CURRENT COMPREHENSIVE APPROACH TO COVID-19

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DUVAR

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ÖNSÖZ

Günümüzde tüm dünyayı etkilemeye devam eden COVID-19 virüsü ve yeni varyantlarının ciddi mortalite artışı, ekonomik ve sosyolojik kayıplara neden olduğu tüm sağlık kurumları tarafından kabul edilmiştir. COVID-19 pandemisi, zaman içinde öğrenmemiz gereken pek çok yönlerin ve yeni bilgilerin olduğunu bizlere fazlasıyla göstermiştir. Bazı ülkeler bu pandemi sürecinde aşı, karantina, maske, ilaç, kapanma ve seyahat ile ilgili ciddi önlemler almış ve güncel olarak sürekli gündeminde tutmuştur. Bu süreçte ülkemizde, kendi üretimimiz olan "TURCOVAC" aşısına sahip olmuş ve yeni bilimsel tecrübeler kazanmıştır. Bu sürecin ne kadar devam edeceği, başka viral salgınların çıkıp çıkmayacağı ise herkesin merak ettiği ve endişe duyduğu konu haline gelmiştir. Şunu da özellikle belirtmek isteriz ki; ülkemiz ve tüm dünya da pandemiyle mücadele de sağlık çalışanlarının rolü ve emeği çok büyük olmuştur. Sağlık çalışanları gece gündüz demeden özveriyle çok fazla emek harcamış ve halen de harcamaktadırlar. DSÖ, Sağlık Bakanlığımız, sağlık çalışanları, hastanelerimiz ve tüm bilim insanları, süreci anlamak, sonlandırmak ve refaha kavuşturmak için var güçleriyle çalışmaktadırlar. Bu amaçla hazırlanmış olan kitabımızın; gerek COVID-19 ve gerekse de gelecekte çıkabilecek yeni varyantlara karşı mücadele de tüm insanlığa bilimsel katkılar sunmasını temenni ederiz. Karamanoğlu Mehmetbey Üniversitesi Tıp Fakültesi ve diğer Tıp Fakültelerinden öğretim üyelerinin COVID-19 ile ilgili olarak özenerek hazırlamış olduğu bu kitabın, bundan sonra yapılacak çalışmalara ışık tutacağı inancındayız.

Değerli Öğretim Üyelerimizin pandemi sürecinde elde ettikleri güncel tecrübelerini katarak hazırladıkları bu kitabın, COVID-19 pandemisi hakkında hazırlanan bilimsel yayınlar içinde en önde gelenlerden biri olduğunu rahatlıkla söyleyebiliriz. Kitabın hazırlanmasında emeği geçen tüm kıymetli Öğretim Üyelerimize verdikleri katkıdan dolayı çok teşekkür ediyor, sağlıklı, mutlu ve huzurlu güzel günler diliyoruz.

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Haziran, 2022

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CONTENTS

CHAPTER 1

A COMPREHENSIVE OVERVIEW OF COVID-19'S CELL BIOLOGY EFFECT MECHANISM: VARIABLE VARIANTS

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INTRODUCTION

When I started writing this article on April 05, 2022, the number of COVID-19 cases worldwide was 485 million and the number of deaths was 6.13 million, according to WHO official results. In Turkey, the number of cases is 14.8 million and the number of deaths is 97.924 B.

Coronaviruses (CoV) are a large family of viruses that can cause more serious infections such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS), from common, self-limiting mild infections such as colds. There are various subtypes of coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63 and HKU1-CoV) that are easily transmitted from person to person. These subspecies circulating among humans are mostly viruses that cause colds. However, there are many subspecies of coronavirus detected in animals and it is known that these viruses can pass from animals to humans, causing severe diseases in humans. As a result of detailed investigations, it was revealed that SARS-CoV is transmitted to humans from musk cats and MERS-CoV from single-humped camels. SARS-CoV, was the first international health emergency of the 21st century, in 2003, in the form of a previously unknown virus (1,2).

On December 31, 2019, the World Health Organization (WHO) China Country Office reported cases of pneumonia with unknown etiology in Wuhan, Hubei Province, China. On January 7, 2020, the factor was identified as a new coronavirus (2019-nCoV) that had not previously been detected in humans. Later, the name of the 2019-nCoV disease was recognized as COVID-19, and the virus was named SARS-CoV-2 due to its close resemblance to SARS CoV. The World Health Organization classified the COVID-19 outbreak as an "international public health emergency" on January 30th and identified it as a global pandemic on March 11 due to the spread and severity of COVID-19 cases in 113 countries other than China, where the first outbreak began. In our country, COVID-19-related studies started in 2020, on January 10th and on January 22nd. the first meeting of the Scientific Advisory Board of the Ministry of Health was held, and with the measures taken, the first case of COVID-19 was seen on March 11th after our neighboring countries such as Europe and Iran (3).

This book section covers the origin, cellular structure, cell binding and mechanism of action, pathogenesis, and phylogeny of COVID-19. At the end of the section, information is provided about the classification, naming, and countries where COVID-19 variants are seen.

1. GENERAL INFORMATION

A new coronavirus (nCoV), designated "SARS-CoV-2" by the World Health Organization (WHO), emerged in Wuhan (China) in December 2019. Reported as the spread and cause of COVID-19 (4,5). The incidence of SARS-CoV (Severe Acute Respiratory Syndrome-coronavirus) and MERS-CoV (Middle East Respiratory Syndrome-coronavirus) in 2002 and 2003 (6). A total of seven human coronaviruses (HCoVs) have now been discovered, including HCoV229E, HCoV-OC43, HCoV-NL63, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2. Over the past two decades, SARS-CoV and MERS-CoV have caused epidemics with mortality rates of approximately 9.5% and 34.4%, respectively (7).COVID-19 was the third extremely epidemic outbreak. The disease and MERS to detect with a lower death rate than SARS differ from country to country. According to WHO statistics, there are 45.678.440 (November 1, 2020) confirmed cases of SARS-CoV-2 (8). İn 219 countries due to its high transmission capacity. Therefore, characterizing acute infection in humans as a result of SARS-CoV-2, scientists and governments urgently took decisive action to monitor and conduct the epidemic through etiological research (9).

With the developments in molecular technologies, different viable vaccines have been developed (Biontech, Moderna, Sinovac, and Turcovac, etc.) (10,11). Higher transmissibility, diverse clinical manifestations, and lower pathogenicity of COVID-19; may be due to the diversity in the biology and genome structure of the virus. This has been compared with SARS-CoV-2 by comparing SARS-CoV

and MERS-CoV(12,13). This section comprehensively covers the biology of the virus and the characteristics of the variants for effective prevention and reduction of COVID-19 mortality.

Shortly after the outbreak of COVID-19, in January 2020, the virus was isolated from samples taken from the lungs of patients in Wuhan, and genome analysis was performed. First, on January 12, 2020, the genome sequencing of SARS-CoV-2 was shared by Chinese researchers in open databases accessible to researchers from all over the World (14). Rapid identification and sharing of genome sequences of SARS-CoV-2; understanding the structure of the virus and the mechanism of disease creation has been of great benefit in terms of determining diagnostic methods, monitoring the ongoing outbreak, and developing potential treatment options.

2. GENOMICS STRUCTURE AND BIOLOGICAL FEATURES OF SARS-COV-2

Coronaviruses belonging to the order Nidoviridales are in the family coronaviridae. They are divided into two subfamilies Coronavirinae and Torovirinae. The Coronavirinae subfamily is divided into four genera: Alpha, Beta, Gamma, and Deltacoronavirus (15). Phylogenetic analysis results revealed that SARS-CoV-2 is closely related to beta-coronaviruses. The genome of SARS-CoV-2 is similar to other coronaviruses as positive-sense single-stranded RNA [(+) ssRNA] with a 5' cap, 3'-UTR poly(A) tail. The SARS-CoV-2 genome is less than 30 kb in length and contains 14 open reading frames (ORFs), spike (S), envelope (E), and membrane/matrix encoding non-structural proteins (NSPs) for virus replication and assembly. processes. (M) and structural proteins, including nucleocapsid (N) and accessory proteins **(Figure 1)** (16-18). The initial ORF contains approximately 65% of the viral genome and is converted to the polyprotein pp1a (nsp1-11) or pp1ab (nsp1-16). Among them, six NSPs (NSP3, NSP9, NSP10, NSP12, NSP15, and NSP16) play crucial roles in viral replication. Other ORFs encode structural and accessory proteins (19,20). The S protein is a transmembrane protein that facilitates the binding of the viral envelope to angiotensin-converting enzyme 2 (ACE2) receptors expressed on host cell surfaces. Functionally, spike protein consists of receptor binding (S1) and cell membrane fusion (S2) subunits (21). The N protein binds to the viral genome and is involved in RNA replication, virion formation, and immune evasion. The nucleocapsid protein also interacts with the nsp3 and M proteins (22). The M protein is one of the most abundant and best-conserved proteins in the virion structure. This protein interacts with N and accessory proteins, 3a and 7a, promoting viral particle formation and budding (23,24). The E protein is the smallest component in the SARS-CoV-2 structure associated with production, maturation, and release by virions (19).

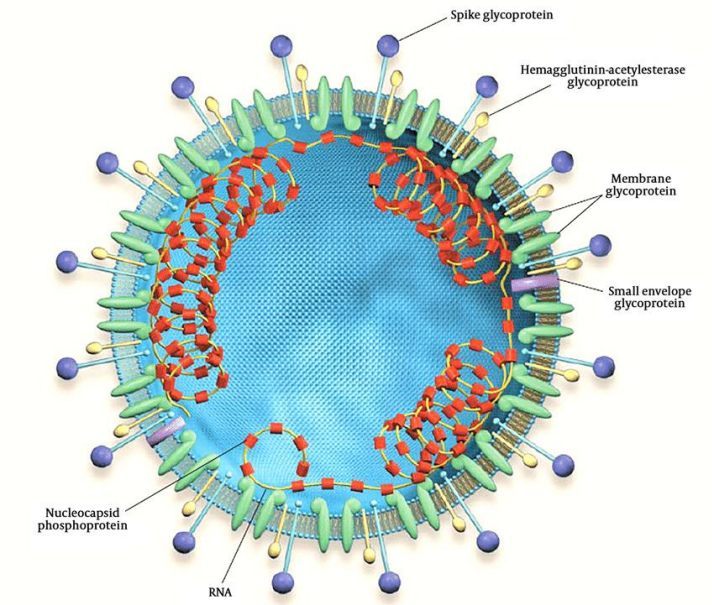


FIGURE 1 Schematic diagram of the Coronavirus structure (doi: 10.5812/archcid.102624) (18).

In the coronavirus genome, the receptor binding domain(RBD) found in the spike protein is known for its most complex structure (25,26). Six RBD amino acids are required for binding to the ACE2 receptor and for harboring SARS-CoV-like coronaviruses. The SARS-CoV and SARS-CoV-2 genetic sequences are quite different from each other when compared. SARS-COV has sequences Y442, L472, N479, D480, T487, and Y4911. SARS-CoV-2 has sequences L455, F486, Q493, S494, N501, and Y505 (27). The human-derived genome sequences of the SARS-CoV strain are quite similar to those found in bats. The dissimilar parts show differences between the gene sequences of the S gene decoding the binding, fusion, and replication proteins and the ORF3 and ORF8 gene sequences, respectively (28). MERS-CoV strains (specifically derived from Camels) have also been proven to be the same, except for differences between the human-decanted genomic regions S, ORF4b, and ORF3 (29). Laboratory studies based on genome sequencing phylogenetically associate the human-CoV strain with bats. As for the S-protein, the strain has a similar genome and protein (30). For recombinations of orf1ab and S-coding genes, up to hundreds of genetic elements have been deciphered among coronaviruses of MERS-CoV, camels, and bats. For this, as a result, SARS-CoV-2 and SARS-CoV, in general, are 96% similar (30,31). ACE protein is found in the tissues of many mammals. The main places where it is located are the lungs, kidneys, gastrointestinal tract, heart, liver, and blood vessels (32).

ACE2 receptors are vital in regulating the renin-angiotensin-aldosterone system pathway. As a result of molecular and biochemical experiments, in SARS-CoV2, homologous receptors of ACE2; appear to have enhanced potent RBD in humans, cats, ferrets, and other organisms (33). In January, the genome sequence of SARS-CoV-2 was 96% with the bat coronavirus (BatCoV) RaTG13 genome of 2020, and SARS-CoV It has been observed that it is 80% identical to the CoV genome (34). The amino acid structures seen as the difference between them are as follows. For example, the protein 8a sequence in the SARS-CoV genome is absent in 2019-nCoV, and the protein 8b sequence of SARS-CoV-2 is 37 amino acids longer than that of SARS-CoV. The CoV genome shows that non-structural and structural proteins are 60% and 45% identical, respectively, among the various CoV strains (35). These data suggest that nsps are more conservative than structural proteins. RNA viruses have a higher mutational burden as a result of their shorter replication times **(Figure 2)** (36). Comparative genomic studies have identified 380 amino acid substitutions in the nsps genes and 27 mutations in the genes encoding the spike protein S of SARS-CoV-2 between SARS-CoV-2 and SARS-like coronaviruses. This variation may explain the difference between SARS-CoV-2 to SARS-CoVs (37). The primary N501T mutation in the Spike protein of SARS-CoV-2 is a good example that increases its binding affinity to ACE2 (38).

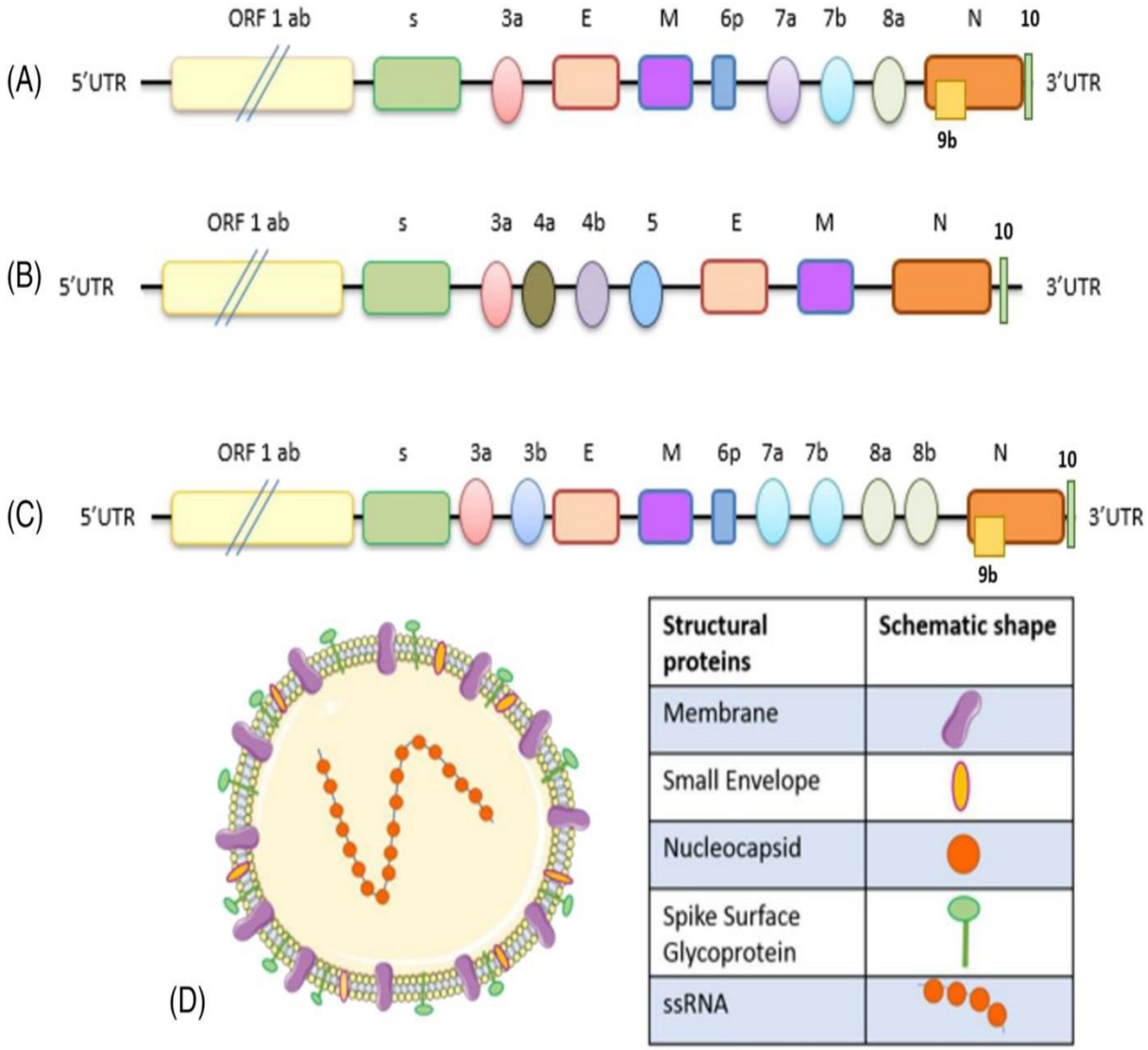


FIGURE 2 Schematic genomic structure of the coronavirus. (a) COVID-19. (b) MERS-CoV. (c) SARS-CoV. (d) Coronaviruses encode structural proteins, spike, envelope, membrane, and nucleocapsid genes. Four structural genes (3a, 3b, 6, 7a, 7b, 8b, 9b and ORFs), including auxiliary proteins (https://doi.org/10.1002/jgm.3303) (36).

2.1. Spike Glycoprotein

The entry of coronaviruses into host cells is mediated by spike glycoprotein (S protein) (39-41). The transmembrane spike glycoproteins form homotrimers that protrude from the viral surface. The spike glycoprotein is crucial for the entry of coronaviruses and therefore an attractive antiviral target. The S protein consists of two functional subunits, including the S1 and S2 subunits. The S1 subunit consists of the N-terminal domain (NTD) and the receptor-binding domain (RBD). The function of the S1 subunit is to bind to the receptor on the host cell. The S2 subunit contains fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic tail (CT) **(Figure 3A)**(42). The function of the S2 subunit is to fuse the membranes of viruses and host cells. The dividing region at the boundary between sub-units S1 and S2 is called the S1/S2 protease dividing region. For all coronaviruses, host protease breaks down spike glycoprotein in the S2' dividing region to activate proteins critical for fusing the membranes of viruses and host cells through irreversible conformational changes. N-bound glycans are critical for proper folding, neutralization of antibodies, and comprehensive decor of spike protein trimers (43,44).

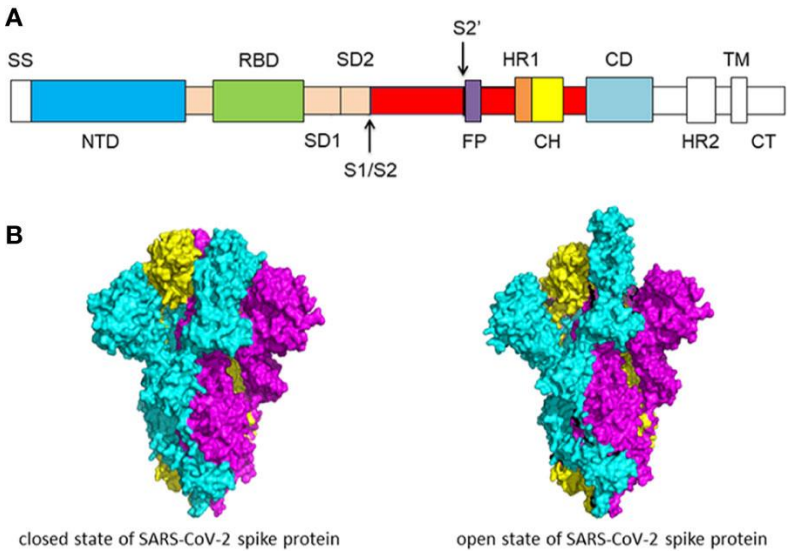


FIGURE 3 (A) Schematic of SARS-CoV-2 spike protein primary structure. Different domains are shown in different colors. (B) Cryo-EM structure of the SARS-CoV-2 spike protein. The closed state of the SARS-CoV-2 S glycoprotein (left) and the open state of the SARS-CoV-2 S glycoprotein (right). (https://doi.org/10.3389/fcimb.2020.587269) (42).

Overall, the structure of the SARS-CoV-2-S protein is similar to the closely related SARS-CoV-S protein. In prefusion conformation, the S1 and S2 subunits do not remain covalently bound. Different types of coronaviruses use special domains in the S1 subunit to recognize different entry receptors. In the case of SARS-CoV and SARS-CoV-2, they recognize the receptor angiotensin-converting enzyme 2 (ACE2) on host cells via the receptor-binding domain (RBD) to penetrate host cells. The S protein has two structural forms, including the closed state and the open state **(Figure 3B)**(42). In the closed state, the three recognition motifs do not protrude from the interface formed by three spike protein protomers. In the open state, the RBD is in the "top" conformation. The open state is necessary for the fusion of SARS-CoV-2 and the host cell membranes, which facilitates SARS-CoV-2 entry into the host cells (43).

2.2. HR1 and HR2

HR1 and HR2 are formed by six helical bundles (6-HB). This structure is very important for membrane fusion dominated by the SARS-CoV or SARS-CoV-2 spike protein. This makes HR1 and HR2 an attractive drug targets (45,46). The difference between the 6-HB of SARS-CoV-2 and SARS-

CoV can stabilize the 6-HB conformation of SARS-CoV-2 and enhance the interactions between HR1 and HR2, resulting in increased infectivity of SARS-CoV-2. The HR1-L6-HR2 complex contains most of the parts of the HR1 and HR2 domains and a linker (47). This fusion protein has a rod-like shape and is the standard structure of 6-HB. Three HR1 domains come together to form a spiral ball trimer in parallel. Three HR2 domains are intertwined antiparallel around the coiled-coil center, which is mainly mediated by hydrophobic forces. Hydrophobic residues on the domain of HR2 bind to the hydrophobic furrow, which is formed by two adjacent HR1 helices each. The 6-HB forest of SARS-CoV and SARS-CoV-2 is very similar, especially the S2 subunit (47). The identity of the HR1 of SARS-CoV and SARS-CoV-2 is 96% and HR2 100%. There are eight distinct remnants in the core fusion region of the HR1 domain. In the HR1 domain of SARS-CoV, lysine 911 binds to glutamic acid 1176 in the HR2 domain via a salt bridge. In SARS-CoV-2, the salt bridge is replaced by a strong hydrogen bridge between serine 929 in HR1 and serine 1,196 in HR2. In SARS-CoV HR1, glutamine 915 has no interaction with HR2. However, in SARS-CoV-2, there is a salt bridge between lysine 933 in HR1 and asparagine 1,192 in HR2 (47). In SARS-CoV, HR1 has a weak salt bridge between glutamic acid (918) and arginine (1,166). However, aspartic acid (936) and arginine (1,158) in HR1 of SARS-CoV-2 use a salt bridge pathway. In SARS-CoV, lysine (929) binds to glutamic acid (1.163) at the HR2 domain via a salt bridge. It does not bind to threonine (925) and glutamic acid (1.163). Also, serine (943) and lysine (947) in SARS-CoV-2 are linked to glutamic acid (1,182) in HR2 via a hydrogen bond and a salt bond. The increased contagiousness of SARS-CoV-2 is due to these differences (47).

2.3. The Receptor Binding Domain (RBD)

An RBD segment that specifically recognizes the ACE2 receptor is found in the spike protein of SARS-CoV-2. Antiviral compounds and antibodies bind from the RBD region (48). There are two structural domains in SARS-CoV-2 RBD: core and outer subdomains. The core subdomain is heavily protected. It has five antiparallel β-strands and a disulfide bridge between two β-strands. The outer subdomain is controlled by the loop stabilized by the disulfide bond (49). The SARS-CoV-2 RBD core consists of five antiparallel β leaflets interconnected by loops and short helices. Between the antiparallel β4 and β7 strands is the receptor-binding motif (RBM), which consists of short β5 and β6 strands, as well as loops and α helices. RBM contains most of the binding sites for SARS-CoV-2 and ACE2. Eight of the nine Cys residues in RBD form four double disulfide bonds. The core of the RBD has three disulfide bonds (C336-C361, C379-C432, and C391-C525) that increase the stabilization of the β leaflet. Regarding the remaining disulfide bond (C480-C488), it establishes the link between the loops in the RBM. The peptidase domain at the N-terminus of ACE2 contains the binding site formed by the two lobes of RBM and ACE2. The RBM is interconnected with the lower lobe of ACE2. The surface of the RBM is slightly concave for binding, leaving no room for ACE2 (50).

A study of spike protein trimmer with an RBD in the "up" conformation (recipient accessible state) also revealed a 3,5 Å resolution construct. When there is receptor binding, the prefusion structure destabilizes. The S1 subunit dissociates and the S2 subunit folds back into a stable post-fusion conformation captured in SARS-CoV. RBD undergoes conformational transitions like a hinge. This causes determinants of the spike protein to be either hidden or exposed to attack a host cell receptor. This process will create two states: the "lower"-conformation and the "upper"-conformation. In the "down" conformation, SARS-CoV-2 failed to recognize ACE2 in host cells. The structure of SARS-CoV-2 is very similar to that of SARS-CoV. One of the bigger differences lies in the inferior conformation. The angle of SARS-CoV-2-RBD is close to the central cavity of the spike protein corrector. SARS-CoV-2 and SARS-CoV are very similar in the alignment of the corresponding individual structural domains (44). The overall structure of the SARS-CoV-2 RBM is the same as in other studies, except for only one difference observed at the distal end (50).

2.4. RBD-ACE2 Complex

Understanding the receptor recognition mechanism of SARS-CoV-2; is an important marker that determines the infectivity, host range, and pathogenesis of the virus. In humans, ACE2 is recognized by both SARS-CoV-2 and SARS-CoV (39,40,51). The crystal structure of SARS-CoV-2 RBD bound to ACE2 was determined **(Fig. 4A)**(42). The general combination mode of the SARS-CoV-2 RBD-ACE2 complex is highly similar to the SARS-CoV RBD-ACE2 complex identified in the previous work. Of the 20 residues of ACE2 that interact with the RBD of SARS-CoV and SARS-CoV-2, 17 are identical.

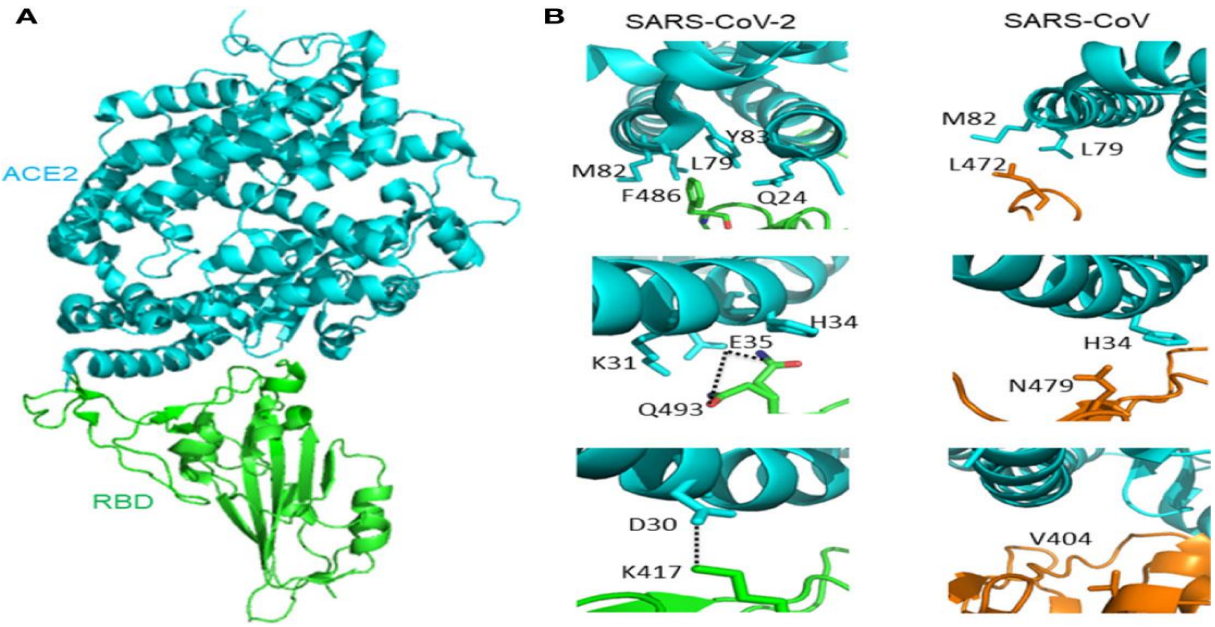


FIGURE 4(A) The structure of SARS-CoV-2 RBD bound with ACE2. (B) Different interactions between SARS-CoV-2 RBD/ACE2 and SARS-CoV RBD/ACE2 contribute to binding affinity differences. (https://doi.org/10.3389/fcimb.2020.587269) (42).

Still, subtle, distinct ACE2 interactions are known that lead to differences in binding affinity from SARS-CoV-2 and SARS-CoV RBD to ACE2. The affinity between ACE2 and SARS-CoV-2 is high. The affinity between ACE2 and SARS-CoV is low. At the F486/L472 position, SARS-CoV-2 interacts with F486, ACE2 Q24, L79, M82, and Y83. SARS-CoV L472 interacts only with ACE2 L79 and M82. SARS-CoV N479 interacts only with ACE2 H34. Outside of the SARS-CoV-2 RBM, there is a salt bridge between ACE2 D30 and SARS-CoV-2 K417 **(Fig. 4B)**. However, SARS-CoV V404 failed to participate in ACE2 binding (42,50,51).

Studies reveal the crystal structure of the SARS-CoV-2 RBD-ACE2 chimeric complex. It uses the SARS-CoV-2 RBM as a functional unit and as a crystallization scaffold. Genetically engineered chimeric RBDs containing the SARS-CoV RBD core may facilitate crystallization. Thus, the SARS-CoV-2 side ring (away from the main binding site) provides a salt bridge between RBD R426 and ACE2 E329. This side circuit further improves crystallization. The structure of the chimeric RBD-ACE2 complex is very similar to the wild-type RBD-ACE2 complex presented above, particularly in the RBM region. There is an N-O bridge between R439 of the chimeric RBD and E329 of ACE2. The N-O bridge is non-natural, resulting from the SARS-CoV-based chimera. The binding affinity between chimeric RBD and ACE2 is higher than the binding affinity between wild-type SARS-CoV-2 RBD and ACE2. The ACE2-binding affinity of SARS-CoV RBD is lower than SARS-CoV-2 and chimeric RBDs (52).

