killers (The Coronaviruses).

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