2.5. Furin Cleavage Site of the Spike Protein

The S1/S2 boundary of the SARS-CoV-2 spike protein forms the cleavage site for the subtilisin-like host cell protease furin, which distinguishes SARS-CoV-2 S from SARS-CoV S. The furin cleavage site contains four residues. P681, R682, R683, and A684. It is located at the boundary between the S1 and S2 subunits. Functionally, R682, R683, A684, and R685 form the minimally polybasic furin cleavage site RXYR, where X or Y is a positively charged arginine or lysine (53). Such polybasic cleavage sites are absent in human SARS-CoV and SARS-CoV-related group 2b beta coronaviruses; this may contribute to the high virulence of SARS-CoV-2 as a result of furin proteases required for proteolytic activation of S. It is ubiquitously expressed in humans, conferring extended tissue tropism and pathogenesis (54).

2.6. The RNA-Dependent RNA Polymerase (RdRp)

SARS-CoV-2 replication is dominated by the multi-subunit replication/transcription complex. The complex consists of viral nonstructural proteins (nsp). The core of the complex is RdRp in nsp12. The functions of nsp12 require additional factors including nsp7 and nsp8. Nsp12 alone has little activity. The presence of nsp7 and nsp8 significantly increased the combination of nsp12 and template primer RNA. The crystal structure of the nsp12-nsp7-nsp8 complex has been identified. The RNA-dependent RNA polymerase, which catalyzes viral RNA synthesis, is a critical component of coronavirus replication/transcription. RdRp is an important antiviral drug target. The constructs on SARS-CoV-2 nsp12 contain a norovirus-unique N-terminal extension domain containing a nidovirus-RdRp-associated nucleotidyltransferase (NiRAN) construct and a "true" RNA-dependent RNA polymerase domain at its C-terminus assumes These two domains are connected by an interface domain. A unique β-hairpin is observed in the region of the N-terminal extension. The β hairpin creates a tight stance to stabilize the overall structure. The RNA-dependent RNA polymerase domain includes three subdomains: 1. finger subdomain, 2. palm subdomain, and 3. thumb subdomain. The β-hairpin structure fits into the compression groove formed by the palm base and NiRAN area. In the Plam domain, the highly conserved polymerase motifs A-G form the active site chamber of the SARS-CoV-2 RdRp domain. RdRp motifs mediate the synthesis of template RNA in a central cavity via four positively charged, solvent-accessible pathways, including the template entry pathway, the primer entry pathway, the NTP entry channel, and the nascent strand exit pathway(55). In a recent study, the cryo-electron microscopic structure of the nsp12-nsp7-nsp8 complex is observed in its active form (56).

When a minimal RNA hairpin substrate is added, the nsp12-nsp7-nsp8 complex exhibits RNA-dependent RNA elongation activity. The structure of the RdRP-RNA complex consists of the nsp12-nsp7-nsp8 complex linked by more than two rounds of duplex RNA. RdRp RNA structure is similar to a free enzyme with some unique features. Compared to the free enzyme, the RdRp-RNA complex contains a long protein region and an overhanging RNA in nsp8. The nsp12 subunit binds to the first round of RNA between thumb subdomains and finger subdomains. The Palmar subdomain contains the active site formed by the five nsp12 motifs AE. Motif C interacts with the 3' end of RNA and contains 760 and 761 aspartic acids. nsp12 motifs F and G are located in the finger subdomain. It also has the function of positioning the RNA template. When the RNA duplex leaves the RdRP cleft, it forms a second helical turn protruding from the nsp12 surface. No structural factors in RdRP limit RNA duplex elongation. Between the α-helical extensions is the RNA duplex. The highly conserved N-terminal regions in the two nsp8 subunits form α-helical extensions. These nsp8 extensions use positively charged residues to interact with RNA backbones. Nsp8 can act as "sliding poles" that slide along the overhanging RNA to prevent RdRP from dissociating prematurely during replication. The triphosphate binding site is preserved. Residues D623, S682, and N691 probably interact with the 2'-OH group of the triphosphate (NTP). Thus, they make RdRP specific for RNA synthesis, not DNA (57).

2.7. The Main Protease

The main protease (M^{pro}) of SARS-CoV-2 plays a crucial role in viral gene replication and transcription. M^{pro} hydrolyzes at least 11 conserved regions of the polyprotein. It begins with the breakdown of pp1a and pp1b of M^{pro}. With the functional importance of the main protease in the life

cycle of the virus and the absence of this part in humans, the main protease functions as an attractive antiviral target. Crystallographic symmetry indicates that M^{pro} forms a homodimer (protomer A and protomer B). Each protomer contains three subdomains, domain I, domain II, and domain III. A long loop connects area II and area III. The cleft between domain I and domain II is located in the substrate-binding pocket containing His41 and Cys145 catalytic dyad residues (58). As for all coronaviruses, the active sites of M^{pro} are highly conserved and consist of four sites: S1', S1, S2, and S4. At the S1' site, a thiol of cysteine anchors the inhibitors with a covalent bond. For inhibitors, covalent bonding is critical to maintaining its antiviral activity (59).

The spike protein is critical in the process of invading SARS-CoV-2 host cells. The main protease and RdRp have important functions in the replication of SARS-CoV-2. In conclusion, spike protein, major protease, and RdRp are important anti-SARS-CoV-2 drug targets and provide ideas for the development of antibodies, drugs, and vaccines.

3. PATHOGENESIS OF SARS-COV-2

The introduction of SARS-CoV-2 into host cells and the release their genomes into target cells depends on some steps. It uses the protein spike, which is important to assess virus, tropism, and virus transmission. In addition, SARS-CoV-2 targets even human respiratory epithelial cells with ACE2 receptors, this indicates an RBD structure similar to SARS-CoV(60). After the binding of S1-RBD to the ACE2 receptor, host cell surface proteases such as TMPRSS2 (transmembrane cool protease 2) affect a critical dividing region in S2.38 This results in membrane fusion and viral infection. Following the introduction of the virus, uncoated genomic RNA is converted into polyproteins (pp1a and pp1ab) and then combined into replication/transcription complexes with virus-induced double-membrane vesicles (DMVs). Next, this complex replicates and synthesizes a nested sub-set of genomic RNA through genome transcription, which encodes structural proteins and some auxiliary proteins. Newly formed virus particles are combined by mediating the endoplasmic reticulum and Golgi complex. Finally, virus particles are budding and released into the out-of-cell media compartment. Thus, both the viral replication cycle and the progression begin (61).

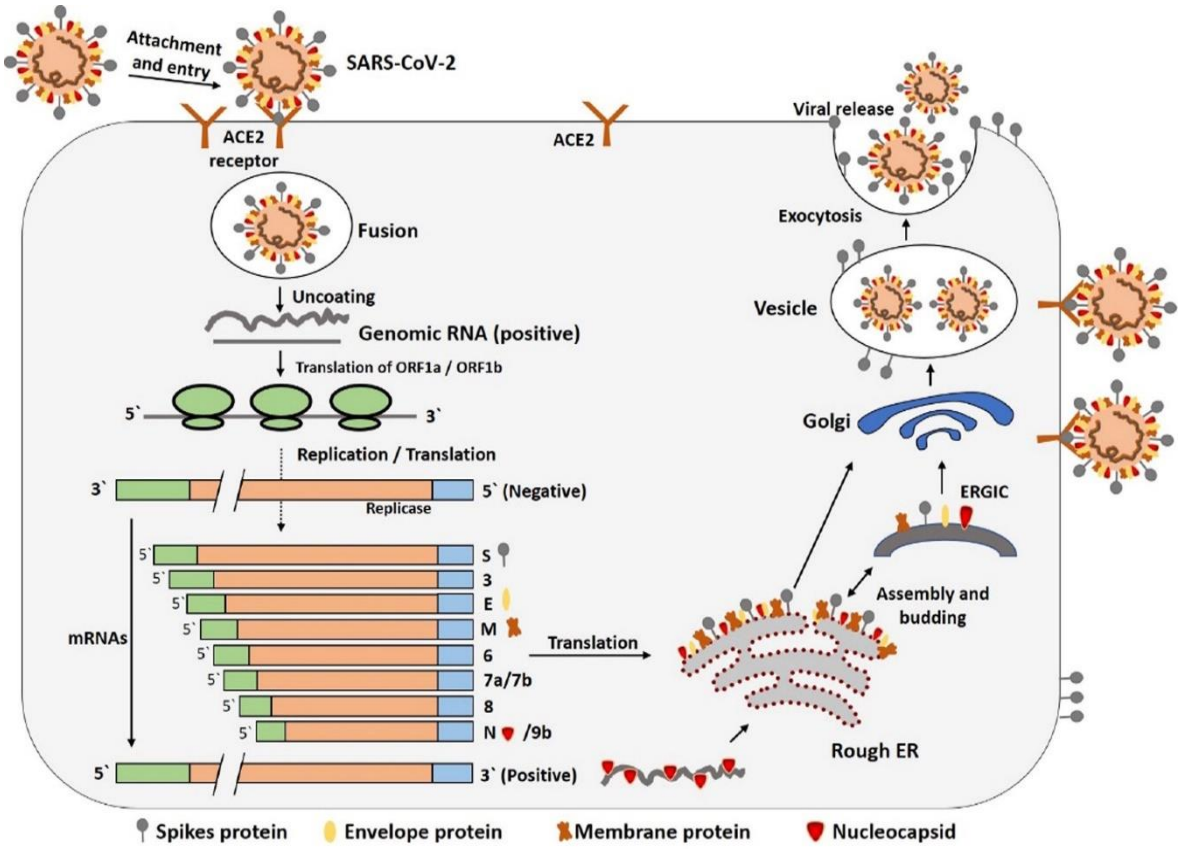


FIGURE 5 The life cycle of SARS-CoV-2 in host cells (https://doi.org/10.1016/j.jare.2020.03.005). (65)

The life cycle of the SARS-CoV-2 virus consists of four steps. SARS-CoV-2 binds to angiotensin-converting enzyme-2 (ACE2) via the spike (S) protein on the outer surface and allows SARS-CoV-2 to enter and infect cells. SARS-CoV-2 uses the TMPRSS2 protease to complete its entry into the cell (62). After the S protein binds to the receptor, its structure changes, making it easier for the virus to fusion into the cell and unsheathing, and the SARS-CoV-2 RNA is released inside the cell. The RNA is then translated into viral replicase polyproteins and broken down into smaller fragments by viral proteinases. As a result of continuous transcription by the polymerase chain reaction, a series of mRNAs is produced and translated into viral proteins. Viral proteins and the RNA genome assemble within virions in the endoplasmic reticulum and Golgi body and are excreted out of the cell (63). SARS-CoV2 is a combination of bat SARS-CoV and an unknown β-CoV virus, the S protein of SARS-CoV2 that helps protect Van der Waals bonds. contains the 3D receptor binding portion (Receptor Binding domain RBD)(64,65)(Figure 5).

Inside host cells, the survival of SARS CoVs is protected by multiple strategies to evade the host immune mechanism, which can be generalized to SARS-CoV-2. From the first step of SARS-CoV infection, they are not recognized by model recognition receptors of the host immune system (26). Nsp1 can block interferon (IFN)-I responses through various mechanisms such as silencing the host translation system, inducing host mRNA corruption, and suppression of transcription factor signal transducer and transcription (STAT)1 phosphorus. Nsp3 antagonizes interferon and cytokine production by blocking phosphorylation of interferon editing factor 3 (IRF3) and blocking the nuclear factor-cappa B (NF-EB) signal pathway. NSPs 14 and 16 collaborate to create a viral 50 titles similar to the hosts. Therefore, the viral RNA genome is not recognized by immune system cells (66). ORF3b and ORF6 auxiliary proteins can disrupt the IFN signaling pathway by inhibiting IFNβ expression due to IRF3 and NF-KB respectively and blocking the JAK-STAT signaling pathway. In addition, the IFN signal is flattened by structural proteins M and N, which causes a deterioration in tank connector kinase 1 (TBK1)/IKB kinase

ε and TRAF3/6-TBK1-IRF3/NF-EB/AP1 signals (23,67). Since the D614 G mutation is found in the virus's outer spike protein, this attracts a large amount of attention from the human immune system and therefore can impair SARS-CoV-2's ability to prevent vaccine-induced immunity. Although the D614 G is involved in the interaction between the hydrogen bond and separate spin mp protomers that regulate mature trimeric forms on the virion surface, it is not in RBD (68). Korber et al. reported that the SARS-CoV-2 variant in the D614 G spike protein was effective worldwide. Clinical and in vitro evidence, Although D614 G indicates that the virus alters phenotype, the effect of the mutation on replication, pathogenesis, vaccine, and therapy development is relatively unknown (69). From in vitro and clinical evidence, it is not clear whether D614 G has a different phenotype from D614 G, but whether this is the result of verified adaptation to human ACE2, as well as whether it increases contagion or has a significant impact (69).

4. SYMPTOMS AND DIAGNOSIS OF THE DISEASE

Fever, cough, severe muscle pain, increased mucus production, shortness of breath, sore throat, loss of smell and headache are common clinical findings in people diagnosed with COVID-19 (71). The patient's age, immunity status, and chronic diseases (chronic obstructive pulmonary disease, diabetes, hypertension, etc.) cause more close observation of these symptoms. It can also lead to serious and sudden clinical manifestations such as acute respiratory failure, septic shock, or clotting disorder (70). But in addition to symptomatic cases, asymptomatic cases are also high in the COVID-19 pandemic. Pandemic and molecular diagnostic procedures have shown studies that confirm this (71). The most effective and valid way to combat the COVID-19 pandemic is to control the spread of the virus as much as possible. This is possible with diagnostic methods that make it possible to identify all symptomatic and/or asymptomatic people who carry the virus as quickly and accurately as possible. Nucleic acid amplification methods (real-time polymerase chain reaction, RT-PCR), serological tests, and computed tomography (CT) findings are commonly used methods for laboratory diagnosis of COVID-19 worldwide (72-74). In addition, some difficulties and limitations in existing diagnostic methods in the COVID-19 pandemic; have increased interest in alternative and innovative techniques, especially CRISPR-based diagnostic approaches.

5. GENERAL SEQUENCE CHARACTERISTICS OF 2019-NCOV COMPARED TO SARS-COV

Studies have found that SARS-CoV-2 is quite similar to SARS CoV at the SARS-CoV-2 amino acid level, but in some shape 3 proteins in proteins 8a, 8b, and 3b. All genome-based sequence analysis shows that SARS-CoV-2 is closer to SARS-like bat CoVs than SARS CoVs. At the entire genome level, SARS-CoV-2 is less genetically similar to SARS-CoV (70%) and MERS-CoV (50%) but shares an 87.23% sequence similarity with Bat-SL-CoVZXC2 and an 87.99% sequence similarity to bat-SL-CoVZC45. At the protein level, the lengths of most proteins encoded by SARS-CoV-2 and bat-SL-CoVZC45, and bat-SL-CoVZXC21 are similar with only a few minor insertions and deletions (75).

Spike glycoprotein is responsible for the penetration of viruses into host cells by forming spikes on the surface of coronaviruses. The receptor-binding area (RBD) in the spike glycoprotein molecule directly binds the receptors on the surface of the host cells. 2019- nCoV genomics sequence can be accessed from the Gen Bank database (MN908947.3). DNA and protein sequences are compared with the BLAST program. Data were determined for the percentage of coverage and sequence similarity percentage for the entire genome and coding region of Spike glycoprotein and the RBD of this glycoprotein. The closest homolog of 2019-nCoV is sars-like coronavirus isolated from the bat (MG772933.1) with a sequence similarity of 99%/87.99% (76). GenBank access numbers are AY274119 for SARS, AY525636 for SARSv, AY304486 for musk cat, AGZ48806.1 for bat, and MN908947.3 for 2019-nCoV (76).

6. THE ORIGIN AND EVOLUTION OF SARS-COV-2

Bioinformatics analyzes say that the SARS-CoV-2 coronavirus family has features unique to the Betacoronavirus 2B strain (77). Early in the pneumonia epidemic in Wuhan, scientists obtained complete genome sequences from five patients infected with SARS-CoV-2. These genome sequences are 79.5% similar to SARS-CoV. Therefore, it appears that there is a new beta coronavirus infecting humans (78). Again, genetic sequencing studies show the closest association of SARS-CoV-2 with the bat SARS-like coronavirus strain BatCov RaTG13 with 96% identity. These studies suggest that SARS-CoV-2 may originate from bats and that SARS-CoV-2 may have evolved naturally from the bat coronavirus RaTG13 (79,80).

In a study, Xiong C. et al. analyzed the genomes of SARS-CoV-2 and similar isolates from GISATD and NCBI. The results compared the different genetic distances of the genome of isolation numbered EPI_ISL_403928 and different phylogenetic trees of the entire length. They found the difference between spike protein (S), nucleoprotein (N), and polyprotein (P) coding sequences from other SARS-CoV-2. 4, 2, and 22 variations were found in S, N, and P at the level of amino acid residues, respectively. These results indicate that at least two SARS-CoV-2 strains were involved in the outbreak (81).

After encoding sequences (CDSs) are aligned based on protein alignments, SARS-CoV-2's open reading frame 8 (ORF8) and open reading frame 10 (ORF10) are different from other viruses. However, most ORFs with annotations from SARS-CoV2 are preserved. The general genomic nucleotide identification between SARS-CoV-2 and SARS-like coronavirus strain BatCov RaTG13 is 96%. Compared to other viruses, SARS-CoV-2 differs in the neutral zone by 17%, much more than previously evaluated. The Spike gene exhibits larger dS (synonymous substitutes per synonym region) values than other genes; this can be due to natural selection or a high mutation rate, which accelerates synonymous substitutes. Researchers obtained 103 SARS-CoV-2 genomes to recognize genetic variants (82). A total of 149 mutations have been identified among the 103 strains, and population genetic analysis shows that these strains are mainly divided into two types. The results show that 101 of the 103 SARS-CoV-2 strains showed significant connectivity between two single nucleotide polypeptides (SNPs). The main SARS-CoV-2 types (type L and type S) are distinguished by two SNP regions, 8,782 and 28,144. Type L accounts for 70% of 103 strains and 30% of type S, suggesting that type L is more common than type S. But type S is the ancestral version of SARS-CoV-2 (82).

In early 2020, 13 mutations in the Spike protein were identified. The D614G mutation is a special mutation. In early February 2020, the Spike D614G mutation began to spread predominantly in Europe (83). One study provides evidence that the D614G mutation in the SARS-CoV-2 spike protein may be increased, showing that in multiple cell lines, SARS-CoV-2 carrying the D614G mutation is eight times more effective at transforming cells than the wild-type spike protein. Transduction of multiple human cell types (84). The D614G mutation may also reduce neutralization sensitivity to the serum of convalescent COVID-19 patients (85)

Bats appear to be the natural reservoir of SARS-CoV-2 (86,87). In one study, betacoronavirus isolated from pangolins has several similarities of up to 99% with the already infected human strain (45). Another study shows that the coronavirus obtained from SARS-CoV-2 and a pangolin in Malaysia have high genetic similarity. Gene similarity between these two viruses in terms of genes E, M, N, and S is 100%, 98.6%, 97.8%, and 90.7%, respectively, suggesting the potential of pangolins to be intermediate hosts (46). Dogs, chickens, ducks, and pigs from animals in close contact with humans are not susceptible to infection. SARS-CoV-2 reproduces efficiently in cats and ferrets (88). SARS-CoV-2 can also be transmitted in the golden hamster (51).

7. SARS-CoV-2 VARIANT CLASSIFICATIONS AND DEFINITIONS

Coronaviruses belongs to the subfamily Coronavirinae in the family of Coronaviridae and the subfamily contains four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (15). **Viruses like SARS-CoV-2 continuously evolve as changes in the genetic code (genetic mutations) occur during the replication of the genome**. A lineage is a genetically closely related group of virus variants derived from a common ancestor. A variant has one or more mutations that differentiate it from other variants of the SARS-CoV-2 viruses. To inform local outbreak investigations and understand national trends, scientists compare genetic differences between viruses to identify variants and how they are related to each other (89,90).

- **Mutation:** A mutation refers to a single change in a virus's genome (genetic code). Mutations happen frequently, but only sometimes change the characteristics of the virus.
- **Lineage:** A lineage is a group of closely related viruses with a common ancestor. SARS-CoV-2 has many lineages; all-cause COVID-19.
- **Variant:** A variant is a viral genome (genetic code) that may contain one or more mutations. In some cases, a group of variants with similar genetic changes, such as a lineage or group of lineages, may be designated by public health organizations as a **Variant of Concern (VOC)** or a **Variant of Interest (VOI)** due to shared attributes and characteristics that may require public health action (91,92).

Many new variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been termed *variants of concern/interest* (VOC/I) because of the greater risk they pose due to possible enhanced transmissibility and/or severity, immune escape, diagnostic and/or treatment failure, and reduced vaccine efficacy. Key Points:

- Ggenetic lineages of SARS-CoV-2 have been emerging and circulating the world since the beginning of the COVID-19 pandemic.
- SARS-CoV-2 genetic lineages in the United States are routinely monitored through epidemiological investigations, virus genetic sequence-based surveillance, and laboratory studies.
- On November 30, 2021, the U.S. government SARS-CoV-2 Interagency Group (SIG) classified Omicron as a Variant of Concern (VOC). This classification was based on the following:
    - Detection of cases attributed to Omicron in multiple countries, including among those without travel history.
    - Transmission and replacement of the Delta variant in South Africa.
    - The number and locations of substitutions in the spike protein.
    - Available data for other variants with fewer substitutions in the spike protein indicate a reduction in neutralization by sera from vaccinated or convalescent individuals.
    - Available data for other variants with fewer substitutions in the spike protein indicate reduced susceptibility to certain monoclonal antibody treatments.

- The SIG Variant classification scheme defines four classes of SARS-CoV-2 variants:
    - **Variant Being Monitored (VBM)**
        - Alpha (B.1.1.7 and Q lineages)
        - Beta (B.1.351 and descendent lineages)
        - Gamma (P.1 and descendent lineages)
        - Epsilon (B.1.427 and B.1.429)
        - Eta (B.1.525)
        - Iota (B.1.526)
        - Kappa (B.1.617.1)

        - 1.617.3
        - Mu (B.1.621, B.1.621.1)
        - Zeta (P.2)
    - **The variant of Interest (VOI)**
    - **The variant of Concern (VOC)**

        - Delta (B.1.617.2 and AY lineages)
        - Omicron (B.1.1.529 and BA lineages)

    - **The variant of High Consequence (VOHC)**
- To date, no variants of high consequence have been identified in the World (93-96).

7.1. HOW VARIANTS ARE CLASSIFIED?

The U.S. Department of Health and Human Services (HHS) established a SARS-CoV-2 Interagency Group (SIG) to enhance coordination among the CDC, National Institutes of Health (NIH), Food and Drug Administration (FDA), Biomedical Advanced Research and Development Authority (BARDA), and Department of Defense (DoD). This interagency group is focused on the rapid characterization of emerging variants and actively monitors their potential impact on critical SARS-CoV-2 countermeasures, including vaccines, therapeutics, and diagnostics (97-99).

The SIG meets regularly to evaluate the risk posed by SARS-CoV-2 variants circulating in the United States and to make recommendations about the classification of variants. This evaluation is undertaken by a group of subject matter experts who assess available data, including variant proportions at the national and regional levels and the potential or known impact of the constellation of mutations on the effectiveness of medical countermeasures, the severity of disease, and ability to spread from person to person. Given the continuous evolution of SARS-CoV-2 and our understanding of the impact of variants on public health, variants may be reclassified based on their attributes and prevalence in the world (100).

- ***Variants being monitored (VBM)***– View current VBM in the world that continues to be monitored and characterized by federal agencies
- ***The variant of interest (VOI)***– Currently, no SARS-CoV-2 variants are designated as VOI
- ***The variant of Concern (VOC)***– View current VOC in the United States that are being closely monitored and characterized by federal agencies
- ***The variant of high consequence (VOHC)***– Currently, no SARS-CoV-2 variants are designated as VOHC

TABLE 1 The labels assigned to each variant are provided in the tables. (100)

WHO Label	Pango Lineage	Date of Designation		
Alpha	B.1.1.7 and Q lineages	**VOC**: December 29, 2020		**VBM**: September 21, 2021
Beta	B.1.351 and descendent lineages	**VOC**: December 29, 2020		**VBM**: September 21, 2021
Gamma	P.1and descendent lineages	**VOC**: December 29, 2020		**VBM**: September 21, 2021
Epsilon	B.1.427 B.1.429	**VOC**: March 19, 2021	**VOI**: February 26, 2021 **VOI**: June 29, 2021	**VBM**: September 21, 2021
Eta	B.1.525		**VOI**: February 26, 2021	**VBM**: September 21, 2021
Iota	B.1.526		**VOI**: February 26, 2021	**VBM**: September 21, 2021
Kappa	B.1.617.1		**VOI**: May 7, 2021	**VBM**: September 21, 2021
N/A	B.1.617.3		**VOI**: May 7, 2021	**VBM**: September 21, 2021
Zeta	P.2		**VOI**: February 26, 2021	**VBM**: September 21, 2021
Mu	B.1.621, B.1.621.1			**VBM**: September 21, 2021

Each variant classification includes the possible attributes of lower classes (for example, VOC includes the possible attributes of VOI); variant status might escalate or deescalate based on emerging scientific evidence. This page will be updated as needed to show the variants that belong to each class. **The World Health Organization (WHO)** external icon also classifies variant viruses as variants of concern and variants of interest; U.S. classifications may differ from those of WHO because the impact of variants may differ by location. To assist with public discussions of variants, WHO proposed using labels consisting of the **Greek alphabet** (for example, alpha, beta, gamma) as a practical way to discuss variants for non-scientific audiences **(Table 1)** (96,100).

7.2. HOW EFFECTIVE IS EACH VARIANT?

SARS-CoV-2 variants are classified according to their lineage and component mutations. As a result, viruses belonging to the same lineage but containing different subsets of mutations can be classified as different variants. Variants are characterized by their transmissibility, disease severity, and ability to evade humoral immunity. Increased transmissibility is demonstrated by the ability of a variant to outcompete other variants and to display a higher effective reproduction rate and/or secondary attack rate compared with other circulating variants (101). Disease severity has been assessed using mortality data and rates of hospitalization (102,103). Variants associated with higher virus levels may be more transmissible and/or cause more severe disease. Evasion of humoral immunity has been assessed by comparing a variant's susceptibility to mAbs, convalescent plasma, and vaccinee plasma with that of other variants (104,105). In the following sections, we summarize the biological, epidemiological, and clinical characteristics of the WHO-defined VOCs and VOIs as of June 2021. **(Figure 6)**(97).

- ***Alpha variant - B.1.1.7:*** First seen in Kent, England. It has been detected in more than 200,000 cases in the UK. It was the factor behind the closure period in late 2020 and early 2021. It has spread to more than 50 countries and different mutated species have also been identified (106).
- ***Beta variant - B.1.351:*** Appeared in South Africa. It has spread to more than 20 countries. However, travel restrictions prevented it from spreading much around the World (107).

- ***Gamma variant - P.1:*** First detected in Brazil. It has spread to more than 10 countries. At one point, Brazil was the worst country in the world in COVID-19 cases and casualties (108-110).
- ***Delta variant - B.1.617.2:*** First seen in India. It was found to be at least 40 percent more contagious. It dominates the UK and is spreading rapidly around the world. "B.1.617.2", first detected in India in October 2020, was the latest of the COVID-19 mutations that WHO categorized as "Alarming Variant" (VOC). The Delta variant, which emerged as a subspecies of the lineage called "B.1.617", shares the Kappa variant, which is referred to as "B.1.167.1", and two mutations called "P681R" and "L452R". Instead of the "E484Q" mutation in Kappa, the Delta variant has a "T478K" mutation. These mutations, which affect the thorn protein that allows the virus to cling and penetrate human cells, increase contagion and reduce the body's antibody resistance (97).

The World Health Organization (WHO) has identified 4 of the mutations of COVID-19 to date as "alarming" and 8 as "must-watch" variants. It warned that mutations it described as "Alarming Variants" (VOCs) could "increase the infectiousness and risk of spreading the virus, alter its lethality or symptoms of the disease, and reduce the effectiveness of prevention and control measures." Who has been tracking the mutations of COVID-19 since January 2020 and decided on May 1st to name variants mentioned in the Greek alphabet with the names mentioned in letters and numbers, except for the names indicated by letters and numbers. Alpha, Beta, Gamma, and Delta in 4 variants designated as "Alarming Variants"; The 8 variants categorized as "Variant to Watch" were named Epsilon (2 variants), Zeta, Eta, Theta, Iota, Kappa, and Lambda. The "Alpha", which was first detected in the UK from the Alarming Variants, was called "Beta" detected in South Africa, "Gamma" detected in Brazil, and "Delta" detected in India (96,97).

- ***"Delta plus" variant:*** India's Ministry of Health announced that the Delta variant was classified as a "worrying variant" of a derivative mutation that first appeared in Nepal in April. Additional mutation lines called "AY.1" and "AY.2" lead to the easier spread of the virus, easier binding to lung cells, and increase resistance to antibody treatment. The variant, called "Delta plus," has been detected in about 40 people in 3 states of India, while it has spread to 9 other countries including the USA, Britain, Portugal, Switzerland, Japan, Poland, Russia, Nepal, and China. Scientists stress that there is not yet enough data to assess the new mutation as the "Alarming Variant " (100).

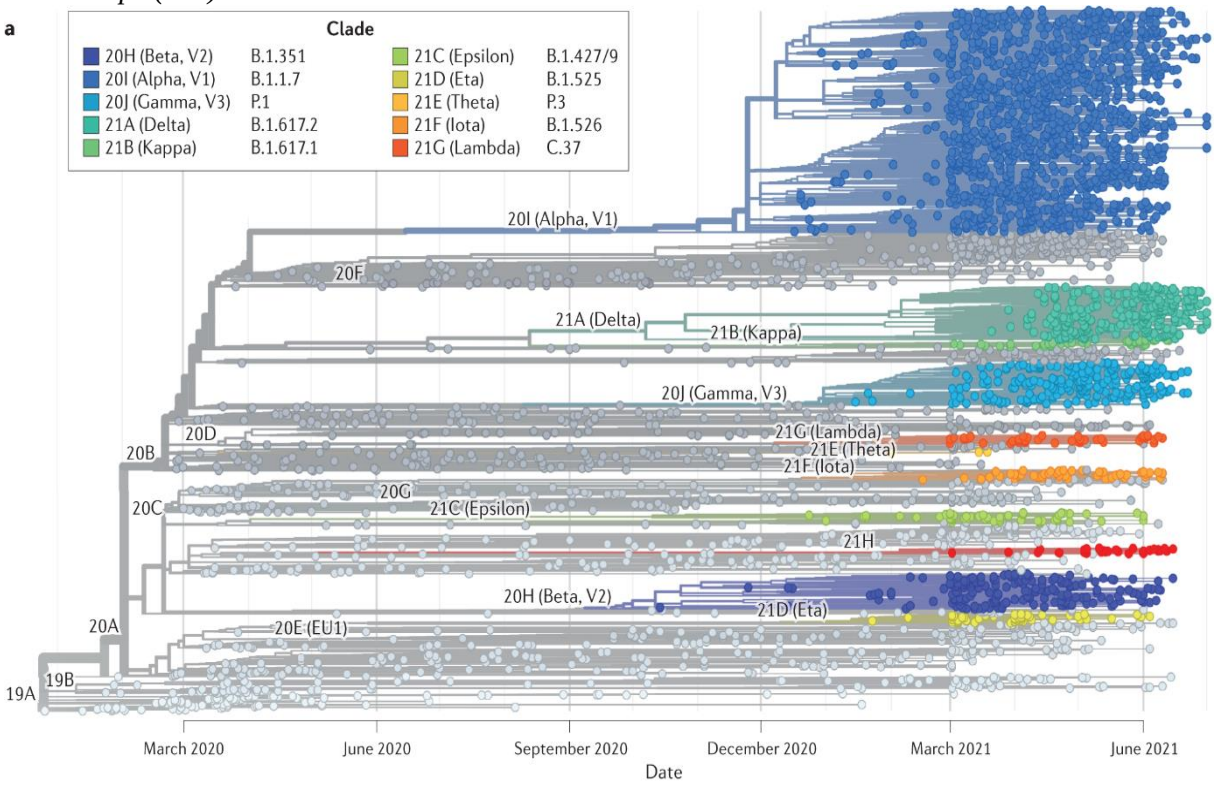
- ***The Omicron variant- B.1.1.529:*** The World Health Organization (WHO) reported that there is no clear evidence that the Omicron variant is spreading faster and causes more severe disease than other variants of Covid-19, including Delta. A written statement from the WHO criticized the introduction of a travel ban on countries in the south of the African continent, where the Omicron variant appeared. "A growing number of countries are imposing flight bans on south African countries due to concerns about the new Omicron variant, while WHO is urging countries to follow science and International Health Regulations," the statement said (100).

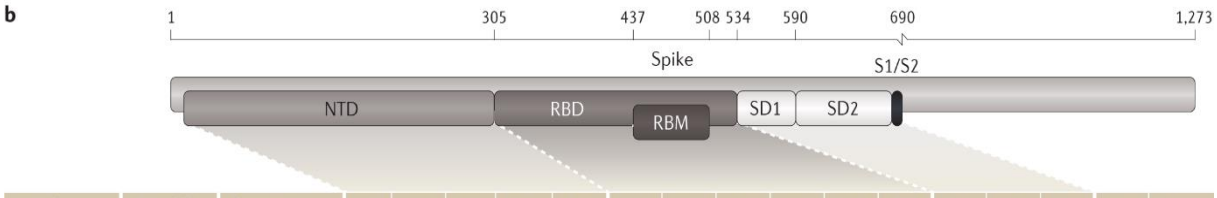
Recently, a new variant of the coronavirus, which has not fallen off the agenda with the Omicron variant, has emerged. The new variant, called 'XE', was previously detected in the UK, the country where the Alpha variant originated. *XE was described as a recombinant with a mutation of the BA.1 and BA.2 Omicron strains. Scientists in the UK said variants mix their genetic material during replication and form a new mutation (111).*

A new sub-variant of Omicron has been detected in China. The new sub-variant sequence, called BA.1.1, has never been found in China or the global variant database. *The new sub-variant, which causes mild symptoms in China, was first detected in Suzhou, near Shanghai. More than 25 million people have been tested for COVID-19 in Shanghai, the country's largest city, as it tries to contain an outbreak caused by cases of Omicron variants in China. The screening aims to uncover the outbreak picture, officials said, adding that the continuation of the gradual quarantine in the city, which began in early April, will be decided according to the screening results (112).*

Omicron, which emerged in South Africa and soon became the dominant species, continues to come up with new sub-variants BA.4 and BA.5, sister variants of the original BA.1 Omicron variant, were added to the watchlist. *We're currently following BA.1 and BA.2, which are dominant worldwide,*

as well as BA.1.1 and BA.3. The WHO announced that it had added these strains to its watch list and said more studies are needed to 'further investigate the effects of BA-4 and BA.5 on the potential for immune escape (113).





WHO	PANGO lineage	Country of first identification	18	69	95	144	242	417	452	478	484	490	501	614	681	701	nsp6: 106	N:203/ 204
			L	H	T	Y	L	K	L	T	E	F	N	D	P	A	S	R/G
Alpha	B.1.1.7	UK		del		del							Y	G	H		del	K/R
Beta	B.1.351	South Africa	F				del	N			K		Y	G		V	del	
Gamma	P.1	Brazil	F					T			K		Y	G			del	K/R
Delta	B.1.617.2	India			I				R	K				G	R			M
Epsilon	B.1.427/9	USA							R					G				
Eta	B.1.525	Nigeria		del		del					K			G			del	
Theta	P.3	Philippines									K		Y	G	H			K/R
Iota	B.1.526	USA			I	del					K			G		V	del	
Kappa	B.1.617.1	India			I				R		Q			G	R			M
Zeta	P.2	Brazil									K			G				K/R
Lambda	C.37	Peru							Q			S		G			del	K/R

FIGURE 6 SARS-CoV-2 variants: evolution and constituent mutations (https://doi.org/10.1038/s41576-021-00408-x) (97).

In this book section, general structural information about coronavirus (SARS-CoV-2), origin, pathogenesis, mutation, and diagnostic cell mechanism is discussed extensively. At the end of the chapter, there is up-to-date information on variants, their structure, and classification.

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CHAPTER 2

COVID-19 EPIDEMIOLOGY

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COVID-19 EPIDEMIOLOGY

According to the World Health Organization(WHO), the emergence of viral diseases poses a serious problem for public health. When we look at the past twenty years, several epidemics have emerged that were caused by viruses, from 2002 to 2003 SARS-CoV and in 2009 H1N1 and in 2012 MERS-CoV. These diseases have had significant impacts on global health (1).

Coronaviruses (CoVs), which are common in the community, range from a mild clinical manifestation of infection to more serious infections such as SARS- CoV and MERS- CoV is a family of viruses that can cause (2). There are several subgroups of coronaviruses (HKU1-CoV, HCoV-229E, HCoV-NL63 and HCoV-OC43) found in humans that can be easily transmitted from human to another human. These subgroups that circulate among humans are mostly viruses that cause the common cold. In addition, there are many subspecies of coronaviruses detected in animals, and it is known that these viruses can easily be transmitted to humans from animals and cause serious illness in humans. At the end of the studies, it was determined that SARS-CoV was infected to human from civet cats and MERS CoV was infected to human from from dromedary camels (3,4).

In China, on December 31, 2019, unknown origin pneumonia patient emerged in Hubei state of Wuhan city, and these cases could not be brought under control and soon spread to other states of China and then to the whole world, causing a pandemic (5).

Initial studies focused on the Huanan sea products bazaar as the origin of the outbreak, with many wild animals for sale, including various poultry, bats and marmots, which 49-66% of patients were associated with (6). It was determined on January 7, 2020 that the causative agent of the disease was a new coronavirus [new CoV (2019-nCoV)]. Later, 2019-nCoV was recognized as CoV disease (COVID-19); The new virus was similar to SARS CoV. Because of this similarity, it was named SARS-CoV-2 (7).

In infectious diseases, the chain of infection is the origin of infection (reservoir), the route of transfer and the host (8). Scientists are constantly working to identify the intermediate vector of the infection, or the reservoir of the host from which it can infect humans. Two snakes have been shown to be possible reservoirs of SARS-CoV2 in previous studies. However, in studies conducted by researchers to date, it has been clearly shown that birds and mammals are reservoirs for coronaviruses (9). Bats are recognized as reservoirs for a variety of coronaviruses, including MERS-CoV, SARS-CoV-1, SARS-CoV-2 as well as viruses from different families. Understanding the adaptation of coronaviruses poses a great challenge because of the complexity of the bat immune system (10).It has been emphasized that pangolins, like bats, may be the source of SARS-CoV-2, but it has not been proven. SARS-CoV-2 virus is not a product for manipulation. The molecular specificity of the virus shows us thisThe receptor binding sequence of SARS-CoV-2 is not optimal like the SARS-CoV-1 receptor binding sequence. This suggests that natural selection is more likely for SARS-CoV-2 (10).Although the main origin of SARS-CoV-2 has not been determined, pangolins and bats have been thought to be the animals most likely to infect humans. Later, it was observed that the source was symptomatic / asymptomatic COVID-19 positive individuals by gaining the feature of transmission from human to another human (11). The first step of infection begins with the binding of SARS-CoV 2 to the receptor presented by the host cell. The virus then integrates with the cell membrane. SARS-CoV2 first targets the epithelial cells of the lung. The interaction between the receptor binding domain at the virus peak and ACE2 has been found to be the main link in transmission fromhuman to another human of the SARS-CoV-2 (9).

COVID-19, is very rapidly contagious feature, had serious effects on the world population and caused the death of 5.7 million people worldwide. COVID-19 is the biggest global health problem the world has faced since the 1918 influenza pandemic (12). Radiological findings consistent with bilateral pneumonic infiltration, fever, shortness of breath were detected in the cases (11).

The COVID-19 outbreak was declared an "International Public Health Emergency" by the WHO on January 30, 2020; It was declared a global epidemic (pandemic) on March 11, 2020, due to the detection of patients with COVID-19 in 113 countries, except for China, the country where the epidemic first started, as well as the severity and rapid spread of the virus. To date, the presence of the virus has been found in all continents of the world (13). The COVID-19 epidemic caused by SARS-CoV-2, which started in Wuhan city of Hubei province of China on 31 December 2019, It went down in world history as the first pandemic caused by coronaviruses (14).

Table1. The number of cases and deaths of the 10 countries with the highest number of covid-19 cases in the World(17)

Country	**Cases - cumulative total**	**Deaths - cumulative total**
United States Of America	79.647.331	978.118
India	43.036.928	521.710
Brazil	30.152.402	661.258
France	26.201.038	140.258
Germany	22.840.776	132.017
The United Kingdom	21.641.008	170.107
Russian Federation	18.018.825	372.245
Republic Of Korea	15.635.274	19.850
İtaly	15.320.753	160.863
Turkey	14.965.58	98.437

The SARS-CoV-2 virus, the causative agent of COVID-19, declared a global pandemic by WHO, spread to 223 countries in total with more than 386 million cases worldwide and caused the death of more than 5.7 million people. COVID-19 has upended many countries around the world and has seriously damaged the health systems of many countries. As a result of the closures that continued for a long time, the pandemic caused ripples and livelihood problems on the world economy (12). Thailand reported the first imported case on 13 January 2020.The case in question is a 61-year-old Chinese woman who traveled from Wuhan to Thailand (15). Later, many countries, especially United States of America (USA), Japan and South Korea, started to report imported cases. Cases and deaths continue to rise in many countries (16). WHO reported 402.044.502 confirmed cases and 5.770.023 deaths worldwide as of 11 February 2022. China, the origin of the epidemic, has announced that it has brought the epidemic under control, and the number of cases is at very low levels. As of 11.02.2022, the number of confirmed cases in China is 143.843, the number of deaths is 5,702, and the number of deaths in the last week is 2. The top three countries with the highest number of confirmed cases are the United States of America (79.647.331), India (43.036.928) and Brazil (30.152.402) as of 12 April 2022 . The top three countries with the highest number of deaths are the USA (978.118), Brazil(661.258) and India (521.710) as of 12 April 2022 (17).

The number of cases and the cumulative incidence show differences in both countries and continents. These differences depend on many variables, including the demographics and population densities of countries, their testing-reporting capacities, and the strategies they implement to contain the disease. It is observed that the risk is higher especially in long-term communal living areas, in nursing homes for the elderly and in areas with low socioeconomic status (18). T.C. Ministry of Health COVID-19 Scientific Committee established on January 10, 2020. The first COVID-19 case was detected on March 11, 2020 in Turkey (7).

The main route from human to another human transmission of SARS-CoV-2 is by exposure to droplets of respiratory carrying the SARS-CoV-2 in a closed environment, or by droplet transmission from people who do not have symptoms carrying the virus, who have not yet started to have symptoms carrying the virus, or who have symptoms carrying the virus. Fomite migration resulting from contamination of inanimate surfaces with the SARS-CoV-2 virus has been successfully analyzed based on several studies reporting whether SARS-CoV-2 can survive on various porous and non-porous surfaces. In experimental environments, it was determined that SARS-CoV-2 was more persistent on stainless steel and plastic surfaces than cardboard and copper surfaces, and the live virus was detected on these surfaces until 72 hours after the surfaces came into contact with the virus (19). Live virus isolation could be performed on non-porous surfaces of objects such as stainless steel and glass at 20°C for 28 days. Conversely, the isolation of SARS-CoV-2 virus from the surfaces of objects with porous surfaces decreased compared to non-porous surfaces (20). As a result of the evaluation of how long the SARS-CoV-2 virus can survive on surfaces and objects, it has been shown that live viruses can be detected in a period of up to 4 hours in copper objects, 1 day in cardboard objects, and 2-3 days in stainless steel and plastic objects. In addition, virus contamination is higher in intensive care units than inpatient services. In addition to the fomite transmission of the SARS-CoV-2 virus, which can be found in trash cans, on floors, in computer mice, on the edges of patient beds, SARS-CoV-2 can be detected up to 4 meters in the air around patients (21). The Centers for Disease Control and Prevention (CDC) also published an update saying that there is a possibility of SARS-CoV-2 transmission through direct contact with people from infected surfaces, but this is unlikely and the main transmission route is not like this (22). There is also the possibility of vertical transmission of COVID-19 from mothers to infants, but this possibility has occurred in a very small proportion of infants born to these mothers (23). Viral RNA begins to appear in blood, saliva and urine from the 5th day after symptoms begin, and continues to be seen for 4-5 weeks in moderate severe cases. It has been determined that it can remain in the feces for more than a month. Although the agent is detected in blood and feces, although it is difficult to prove, infection with fecal-oral contamination has not been clinically defined and according to the joint World Health Organization-China report, it has not been seen as an important factor in the spread of infection (24,25).

The main route for SARS-CoV2 entry into the host cell is via the ACE2 receptor (26). SARS-CoV2 receptors also contain CD4, transmembrane protein serines2 (TMPRSS2), neuropilin-1 (NP-1). The importance of these receptors stems from their role in the pathological progression of the virus and in producing clinical manifestations. To illustrate by way of example, activation of the virus S protein by host cell protease transmembrane protein serines2 is a prerequisite for the spread of SARS-CoV2 in humans (27). To give another example, the sudden decrease in CD4 + T lymphocyte count in patients with COVID-19 is associated with serious clinical course (28).

Just as with other RNA viruses, the genetic structure of the SARS-CoV-2 virus changed over time, while adapting to new human hosts, with the effect of mutations, and new variant viruses that may have different characteristics from their ancestors emerged (12).

As a result of the emergence of new mutated variants of the virus in countries, second, third and fourth waves occurred in the epidemic. With these new waves, the virus has caused serious damage in many countries and as a result in the world. While significant progress in clinical trials and research has

led to a better understanding and clinical management of SARS-CoV-2 and COVID-19, preventing the continued spread of this virus and its emerging new variants has become a matter of increasing concern.

Several variants of SARS-CoV-2 have been identified during this pandemic, and some of them have been recognized as variants of concern by the World Health Organization given their serious impact on the world health system. According to the last epidemiological update made by the WHO on 11 December 2021, since the start of the pandemic, five variants of SARS-CoV-2 have been identified:

- **Alpha (B.1.1.7):** first described in December 2020, in United Kingdom
- **Beta (B.1.351):** first described in December 2020 in South Africa
- **Gamma (P.1):** first described in January 2021 in Brazil
- **Delta (B.1.617.2):** first described in December 2020 in India
- **Omicron (B.1.1.529):** first described in November 2021 in South Africa (12).

According to the epidemiological data presented by the WHO, over 200 countries worldwide have reported the presence of SARS-Co-V-2 variants in their countries. The newest of these variants, Omicron, has been reported by 89 countries so far in November 2021, the date of its first appearance. (29). According to the estimates of the World Health Organization, the mortality rate of COVID-19 is 2.2%. However, case fatality rates differ significantly from country to other country. This is because the average age of countries, pre-existing underlying conditions, and the severity of the disease differ from country to country.

People of all age groups are at risk for contracting or severely ill with COVID-19. However, the elderly over 60 years of age, obese, smokers, cancer patients, patients with chronic diseases (chronic lung disease, chronic kidney failure, diabetes mellitus, cardiovascular disease), and transplant patients have a higher risk of developing a more devastating disease of SARS-CoV-2. People who had a medical condition before they got COVID-19 are about 6 times more likely to be hospitalized during their time with Covid-19 than people without a medical condition. (45.4% vs. 7.6%). The rate of people who lost their lives due to COVID-19 is 12 times higher in those with pre-existing medical problems than in those without medical problems. (19.5% vs. 1.6%) (30). When the data of COVID-19 in different genders were examined, it was seen that the rates of severe disease due to COVID-19 and the death rates due to this disease were higher in men than in women (31,32).

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CHAPTER 3

SARS-CoV-2 (COVID-19) VACCINES

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INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) pandemic emerged on December 31, 2019 in Wuhan, China and declared a global epidemic by the World Health Organization (WHO) on March 11, 2020 (1). Many COVID-19 vaccines have been approved for emergency use by WHO due to the necessity of the pandemic. First mass vaccination practices announced in early December 2020. The number of COVID-19 vaccine doses administered is updated daily by WHO on the dashboard. Globally, as of March 12, 2022, there were 6,029,852 deaths and 452,201,564 confirmed cases of COVID-19 reported to WHO, with a total of 10,704,043,684 doses of vaccine administered. According to the Global Initiative for the Sharing of Influenza Data (GISAID), as of March 12, 2022, a total of 2,184,933 Omicron genome sequences from 164 countries variants can be monitored up-to-date with the data shared.

Table 1: As of January 12, 2022, Vaccines of COVID-19 approved by WHO (2).

Organization	Vaccine	Technological Platform	Type Of Vaccine	Approval Date
BioNTech/Fosun Pharma/Pfizer ***Germany and USA***	3 LNP-mRNAs	RNA	3 LNP-mRNAs	31.12.2020
SII/COVISHIELD University of Oxford/AstraZeneca ***UK***	AZD1222	Non-replicating viral vector	ChAdOx1-S	16.02.2021
Johnson & Johnson and Janssen ***Netherlands and USA***	Ad26.COV 2.S	Non-replicating viral vector	Ad26.COV 2.S	12.03.2021
Moderna/ NIAID ***USA***	mRNA-1273	RNA	LNP-encapsulated mRNA	30.04.2021
Beijing Institute of Biological Products/Sinopharm ***Chinese***	Vero cells	Inactivated	Inactivated	07.05.2021
Sinovac ***Chinese***	CoronaVac	Inactivated	Inactivated	01.06.2021
Bharat Biotech ***India***	BBV152 COVAXIN	Inactivated	Inactivated	03.11.2021
Covovax ***India***	NVX-CoV2373	Protein subunit	Recombinant	17.12.2021
Novavax ***USA***	NVX-CoV2373	Protein subunit	Recombinant	20.12.2021

The WHO Emergency Use Listing process thoroughly examines all available data within a product safety and efficacy framework to determine whether its use is advisable. Vaccines are extensively evaluated for clinical trial data, manufacturing and quality control processes, and standards for quality, safety, and efficacy. It weighs the benefits to be derived from the use of the product against the possible risks, as well as the priority of the threat posed by the emergency in the assessment. Each country has its own autonomy to grant emergency use authorization for vaccines in accordance with its national regulations and legislation. Domestic emergency use permits are issued at the discretion of countries and are not subject to WHO approval (2, 3).

Among the COVID-19 vaccines, those of Janssen, Moderna and Pfizer-BioNTech have been approved by the Food and Drug Administration (FDA). There are 16 vaccines whose Phase 3 studies have not been approved by WHO, but have been approved in their own country or in other countries.

Table 2: Vaccines have been approved by their own country or in other countries (2).

Organization	Vaccine	Technological Platform	Type Of Vaccine	Use Date
Sputnik V ***Russia***	Gam-COVID-Vac	Non-replicating viral vector	Viral Vector	11.08.2020
Sputnik Light ***Russia***	Gam-COVID-Vac	Non-replicating viral vector	Recombinant	20.08.2020
EpiVacCorona ***Russia***	Aurora-CoV	synthetic peptide-based vaccine	Antigens based vaccine	14.10.2020
Convidicea ***Chinese***	Ad5-nCOV	Non-replicating viral vector	Viral Vector	17.03.2020
WIBP-CorV ***India***	WIBP-CorV	chemically-inactivated whole virus	Inactivated	14.04.2020
CoviVac ***Russia***	AYDAR-1	An inactivated virus-based	İnactivated	20.02.2021
ZF2001 ***China/Uzbekistan***	Zifivax	Protein subunit	Recombinant	01.03.2021
QazVac ***Kazakhistan***	QazCovid-in	İnactivated	Inactivated	01.06.2021
COVIran Barekat ***Iranian***	BIV1-CovIran	İnactivated	Inactivated	17.12.2020
Abdala ***Cuba***	CIGB 66	Protein subunit	Recombinant	02.05.2021
Soberana 02/Soberana Plus	FINLAY-FR-2	Spike protein conjugated toxoid	Conjugated	11.04.2021

Cuba/Iranian				
MVC-COV1901 ***Iranian***	MVC-COV1901	Protein subunit	Recombinant	26.10.2021
ZyCoV-D ***India***	ZyCoV-D	DNA plasmid based	DNA	20.08.2021
Spikogen ***Iranian***	COVAX-19	Protein based	Recombinant	10.08.2021
FAKHRAVAC ***Iranian***	MIVAC	Inactivated	Inactivated	09.09.2021
NVX-CoV2373 ***USA***	TAK-019	Protein subunit and a virus-like particle	Recombinant	10.10.2020
Turkovac ***Turkey***	ERUCOV-VAC	Inactivated	Inactivated	25.11.2021

1. CELL ENTRY MECHANISM OF SARS-COV-2

The SARS CoV-2 virus contains four structural proteins called nucleocapsid (N) protein, membrane/matrix (M) protein, envelope € protein, and spike (S) protein. The S protein portion consists of two subsections, S1 and S2. It is an S protein and binds to receptor angiotensin converting enzyme 2 (ACE2) in cells to cause infection. The S1 unit contains the specific receptor binding domain (RBD) and performs initial binding to host cells. The S2 subunit supports the viral fusion of the cell that initiates the infection. Then, viral RNA enters the host cell by fusion with TMPRSS2 and TMPRSS4 in the presence of S protein, host cell proteases, serine proteases and furin. In addition, the S protein influencely stimulates the T-cell immune response. Therefore, these are the most important target antigens when designing vaccines (4).

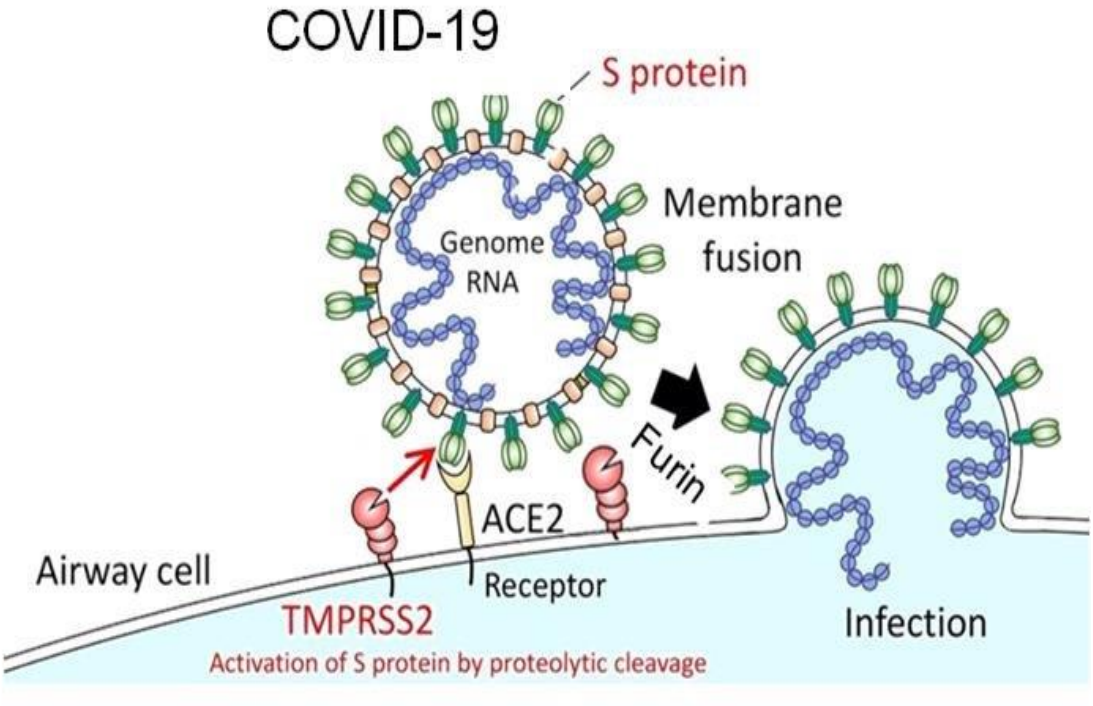

Figure 1: Cell entry mechanism of SARS-COV-2 (5) .

SARS-CoV-2 eliminates or inactivates innate immunity in the immune response. For the body to respond appropriately, it must activate the adaptive immunity composed of B and T cells. It produces antibodies (humoral mediated antiviral response) that prevent the spread of the infection with antibodies produced from B cells and Immune globulins that bind to virus particles on mucosal surfaces. SARS-CoV-2 first induces IgM response and then IgG and IgA antibody responses. Infection seroconversion occurs approximately after seven days in half of patients and around the 13-14th days in all of patients (5). On average, it has been reported that the IgM response peaks at 7-10 days and the IgG response peaks at about three weeks later infection (6). However, mature T cells in the thymus kill virus-infected cells via the T cell receptor (TCR), which mediates recognition of viral small peptides and antigens. T cells recognize viral antigens by their presentation by major tissue compatibility class I (MHC I) and class II (MHC II) molecules. Activated T cells proliferate rapidly to produce large numbers of T cells with the same TCRs. CD8+ T cell clones cleave directly by releasing perforin and granzyme to eliminate virus-infected cells (7).

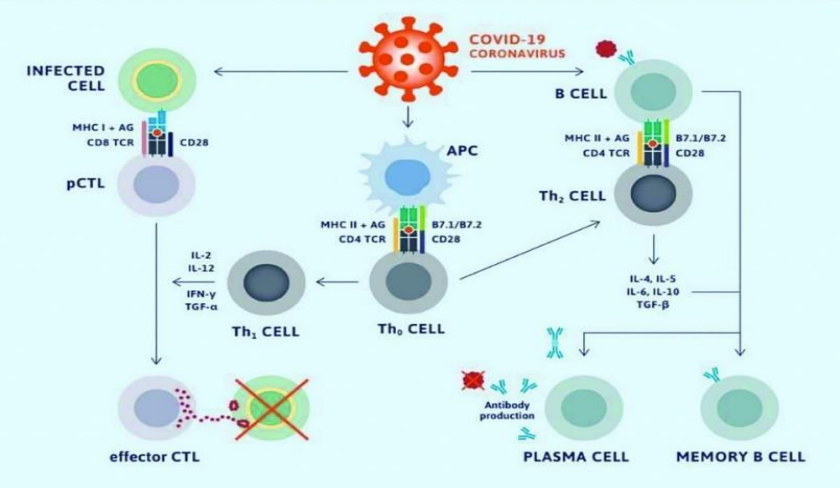

Figure 2: T cells and B cells in immunity to SARS-CoV-2 (8).

2. COVID-19 VACCINATION

Activation of both cellular and humoral immunity is targeted in the design of SARS-CoV-2 vaccines (8). Due to the pandemic, the discovery and introduction of COVID-19 vaccines have occurred at an incredible speed, unprecedented in vaccine history. According to the latest data, there are more than 190 vaccine candidates in the preclinical stage and more than 14o vaccine candidates in the clinical development stage (9). The protection developed by vaccines against viruses is mainly based on virus neutralizing antibody activities. These antibodies act by blocking the communication of COVID-19 with its cellular receptor or by preventing the fusion of viruses (10).

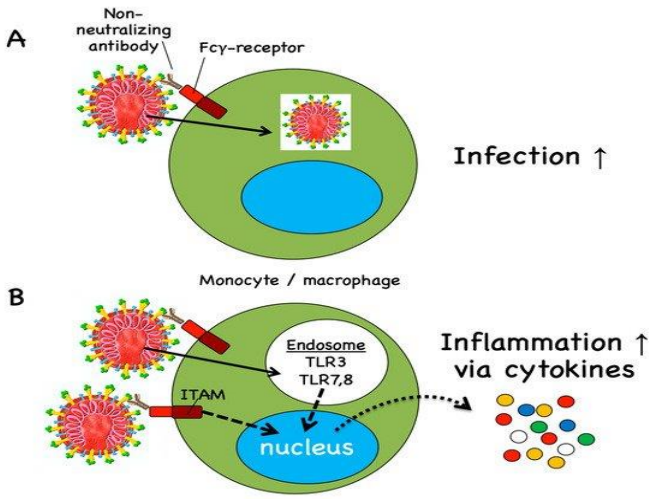

Figure 3: Neutralizing antibody and nonneutralizing activity (10).

3. COVID-19 VACCINES APPROVED BY THE WHO

For now, the nine COVID-19 vaccines approved by WHO are divided into four main groups:

A-Whole virus vaccines (attenuated, inactivated): Sinovac, Sinopharm - Bharat Biotech vaccines are inactivated whole virus SARS-CoV-2.

B-Nucleic acid vaccines (DNA and mRNA): Of the vaccines developed for COVID-19: Moderna - Pfizer-BioNTech are messenger RNA (mRNA) vaccines.

C-Viral vector vaccines: The Astra-Zeneca, Janssen-Johnson & Johnson vaccines are obtained using adenovirus vectors.

D-Recombinnt protein subunit vaccines: Covovax and Novavax are recombinant protein subunit vaccines.

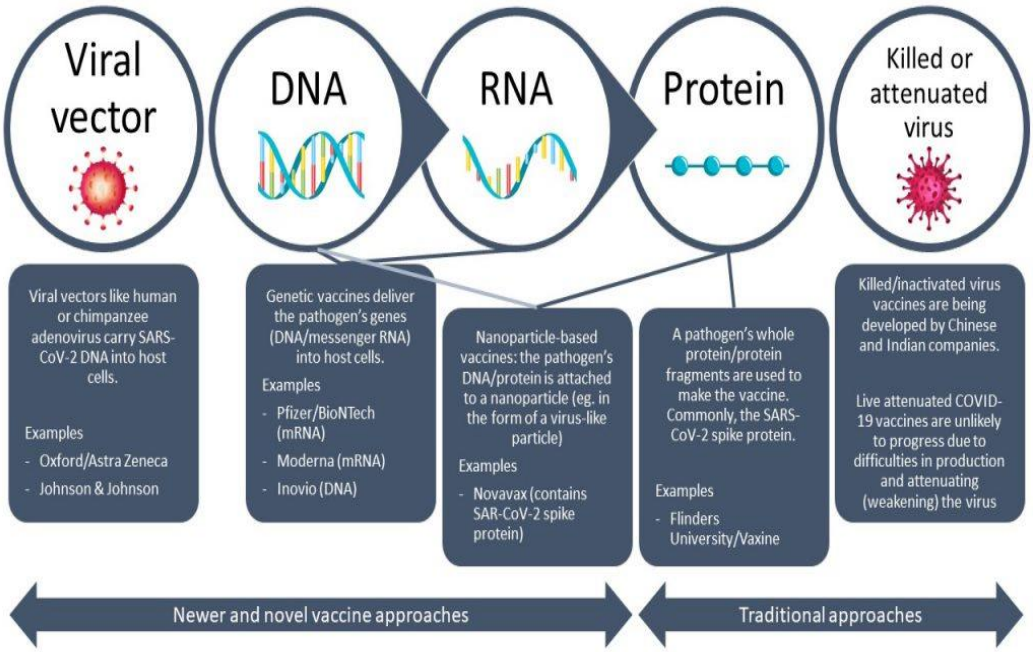

Figure 4: COVID-19 vaccine platforms (11).

A-Whole Virus Vaccines

Whole virus vaccines are non-infectious and incapable of replication in host cells, but they can elicit an immunogenic response. It forms the basis of the classical virus vaccines used today. These vaccines are available in SARS-CoV-2 attenuated or inactivated forms. It has been proven that whole SARS virus vaccines can induce the production of neutralizing antibodies in humans (12).

A.1. Live Attenuated Vaccines

Live attenuated vaccines consisted of weakened viruses that were able to replicate but did not cause disease. Attenuated live viruses mimic natural infection for a prolonged immune response, but without the risk of pathogenicity. Live attenuated vaccines can easily present viral antigens to the host immune system with antigen presenting cells (APCs). Thus, it potently induces cytotoxic T-cell responses and long-term immune memory. The advantages of live attenuated SARS-CoV-2 vaccines are that they induce strong mucosal and cellular immunity. However, the disadvantage of SARS-CoV-2 vaccine is fecal excretion. that is a risk of SARS-CoV-2 transmission to the unvaccinated people (13).

•COVI-VAC vaccine: The COVI-VAC live attenuated vaccine, developed in collaboration with Codagenix - the Serum Institute of India on February 20, 2021. This vaccine can recognize the entire virus and administered by intranasally. It has not yet been approved by the WHO (14).

There are two vaccines that are still under trial in Turkey and India:

•Indian Immunologicals Ltd and Griffith University

•Mehmet Ali Aydınlar University, Turkey (15).

A.2. Inactivated Coronavirus Vaccines

Vaccines based on dead viruses (inactivated) use traditional technology. Vaccines produced by this method are more stable than other vaccines, but elicit short-term immunity while requiring higher amounts of virus to combine with a strong adjuvant. The resulting immune response is against the S protein, and many other antigens of SARS-CoV-2. Although the induced response is weakly, but the vaccine use easyly, cheapest and safely (15). Inactivated vaccines contain genetic materials destroyed by chemicals, radiation or heat can't infect human cells, but trigger immunity (16,17).

•Sinopharm COVID-19 vaccine (BBIBP-CorV; SV), May 7, 2021.

The SV inactivated vaccine is produced by the Chinese company Sinopharm Group and is marketed by the United Arab Emirates (UAE). It is administered by intramuscular injection in two doses at 3-week intervals. It has been reported to have an efficacy of more than 79% in China and 86% in the UAE (18,19).

•Sinopharm-Wuhan Vaccine (SWV): SWV is the Wuhan version of the Sinopharm vaccine prepared WIV-04 strain by the Wuhan Institute of Biological Products. This vaccine is given in two doses at intervals of 3-4 weeks. A third lower dose is recommended for people with weakened immunity (20).

•CoronaVac - Sinovac vaccine (formerly PiCoVacc; CV), June 1, 2021.

The CoronaVac vaccine was produced in collaboration with the Beijing company SinoVac Biotech and Brazilian Butantan. The CV vaccine is given by intramuscular injection in two doses with an interval of two weeks (21). It showed higher efficacy of more than 91% and more than 65% in Turkey (22) and Indonesia (23) respectively. This vaccine elicits moderate immune responses with lower antibody levels compared with patients recovering from COVID-19. Therefore, coronaVac requires a strong adjuvant to potentiate the immune response, making it unsuitable for respiratory applications (17). CoronaVac did not show serious side effects. It can be stored at 2-8 °C and is suitable for worldwide distribution (20,24).

•Covaxin vaccine (Bharat Biotech Vaccine, COV, BBV152)

The Covaxin vaccine was produced in collaboration with the Indian Bharat Biotechnology Company, the Medical Research Council and the National Institute of Virology (25). The Indian company signed a marketing agreement with the Pennsylvanian company Ocugen. This vaccine was given in two doses with an interval of four weeks and its effectiveness was found to be 81%. It can be stored at room temperature for one week, it suitable for use in hot countries (20,26).

- **Turkovac (ERUCOV-VAC):**

Turkovac (ERUCOV-VAC) is an inactivated COVID-19 vaccine. It is developed with the Turkish Ministry of Health and Erciyes University. Studies comparing with Turkovac and Sinovac vaccines (27).

B-Nucleic acid vaccines (mRNA and DNA)

Some types of SARS-CoV-2 vaccines use mRNA and DNA vaccine technology (28). In order for DNA or RNA to enter the human cell, it must be transported in a special method (29). DNA and RNA vaccines are safer because they do not required a live virus. Nucleic acid vaccines can induce systemic immune responses quite effectively (29).

B.1. DNA vaccines

DNA vaccines are preferred because of their simple, stable and easy production properties (30). They are safer in terms of pathogenicity risk than live attenuated viruses or inactivated viruses (31). DNA vaccines induce immunity through recombinant plasmids that encode genes for foreign antigens. In the nucleus, viral genetic material is expressed (32). The DNA vaccine enters the cell through endocytosis and activates the innate immune system thanks to the Toll-Like Receptor 9 (TLR9) found in endosomes (33). APCs are directly stimulated by the injected genetic materials. MHC I and MHC II molecules are loaded by expressed antigens. After expression, antigens elicit recognition by APCs and cytotoxic or humoral immune responses. APCs are involved in activating cellular immune responses by presenting antigens to naive CD4+ T cells and CD8+ T cells. Thus, the antigens produced reach the secondary lymphoid organs and activate the B cells to produce antibodies (34). Co-administration with adjuvants will further enhance adaptive immunity (35). Although there are several animal DNA vaccines in use today, no human DNA vaccine has yet been approved (36). They can be freeze-dried for long-term storage. The Zika vaccine (GLS-5700) and MERS-CoV vaccine INO-4700 are clinical-stage DNA vaccines (37,38).

•**INO-4800 vaccine**: This DNA vaccine encoding the SARS-CoV-2 S protein has been tested in guinea pigs and mice and virus neutralizing antibodies have been observed. Electroporation CELLECTRA was used in the design of the intradermal SARS-Cov-2 vaccine candidate INO-4800. They are still clinical trial phase (39).

B.2. mRNA vaccines

The use of mRNA vaccines in the fight against the rapidly spreading COVID-19 has come into use very quickly. Before the COVID 19 vaccines, there was no mRNA vaccine in use. This technology uses single-stranded mRNA designed to give human cells for the production of S proteins (29). The advantages of mRNA vaccines are immunogenicity, low preparation and production costs, high safety profiles, and rapid production. The disadvantages are that they require special storage and distribution systems. They are also superior to protein subunits as they bypass the purification and standardization steps of viral proteins. The mRNA vaccine induces the cell to temporarily produce the antigen protein encoded by the mRNA. Usually in COVID-19 mRNA vaccines, the target antigen encoded by the mRNA is mostly S protein, variants or fragments. The mRNA doesn't stay in the cells for approximatelt 48 hours. This type of vaccine is carried by mRNA liposomes to prevent the breakdown of body cells and to add stability to mRNA, a fragile molecule. However, the requirement for the production of RNA vaccines is by additions to a poly(A) tail at the 5' and 3' ends respectively. Naked mRNA is highly sensitive to ribonuclease in the cytoplasm. Therefore, lipid nanoparticles are required to package mRNA in a stable form (40). These types of vaccines need to be stored at ≤ -20 °C the long-term storage requirements. After transmitting its instructions to body cells, it is broken down by enzymes called ribonucleases (RNases). Therefore, it is impossible for mRNA to move into the cell nucleus; it cannot integrate of the DNA and induce genetic changes. After vaccination, both cellular (mediated by CD4+T and CD8+ T cells) and humoral (antibodies) immunity is initiated. Neutralizing antibodies prevent the S protein or fragments from binding to cells. T cells (CD8+) in vaccinated individuals can recognize and destroy cells infected with the coronavirus (41). It offers rapid antigen production within the host cell and therefore cellular and humoral immune responses are rapidly induced. It provides equivalent long-term protection at a much lower dose. Because the S protein is produced using host cells in most cases, mRNA vaccines are capable of inducing both T-cell responses and antibody production. However, the antigenic expression that occurs after vaccination is temporary, its persistence in the human response is limited, and it requires repeated administration at regular intervals (42).

•Pfizer/BioNTech Comirnaty vaccine (PBV), 31 December 2020.

The PBV vaccine was co-produced by Pfizer and BioNTech. This vaccine with the generic name of tozinameran (Comirnaty) is given in two doses three weeks apart. It is also recommended that a reminder dose be given once within 12 months and in subsequent years (43). The vaccine is provided in vials containing five doses of liquid and was found to be 95% effective in prevention. It is reported to be able to generate strong T cell immune response and antibody responses. Although this vaccine does not cause serious side effects, it can often cause temporary reactions; such as pain at the injection site, mild fever, fatigue and muscle pain (20,44). Disadvantages of the PBV, it requires of -70 °C for distribution and storage, and difficulties in its use in poor countries with insufficient infrastructure have been observed (45).

•Moderna COVID-19 vaccine (mRNA 1273, MV), April 30, 2021.

The mRNA-based vaccine MV is manufactured by Moderna and the US National Institute of Health. This vaccine is also an (mRNA-1273) encapsulated in a lipid nanoparticle (LNP) (46). An advantage of this vaccine over the PBV is that it can be stored in -20 °C, making it easy to transport to rural areas (47). Although data from MV Manufacturers and FDA report 94.1% effectiveness in adults, there is no safety risk as two applications are required four weeks apart (48).

•CureVac CVnCoV Vaccine (CVV): The CVV was produced in partnership with Bayer and CureVac. This vaccine is considered a competitor to leading mRNA vaccines from Moderna and Pfizer-BioNTech, with lower doses and 95% efficacy (49). It can also be stored at 5 °C and is stable for three months at refrigerator temperatures, making it suitable for use in poor countries (50).

C-Viral Vector-Based Vaccines

Viral vector-based vaccines elicit an immune response without causing infection. Such vaccines use attenuated bioengineered products. Vector vaccines use either non-pathogenic organisms or plasmids (vector). Vector vaccines may or may not be replicated (51,52). Viral vectors are chosen to easily infect human cells and to be detected by APCs (53). Accidental integration of the viral genome with the host genome can lead to uncontrolled replication with disastrous results. They currently use adenoviruses to transport and deliver SARS-CoV-2 RNA encoding the S-protein antigen (54). The adenoviruses used are the human adenoviruses Ad5, Ad26 and chimpanzee adenovirus ChAdOx1 (55). After injection of adenovirus vectors enter the cells but cannot replicate intracellularly. The delivered genetic material leaves the vectors and goes to the nucleus (20,56). The mRNA is then transcribed and leaves the nucleus; these proteins aggregate on infected cells. S proteins or particles are recognized by the immune system and alert signals are sent to produce activated T cells (CD4+ and CD8+) and specific neutralizing antibodies. Protection from these vaccines varies between 76% and 100% (57). These vaccines can be stored in 2-8 °C for 6 months (58). Furthermore because these vaccines contain their own pathogen-associated molecular models (PAMPs) and they can induce robust immune responses without the need for strong adjuvants (20,59). The only FDA-approved viral vector vaccine is the ERVEBO: Ebola vaccine; at November 2020 (60).

•SII/COVISHIELD and AstraZeneca/AZD1222 vaccines (OAV; AZD 1222; Vaxzevria), February 16, 2021.

OAV (Vaxzevria) was produced in collaboration with AstraZeneca and Oxford University. An Indian version called Covishield was also produced. (61). When the effects of viral vector vaccines containing

S protein and N protein are examined separately, it has been shown that different antibodies can have positive and negative effects. Rats immunized with the N protein had increased proinflammatory cytokine secretion, neutrophilic and eosinophilic infiltration, and more severe cytopathic effects were observed. It has been stated that different epitopes of the S protein may have different effects. It has been shown that antibodies developed against the RBD and HR2 domains of the S protein may be more protective in non-human primates. On the other hand, antibodies against some other S protein epitopes have been observed to cause ACE 2. Neutralizing antibodies that specifically target the Heptad Repeat 2 region can prevent S protein-mediated viral fusion (62). Since this vaccine can be kept for at least six months at between 2-8 °C, it is easy to store, transport and distribute (63). While OAV activates a strong humoral and cellular immune response, it cause mild side effects, such as fatigue and headache (64). Thrombocytopenia syndrome (TTS) has been diagnosed within three weeks of vaccination with the Vaxzevria, so it has been reported in some countries as a contraindication not to administer the Vaxzevria vaccine to thrombocytopenic individuals. It is also thought to trigger heparin-induced thrombocytopenia (HIT) (65).

•Janssen/Ad26.COV 2.S vaccine was developed by Johnson & Johnson (J&J V; JNJ-78436735), March 12, 2021.

Manufactured by Janssen Pharmaceutical and Johnson & Johnson. It is a single-dose vaccine that can produce an effective neutralizing antibody response in approximately 90% of those vaccinated (66). It can be kept for up to three months at 2–8 °C and for up to two years at -20 °C (67).

•Sputnik-V vaccine (SVV; August 11, 2020): This is a vaccine of the Russian vector-based SVV vaccine administered intramuscularly at three-week intervals using Ad5 and Ad26. It has been reported that the vaccine has 91.6% efficacy after two doses (68). The first dose uses the Ad26 and the second dose the Ad5 vector. It can be kept at -20 °C (69). The SVV is still awaiting approval.

D-Recombinant Protein Subunit Vaccines

Subunit vaccines consist of either synthetic peptides or recombinant proteins. Because these vaccines are made up of specific immunogenic parts rather than whole viruses, they are highly effective, safer and have less risk of side effects. Generally, peptide subunits should be given in booster doses or with appropriate adjuvants to elicit a desired level of immunogenic response. In addition, the highly protective efficacy of mucosal vaccination. It also requires adjuvants and repeated administration as it is poorly immunogenic (70-72). Considering the effectiveness of cellular immunity in eliminating coronavirus infections, the use of the COVID-19 subunit vaccine in combination with other vaccine platforms are recommended (73).

•Covovax (NVX-CoV2373) vaccine, December 17, 2021

The vaccine, developed by Novavax, USA. It is produced by the Indian Serum Institute of under the name Covovax. It is also a vaccine that uses a recombinant protein-based modified baculovirus. It was applied with an adjuvant called Matrix M (74).

•Nuvaxovid (NVX-CoV2373) vaccine, December 20, 2021

The NVX vaccine is produced in collaboration with Novavax, GSK and Sanofi by adding viral proteins to a nanoparticle carrier (75). It is administered by intramuscular injection in two doses, three weeks apart. They are reported to produce a stronger antibody response and T cell activation than patients recovering from COVID-19 (76). Stable at refrigerator temperatures, this vaccine has an efficacy of between 89.3%–96% (77,78). In addition, the administration of this vaccine in combination with the flu vaccine is under investigation (79).

•**EpiVacCorona vaccine (EVCV):** The EVCV vaccine produced by the Russian Vector Institute uses fragment**s** of synthetic viral peptides that reflect SARSCoV-2 on October 14, 2020 (79). When given to adults by two doses of intramuscular injection, three weeks apart, all volunteers formed specific antibodies against their antigens. It can be stored at refrigerator temperatures for up to two years and is still awaiting approval (80).

•**ZF 2001 (RBD Dimer) vaccine**: This vaccine are developed by the Chinese Academy of Military Medical Sciences on March 1, 2021. The vaccine is considered quite safe as it uses a portion of the S protein called RBD combined with an adjuvant (81). The ZF 2001 vaccine is administered by intramuscular injection in three doses at 4-week intervals (82). It has been approved for emergency use in Uzbekistan and China (83).

4. MUTANT VARIANTS OF SARS-COV-2 AND VACCINES

SARS-CoV-2, which is one of the RNA viruses, also creates mutant variants through the development of genetic evolution over time. Various variants of SARS-CoV-2 have been identified during the pandemic. Based on the latest the WHO epidemiological data, five major SARS-CoV-2 variants have been identified since the start of the pandemic:

Alpha (B.1.1.7): The first type of mutant variant declared in the United Kingdom (UK) in late December 2020.
Beta (B.1.351): First reported in December 2020 in South Africa.
Gamma(P.1): First reported in Brazil in early January 2021.
Delta (B.1.617.2): First reported in India in December 2020.
Omicron (B.1.1.529): First reported in South Africa in November 2021 (84).

According to GISAID data, It was stated that 93 percent of the cases belonged to Omicron, 6.7 percent to Delta and the rest to other variants. In Turkey, totaly 84,975 cases confirmed of COVID-19 and 4,854 cases belonged to omicron.

All over the world, alpha, gamma, beta and Omicron variants continue to be tracked, Since COVID-19 vaccinations have begun, there has been a reduction in severe illness, hospitalizations, and deaths (85,86). As in other countries SARS-CoV-2 mutations have spread rapidly in Turkey. The first type and date of mutant variants declared in Turkey are as follows:

Alpha (B.1.1.7): First reported in Turkey on January 4, 2021 (87).
Beta (B.1.351): First reported in Turkey in January 31, 2021 (88).
Gamma (P.1-GR/501Y.V3): First reported in Turkey on March 2, 2021 (89).
Delta (B.1.617.2): First reported in Turkey on April 28, 2021 (90).
Omicron (B.1.1.529): First reported in Turkey on December 11, 2021 (91).

Table 3: Turkey GISAID data 16.02.2022 (3).

Variants	Number
Alpha	1,917
Beta	503
Gamma	258
Delta	59,024
Omicron total	4,854
BA.1	4,528
BA.1.1	318
BA.2	8
Non-VOC/VUI	18,294
Total	84,975

The Delta (B.1.617.2) variant is contagious as previous variants (92). Although research suggests that SARS Cov-2 vaccines are effective against the variants, the Moderna, Pfizer-BioNTech, and Janssen/Johnson & Johnson SARS Cov-2 vaccines have shown that they still provides protection against severe SARS Cov-2 (93). The Omicron (B.1.1.529) variant can spread most easily (94). However while Omicron is said to not cause more severe disease, SARS Cov-2 vaccines are predicted to be effective in preventing serious illness (95). According to the European Medicines Agency, early research also shows that a booster dose of SARS Cov-2 mRNA vaccines improves protection against serious diseases caused by Omicron. So far, Omicron has shown a mild course of infection in terms of reported intensive care unit admissions and the number of ventilated patients (96). Vaccination and a high neutralization capacity play a big role in this. However, it has been reported that in cases of low neutralization capacity, the vaccine may be avoided. This study also supports the idea that if a high neutralization capacity is provided by vaccination, reasonable protection against Omicron will be provided (97). A retrospective case-control analysis study found that receiving three doses of an mRNA vaccine provided protection against both Omicron and Delta variants, but less protection for Omicron than Delta, compared with those who were unvaccinated and those who received two doses (98). In a study carried out on individuals who had previously been infected or were vaccinated with the Pfizer vaccine, it was stated that a reminder dose would provide significant protection from the Omicron variant (99). It was reported by the United Kingdom Health Safety Agency that there was 20-40 fold decrease in the neutralizing antibody titer for Omicron in serum samples obtained from those who received two doses of Pfizer vaccine (100). According to data from the Centers for Disease Control and Prevention (CDC), those who are not vaccinated are five times more likely to be infected, 10 times more likely to be hospitalized, and 11 times more likely to die than those who are fully vaccinated (101). In another study conducted between April 4 and December 25, 2021, it was found that the risk of infection in unvaccinated people was more than 13 times higher and the risk of death related to SARS Cov-2 was more than 53 times higher during the emergence of the Delta and Omicron variant (101).

Vaccines are still our most effective weapon in the fight against all mutant variants of SARS CoV-2, Although they require a reminder dose of currently available vaccines. It helps contain the epidemic, It is also necessary to make a new sustainable plans. With the first such accelerated development of COVID-19 vaccines, the public health response was in time. The development of heat-resistant vaccines in order to be prepared for future epidemics is important for all countries in the world to access the vaccine under equal conditions, regardless of their political ideologies. Many more promising vaccines are being developed in the fight against SARS-CoV-2 with WHO approval. It is expected that the transmission of COVID-19 will be significantly under control by the impact of vaccines.

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CHAPTER 4

HISTOPATHOLOGICAL EFFECTS OF COVID-19 INFECTION ON BRAIN TISSUE

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes coronavirus disease 2019 (COVID-19), with approximately 500 million confirmed cases and more than six million deaths worldwide (1). While many people infected with SARS-CoV-2 do not show any symptoms or only experience respiratory symptoms; extrapulmonary symptoms, including neurological symptoms, are gradually increasing (2). In the early days of the COVID-19 pandemic, the focus was on treating damage to the lungs and circulatory system. However, different neurological and psychiatric symptoms such as loss of smell, headache, encephalitis, and paralysis were also observed in patients (3). Confusion decreased environmental awareness, and agitation was observed in some individuals hospitalized with COVID-19 infection, and delirium was diagnosed (4, 5). The first report of a patient with COVID-19 with swelling and inflammation of brain tissues was published in Japan in April 2020 by a group of researchers (6). As a result of molecular analysis, no RNA or protein specific for SARS-CoV-2 could be detected in the brain. The reason for this is focused on the possibility of early neuroinvasion, which is resolved later. In immunohistochemical examinations, on the other hand, cellular deteriorations confirm that the barrier cells of the choroid plexus sense and transmit peripheral inflammation to the brain, and the presence of peripheral T cells in the brain parenchyma draws attention. It is known that abnormal T cell leakage is sufficient to promote neuroinflammation and impair neurogenesis (3, 7).

Initially, autopsies for COVID-19 patients were limited due to uncertainties in the infectious nature of SARS-CoV-2, and limitations in personnel and personal protective equipment supply. Detailed neuropathological reports lagged behind general autopsy series due to the greater focus on lung pathology and the preference for longer (2-3 weeks) formalin fixation before cutting brain tissues. Additionally, some agencies may be reluctant to remove brain tissue in cases of COVID-19 due to concerns about aerosols from electric bone saws; although this can be controlled using hand saws or vacuum filters (2). However, cell and tissue-level approaches are extremely important to understanding the neuropathogenesis of COVID-19 and developing preventive treatment strategies.

HISTOPATHOLOGICAL ALTERATIONS IN BRAIN

Hypoxic changes, including red neurons, a typical marker of hypoxic injury of the central nervous system (CNS), are the most frequently reported changes in brain tissue in cases of COVID-19. This is followed by ischemic and hemorrhagic lesions, reactive astrogliosis, and microgliosis (8). Reichard et al. were one of the first to publish the neuropathological results of an autopsy performed on a patient who died from COVID-19. In the study, brain swelling and diffuse bleeding in some areas were determined as the main macroscopic findings. Microscopically, hypoxic changes, few infarctions, intraparenchymal hemorrhage foci with peripheral macrophages, diffuse reactive gliosis, apoptosis of oligodendrocytes, and axonal damage were observed due to loss of myelin (9).

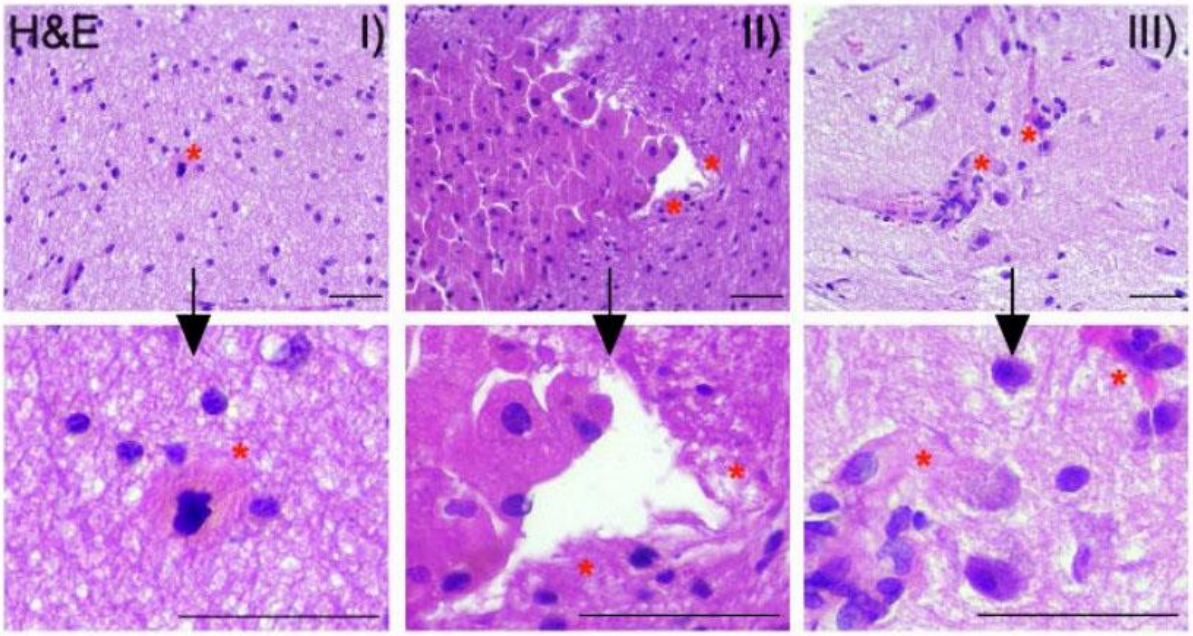

Figure 1. Histopathological images of brain tissue from individuals who died due to Covid-19, stained with hematoxylin-eosin. (I) areas of necrosis, cytopathic damage (enlarged, hyperchromatic, atypical-appearing nuclei), (II) vessels with leukocytes and thrombus margins, and (III) infiltration of immune cells into tissue are shown. Changes are marked with red stars. Images were obtained at 400x magnification and the scale bar indicates 50 µm (Crunfli F et al.) (18).

Myelin is a sheath that surrounds and protects the axon. Oligodendrocytes in CNS; in the peripheral nervous system (PSS), it consists of the cell membrane of Schwann cells. In addition to protecting the axon, myelin is responsible for accelerating signal transmission, and therefore, damage to myelin disrupts signal transmission (10, 11). In May 2020, a patient with COVID-19 was reported with deterioration in the structure of the myelin sheath, which is known to be irreversibly damaged in neurodegenerative diseases as a result of magnetic resonance imaging (MRI) examination. It has been suggested that the delay of the immune response by SARS-CoV-2 is a factor that destroys the myelin sheath (12). Brun et al. detected bilaterally asymmetrical lesions showing white matter involvement in the brain MRI images of a 54-year-old female patient, and they associated this condition with acute demyelination (13). Another study described a COVID-19 patient with symmetrical demyelinating lesions on the bilateral posterior inner capsules. The absence of damage, bleeding, or cavitations in the gray matter suggests the presence of demyelination rather than encephalomyelitis (14). There are studies with similar results in the literature, and the data obtained confirm that SARS-CoV-2 infection is a risk factor for demyelination in both the peripheral and central nervous systems (15-17).

Astrocytes are the most abundant glial cells in the brain and have multiple functions, including providing nutrients to neurons. Andrews et al. reported that SARS-CoV-2 preferentially infects astrocytes over other brain cells. They observed that when they exposed brain organoids (miniature brain-like structures produced from stem cells in a laboratory environment) to the virus, the virus mostly infects astrocytes (19). Brain samples from 26 people who died of COVID-19 in February 2021 were analyzed. Brain cells of five of these individuals were proven to have SARS-CoV-2 infection (Figure 1), and 66% of the cells affected by the infection were found to be astrocytes (18). Variable degrees of reactive astrogliosis was observed as a result of immunohistochemical staining for GFAP (Glial Fibrillary Acidic Protein), an astrocytic marker, in various regions of post-mortem brain tissues (Figure 2). The presence of reactive astrocytes contributes to the development of demyelination (20).

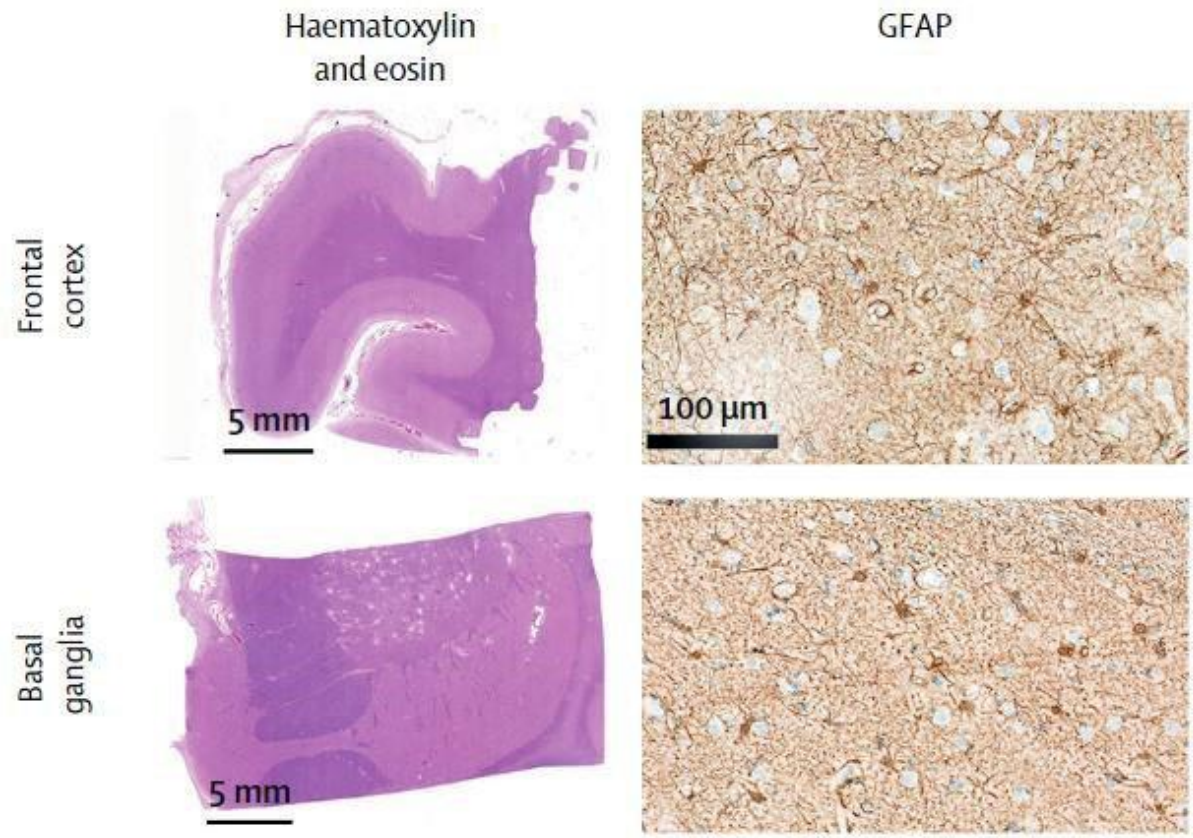

Figure 2. On the left, the general appearance of different parts of the brain is shown by hematoxylin-eosin staining. On the right side, immunohistochemical staining for the astrocytic marker GFAP showed variable reactive astrogliosis (brown) in these brain regions. GFAP; glial fibrillary acidic protein (Matschke J et al.)(20).

The increased reactivity of microglia, which are macrophages residing in the brain, sets the stage for the excessive release of neuroinflammatory cytokines following activation of the peripheral innate immune system (21). Reactive microglia; the production of myelin-forming oligodendrocytes disrupts cellular homeostasis mechanisms such as myelin plasticity and new neuron formation in the hippocampus. Secretion of local microglial cytokines plays a role in at least some of this dysregulation. Microglia also induces neurotoxic astrocyte reactivity via cytokine signaling. Reactive astrocytes can cause the death of oligodendrocytes and neuronal cells through the secretion of saturated lipids in lipoprotein particles (22). Hanley et al. stated that microglial activation is the most prominent pathological feature in COVID-19 cases. They also reported that there are variable levels of ischemic changes in neurons and white matter in the cerebral cortex (23). Fabbri et al. noticed that activation of microglia and astrocytes occurs mainly in the brain stem. In addition, the presence of microthrombus (Figure 3A) and cortical ischemic areas (Figure 3B) was detected in cortical or deeply located vessels (in the basal ganglia and along the brain stem) (24).

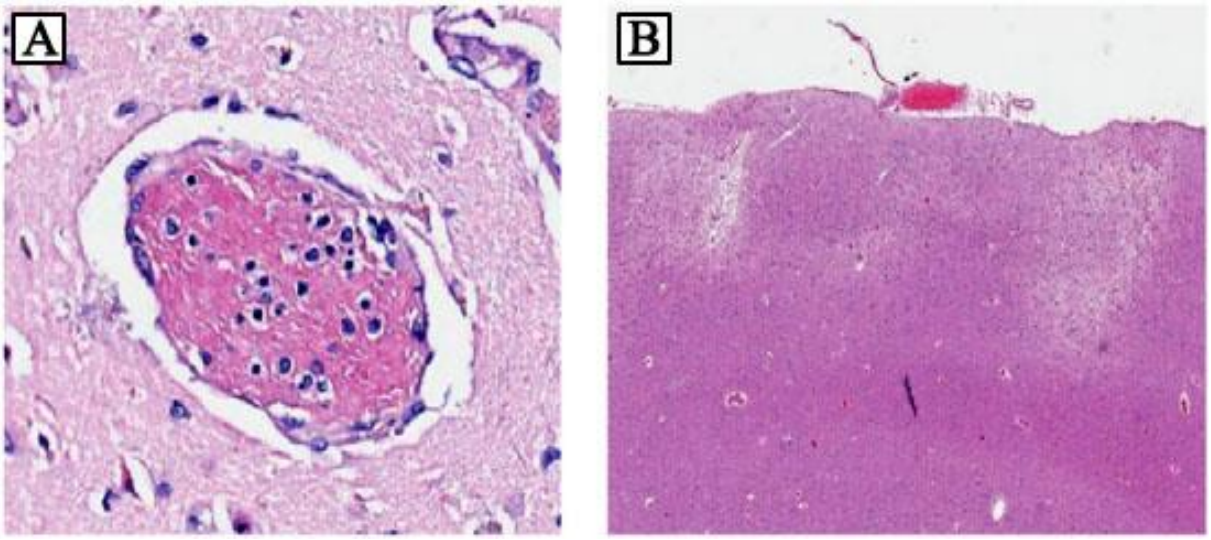

Figure 3. (A) Microthrombus is seen in the small intraparenchymal vessels in the brain stem of the SARS-CoV-2 positive case. (B) Cortical microscopic ischemic areas are noticed in the occipital cortex. Sections were stained with hematoxylin-eosin (Fabbri VP. et al.) (24).

Penetration of blood contents in the body into the brain can cause viral particles to directly enter and damage neurons. It has been previously described that other types of coronavirus can enter the brain directly. In the pathological study performed with postmortem electron microscopy, SARS-Cov-1 was detected in some neurons of patients with SARS-ARDS (Acute Respiratory Distress Syndrome) (25). Given their high level of genetic similarity, SARS-Cov-2 is also likely to play a direct role in demyelination or neurodegeneration (26, 27).

Although it is not known exactly how SARS-CoV-2 reaches the CNS, it is assumed that it can pass through the nerves in the olfactory mucosa, which is a part of the nasal mucosa. Kirschenbaum et al. published the autopsy results of two male patients, aged 70 and 79, who died of SASR-CoV-2 infection. Before death, only one patient had anosmia. On histological examination, prominent leukocyte infiltrates and focal atrophies at the level of the lamina propria in the olfactory mucosa of both patients; As a result of immunohistochemical analyzes, "digestive chambers", which are thought to be indicative of axonal damage in olfactory nerves, were observed (Figure 4). In addition, perivascular lymphocyte infiltration and intravascular microthrombus, mostly in the basal ganglia, were detected in both brains (28).

On the other hand, it has been stated that if the endothelial cells in the blood-brain barrier or the epithelial cells in the blood-cerebrospinal fluid barrier in the choroid plexus are infected with a virus, there may be an increase in the permeability of the blood-brain barrier due to the cytokines released into the environment (29). In the electron microscopic findings of Paniz-Mondolfi et al., viral particles were found in the frontal lobe and endothelial cells of the brain (30). Varga et al. obtained evidence of extensive endothelial inflammation and that SARS CoV-2 directly infects endothelial cells in a variety of tissues. This endothelial dysfunction can cause vasoconstriction and damage to the blood-brain barrier along with cerebral ischemia and inflammation (13). SARS-CoV-2 infects the host using the angiotensin-converting enzyme 2 (ACE2) receptor, which is expressed in various organs and helps lower blood pressure under normal conditions. ACE2 receptors are also expressed by endothelial cells (31).

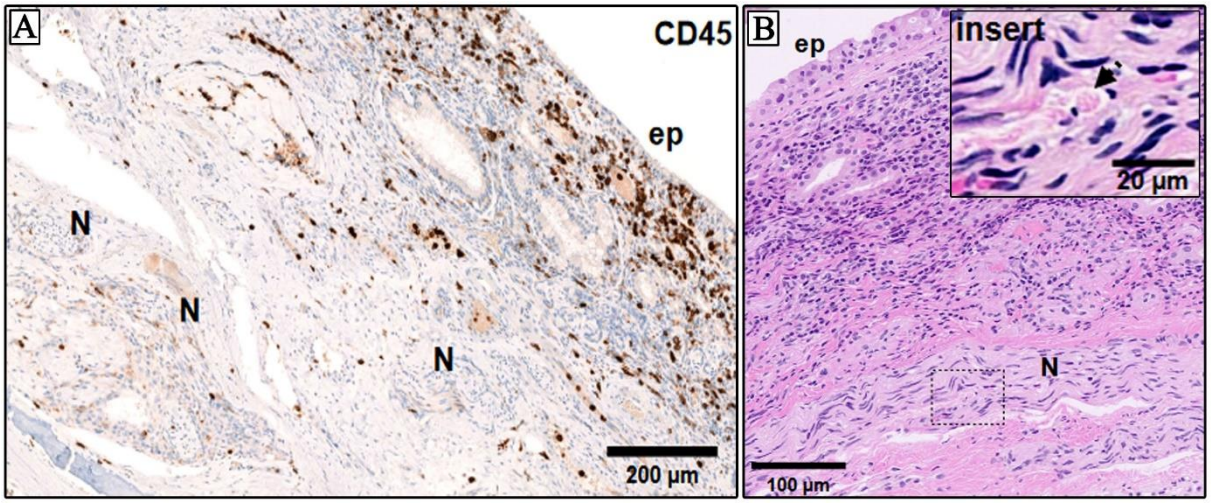

Figure 4. Autopsy brain tissues of patients with COVID-19 infection are seen. (A) Significantly differentiated leukocyte infiltration in the lamina propria of the olfactory mucosa was detected by CD45. (B) As a result of magnification of the area indicated by dashed lines, the presence of digestive chambers (black arrow) in olfactory nerve fibers is remarkable. The section was stained with hematoxylin-eosin. N; nerve fibers, ep; olfactory epithelium (Kirschenbaum D et al.) (28).

Pericytes are cells found in the wall of capillaries throughout the body, including the brain. They play a role in regulating blood flow, sealing the vessel walls, and thus protecting neurons. First, it was reported that SARS-CoV-2 can infect pericyte-like cells in brain organoids (32). In a different study published later, it was observed that SARS-CoV-2 caused the narrowing of the capillaries in the tissue by blocking the functioning of the receptors in the pericytes (33,34).

CONCLUSION

One of the most important issues regarding COVID-19 infection is the mechanisms underlying the very different symptoms that the disease causes in the brain. Exposure to the disease is likely to affect the brain in many ways, including the psychological and systemic impact. In addition, studies on whether the virus directly affects the brain draw attention (35). Current findings show that individuals with COVID-19 are at high risk for the development of neurological diseases, especially Alzheimer's.

The few studies to investigate the histopathological features of COVID-19 nerve involvement did not highlight any specific damage to brain tissue directly caused by the virus. According to the current neuropathological findings, the possibility of a reactive response of CNS components due to systemic inflammation and coagulopathy caused by SARS-CoV-2 and frequently causing infarction, hemorrhage and microthrombus formation in various brain regions of infected individuals seem more likely (8).

Autopsy-based histopathological analyzes are extremely important to understand the neuropathogenesis of SARS-CoV-2 infection and to design effective strategies to control the lethal consequences of the disease.

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CHAPTER 5

EVALUATION OF CLINICAL CHARACTERISTICS OF SARS COV-2 (COVID-19) AND SARS COV-2 VARIANTS

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INTRODUCTION

As of February 2020, covid-19 (SARS-CoV-2), which is accepted as a worldwide pandemic by the World Health Organization (WHO), has been met with concern all over the world, as there are many unknowns and high death rates.

Coronavirus; was first detected in December 2019 in Wuhan, China, with 3 cases of severe pneumonia. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative pathogen of coronavirus disease (COVID-19), caused 5.3 million deaths worldwide as of November 2021 (1). Adaptive mutations in the viral genome can change the pathogenic potential of the virus (2). Many vaccines have been developed over the years. The SARS-CoV-2 virus showed simultaneous immune-escaping ability, the emergence of new mutant SARS-CoV-2 strains, and an increase in infective abilities (3). Covid-19 virus and its variants clinically manifest themselves with different features in humans.

CLINICAL FEATURES OF SARS-COV-2

The mean incubation period for SARS-CoV-2 is 5.1 days, and the disease manifests itself within 11-12 days (4). It is thought that 17.9% to 33.3% of infected people are asymptomatic groups (5,6). Patients with SARS-CoV-2 infection may present with different clinical manifestations, such as septic shock and multi-organ failure, which progress from asymptomatic to respiratory failure and have more severe clinical manifestations. The majority of patients with symptoms usually present with fever, cough, and shortness of breath, and less frequently, they may present with a sore throat, anorexia, taste and smell disturbance, weakness, myalgia, and diarrhea. A study by Stokes et al. reported that of 373,883 confirmed symptomatic COVID-19 cases in the USA, 70% experienced fever, cough, and shortness of breath, 36% reported muscle pain and 34% reported headache (7). Severe cases are associated with respiratory diseases such as pneumonia and acute respiratory distress syndrome (ARDS). Postmortem studies have documented a link between aspects of respiratory distress syndrome, particularly diffuse alveolar damage and SARS-CoV-2 infection (8-10).

The disease caused by SARS-CoV-2 primarily affects the respiratory system, as it affects many systems. It spreads from person to person, mostly through respiratory particles from activities such as coughing and sneezing. Most transmission results from close contact with presymptomatic, asymptomatic, or symptomatic carriers. Contamination by aerosol-generating procedures and contamination of inanimate surfaces with SARS-CoV-2 also play a role in the spread of COVID-19 (11).

EFFECTS ON OTHER SYSTEMS

Cardiac effects: May manifest as Myocardial ischemia/infarction (MI) or myocarditis, more often with damage to myocardial tissue. Other common cardiac disease manifestations include arrhythmias, cardiogenic shock, and cardiomyopathy. In a study, it was reported that 27.8% of patients showed myocardial damage with elevated troponin levels. This study also shows that high troponin levels may increase disease severity and increase mortality compared to normal troponin levels (12).

It can cause Guillain-Barré syndrome (GBS), which manifests itself with peripheral nervous system damage as well as neurological findings such as headache, unconsciousness, epileptic seizure, ischemic cerebrovascular disease, and anosmia (13,14).

Hematological effects: Lymphopenia is a finding in the majority of patients with COVID-19. However, thrombocytopenia, leukopenia, high sedimentation levels, C-reactive protein (CRP), lactate dehydrogenase (LDH), and leukocytosis may be seen. COVID-19 is also associated with a state of coagulopathy as evidenced by the high prevalence of venous and thromboembolic events. Significantly elevated D-dimer, fibrinogen levels, prolonged prothrombin time (PT), and partial thromboplastin time (aPTT) are associated with COVID-19 patients at risk of developing arterial and venous thrombosis. Especially pulmonary embolism, deep vein insufficiency, ischemic stroke, and myocardial ischemia are examples of thromboembolic diseases (12,15).

Renal effects: Acute renal failure is one of the most common symptoms outside the respiratory system in Covid 19 patients and has a high mortality relationship (16,17). In studies conducted, acute kidney injury (AKI) occurs due to many factors related to hypervolemia, drug-induced toxicity, as well as cytotoxicity caused by the possible virus in hospital-followed covid 19 patients (18). Hirsch et al. In their study with 5449 covid-19 patients, reported that 14.3% of them required renal replacement therapy (RRT) (18). Other clinical and laboratory symptoms include electrolyte abnormalities such as proteinuria, hematuria, hyperkalemia, hyponatremia, and acid-base balance disorders such as metabolic acidosis. is located (12,15).

Gastrointestinal effects: Elmunzer et al. In a meta-analysis study conducted by; reported that 1992 patients (53%) experienced GI symptoms, the most common symptoms being diarrhea (34%), nausea (27%), vomiting (16%), abdominal pain (11%)(19). Elevated liver function tests, COVID-19 occurs frequently in 14% to 53% of patients with infection (20). Elevated liver enzymes are more common in severe cases. However, cases of acute mesenteric ischemia and portal vein thrombosis due to coagulation disorders have also been described (21).

Endocrinological effects: It is emphasized that patients with chronic diseases such as diabetes mellitus increase in the severity of the disease, uncontrolled glucose levels, abnormal blood sugar levels, and clinical manifestations such as euglycemic ketosis and diabetic ketoacidosis may develop in Covid 19 patients (12). However, pituitary dysfunction and thyroid dysfunction are endocrinological diseases that can be seen in covid 19 patients (22). Viruses mutate in host cells during their replication cycle. With evolution, this high mutation rate allows for rapid adaptation (23).

Given their clinical impact on populations, multiple variants of SARS-CoV-2 have been identified, several of which are considered variants of concern (VOCs). Enhanced infectivity or virulence of variants is associated with a decrease in immunization neutralization, the ability to evade detection, or a decrease in therapeutics or vaccination efficacy by antibodies obtained by natural transmission or vaccination. According to the WHO epidemiological update of 11 December 2021, five variants of SARS-CoV-2 have been identified since the beginning of the pandemic (24).

SARS-COV-2 VARIANTS ARE CLASSIFIED UNDER TWO HEADINGS (1,24)

1-Variants to consider (Variant of Interest; VOI)
2-Variants of Concern (Variant of Concern; VOC)

- Increased contagion or disease severity
- Significant reduction in susceptibility to one or more treatment classes
- Significant reduction in neutralization by antibodies produced by previous infection or vaccination
- Variants with decreased effectiveness of current diagnostic tests
- In addition, a definition was made as variants with severe consequences (Variant of High Consequence; VOHC) (1). Variants with severe consequences (Variant of High Consequence; VOHC) In addition to the possible features of a VOC variant;
- Proven failure of diagnostic test targets
- A significant decrease in vaccine efficacy,
- The disproportionately high number of infections in vaccinated persons

- ❖ Significantly reduced sensitivity to therapeutic agents,
- ❖ Defined as variants causing more severe clinical disease and increased hospital admissions.

1. **Alpha (B.1.1.7):** The first type of concern was declared in the UK (UK) in late December 2020
2. **Beta (B.1.351):** First reported in December 2020 in South Africa.
3. **Delta (B.1.617.2):** First reported in India in December 2020.
4. **Gamma(P.1):** First reported in Brazil in early January 2021.
5. **Omicron (B.1.1.529):** first reported in South Africa in November 2021.

PROPERTIES OF VARIANTS

1. Alpha (B.1.1.7): It has been reported to be descended from B.1.1.7, the SARS-CoV-2 worrying strain. The variant described in the United Kingdom (UK) was reported to be 43% to 82% more contagious. The three mutations with the highest potential to affect the infectiousness of the virus have been reported as H69-V70del, N501Y, and P681H (25,26).

Data from epidemiological studies show that the B.1.1.7 variant is more contagious than pre-existing variants (27,28). A reported large-scale study showed that individuals infected with the B.1.1.7 variant were more likely to report cough, sore throat, fatigue, muscle pain, and fever. It was reported 7 days before the test that it was less likely to report loss of taste or smell. In addition to its rapid spread, clinical symptoms such as nasal discharge, congestion, and cough were more prominent at the first onset of the disease (29). Studies show that the B.1.1.7 variant increases the risk of hospitalization and death (30). Although the effect of this worrying variant is not clearly understood, especially in children, Menger et al. have reported the chıld patıent wıth pnomonıa and pulmonary bleedıng of a 4-year-old chıld patıent ınfected wıth B.1.1.7 (31). acer et al. ın the comparıson of alpha and delta varıant, patıents wıth a vıral load especıally ın mortalıty are reported to be fatal. However, it has been reported that patients over the age of 40 are affected and the course of the disease continues moderately and severely. They emphasized that there is no sex difference in the B.1.1.7 variant (32,33).

2. The B.1.351, or Beta variant, appeared probably just after the first wave of the Covid-19 outbreak in South Africa in October 2020. At the end of November 2020, it spread to the Western and Eastern Cape Provinces and became the dominant virus strain (34). The variant with a high vaccine escape has been reported to be more likely to cause re-infection. They emphasized that (35,36). In addition, B.1.351 cases, including those <60 years old, were found to have higher rates of hospitalization and admission to intensive unit care compared with cases without VOCs[35]. Funk et al. When comparing the cases developing SARS-Cov2 and the Beta variant, report that B.1.351 cases, including those <60 years old, have higher hospitalization rates and admission rates to intensive unit care (37). The beta variant is highlighted (38).

3. Gamma variant; The P.1 variant, also known as the gamma variant or GR/501Y.V3, was identified in Brazil in December 2020 and was first detected in the USA in January 2021[4]. They report in one study that "reinfection with the P.1-linked variant is common and more frequent than detected by traditional epidemiological, molecular and genomic surveillance of clinical cases." Dejnirattisai et al. reported that the percentage of serious disease development in young patients increased, especially with the spread of the Gamma variant[39]. However, studies have reported that the Gamma variant may be responsible for an increase in the mortality rate, an increase in the severity of the disease in people without known SARS-CoV-2 risk comorbidities, and a decrease in the time from the onset of symptoms to hospitalization (39-42). When the gamma variant emerged, it was reported that the people in the Amazon region encountered nearly 100% of the virus (43).

4. Delta variant, Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) B.1.617.2 (delta) variant was first detected in India in December 2020, after the 2019 coronavirus disease (COVID-19)

global pandemic. It became a more dominant variant (44). Due to its rapid spread in Korea, the number of cases increased very quickly within 5 months (45,46). Compared to other variants, hospitalization, mortality rates, and admission to intensive care unit (47-49). Delta variant has a higher degree of viremia and a more active inflammatory response (50,51).

5. Omicron (B.1.1.529) has been reported that the Omicron variant, which is one of the variants of SARS-CoV-2, is mutated with 32 mutations only in the spike protein of 50 mutations (52). The clinical features of the "Omicron variant" infection consisted mostly of mild symptoms. Although its prevalence is high, patients usually present with a mild cough, fever, generalized muscle pain, weakness, scratchy but not sore throat, headache, body aches, and moderate to severe fatigue (53,54). In a study, it is reported that the virus reproduction rate is 3.8 times higher than the delta variant (55).

The variant of Omicron is a highly contagious variant with a doubling over a 2–3 day period. Data from its Study reported by ZOE and King's College London show that Omicron symptoms predominantly come from the upper respiratory tract and include runny nose, sore throat, headache, fatigue, and sneezing (56). Loss of smell and taste, which was previously one of the common symptoms of COVID-19 infection, is reported to be rare during Omicron infection. Early data also suggest that Omicron infection is associated with a lower risk of hospitalization, serious illness, and death (57,58). Report that the omicron variant, in particular, is a symptom of odynophagia in a case series presented (59).

As a result; SARS-CoV-2 and its variants showed themselves in different waves with different symptoms. Communities gained immunity by exposure to the agent or by vaccination.

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CHAPTER 6

THE IMPORTANCE OF PHOTOTHERAPY AGENTS IN THE COVID-19 PANDEMIC

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INTRODUCTION

A novel coronavirus (SARS-CoV-2), causing an emerging coronavirus disease (COVID-19), was first detected in Wuhan City, Hubei Province, China, which has taken a catastrophic turn with high toll rates in China and subsequently spreading across the globe. The rapid spread of this virus to more than 210 countries while affecting more than 25 million people and causing more than 843,000 human deaths, it has resulted in a pandemic situation in the world. The SARS-CoV-2 virus belongs to the genus Betacoronavirus, like MERS-CoV and SARS-CoV, all of which originated in bats. It is highly contagious, causing symptoms like fever, dyspnea, asthenia and pneumonia, thrombocytopenia, and the severely infected patients succumb to the disease. Coronaviruses (CoVs) among all known RNA viruses have the largest genomes ranging from 26 to 32 kb in length. Extensive research has been conducted to understand the molecular basis of the SARS-CoV-2 infection and evolution, develop effective therapeutics, antiviral drugs, and vaccines, to design rapid and confirmatory viral diagnostics as well as adopt appropriate prevention and control strategies. To date, Auguon st 30, 2020, no effective, proven therapeutic antibodies or specific drugs, and vaccines have turned up. This review focuses on potentially effective plants and their effects apart from routine drugs. Lundstrom has stated that more than 300 potential therapies and at least 150 vaccine studies are in progress at various stages of preclinical or clinical research. Also, Amir et al. stated that natural products and their derivatives have potential activities in the treatment of viral infections. Until now, several herbal extracts or their derivatives have shown potential antiviral efficacy. However, there are no adequate studies on the development of anti-COVID-19 agents from herbal extracts. Such herbal extracts are important to prevent and combat COVID-19. For example, Maideen has stated that various randomized controlled trials, pilot studies, case reports, and in vitro and in vivo studies confirmed that Nigella sativa has antiviral, antioxidant, anti-inflammatory, immunomodulatory, bronchodilatory, antihistaminic, antitussive activities related to a causative organism and signs and symptoms of COVID-19. Maidenn stated that N. sativa could be used as an adjuvant therapy along with repurposed conventional drugs to manage the patients with COVID-19 (1-4).

ANTIVIRAL HERBAL THERAPIES AGAINST COVID-19

The application of phytomedicines has increased due to their therapeutic value when compared to allopathic medicines as these bio-compounds exhibit fewer side effects. The possibility of using plant-derived phytochemicals (particularly polyphenols) with putative active substances (e.g., flavonoids, gallates, and quercetins), which are potent agents prohibiting the proliferation of the COVID-19-inducing coronavirus has recently been reviewed by. They can be used as pharmaceutical preparations or functional foods. Herbal plants such as Artemisia kermanensis, Eucalyptus caesia, Mentha spp., Rosmarinus officinalis, Satureja hortensis, Thymus spp., and Zataria multiflora is typical examples of rich sources of phenolic compound. Biflavonoids from Torreya nucifera inhibited the replication of SARS-CoV 3CLpro. Senna L. is a large genus of flowering plants in the legume family (Fabaceae) othe f subfamily Caesalpinioideae, comprised of 300–350 species. It is a widespread and diverse genus. Many species of Senna are commonly used in foods and herbal medicine. In different parts of the world, the whole Senna alata plant is currently being used in the treatment of flu, fever, malaria, a and large number of other medical conditions due to the presence of bioactive compounds in the plant, including quinones, alkaloids, and terpenes. The leaf extract of Senna alata considerably inhithe bited 3D7 strain of the Plasmodium falciparum parasite in vitro. Recently, it was determthe ined that use of an aqueous extract obtained after boiling 5 g of Senna leaves in 500 mL water for 10 min provided relief from virüs symptoms in COVID-19 patients (unpublished data). After boiling, it is recommended that half of the aqueous leaf extract can be used immediately while the second half can be used after 8–10 h to avoid toxicity. In the intervening time, water taken frequently can avoid toxicity. In Tooid the bitter taste of aqueous leaf extract, honey can be added, which has also an antimicrobial effect. As Senna alata plants

are currently being used to treat different medical conditions in different parts of world, incluthe ding Africa and Asia, there is a need to confirm Senna leaf aqueous extracts in COVID-19 patients as a herbal therapy. Lianhuaqingwen (LH), a Traditional ChineseMedicine formula composed of a combination of 13 herbs was shown to suppress SARS-CoV-2 replication, reduced pro-inflammatory cytokine production, and changed the morphology of SARS-CoV-2 cells. It was noteworthy that transmission electron microscopy (TEM) revealed that the number of virus particles in infected patients was greatly reduced in cells infected with SARS-CoV-2 that were treated with LH at 600 μg/mL. LH is widely used for a variety of respiratory virus infections, including influenza virus. The precise mechanism of action of LH to reduce virus infection remains unknown, although it has been shown to also reduce cytokine release from infected cells, suggesting that multiple levels of action are taking place. Chinese health authorities in 23 out of 31 provinces have issued herbal remedyprograms to prevent the spread of COVID-19. The top two herbal formulas used were Radix astragali (Huangqi) and Glycyrrhizae radix Et Rhizoma (Gancao). Another study recommends the use of tender leaf of Toona sinensis Roem, a popular Chinese vegetable that is already used safely. In vitro studies show that the tender leaf of Toona sinensis Roem, can inhibit SARS-CoV. It is expected to also block SARS-CoV-2. As mentioned above, licorice root has some bioactive properties, including antitumoral, anti-inflammatory, and antiviral effects on health. Glycyrrhizic acid (glycyrrhizin, GL) and its aglycone glycyrrhetinic acid (GLA) are active against a broad spectrum of viruses, including herpes viruses, flaviviruses, Hepatitis C virus, human immunodeficiency virus, and SARS coronavirus (SARS-CoV) in vitro. Virus inhibition has been demonstrated in Vero cells and in patients. Further dissection of glycyrrhizic acid indicated that sugar moieties are responsible for the anti-SARS activity, as a replacement of these with functional groups resulted in a loss in activity. Earlier studies using herbs to block SARS could also successfully inhibit SARS-CoV2 infection. For example, Wen et al. examined extracts of over 50 traditional Chinese medicinal (TCM) herbs on anti-SARS-CoV activity using a Vero E6 cell-based cytopathogenic effect (CPE) assay. The authors were able to demonstrate that six novel herbal extracts may be used as potential SARS drug targets. The herbal extracts were derived from Rhizoma cibotii, Gentianae radix, Dioscoreae rhizoma, Cassiae semen, and Loranthi ramus; all of them inhibited SARS-CoV replication, and two inhibited virus protease activity. Studies using phlorotannins isolated from the edible brown algae Ecklonia cava found that several of these bioactive compounds were able to inhibit SARS-CoV activity by functioning as protease inhibitors. One of these, dieckol, had the most potent antiviral activity; this took place through competitive binding at the catalytic site of the protease. It is feasible that dieckol would also have an inhibitory effect against SARS-2 (5-18).

POTENTIAL THERAPEUTIC AGENTS FOR COVID-19 PANDEMIC (19).

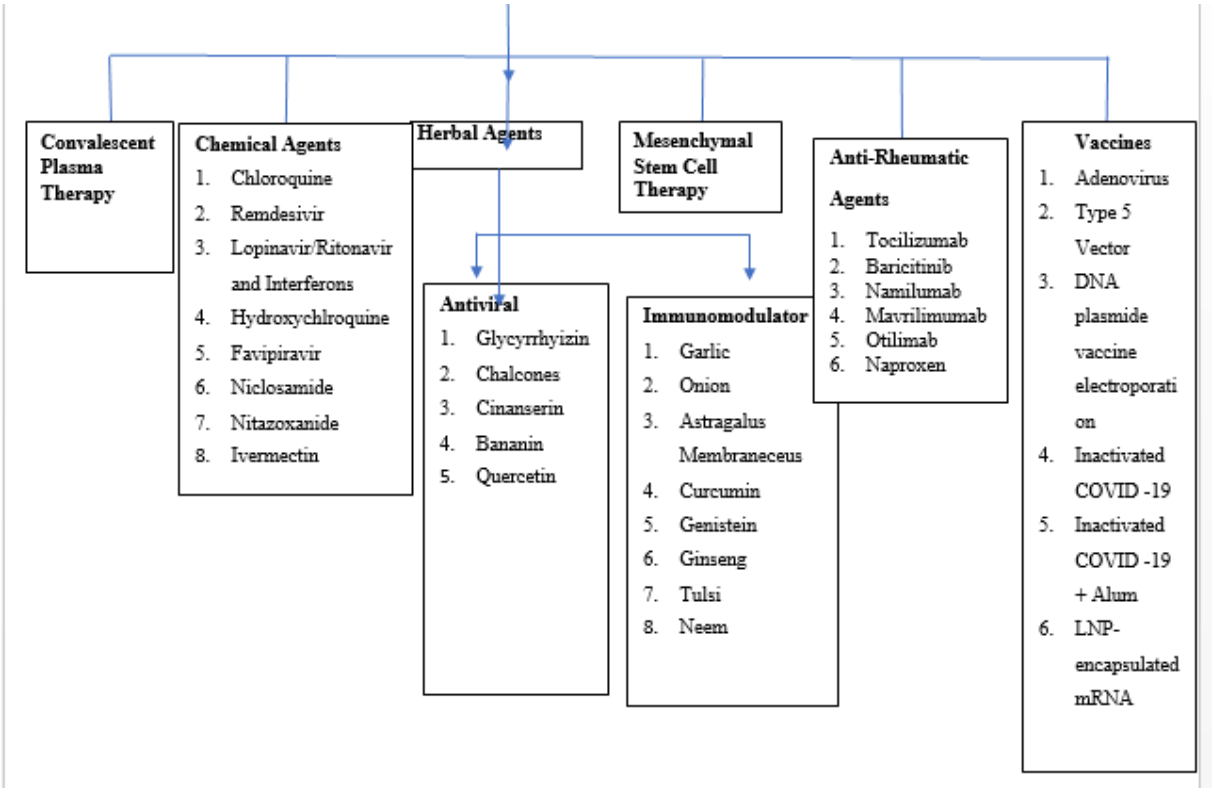

Figure 1. Potential Therapeutic Agents For Covid-19 Pandemic.

Bhandari et al. summarized some of the studies as follows: a study done on glycyrrhizin which is a major active constituent of licorice root used seldom as a herb in Traditional Chinese Medicine has shown its potential to inhibit the replicating isolates of the SARS virüs. The activity of various herbs has been evaluated for activity against COVID-19. Glycyrrhizin causes inhibition of SARS-associated coronavirus penetration, adsorption, and replication. Chalcones containing perhydroxyl group from Angelica keiskei exhibit potent inhibitory activity against SARS-CoV proteases. Cinanserin is a serotonin antagonist obtained from the Houttuynia cordata of family Saururaceae. Being another example of an agent that causes inhibition of SARS-CoV replication, it may inhibit the protease enzyme as well. Bananins are a type of compounds which have a trioxa-adamantane moiety bounded with a pyridoxal moiety. Bananin was also found to inhibit SARS-CoV replication in fetal rhesus kidney 4 cells with over 300 μ M of 50% cytotoxicity concentration. Quercetin is an aglycone found in onions that possess a virucidal activity against a large variety of viruses. In a study done on quercetin flavonoid obtained from Houttuynia cordata, a successful inhibition of murine coronavirus was seen (19). Also, Benarba and Pandiella have shown that possible anti SARS-CoV 2 actions of natural products (Figure 2) (20).

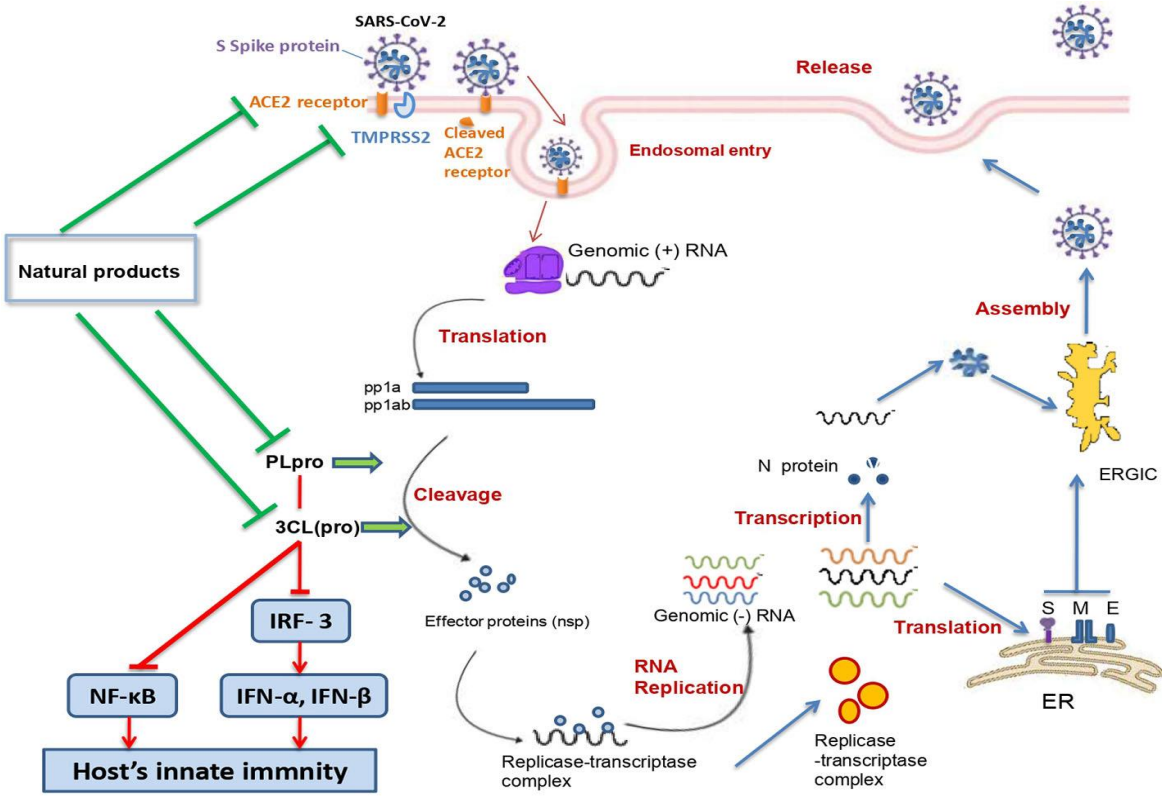

Figure 2. Benarba and Pandiella have shown that possible anti SARS-CoV 2 actions of natural products (20).

Alami et al. announced that they identified a total of 23 medicinal plant species belonging to 11 botanical families used during the Covid-19. Alami et al explained as follows: the most important family is that of the Lamiaceae represented by seven species (Thymus maroccanus, Thymus satureioides, Mentha suaveolens, Mentha suaveolens, Rosmarinus officinalis, Lavandula dentate and Lavandula dentate), followed by the family of Cupressaceae with three species (Tetraclinis artusulate, Juni phoenicea and Juniperus oxycedrus) and the family of Zingiberaceae (Zingiber officinale, Alpinia officinarum and Curcuma xanthorrhiza). The family of Apiaceae is represented by two species (Pimpinella anisum and Foeniculum vulgare) and the family of Liliaceae is represented by Allium cepa and Allium Sativum. The other six families are only represented by a single species (Asteraceae: Artemisia herba-alba; Myrtaceae: Eucalyptus globules; Ranunculaceae: Nigella sativa; Oleaceae: Olea europaea; Arecaceae: Phoenix dactylifera; Brassicaceae: Lepidium sativum). In general, the infusion or decoction of areal parts of the Lamiaceae and Asteraceae species, the infusion of seeds of the Apiaceae species, the decoction of Zingiber officinale and Alpinia officinarum rhizomes, and the powder obtained by drying young twigs of the Cupressaceae species are given orally. The bulb of the Liliaceae species, fruits of Phoenix dactylifera, seeds of Lepidium sativum and of Nigella sativa, the rhizome powder of Curcuma xanthorrhiza and the oil of Olea europaea are taken also orally. Hot infusion of Eucalyptus globulus is used for inhalation (21).

The use frequencies of the main medicinal plants (use frequency > 10%) used during the Covid-19 are shown in the Figure 1. The most used plants (use frequency > 40%) were Allium Sativum (80.9%), Olea europaea (72.7%), Allium cepa (66.7%), Zingiber officinale (66%), Thymus maroccanus (59.2%), Eucalyptus globules (56.5%), Foeniculum vulgare (54.3%), Curcuma xanthorrhiza (50%), Phoenix dactylifera (50%), Rosmarinus officinalis (47.9%), Thymus satureioides (41.9%), Mentha pulegium (41.3%) and Pimpinella anisum (40%). Alami et al. showed the plants used against covid 19 and the rate of use of these plants in figure 3 (21).

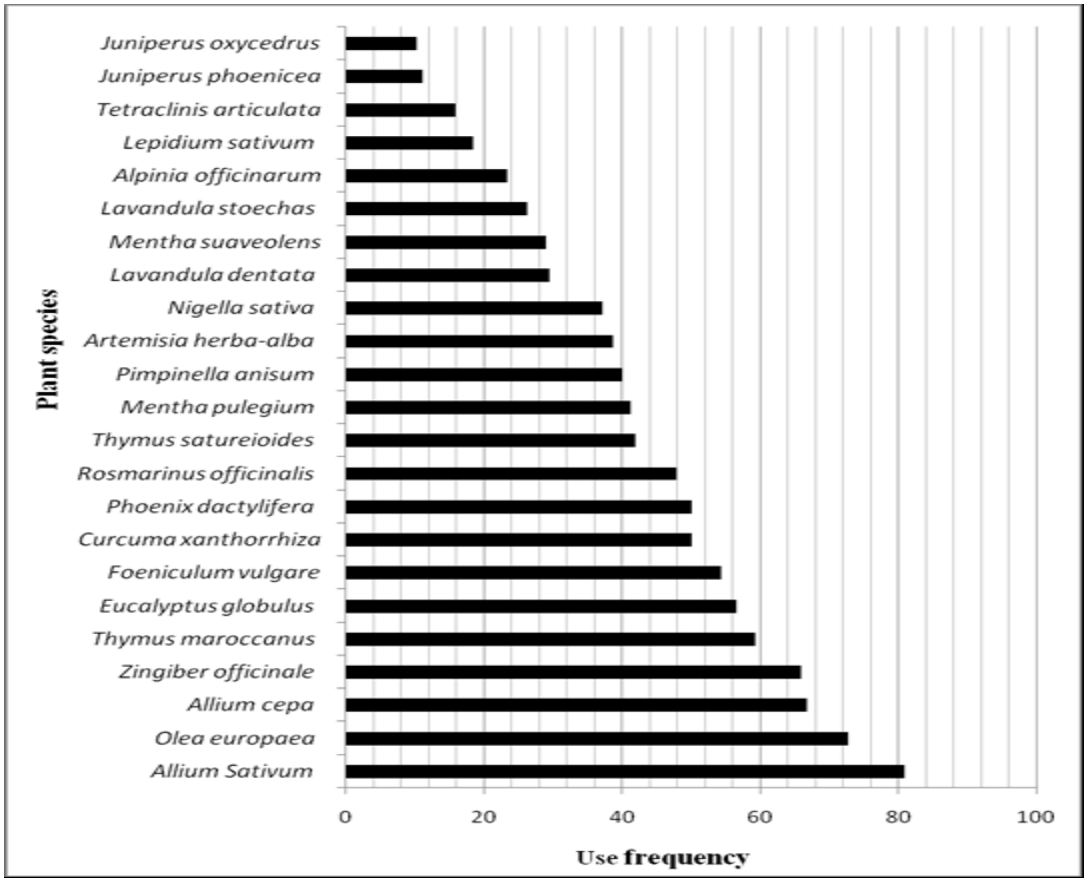

Figure 3. Alami et al. showed the plants used against covid 19 and the rate of use of these plants (21).

However these plants may contain toxic substances which can cause various overdose intoxications and disorders. The majority of medicinal plants used for prevention purposes during the Covid-19 pandemic may contain toxic substances such as phenols, colchicine, carvone, anisatin, neoanisatin, anethole, sesquiterpene lactones, cyanogenic glycosides, myristicin, safrole, Coumarin, Cinnamaldehyde, Elemicine and Nigelline which in overdose can cause a wide variety of disorders and intoxications (21).

Benarba and Pandiella also explained that the most frequently six herbal species were found to be the most frequently used including Astragalus mongholicus Bunge, Glycyrrhiza glabra L., Saposhnikovia divaricata (Turcz. ex Ledeb.) Schischk., Atractylodes lancea (Thunb.) DC., Atractylodes macrocephala Koidz., Lonicera japonica Thunb., and Forsythia suspensa (Thunb.) Vahl. These species are the ingredients of the Chinese traditional medicine Yupingfeng powder. On the other hand, the ethanol extract of Sambucus javanica subsp. chinensis (Lindl.) Fukuoka stem exerted promising anti-human coronavirus NL63 effects with IC50 ranging from 1.17 (virus yield) to 15.75 μg/ml (virus attachment). The extract significantly decreased virüs yield, plaque formation, and virus attachment. Furthermore, three of its major phenolic acids (caffeic, chlorogenic, and gallic acid) were shown to inhibit the NL63 replication and virus attachment. Caffeic acid was the most potent phenolic acid. Phenolic acids are characterized by their metabolizing ability by the microbiota enhancing their bioavailability. Moreover, their antiviral potential could be increased with the alkyl chain length. However, their efficacy is still controversial due to their low absorption and instability in alkaline and neutral media, which could limit their use in pure form. Therefore, the clinical utility of phenolic compounds as anti-SARS-CoV-2 agents remains debatable since their bioavailability, delivery mechanisms and efficient doses should be further studied using in vivo models (20). Main purpose is; to increase the immunity before the disease and to increase the patient's compliance to the treatment by reducing the expected / possible side effects of chemical drugs during the disease. At the phytotherapeutic level in the treatment of COVID-19 infection, Curcuma Longa, Vitamin D, Vitamin

C, Vitamin A, Zinc (Zn), Iron (Fe), Propolis, Scutellaria baicalensis, Glycyrrhiza glabra, Allium cepa, Malus domestica, Solanum lycopersicum, Fragaria, Matricaria recutita, Petroselinum crispum, Apium, Allium sativum, Quercetin, Astragalus membranaceus, Mycelium Mushroom Extract, Mentha piperita, Andrographis paniculata, Rheum Palmatum, Aloe vera, Salvia officinalis, Melissa officinalis, Rosa canina, Nigella Sativa, and Rhus typhina can be used. In Traditional Chinese Medicine (TCM), Saposhnikoviae divaricata, Rhizoma Atractylodis Macrocephalae, Lonicerae Japonicae Flos, Fructus forsythia, Atractylodis Rhizoma, Radix platycodonis, Agastache rugosa, Cyrtomium fortune J. Sm, Shu Feng Ji eDu and Lian hua qing wen capsules can be recommended (22). In conclusion, based on historical records and clinical evidence of SARS and H1N1 infl uenza prevention, Chinese herbal medicine formula could be an alternative approach for the prevention of COVID-19 in high-risk population while waiting for the development of a successful vaccine. Prospective well design population studies are needed to evaluate the preventive effect of Chinese medicine (23). Also Weng et al. states that the antiviral activity of Sambucus FormosanaNakai stem ethanol extract and some phenolic acid constituents against HCoV-NL63. The extract was less cytotoxic and concentration-dependently increased anti-HCoV-NL63 activities, including cytopathicity, sub-G1 fraction, virus yield (IC50 = 1.17 μg/ml), plaque formation (IC50 = 4.67 μg/ml) and virus attachment (IC50 = 15.75 μg/ml). Among the phenolic acid constituents in Sambucus FormosanaNakai extract, caffeic acid, chlorogenic acid and gallic acid sustained the anti-HCoV-NL63 activity that was ranked in the following order of virus yield reduction: caffeic acid (IC50 = 3.54 μM) > chlorogenic acid (IC50 = 43.45 μM) > coumaric acid (IC50 = 71.48 μM). Caffeic acid significantly inhibited the replication of HCoV-NL63 in a cell-type independent manner, and specifically blocked virus attachment (IC50 = 8.1 μM). Therefore, the results revealed that Sambucus Formosana Nakai stem ethanol extract displayed the strong anti-HCoV-NL63 potential; caffeic acid could be the vital component with anti-HCoV-NL63 activity. The finding could be helpful for developing antivirals against HCoV-NL63 (24). Also, plant phenolics are considered to be a vital human dietary component and exhibit a tremendous antioxidant activity as well as other health benefits. Epidemiology evidence indicates that a diet rich in antioxidant fruits and vegetables significantly reduces the risk of many oxidative stress related diseases viz. cancers, diabetes and cardiovascular. The number and position of hydroxyl group in a particular phenolic compound leads to the variation in their antioxidant potential. Polyphenols are the main source of dietary antioxidants, and are effortlessly absorbed in the intestine. Phenolic acids, a sub class of plant phenolics, possess phenol moiety and resonance stabilized structure which causes the H-atom donation results in antioxidant property through radical scavenging mechanism (25).

Cho et al. (26) identified five new geranylated flavonones, tomentin A, tomentin B, tomentin C, tomentin D, tomentin E from the ethanolic extract of Paulownia tomentosa (Thunb.) Steud. fruits. These flavonoids besides seven other knowns resulted in significant inhibition of SARS-CoV PLpro in a dose dependent manner with IC50 of 5.0 and 14.4 mM. Tomentin E exhibited the highest inhibitory effect with an IC50 of 5.0 ± 0.06 μM. It has been found that molecules with 3,4-dihydro-2H-pyran moiety possessed higher inhibition. The P. tomentosa flavonoids were found to be reversible, mixed inhibitors. Also Park et al. (27) stated that the inhibitory potential of nine alkylated chalcones (isobavachalcone, 4-hydroxyderricin, xanthoangelol, xanthoangelol F, xanthoangelol D, xanthoangelol E, xanthoangelol B, xanthoangelol G, xanthokeistal A) and four coumarins extracted from the ethanolic extract of Angelica keiskei (Miq.) Koidz. The alkylated chalcones inhibited SARS-CoV PLpro in a significant dose-dependent manner with IC50 ranging from 1.2 ± 0.4 to 46.4 ± 7.8 μM. On the other hand, the analyzed coumarins did not show a significant inhibitory effect toward SARS-CoV PLpro. Also they stated that Kim et al explained study that ethanolic extract of Cullen corylifolium (L.) Medik. Seeds showed an important inhibitory effect of SARS-CoV PLpro with an IC50 of 15 μg/ml. Furthermore, six flavonoids present in the extract (Bavachinin, neobavaisoflavone, isobavachalcone,40 –O-methylbavachalcone, psoralidin, and corylifol A) inhibited SARS-CoV PLpro activity in a dosedependent manner with IC50 estimated to be 4.2–38.4 μM. The highest inhibitory effect was exerted by psoralidin (IC50 = 4.2 ± 1.0 μM) and isobavachalcone (IC50 = 7.3 ±0.8 μM). Finally Nguyen et al. (28) explained Seven flavonoids

(Quercetin, Puerarin, Daidzein, gallocatechin gallate, epigallocatechin gallate, epigallocatechin, ampelopsi) were evaluated for their inhibitory effects of SARS-CoV-3CL(pro) expressed in Pichia pastoris GS115. At 200 μM, gallocatechin gallate, epigallocatechin gallate, and quercetin were able to inhibit the SARS-CoV-3CL(pro) activity by 91, 85, and 82%, respectively. Gallocatechin gallate was found to be a competitive inhibitor of SARS-CoV-3CL(pro) with IC50 of almost 47μM (20).

Jalali et al. summarized that some possible medicinal plants/components with the potential to interfering with covid-19 pathogenesis sign and symptom therapy of covid-19 in below table 1 and table 2 (29).

TABLE 1. POSSIBLE MEDICINAL PLANTS/COMPONENTS WITH THE POTENTIAL TO INTERFERING WITH COVID-19 PATHOGENESIS (29)

No	Scientific Name/family	Antiviral Activity Type	Main Component	Mechanism/Outcome	Study
1.	Alnus japonica (Thunb.) Steud./ Betulaceae	B	Diarylheptanoid (Hirsutenone)	PLpro inhibition/ IC50 = 4.1 μM	-
2.	Andrographis paniculata (Burm.f.) Nees/Acanthaceae	A B	Andrographolide Andrographiside	ACE inhibition SARS-3CLpro inhibition	3 3
3.	Artemisia annua L./ Compositae	C	-	-/inhibition of infection [EC50 = 34.5 ± 2.6 μg/mL]	1
4.	Asparagus racemosus Willd./Liliaceae	A	-	ACE inhibition/ C50 = 82.88%	1
5.	Camellia sinensis (L.) Kuntze/Theaceae	A B	Polyphenol (Rutin) Phenol (theaflavin-3,30-digallate, tannic acid, [−]-epigallocatechin gallate)	ACE inhibition: Green <oolong <white <black <dark teas SARS-3CLpro inhibition	1,3 3
6.	Cibotium barometz (L.) J.sm./ Cibotiaceae	B	-	SARS-3CLpro inhibition/ IC50 = 39 μg/mL	1
7.	Citrus Spp./Rutaceae	B	Hesperetin, hesperidin Rhoifolin, Neohesperidin	SARS-3CLpro inhibition in dose-dependent manner	1,3
8.	Cynara scolymus L./ Compositae	A	Cynaroside	ACE inhibition/ IC50 = 49.7%	1,3
9.	Dioscorea batatas/ Dioscoreaceae	B	-	SARS-3CLpro inhibition/ IC50 = 44 μg/mL	1
10.	Erigeron abajoensis Cronquist/	A	Flavone (Scutellarin)	ACE inhibition	1

	Compositae				
11.	Equisetum hyemale L./Equisetaceae	A B	- Herbacetin	ACE inhibition/IC50 > 40% Positive 3CL pro inhibitory activity	- 3
12.	Glycyrrhiza glabra L./ Leguminosae	A	Glycyrrhizin	ACE inhibition/IC50 > 40%	-
13.	Hibiscus sabdariffa L./ Malvaceae	A	Anthocyanins Delphinidin-3-Osambubioside Cyanidin-3-Osambubioside	ACE inhibition/#serum angiotensin-converting enzyme, #plasma aldosterone	4
14.	Lindera aggregata (Sims) Kosterm./ Lauraceae	C	-	-/antiviral [EC50 = 88.2 μg/mL]	1
15.	Linum usitatissimum L./Linaceae	A B	Secoisolariciresionol Diglucoside Herbacetin	ACE inhibition SARS-3CLpro inhibition	2
16.	Fjfujg-Lycoris radiate —Amaryllis	C	Lycorine	-/inhibition of viral infection [EC50 = 15.7 ± 1.2 nM]	1
17.	Hancornia speciosa Gomes/ Apocynaceae	A	Chlorogenic acid	ACE inhibition	2,3
18.	Houttuynia cordata Thunb./ Saururaceae	B	-	SARS-3CLpro inhibition	1
19.	Isatis indigotica/ Brassicaceae	B	Phenol (indigo, sinigrin, aloe emodin, hesperetin, Sinigrin), 2,2-Di(3-indolyl)-3-indolone, Phaitanthrin D	SARS-3CLpro inhibition/ IC50 = 53.8 ± 4.2 μg/mL	-
20.	Psoralea corylifolia L./ Fabaceae	B	Bavachinin, neobavaisoflavone, isobavachalcone,	PLpro inhibition/dose dependent manner (IC50 between 4.2 and	1

			40-Omethylbavachalcone, psoralidin, corylifol A	38.4 μM)	
21.	Rheum officinale Baill./ Polygonaceae	A	Anthraquinone (Emodin)	Positive ACE inhibitor in combination with ACEI/ ARB agents	
22.	Scutellaria baicalensis Georgi/Lamiaceae	A B	Baicalin Cosmosiin	ACE inhibition SARS-3CLpro inhibition	3 3
23.	Paulownia tomentosa Steud./ Paulowniaceae	B	Geranylated flavonoids	PLpro inhibition/ IC50 = 5.0–14.4 μM	1,3
24.	Pyrrosia lingua (Thunb.) Farw./ Polypodiaceae	C	-	-/inhibition of viral infection[EC50 = 43.2 (±14.1) μg/ml]	1
25.	Toona sinensis (Juss.) M.Roem./ Meliaceae	C	-	-/anti SARS-CoV activity (selectivity index 12 _ 17)	1
26.	Torreya nucifera (L.) Siebold & Zucc./ Taxaceae	B	Biflavone [Amentoflavone (9)], Authentic flavones (Apigenin)	SARS-3CLpro inhibition/ 62% at 100 μg/mL	-
27.	Tribulus terrestris L./ Zygophyllaceae	B	Cinnamic amides	PLpro inhibition/ IC50 = 15.8–70.1 μM	1,3
28.	Tripterygium wilfordii Hook. f./ Celastraceae	B	Celastrol	Interferon I up-regulation, downstream interferon stimulation	1

Note: Antiviral activity type: (A) Virus entry inhibition to its host cells, (B) Inhibition of virus replication, (C) Non-specific anti-SARS-COV activity. Study type: 1: In-vitro, 2: In-vivo, 3: In-silico, 4: Clinical trial.

TABLE 2. POSSIBLE MEDICINAL PLANTS/COMPONENTS FOR SIGN AND SYMPTOM THERAPY OF COVID-19 (29)					
No	**Scientific Name/family**	**Sign (Type of complication)**	**Main Component**	**Mechanism/Outcome**	**Study**
	Aloe barbadensis mill./ Asphodelaceae	B	HF1Z (polysaccharide)	Emollient/potent antitussive activity	2
	Althaea officinalis L./ Malvaceae	A	-	-/↓ fever (marshmallow rinse)	4
		B	Mucilage (polysaccharides)	Emollient/potent antitussive activity	2
	Andrographis paniculata (Burm.f.) Nees/ Acanthaceae	A	Andrographolide	Inhibit the expression of IL-1β and IL-1α, ↓IL-6 release/antipyretic	2
		B	Arabinogalactan, Andrographolide	-/potent antitussive	2
	Angelica decursiva (Miq.) Franch. & Sav./ Apiaceae	B	Columbianadin	↓IL-1β,NO/ ↓airway inflammation	1
	Artemisia capillaris Thunb./Compositae	A	Capillarisin (flavone)	↓ TNF-α, IL-1β, IL-1α, and IL-6/antipyretic	5
	Cinnamomum cassia (L.) J.Presl/Lauraceae	A	7-Hydroxycoumarin, 4-allylanisole, Cinnamic acid Ethylester, acetic acid cinnamylester, 20-Hydroxyacetophenone, 2-Hydroxycinnamic acid	↓ IL-1/antipyretic	2
	Cissampelos pareira L./ Menispermaceae	A	-	↓TNF-α /antipyretic	2
	Citrus spp.	A	-	Inhibit the expression of COX-2, iNOS, IL-1β and IL-6/antipyretic	2
		B	Naringin, Naringenin	Opening conductance Ca2+-activated K+ channel/relax tracheal smooth muscle	1

	Eriobotrya japonica (Thunb.) Lindl./ Rosaceae	B	-	↓iNOS and COX-2/	1
	Euphorbia hirta L./ Euphorbiaceae	A	-	↓TNF-α/antipyretic	5
	Cuminum cyminum L./ Apiaceae	A B	- -	Inhibit the expression of COX-2, iNOS, IL-1β and IL-6/antipyretic ↓inflammation/significant antitussive activity	5 2
	Glycyrrhiza glabra L./ Leguminosae	B	Arabinogalactan protein	Spasmolytic activity, protective effects on mucous ↓/ citric acidinduced cough Guinea pigs	2
	Salvia officinalis L./ Lamiaceae	B	Polysaccharide	Emollient/potent antitussive activity	2
	Malva sylvestris L./ Malvaceae	B	Polysaccharides	Emollient/potent antitussive activity	2
	Paederia foetida L./ Rubiaceae	B	-	↓inflammation/similar antitussive activity to the non-narcotic antitussive	2
	Radix Puerariae/	A	Puerarin (Isoflavonoid)	↓TNF-α, IL-1β, and IL-6, PGE2 /antipyretic	2
	Tetrastigma hemsleyanum Diels & Gilg/Vitaceae	A	Galacturonic acid, glucose, mannose, arabinose, galactose, and rhamnose (polysaccharides)	Regulation of cytokine secretion (IL-6, IL-10, and IFN-γ)/positive antipyretic activity (200 and 400 mg/kg. P.O.)	2
	Ziziphus abyssinica Hochst. Ex A.Rich./ Rhamnaceae	A	-	Antipyretic	2

Note: Sign (Type of complication) A: fever, B: Cough. Study type: 1: In-vitro, 2: In-vivo, 3: In-silico, 4: Clinical trial, 5: Traditional medicine.

Boozari and Hosseinzadeh have shown that COVID-19 prevention with natural products in below figure 4 (30).

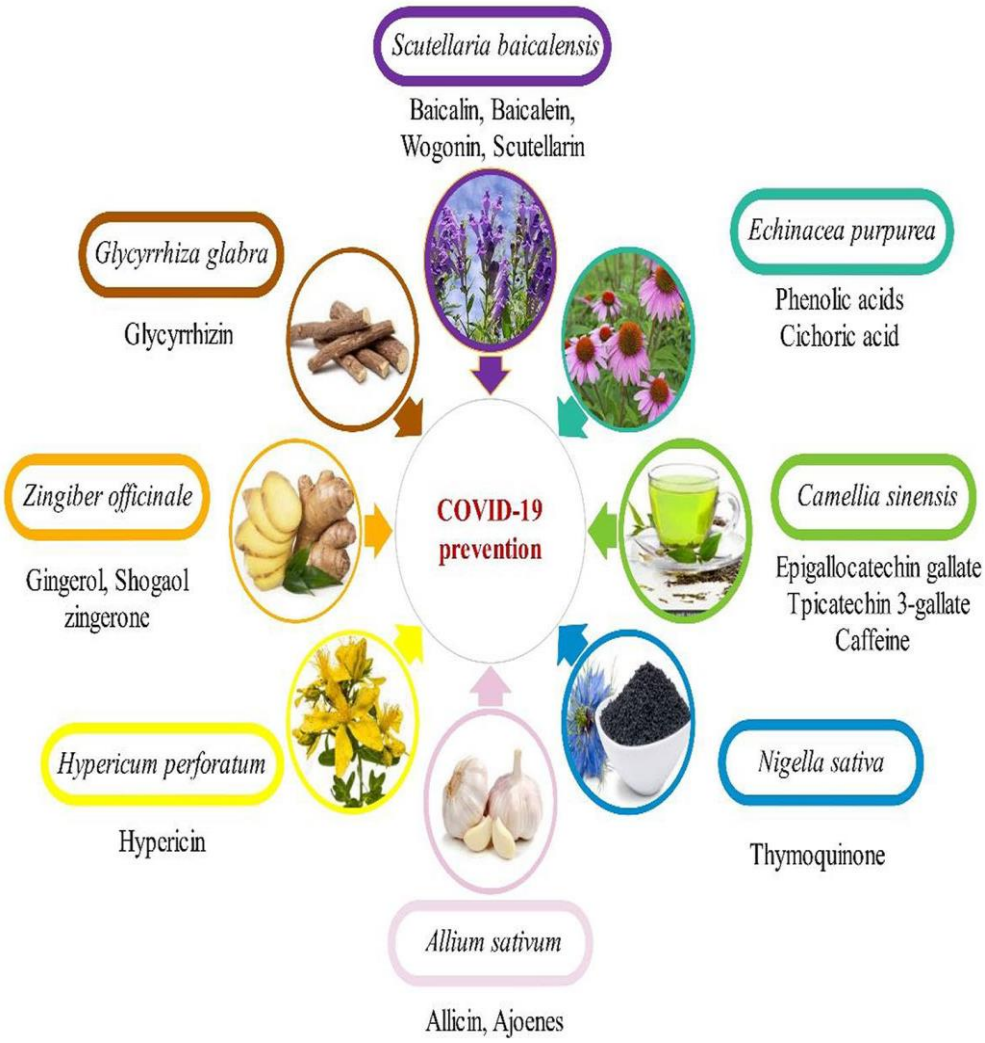

Figure 4. COVID-19 prevention with natural products (30).

CONCLUSION

The herbs mentioned above have numerous benefits due to the variety of secondary metabolites they contain. The majority of these compounds, especially essential oils, are well known for their positive biological effects on respiratory functions. But some plants may contain toxic substances which can cause various overdose intoxications and disorders. In line with these advantages, it is very important to conduct more preclinical and clinical studies on plants (21).

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CHAPTER 7

DETECTION AND DIAGNOSTIC TECHNOLOGIES IN COVID-19 INFECTION, RECENT MOLECULAR BIOLOGICAL DEVELOPMENTS: qRT-PCR

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INTRODUCTION

In December 2019, an undetermined case of pneumonia was reported in the city of Wuhan in China, and the disease was subsequently named COVID-19. In March 2020, COVID-19 was declared a pandemic by the World Health Organization (WHO) (1,2).

Worldwide, the COVID-19 internet information led by the health organization shows that the number of cases as of April 05, 2022, is 491,441,483 and the number of deaths is 6,152,898. The COVID-19 outbreak has increased again in China and the UK as of this date. In these countries, measures are taken regarding high health and safety measures. While the number of deaths in the COVID-19 pandemic has exceeded 6.1 million all over the world, diagnosis and diagnostic methods have become important in preventing infection and controlling the epidemic. This book chapter introduces the molecular diagnostic methods necessary for the detection of the SARS-CoV-2 virus in the pandemic. Especially RT-PCR molecular diagnosis method is widely used all over the world. That's why this method is explained in detail.

The pandemic of coronavirus disease 2019 (COVID-19); Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused a sudden and significant increase worldwide in hospitalizations for the multiorgan disease pneumonia. It has been stated by WHO that the polymerase chain reaction (PCR) technique can be applied for diagnosis in asymptomatic or mildly symptomatic cases. In our country, real-time PCR (RT-PCR) applications for the definitive diagnosis of SARS-CoV2 are carried out as specified in the WHO guideline and approved by the Ministry of Health. Here, the process of detecting the SARS-COV-2 virus, which causes COVID-19 disease, by RT-PCR is summarized. The COVID-19 pandemic, which has killed more than one million people worldwide; demonstrated the critical importance of microbiological diagnostic methods in the prevention of infectious diseases and control of epidemics. The most effective way to combat SARS-CoV-2 is to reduce the spread of the virus as much as possible. Reducing the spread of the virus is possible with diagnostic methods that ensure that all symptomatic or asymptomatic individuals carrying the virus are detected in the shortest time and accurately. Various microbiological methods (serological tests, nucleic acid amplification methods) recommended by international health authorities are widely used in the diagnosis and follow-up of COVID-19 disease. The difficulties experienced in the application of different diagnostic methods, including the real-time polymerase chain reaction (RT-PCR) method, which is specified as the gold standard method in the laboratory diagnosis of COVID-19 by the World Health Organization, were once again understood during the pandemic process. To achieve higher success in the fight against infections, it is necessary to determine the application and evaluation standards of existing microbiological diagnostic methods and to develop new methods that are easy to apply and cost-effective, giving accurate results in a shorter time (3).

The need for a rapid and accurate self-test tool for the diagnosis of COVID-19 has made it important to fully understand the number of cases worldwide and to cooperate with medical treatment and government accordingly. SARS-CoV-2 (formerly 2019-nCoV) infection was first reported in December 2019 in Wuhan (China). It then quickly spread around the world. It has caused ~487 million active cases with ~6.1 Mn deaths as of April 2022. The diagnostic tools available so far are built on a) Viral Gene detection, b) Human Antibody Detection c) Viral Antigen Detection. Among them, viral gene detection by RT-PCR was found to be the most reliable technique (3).

1. GENERAL INFORMATION ABOUT PCR

The Polymerase Chain Reaction (PCR) method was invented in 1993 by scientists Kary Mullis & Michael Smith. It is considered one of the revolutionary developments in the scientific world. PCR and thanks to other developments, a lot of information has been obtained about gene diversity and the interaction between genes. PCR method, it is possible to detect many causes that cause various diseases in humans (2,4).

PCR (**P**olymerase**C**hain**R**eaction) testing is a test that is often raised these days due to the SARS-CoV-2 virus outbreak. This test, which is used to diagnose the corona virus, has many uses, especially in the field of health. In this section, topics such as what exactly a PCR test is, for what purposes it is used, how the PCR test is used, and what should be considered when applying it, how it is used in COVID-19, are given in outline (5).

1.1. WHAT IS THE POLYMERASE CHAIN REACTION (PCR) TEST?

PCR is a general name given to reactions applied to enzymatically amplify a specific region in DNA. The PCR method is briefly expressed as a certain number of continuous imitations or repetitions of DNA replication. This method can also be defined as the nucleic acid sequence cloning process in vitro. PCR is an advanced and highly sensitive molecular recognition technique among molecular diagnostic tests (5).

1.2. WHAT KIND OF TECHNIQUE IS A PCR TEST?

The PCR (Polymerase Chain Reaction) technique is based on the separation of the two strands of DNA by exposure to high heat, that is, it becomes a single helix, then the synthetic oligonucleotides bind to the target DNA, allowing the chain to elongate, and this cycle is repeated a certain number of times. Thanks to this feature, it becomes possible to detect even a small number of microbes by multiplying with the PCR test (5).

1.3. IN WHICH AREAS IS THE PCR TEST USED TODAY?

PCR testing is well on the way to standardizing the clinical use of nucleic acid transcription techniques for diagnostic purposes. It also protects against important drug side effects that may occur during the treatment and the detection of the patient's genes related to this drug in personalized drug treatment plans. The normal life cycle of the cell is disrupted and it can divide continuously, resulting in cancer. With the PCR method, differences in genes related to the cell cycle, DNA repair, and growth signal can be detected. PCR test, which has a limited application area in the diagnostic approach for all cancer types at the moment, may contribute to the diagnosis of some cancer types such as bladder cancer, breast cancer, colorectal cancer, and endometrial cancer. The PCR method is also an effective test for the detection of viruses in human body samples. In diseases caused by bacteria, it can contribute to the diagnosis of these diseases in the early stages, especially in diseases that are thought to be caused by bacteria that reproduce late in culture. The PCR test, which can provide diagnostic benefits in animal diseases as well as in human infectious diseases, is also used in veterinary diagnosis and treatment planning. The PCR test is used in many diagnostic research, studies, and determination of unknown features. Many uses of the PCR test in the field of health can be summarized as follows (5).

- **Diagnosis of carrier and patient in hereditary diseases**

Defects in these genes can be detected by amplifying gene sequences in the PCR test. Early diagnosis, even in carriers, is a great advantage of this test.

- **Prenatal diagnosis**

In this way, abnormalities in the fetus can be detected before birth. These abnormalities are usually caused by genetic diseases.

- **Detection of pathogenic organisms in clinical samples**

It has a great contribution to distinguishing pathogenic organisms by multiplying even a small number of microbes. It is a fast and sensitive technique in disease diagnosis. In Corona virus detection, the pathogenic organism can be recognized and diagnosed in this way.

- **Forensic Medicine**

In this method, DNA analysis of criminals can be made by amplifying the gene sequences performed in the PCR test. Therefore, it has great importance in forensic medicine.

• Cancer-related research

Mutations in genes that cause tumors can be detected by PCR testing.

• Paternity test

• Gene expression studies in the creation/cloning of probes

• Creation of large amounts of DNA samples in DNA sequence analysis

• Determination of unknown gene sequences

• Examination of past DNA and elucidation of biological evolution

• Development of vaccines in the field of biotechnology

•Ensuring that the baby is born normally by performing genetic tests before implantation and performing implantation after in vitro fertilization in a single cell.

• Development of techniques used in the diagnosis of genetic relatednesses in many plants and animals, such as PCR-derived RAPD, AFLP, SSR, and ISRR

• Investigation of DNA protein interaction (footprinting)

The PCR test is a technique that has many uses in sectors such as food and agriculture, apart from the above-mentioned uses in the field of health. Food safety is one of the factors that significantly affect health, and serious illness can occur as a result of consuming contaminated or virus-infected foods. The PCR method can also be used to detect these factors that may be food health-related disease factors (5).

1.4. WHICH DISEASES CAN BE USED FOR THE DIAGNOSIS OF PCR TEST?

The PCR test is an examination that has an important area of use in the diagnosis of disorders caused by microbial agents. PCR testing can be useful in the diagnosis of diseases caused by various bacterial, viral, parasitic, or fungal (fungal) diseases (5):

• Use of PCR Test in the Diagnosis of Bacterial Diseases

In addition to detecting bacterial pathogens, PCR testing may also be useful in detecting resistance genes against antibiotics used in these organisms. For example, by taking a swab sample from the throat in throat infections caused by *Streptococcus* bacteria, or for *Clostridium difficile*, which can cause diarrhea after antibiotic use, and E.coli, which can cause intestinal infections, PCR examination of stool samples can result in a shorter time compared to cultural production. Apart from these bacteria, the PCR test can also be used in the diagnosis of lung infections caused by bacteria such as mycoplasma, chlamydia, and legionella, which are the causative agents of pneumonia with an atypical course. In infections caused by atypical pneumonia agents, culture results of disease-causing bacteria are quite time-consuming. These bacteria can be detected in a shorter time with the PCR test from throat swab samples taken from these patients (5).

• Use of PCR Test in the Diagnosis of Viral Diseases

One of the earliest uses of PCR testing was to diagnose viral diseases. Herpes simplex virus (HSV), which causes the development of lesions known as herpes, causes clinical manifestations involving the genital area, skin, and central nervous system. If this disease affects the brain structures and the patient does not receive appropriate treatment, the probability of the disease being fatal can increase up to 70%. Thanks to the investigation of some genes in the DNA of this virus by PCR test, the presence of this virus in the person can be detected 1 day after the onset of clinical symptoms.

Apart from this virus, with PCR test, Varicella-Zoster virus, which is the causative agent of Chickenpox, Epstein-Barr virus, which can trigger the development of Lymphoma, JC and BK viruses,

which can cause significant damage to the kidney and brain, Parvovirus B19 in children and respiratory tract disease factors such as influenza, adenovirus and coronavirus can be determined (5).

1.5. WHAT IS THE ROLE OF THE PCR TEST IN THE DIAGNOSIS OF COVID-19?

In the COVID-19 disease caused by SARS-CoV-2, which affected the whole world in a short time, the PCR test is a very important examination for the control and prevention of the epidemic. Real-time RT-PCR (Reverse Transcriptase-PolymeraseChainReaction) test is very helpful, especially in the diagnosis of early disease. The basis of suspicion about this test is the risk of false-positive or false-negative results. The results of the majority of PCR tests performed for the diagnosis of COVID-19 do not cause any problems. This test may result in false-negative results, as the patient has not yet developed a detectable viral load in tests performed at a very early stage. For this reason, samples must be taken correctly and at the right time for the PCR test to give accurate results (3,6).

Coronavirus diagnostic tests are of two types, molecular and serological tests. The tests used in the diagnosis of COVID-19 can be made with the help of the genetic material of the virus (it can be DNA or RNA) or the antibodies that the defense cells create against the virus by activating the immune system of the antigenic material. While the tests that detect the genetic material of the virus are called molecular tests, the tests that try to detect the antigen-antibody of the virus are called serological tests. Antibody-antigen tests, that is, serological tests, are tests used to detect the stages of viral infection that have been or are currently being passed. Real-time PCR testing can be used to confirm serological tests in the Corona virus. The tests to be used may vary according to the stage of the disease (3).

The PCR test, which is an important part of the prevention and control studies of the Coronavirus epidemic, can provide an early diagnosis of this disease thanks to its convenience and specificity. One of the problems in the use of PCR test in the diagnosis of COVID-19 is the detection of false negatives or false positives. For this reason, the compatibility of the patient's signs and symptoms with the PCR test can be considered in the clinical diagnosis of COVID-19 (3).

PCR test; it detects the RNA polymerase enzyme gene, that is, the genetic material of SARS-CoV-2, which can be found depending on RNA. This test should be administered by microbiologists and biologists. Otherwise, it can lead to very wrong results. As a result of false results, the disease may not be diagnosed. This may allow the virus to spread further. If the results are negative, the possibility of incorrect application of the test should be considered. For the test to give accurate results, it is very important when the sample is taken and whether the sample is taken correctly. The performance of the PCR test depends on the abundance of RNA in the sample, as it makes a diagnosis from RNA detection. Therefore, the quality of the sample taken is also very important for the efficiency of this test. In addition, improper transfer of the sample taken may also cause inaccuracy in the results. Technical errors that are not caused by the person performing the test, such as virus mutation or PCR test inhibition, are among other errors that may occur during the application of the test. At this time, there are two types of applications for PCR. These types are PCR tests that give real-time results and give fast results. These are real-time PCR and PCR with fast results. While the RT-PCR result can be obtained within 1-3 hours, the results can be obtained from 15 minutes to 1,5 hours for those with fast results. RT-PCR test of these types is used in Turkey. Unlike conventional PCR, in RT-PCR, the amount of DNA is measured after each cycle using fluorescent dyes that give a fluorescent signal that increases in direct proportion to the number of molecules amplified by PCR. **(FIGURE 1)** (6,7).

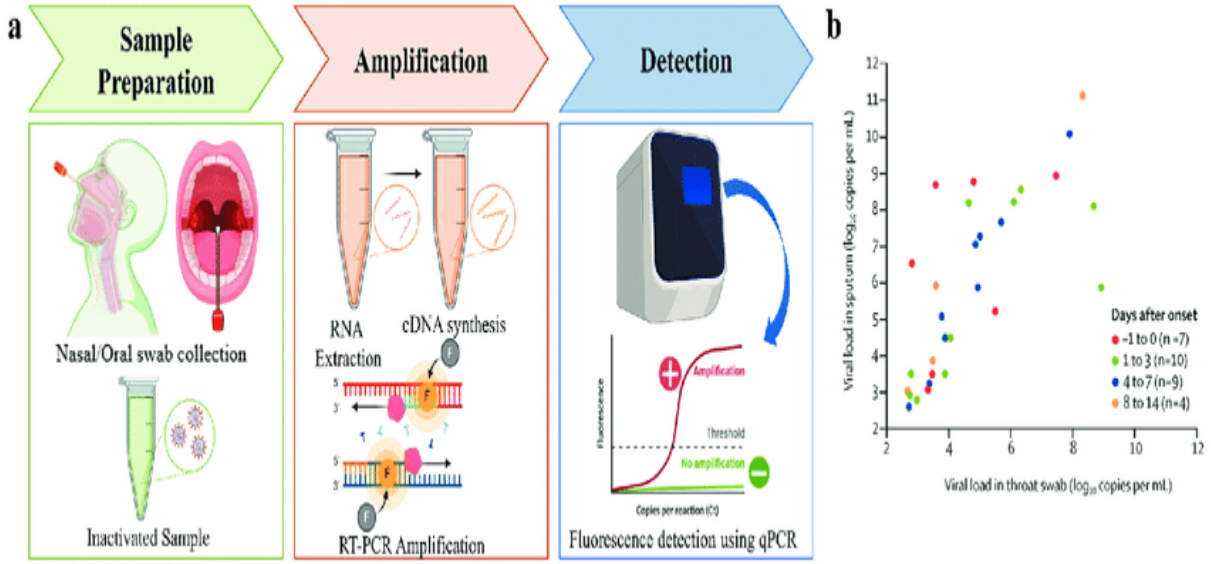

FIGURE 1 Schematic workflow of COVID-19 Diagnostic Test using RT-PCR. A) Nasal or oral swabs are collected from infected patients containing upper and lower respiratory tract fluids. The collected samples are preserved at specific temperatures and sent to labs for testing. Viral samples are deactivated by heat, and RNA is isolated using an RNA extraction kit and amplified using specific primers targeting segments of different genes (E, N, ORF1ab, and RNase P). Based on the amplified fluorescent signal, test results are interpreted. B) Correlation of a high number of viral loads present in sputum samples than throat swabs on different stages of infection for hospitalized patients (https://doi.org/10.1080/14737159.2021.1894930) (7).

For PCR testing, a sample should be taken from a respiratory tract organ such as a nasopharyngeal swab (swabs from the nose and throat) or a sputum sample from the lower respiratory tract. Different methods can be used to take this sample. Taking samples with throat sticks, which are among these methods, is a method that can give accurate results for the first week. In the continuation of the disease, that is, if the patient has pneumonia, the virus spreads rapidly in the lungs. In the throat, the number may decrease and disappear. For this reason, the sample taken from the throat may not be useful for diagnosis. Therefore, since the disease progresses after the first week of the disease, the diagnosis can be made on sputum, which is a cough material (6).

After the samples are taken at the right times and in the right way, the samples are placed in a liquid viral transport medium that can allow the virus RNA to pass into the solution. Then, the RNAs that pass from the sample to the solution are separated from this solution and then this RNA is amplified to facilitate identification. The RNA of the virus type is determined by several tests performed in the laboratory through the replicated RNA, and thus the diagnosis is made. PCR; Denaturation, that is, the separation of two strands of DNA exposed to high temperatures, between 94 °C - 98 °C, annealing between 37 °C and 65 °C, that is, attachment of synthetic oligonucleotides to the target DNA (hybridization), and 72 °C It consists of 3 stages, including elongation stages, and is performed by repeating these stages in a certain cycle, a certain number of times (4).

2. DIAGNOSIS OF COVID-19

Early identification and isolation of suspected patients play a critical role in containing this epidemic. Therefore, this section discusses different diagnostic methods to determine the specificity and susceptibility of COVID-19 (8).

A. Clinical presentation

Symptoms of COVID-19 are observed around 5 days after the incubation period (9). Individuals infected with the virus show symptoms for around 11,5 days (10). This period is closely related to the patient's age and immune system. Gastrointestinal symptoms include diarrhea, vomiting, and anorexia, which are noted in approximately 40% of patients (11,12). Approximately 10% of patients with this symptom show no signs of fever or respiratory infections. It can damage various organs. When there is a dysfunction of the extrapulmonary system, including the circulatory and digestive systems; the septic shock is possible and the mortality rate increases significantly (13). COVID-19 is also associated with hypercoagulation disease, which increases the risk of venous thrombosis (14). There are also records of neurological symptoms (such as fatigue, dizziness, and impaired consciousness), ischemic and hemorrhagic strokes, and muscle damage (15). Children tend to have a hyper-inflammatory response to COVID-19, similar to Kawasaki disease (16). In addition, significant research supports COVID-19 in people aged 25-89 years. Many elderly patients infected with this virus were between the ages of 35 and 55. Less cases are seen in newborns and infants. An analysis of the dynamics of initial transmission of the virus showed that the average age of patients was 59 years. Most of the patients (59%) were male (11).

B. Other screening tests for COVID-19

Blood test findings are usually nonspecific but can help identify the causes of the disease. A complete blood count typically shows a normal or low white blood cell count and lymphopenia. C-reactive protein (CRP) and erythrocyte sedimentation rates generally increase in disease. These tests need to be repeated frequently, especially in follow-up patients (17-19). Creatine kinase plus myoglobin, aspartate aminotransferase, lactate dehydrogenase, D-dimer, and creatine phosphokinase levels may be increased in severe forms of COVID-19 disease. Procalcitonin levels may be elevated during viral-bacterial coinfections (20,21). In a systematic meta-analysis study, accessible laboratory results were sought among 2361 SARS-CoV2 patients, and the results showed 26% leukopenia, 13.3% leukocytosis, and 62.5% lymphopenia (22). Wuweian et al. plasma cytokines/chemokines tumor necrosis factor (TNF)-α and interleukin (IL)-1β, IL1RA, IL2, IL4, IL5, IL-6, IL-10, IL13, IL15, and IL17A were measured to investigate the effect of coronavirus during the acute phase of the disease (17,23). One study showed that macrophages and dendritic cells play crucial roles in an adaptive immune system. These inflammatory reactions may cause systemic inflammation (24). Therefore, stool and urine tests have been recommended to patients and healthcare professionals to detect possible alternative transmission. Consequently, the development of tools to identify different modes of transmission, including stool and urine samples, is very useful. It is urgently warranted to develop treatments to control the disease, as well as develop strategies to prevent and minimize transmission (25).

C. Radiological findings

Chest X-ray examination shows features or patterns that differ in COVID-19 patients. Imaging results vary with the patient's age, disease stage at the time of screening, adequacy, and drug therapy schedules. and essential for therapeutic efficacy. According to the Diagnosis and Treatment Protocols Regulation (DTPR), it can be re-examined 1 to 2 days after admission (26). The most important feature of COVID-19 is multiple, bilateral, posterior, and peripheral ground-glass opacities with or without pulmonary consolidation and infiltrating in severe cases (27). Autopsy analysis of a COVID-19 patient showed fluid accumulation and hyaline membrane formation. alveolar walls, which may be the primary pathological driver of ground-glass opacity (28). Further studies, however, have shown that COVID-19 patients often show signs of small patchy shadows, pleural changes, subpleural curved lines, and inverted halo. Intra-lobular lines and thickened interlobular compartments are frosted and displayed in a crazy tiling pattern on a glass opacity background (29,30).

Evidence has indicated that an initial chest CT has a higher detection rate (approximately 98%) compared to reverse transcriptase-polymerase chain reaction (RT-PCR) (approximately 70%) in infected patients. Xie et al. demonstrated that about 3% of patients have no primary positive RT-PCR but have a positive chest CT; therefore, both tests are recommended for COVID-19 patients. CT of the chest comprises an urgent and simple method for detecting initial COVID-19 infection with high sensitivity for prompt diagnosis and disease progression monitoring in patients. Particular notice should be paid to the role of radiologists in finding novel infectious diseases (31).

D. Molecular diagnosis

The clinical diagnosis of COVID-19 relies on epidemiological data, clinical symptoms, and some assistive technologies such as nucleic acid detection and immunoassays. Isolation of SARS-CoV-2 requires a highly secure environment (biosafety level-3). There are three main topics in molecular diagnostic testing for COVID-19: (a) reducing the number of false negatives by detecting minimal amounts of viral RNA; (b) avoiding the number of false positives by correctly distinguishing positive signals between different pathogens; and (c) high capacity for fast and accurate testing of large numbers of samples in a short time (32).

2.1. NUCLEIC ACID DETECTION

Two technologies commonly used for SARS-CoV-2 nucleic acid detection are real-time RT-PCR (rRT-PCR) and high-throughput sequencing.

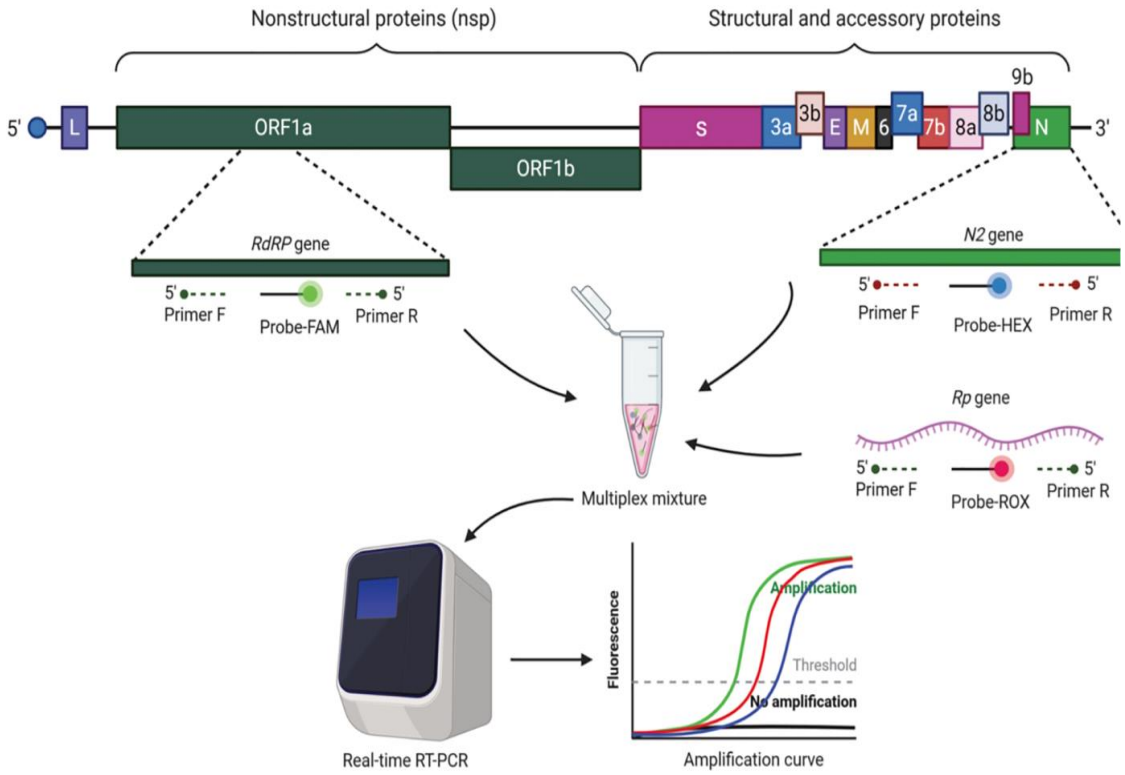

FIGURE 2 Genome structure of SARS-CoV-2 and the targeted genes in the multiplex rRT-PCR assay. (https://doi.org/10.1038/s41598-022-06977-z) (33).

Sequencing systems are rarely used in clinical diagnosis due to high cost and equipment limitations. Thanks to the sequencing system, access to the entire genome structure of the SARS-CoV-2 virus has been very helpful for the design of specific primers and the creation of PCR working protocols (10,34).

Initial published reports on the application of rRT-PCR in the diagnosis of COVID-19 report remarkable specificity and limited sensitivity of the spike gene region (S) of SARS COV-2 (27).

Next, the sensitivity in this technique is RNA in the ORF1ab region. dependent RNA polymerase (RdRp), Nucleocapsid (N), and Envelope (E), including for other viral-specific genes. Two measures were taken in the study to prevent cross-reaction with other human coronaviruses and to prevent possible genetic drift of SARS CoV-2. The first is to identify a non-specific target for detecting other CoVs and the second is to identify a specific target for SARS-CoV-2. A comparison of all results from the study of the RdRp gene confirmed that the RdRp gene was the most suitable target with the highest sensitivity. RdRp tests have also been validated in approximately 30 European laboratories (32). Chan et al. proposed a new RT-PCR assay targeting the low SARS-CoV-2 load in the upper respiratory tract, plasma, and saliva samples, and the RdRp/Hel sequence without any cross-reactivity with other common respiratory viruses (35). WHO recommends E gene testing as first-line screening followed by RdRp gene testing as confirmatory testing. The QIAstat-Dx SARS-CoV-2 panel, the RT-PCR system studied in this regard, is very sensitive **(FIGURE 2)** (33).

In general, quantitative (RT-PCR) RT-qPCR testing is considered the gold standard for the complete diagnosis of COVID-19. However, the mechanism of susceptibility varies according to viral load, RNA extraction technique, sample source, and disease stage. Indeed, RT-PCR false-positive results are related to cross-contamination of samples and processing errors. Conversely, inaccuracies at any stage of collection, storage, and processing of samples can lead to false-negative results. Some studies have revealed that samples from the upper respiratory tract (bottom of the nostrils and oropharynx) are more desirable for RT-PCR testing as a result of multiple viral replications (37). Molecular diagnostic techniques for COVID-19 currently include methods based on isothermal amplification. The loop-mediated isothermal amplification (LAMP) technique uses DNA polymerase and 4 to 6 different primers that bind to different sequences on the target genome (38). In LAMP reactions, amplified DNA is indicated by a blur caused by a byproduct. The reaction can be detected by a color produced by a pH-sensitive dye or by fluorescence irradiation produced by a fluorescent dye (39). The approximation takes place at a single temperature, in less than 1 hour, and with minimal background signal. The LAMP diagnostic test for COVID-19 is more specific and sensitive compared to traditional RT-PCR tests. It is not dependent on special laboratory equipment such as a thermocycler. However, as a result of the multiplicity of primers used in this method, optimizing the reaction conditions poses a major challenge (40,41).

2.2. MICROARRAY-BASED TECHNIQUE

The microarray-based technique is a rapid and high-throughput method to test for COVID-19 (42). In the protocol, cDNA labeled with specific probes is produced by reverse transcription before SARS-CoV-2 RNA. Complementary DNA is then generated from coronavirus RNA templates followed by reverse transcription labeling with specific probes. Labeled targets hybridize to the probe microarray. Free DNA is removed by washing the solution. Finally, specific probes detect COVID-19 RNA (42). Shi et al. SARS CoV in patient samples and Xu et al. successfully studied various spike gene polymorphisms (43,44). Jiang et al. prepared a SARS-CoV-2 protein microarray of 18 of the 28 expected proteins and applied it to 29 recoveries to characterize serum immunoglobulin IgG and IgM responses. All of these patients were found to have IgM and IgG antibodies that recognize and bind to SARS-CoV-2 proteins, particularly S1 and N proteins (45).

E. Immunological diagnosis

Serological test methods are mostly used in cases where nucleic acid cannot be detected, to detect asymptomatic cases, and to monitor the immune response in people who have had an infection (46). These tests are also used as antigen/antibody tests; It is based on the principle of determining the viral antigen or the antibody response in the body against the virus. Studies indicate that after the SARS-CoV-2 virus is taken into the body, it can take about a week for the development of an antibody response to the virus. For this reason, these tests are not recommended for early diagnosis, they are mostly used in mass screening (47,48).

S proteins, which are the structural N and antigenic regions of SARS-CoV-2, or these antigenic detect antibodies developed against these structures in the body. There are different serological tests for SARS-CoV-2 nucleocapsid protein (N); that takes part in virus replication. The highest N level protein seen in serum and urine in the first 14 days of COVID-19 patients is an important antigen used in early diagnosis (49,50). Another important structural protein of the virus, the S glycoprotein, enables the virus to bind to the corresponding receptor on the host cell. S protein and these antigenic Neutralizing antibodies and vaccines are more important than detecting antibodies against their structures and diagnosing the disease. They are used in their studies (51-53). Against N antigen tests that detect IgG and IgM antibodies. It has been reported to have higher sensitivity (53). In addition, commercial serological test kits containing the N and S antigens produced by recombinant technology are also available. It has been developed and put into use with the approval of the FDA (American Food and Drug Administration). IgG and IgM antibody response to SARS CoV-2 in the human body are usually 3-6 days after the onset of symptoms. It is known that it occurs and can last for about three weeks. World Health Organization to detect SARS-CoV-2 in cases where serological tests for only a single serum sample is to be taken from the patient; at least three weeks after the onset of symptoms recommends testing with the sample taken (54,55).

Serological tests are negative cross-reacting tests whose sensitivity and specificity are known to be variable. Since there are test methods that can give results, it is recommended to be used alone in the early diagnosis of COVID 19 disease **(FIGURE 3)** (56,57).

More than antigen-antibody tests in mass screening, disease in a particular population. In determining the level of immunity against the disease, the follow-up of contacts during the epidemic process, in the detection of individuals who may carry therapeutic or neutral antibodies is used.

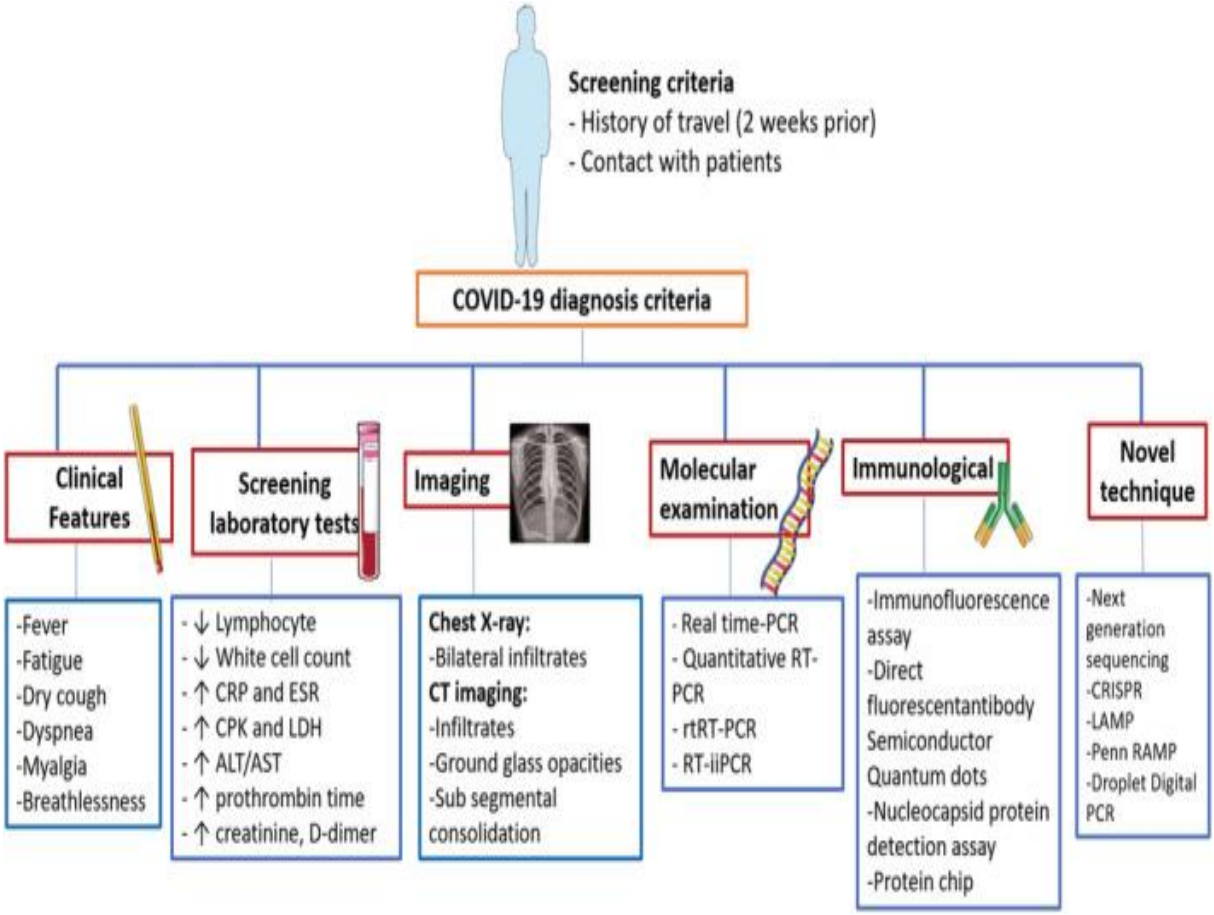

FIGURE 3 Diagnostic protocol recommended for COVID-19 (https://doi.org/10.1002/jgm.3303)(57).

F. Novel techniques

2.3. CRISPR-BASED DIAGNOSIS

Nucleic acid detection with CRISPR-Cas13a/C2c2 is a highly rapid, sensitive, and specific area of molecular detection that can aid in the epidemiology, diagnosis, and control of the disease. In addition, Cas13a/C2c2 can detect the expression of transcripts in living cells and different diseases.(58,59) Zhang et al. presented a protocol for the detection of COVID-19 using the CRISPR diagnostic-based SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing) technique. RNA fragments of the SARS-CoV-2 virus help detect target sequences of about 100 copies. The assay is performed by isothermal amplification of the extracted nucleic acid of samples from patients followed by Cas13-mediated amplification of the viral RNA sequence (60,61). Huang et al. created a CRISPR-based assay by a custom CRISPR Cas12a/gRNA complex (62). They used a fluorescent probe to identify target amplicons produced by standard RT-PCR or isothermal recombinase polymerase amplification. This method demonstrated specific detection at sites not equipped with PCR systems required for qPCR diagnostic tests in real-time. The assay allows the identification of SARS-CoV-2 positive samples with a test response time of approximately 50 minutes and a detection limit of two copies from each sample to be detected. The findings of CRISPR testing on nose samples collected from people with COVID-19 were comparable to matching data from CDC-approved RT-qPCR testing (62). Broughton et al. described the development of a rapid (< 40 min), simple to administer, and sensitive CRISPR-Cas12-based lateral flow test to diagnose SARS-CoV-2 from an RNA extract from a nasal swab. They validated their processing using artificial reference samples and clinical samples from patients diagnosed with COVID-19 disease and 42 patients with other respiratory diseases. This CRISPR-based approach

provided a visual and faster alternative to the SARS-CoV-2 real-time RT-PCR method used at the US Centers for Disease Control and Prevention, with approximately 100% negative predictive agreement and 95% positive (63).

2.4. REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) TECHNIQUE

Loop-mediated isothermal amplification (LAMP) is a new isothermal nucleic acid amplification method with great efficiency. In this test, it is possible to amplify RNAs and DNAs with high specificity and sensitivity as a result of six specific target sequences identified by four separate primers (64). The LAMP test gives results very quickly. It does not need expensive reagents or equipment. In addition, it is widely used in the gel electrophoresis method to search for undetected parts. Therefore, the LAMP test is highly advantageous to reduce the cost of detecting coronavirus (65). Poon et al. reported a simple LAMP test in the SARS study and demonstrated the applicability of this method for SARS-CoV detection (66). SARS-CoV ORF1b sites were first selected for SARS detection and amplified in the presence of six primers via the LAMP reaction. Then the amplified products were evaluated by gel electrophoresis. The sensitivity and detection levels in the LAMP assay for SARS are close to those of conventional PCR-based techniques. Pyrc et al. effectively applied LAMP to HCoV-NL63 detection in mobile cell cultures and clinical samples with a desirable sensitivity and specificity. Notably, one copy of the RNA template was found to be responsible for the detection restriction. Amplification is observed as a fluorescent dye or precipitate of magnesium pyrophosphate. These techniques can be performed in real time by monitoring the turbidity of pyrophosphate or fluorescence, which accurately overcomes the limitation of endpoint detection (67).

2.5. DROPLET DIGITAL PCR

For the direct identification and quantification of DNA and RNA targets, droplet digital PCR (ddPCR) includes a highly sensitive technique (68). It is widely and efficiently used in infectious disease states, especially due to its ability to accurately identify several copies of viral genomes (69). If the identification of low-level and/or residual viral presence is appropriate, ddPCR quantitative data are much better than those provided by normal RT-PCR tests. The use of ddPCR can provide vital support in the diagnosis of COVID-19 in false-negative results. Despite this, the ddPCR test is still very rarely studied in clinical settings. There is currently no evidence available for European cases (70).

2.6. NEXT-GENERATION SEQUENCING (NGS)-BASED TECHNIQUE

The highly diverse RNA viruses are the etiology of major human and animal infectious diseases (71). RNA viruses constitute the greatest diversity and are the etiological cause of multi-infectious diseases in humans and animals such as SARS, hepatitis, influenza, and IB (avian infectious bronchitis). The use of high-throughput NGS technology is of vital importance in primary and accurate diagnosis (72). Additionally, the NGS method can detect whether various types of viruses contain a pathogen. NGS's rapid novel virus technique, including DNA sequencing and RNA sequencing, has improved the phylogenetic diagnosis of viral diversity (73). Identifying a wide variety of pathogens using NGS technologies is also important for controlling viral infection caused by a novel pathogenic virus (74). In recent years, the advancement of the NGS method through RNA sequencing has seen rapid recognition of new RNA viruses(75). Chen et al. reported a novel duck coronavirus (viral 1b gene from three regions) using RNA sequencing that differs from chicken IBV (infectious bronchitis virus)(74).

As a result, at the end of December 2019, a new coronavirus broke out and spread immediately. It has had a profound impact on medicine, the economy, and public health around the world. Numerous studies have shown that ACE2 receptors contain structural proteins important for virus budding and entry into host cells. Both interspecies transmissions from unidentified intermediate hosts and human-to-human transmission have been recognized. Therefore, early detection and isolation of suspected patients are important in containing this epidemic. Currently, diagnostic methods for COVID-19 are

plentiful; therefore, it is imperative to select an appropriate detection protocol. rRT-PCR and qRT-PCR methods are frequently used in Turkey. Each of the techniques described has its disadvantages and advantages. Both chest CT imaging and RT-PCR tests are recommended for COVID-19 patients. LAMP can be detected with a low number of DNA or RNA templates within 1 hour. Microarray is an expensive method for diagnosing COVID-19 and other newly developed methods will gain importance in the future. Given the increasing number of infected cases, it is valuable to develop vaccines and antiviral treatments and uncover the molecular biological pathways of the virus.

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CHAPTER 8

LABORATORY BIOMARKERS FOR THE DIAGNOSIS AND FOLLOW UP PATIENTS WITH COVID-19

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INTRODUCTION

The SARS-CoV-2 pandemic commonly named coronavirus disease 2019 (COVID-19) has emerged as the most challenging global health problem of this century. The first case was reported from Wuhan in China in December 2019, the World Health Organization on February 11, 2020, officially named this infection, Covid-19, and the virus as SARS-CoV-2. It was declared a pandemic on March 11, 2020 (1).

The virus consists of a positive-sense, single-stranded RNA genome with a capsid and envelope structure surrounding it. Virus particles are spherical with 70-90 nanometer diameters on the outer surface appear to have glycoprotein extensions in the form of spinous protrusions (Figure 1) (2).

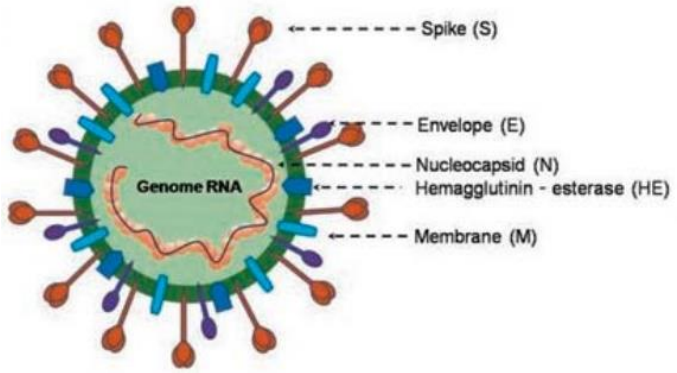

Figure 1: A schematic view of the coronavirus (2).

SARS-CoV-2 infection is defined as a multisystem disease characterized by high mortality in specific patient groups (especially elderly male individuals with hypertension, diabetes, obesity, cancer, and pulmonary, cardiovascular, liver, neurological, and renal disease) (3). Although there are still no definite definitions regarding the physiopathology of the disease, it is an undeniable fact that inflammatory processes occur at the beginning and in the later period. Routine clinical biochemistry laboratory findings guide clinicians in disease management, but it is also known that about COVID-19 has a long way to go in scientific research still there is still present.

In this part of the book, for the readers, we aimed to shed light on the COVID-19 disease in terms of laboratory findings.

OVERVIEW OF SPECIFIC PARAMETERS

1. Hematological Parameters

1.1. Hemoglobin

In a meta-analysis study, it was stated that hemoglobin value decreased significantly in patients with COVID-19 compared to those without severe disease, but between survivors and nonsurvivors, there was no significant difference (4).

In a retrospective study anemia and differential, iron homeostasis was common in hospitalized COVID-19 patients. There was an association between increased mortality and anemia at admission. The researchers of this paper observed that patients with a high ferritin/transferrin ratio required more intensive care unit admission and mechanical ventilation assistance (5).

1.2. White blood cells

Clinical studies had found that in the early stages patients with COVID-19 showed normal or decreased white blood cells count and decreased lymphocyte count. In the etiology of lymphopenia; lysis of the cell by direct binding of the virus to the surface of lymphocytes, cytokine secretion of lymphocytes, acceleration of apoptosis in lymphocytes, atrophy of lymphoid organs, and suppression of lymphocyte production by acidosis may be factors. Guan et al. (2020) examined 1,099 clinical cases in the early stage of COVID-19and found that 33.7% had leukopenia, and 83.2% had lymphopenia (6).

Wu et al. (2020) found that 31% had leukopenia and 42% had lymphopenia in some cases with COVID-19 (7).

In studies, the neutrophil count was found to be high in patients who had COVID-19. This elevation is proportional to disease severity and death (4,8).

İn a study belonging to He Z all Patients with severe disease had just a mild increase in WBC level, while patients who died had a much clinically significant increase in WBC count. As such, in patients with severe disease, a significant increase in WBCs may signify clinical worsening and an increased risk of a poor outcome.

He Z. et al. have also suggested that the increase in WBCs is driven by elevated neutrophils, as decreasing trends were observed for lymphocytes, monocytes, and eosinophils. In patients with severe disease, a decrease in both CD4 and CD8 was observed (9). It can be said that a high neutrophil count is also associated with cytokine storms (4,8). Also, a high neutrophil count may be indicative of secondary bacterial infection added to the viral infection.

2. Inflammatory Parameters

As expected, an increase is observed in acute phase reactants like erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), D-Dimer, and ferritin, in covid 19, as in all viral diseases. In severe COVID-19, higher procalcitonin levels have also been shown, this

suggesting the beginning of bacterial co- or supra-infection in critically ill patients. In addition to biochemical markers of inflammation, hematological findings, high neutrophil-to-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) have prognostic potential (10).

According to some studies, NLR is an important index and it is associated with the prognosis of patients with cardiovascular disease and sepsis. Among COVID-19 patients, the disease may be aggravating in patients with a higher NLR, and an elevated NLR in severe COVID-19 patients suggests a higher risk of death. Therefore, NLR could be better than white blood cell (WBC) count to indicate the infection status in vivo (11).

Odabası et al. found in their study that the most valuable parameter to predict the need for intensive care is the neutrophil count, and the best predictive marker of mortality is the procalcitonin value and the Neutrophil-to-monocyte ratio (NMR). Age, the mean platelet volume (MPV), procalcitonin, CRP, and D-dimer levels were found to be positively correlated with the prolongation of hospitalization (12).

Although activation of the immune system is necessary for the body to defend itself against infectious agents, the release of several inflammatory mediators called cytokines accompanies this process. Abnormally activated dendritic cells, macrophages, lymphocytes, and other immune cells release a large number of cytokines to induce inflammatory cascades or cytokine storms, which manifest as fever, headache, and fatigue in mild patients, or diffuse intravascular coagulation, shock, multiple organ failure, and even death in severe patients. Serum levels of inflammatory cytokines increased in most COVID-19 patients, and were significantly in intensive care unit patients (ICU) than in general patients, indicating that cytokine levels could be associated with the severity of the disease. Large numbers of cases showed that COVID-19 patients with an elevated level of interleukin-6 (IL-6) and tumor necrosis

factor-α (TNF-α) were more likely to receive mechanical ventilation support and patients with COVID-19 had a higher risk of death (13).

3. Coagulative Parameters

The evidence obtained shows abnormal immune responses and excessive pro-inflammatory responses in COVID-19 cause irregularities in multiple biological pathways. These irregularities combine to trigger the development of a profound disorder of hemostasis, manifested as systemic and regional coagulopathies and thrombotic events (13).

This hemostasis disorder is called "COVID-associated coagulopathy" and has been reported that this coagulopathy could be associated with disease severity. However, the pathophysiology of COVID-19 associated coagulopathy is quite complex and differs in important ways from the known mechanisms of thrombosis (14).

In COVID-19, coagulopathy results from thromboinflammation due to viral activity, and the incidence of DIC is low (less than 1%) (15).

3.1. Thrombocytes

Thrombocytopenia is when the platelet count is below 150x109/L. It is announced that COVID-19 infection leads to less thrombocytopenia than other corona viruses SARS and MERS. Thrombocytopenia was found in 40-45% of SARS infections, 36% of MERS infections, and 5-12% of COVID-19 infections (16).

For COVID-19 patients, in following-up, the disease severity and the clinical course, monitoring of platelet levels, and detection of thrombocytopenia can be used as indicators of prognosis and mortality (17). Lippi et al. demonstrated in a meta-analysis study that the patients with severe COVID-19 infection had significantly lower platelet counts. They stated that thrombocytopenia increases the risk of severe COVID-19 disease by 5 times and is associated with mortality (18).

3.2. D-Dimer

D-dimer test is a test used to if thrombosis exists or is absent in the body. A negative result may rule out thrombosis, while a positive result may indicate thrombosis (19). D-dimer levels are associated with prognosis and are indicative of thrombosis. Patients with high D-dimer levels although they don't show clinical symptoms. They should be hospitalized and followed up with anticoagulant therapy (20).

Zang et al. suggested that D-dimer levels are the most valuable test for determining recovery among coagulation tests (21). Yao et al. noted that the D-dimer level correlated with disease severity and was a reliable prognostic parameter for in-hospital mortality (22). In a study of 4103 patients, it was found that age and the CRP and D-dimer levels were the strongest risk factors affecting hospitalization (23).

3.3. Activated Partial Thromboplastin Time (aPTT) and Prothrombin Time (PT)

Mitchell et al. stated that COVID-19 coagulopathy differs from classical DIC because of the presence of high fibrinogen levels, normal or moderately prolonged PT and aPTT values, platelets above 100,000/L, and no significant bleeding (24).

Information on the mechanism of COVID-19 coagulopathy is still inconclusive, but data show that thrombotic coagulation disorder is seen quite frequently in COVID-19 disease. The incidence of thrombocytopenia is relatively lower than septic shock, while D-dimer is more sensitive compared to other coagulative parameters and is more valuable for the measurement of severity. Considering the high incidence of thrombotic events, it can be said that the use of standard anticoagulant therapy may be beneficial in terms of mortality and morbidity, due to the very low probability of significant bleeding in COVID-19 (25).

4. Cardiac Parameters

Cardiac injury is an important factor in disease progression and outcome. Direct viral infection and damage and immune-mediated damage are the two mechanisms proposed for cardiac injury (26).

Viral infection of cardiomyocytes and intracellular replication leads to cardiomyocyte degeneration and necrosis, causing cardiac dysfunction and arrhythmia. The immune-mediated mechanism involves the cytokine storm leading to microcirculation defects, tissue ischemia, and hypoxia. The proinflammatory state is also said to aggravate atherosclerosis and immune complex precipitation which can increase the risk of acute myocardial infarction (27).

Cardiac biomarkers have been studied in diagnosis, triaging, treatment, and prognosis. Raised cardiac biomarkers including creatine kinase (CK), creatinine kinase-muscle/brain activity (CK-MB), Lactate dehydrogenase (LDH), aminotransferase (AST), cardiac troponin I (cTnI), myoglobin (Mb), and N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) have been seen in patients with COVID-19. Among these CK, LDH and AST are not myocardial specific and may be elevated in injury to the liver, kidneys, and lungs. On the other hand, CK-MB, cTnI, Mb, and NT-proBNP are more myocardial injury-specific and increased to varying degrees, especially in severe and critical illnesses. Moreover, higher levels were associated with higher mortality (28, 29).

In some of the patients admitted to the ICU, CK-MB and high sensitive-cTnI levels were found to be significantly higher (30). In addition, patients with COVID-19 and with acute myocarditis were also found to have increased IL-6, IL-1β, and IL-10 levels (31).

Biomarkers reflecting heart and muscle damage are elevated in both severe and fatal COVID-19 patients. Patients who died had significantly elevated cardiac troponin measurements at presentation, thus suggesting potential for viral myocarditis involvement, cardiac injury from progression towards multiple organ failure (MOF), as well as secondary cardiac injury from the organ, targeted pathologies (e.g. renal or liver failure)(32).

5. Biochemical Parameters

5.1. Plasma albumin

Hypoalbuminemia in critically ill patients is multifactorial and is attributed to increased capillary permeability, decreased protein synthesis, increased turnover, decreased serum albumin level, increased distribution volume, and increased vascular endothelial growth factor expression. Although common, the exact temporal association of hypoalbuminemia is yet to be studied. In COVID-19 disease a similar trend was found that the meta-analysis of 11 studies showed that the mean serum albumin on admission was 3.50 g/dl in severe COVID-19 patients, and 4.05 g/dl in non-severe COVID-19, respectively (33).

About 40% of patients presented with increased LDH levels. Elevated LDH levels have been associated with a higher risk of acute respiratory distress syndrome (ARDS), need for intensive care units, and higher mortality (34).

5.2. Urinary albumin

Estimated by elevated urine albumin levels in patients affected by COVID-19 proteinuria developed and was diagnosed in the early stages of the infection. Microalbumin was detected in the urine in most of the patients, that is, in 34% of 59 patients. Characterized by the presence of blood in the urine in 63% of patients proteinuria and hematuria were detected. In studies, blood urea nitrogen was found in only 10.8% of patients. Partial increases were seen in BUN and creatinine values (35).

6. Renal function parameters

Acute kidney injury (AKI) emerges as a serious complication in critically ill patients with COVID-19. The etiology of COVID-19-related AKI is multifactorial and it includes hemodynamic disorder, inflammation, cytokine release, endothelial dysfunction, change in microcirculation, nephrotoxic exposure, and invasive mechanical ventilation effect (36).

A case study of 149 cases of SARS-CoV-2 capability of causing kidney damage showed that 28.8% of COVID-19 patients had elevated serum creatinine levels (37).

7. Liver Functions

COVID-19 disease often presents with upper respiratory tract and pulmonary findings. However, like SARS-CoV and MERS-CoV infections also in the case of COVID-19 disease, liver involvement occurs. Liver involvement in COVID-19 patients has been shown to increase ALT, AST, or bilirubin measurements in studies. In publications, elevated liver enzymes in COVID-19 patients were found in 16.1-53.1% (38).

Guan et al. study found that AST was elevated in 22.2% of patients, ALT was elevated in 21.3%, and total bilirubin was elevated in 10.5%. These rates were found to be significantly higher in patients with a severe course. In severe course and other patients, AST elevation was found in 39.4% and 18.2%, respectively. Elevated ALT was observed in 28.1% of cases with the severe course, and 19.8% of others, and elevation of total bilirubin in 13.3% of cases with the severe course and 9.9% in other clinical courses (39).

8. Erythrocyte Sedimentation Rate (ESR)

Especially with CRP in COVID-19 ESR assessed was detected usually slightly elevated. Tan et al. showed increased ESR levels with increased CRP levels in moderate and severe COVID-19 patients before CT imaging changes (40).

Table 1: Laboratory parameter changes in severe and fatal COVID-19 patients.

	Biochemical parameters
↓	Albumin
↑	Total Bilirubin
↑	Blood Urea Nitrogen
↑	Creatinine
↑	CK (Creatine Kinase)
↑	Creatine Kinase-MB
↑	GPT (Glutamate Pyruvate Aminotransferase)
↑	GOT (Glutamate Asparatate aminotransferase
↑	LDH (Lactate Dehydrogenase)
↑	Myoglobin
↑	Cardiac Troponin I
	Coagulative parameters
↑	D-Dimer
↑	Prothrombin time
	Hematologic Parameters
↓	Platelet Count
↑	WBC (White blood cell) Measurement
↑	Neutrophil Count
↓	Lymphocyte Count
↓	Eosinophil Count
↓	Hemoglobin
	Inflammation Parameters
↑	IL-6
↑	IL-8
↑	IL-10
↑	CRP (C-Reactive protein)
↑	ESR (Eritrosit Sedimentation Rate)
↑	Plasma Ferritin
↑	Procalcitonin

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CHAPTER 9

RHEUMATOLOGIC MANIFESTATIONS OF COVID-19 INFECTION

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INTRODUCTION

COVID-19, is a new illness, first identified in Wuhan, China, in December 2019 and rapidly spread throughout the world. It caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and characterised by fever, dry cough, interstitial pneumonia, fatigue, headache, loss of taste and smell (1). This illness has been declared a pandemic and at the time of writing this article, the global caseload had crossed 490 million, with more than 6 million deaths (2).

Table 1: Autoimmun conditions described with COVID-19 (4).

Guillian-Barré syndrome
Miller Fisher syndrome
Antiphospholipid syndrome
Immune trombocytopenic purpura
Evans syndrome
Systemi lupus erythematosus
Kawasaki disease
Cold agglutinin disease & autoimmune haemolytic anaemia
Neuromyelitis optica
NMDA-receptor encaphalitis
Myastenia gravis
Myositis
Type-I diabetes
Large vessel vasculitis
Medium vessel vasculitis
Small vessel vasculitis
Psoriasis
Subacute thyroiditis
Graves' disease
Sarcoidosis
Inflammatory arthritis

Primarily a respiratory disease, COVID-19 may affect many systems. It may cause gastrointestinal symptoms, kidney and liver injury, myocardial dysfunction and acute coronary syndromes, neurologic complications, and dermatologic findings. While myalgia is a common clinical feature of COVID-19, the other musculoskeletal manifestations of COVID-19 were infrequently described early during the pandemic, there have been increasing reports of neuromuscular and rheumatologic complications related to both the virus and treatment/hospital course (3). Rheumatological manifestations (Table 1) these include inflammatory arthritis (viral arthritis, reactive arthritis, chronic arthritis, rheumatoid arthritis), lupus like syndromes, vasculopathy (Kawasaki-like disease, chilblain lesions, vasculitis) and a chronic fatigue spectrum disorder (4). Musculoskeletal symptoms in COVID-19 patients due to direct effect of virus, endothelial cell damage, tissue damage and increased proinflammatory cytokines.

1. ARTHRALGIA, MYALGIA AND FATIGUE

Arthralgia, myalgia, weakness and fatigue, especially in mild to moderate disease can often be an initial sign of covid-19 illness (5). Therefore, these patients primarily apply to rheumatology clinics. Thereby, physicians should also consider covid 19 infection in the differential diagnosis, especially in these days. the frequency of myalgia has been reported between 15-59% and arthralgia %2,5-31 in studies (5). It is considered that this effect of Covid-19 which mimics rheumatic manifestations caused by providing long-term immune dysregulation (6).

Uncontrolled release inflammatory cytokines such as Interleukin (IL)-1, IL-6, monocytes chemoattractant protein 1 and associated elevated ferritin and decreased natural killer cell functions lead to cytokine storm syndromes (7). Increased IL-6 during cytokine storm has a role in the development of myalgia and arthralgia (8).

Several articles describes fatigue during COVID-19 infection. Some of these, explored fatigue at disease onset and the others during its evolution. Pooled estimate of fatigue as initial symptom was 0.317 (95% CI 0.198–0.464) and pooled estimate of prevalence of fatigue in patients with COVID-19 was 0.356 (95% CI 0.297–0.420) (5).

A meta-analysis shows that muscle pain and fatigue are present respectively in 19 and 32% of patients as initial presentation of COVID-19, while the overall prevalence estimates are 16 and 36% throughout the course of the illness (5).

Arthralgia, myalgia and fatigue are the most common symptoms leading to referral of patients to a rheumatologist. As outlined by our systematic review and metaanalysis, 19% of COVID-19 cases might present muscle pain as initial symptom, while 32% might present fatigue. It is therefore conceivable that, especially for individuals with non-specific or mild complaints and without respiratory distress, a proportion of COVID-19 patients might be referred to the rheumatologist early in the disease course. Rheumatologists should hereafter bear in mind COVID-19 as a possible differential diagnosis (5).

2. INFLAMMATORY ARTHRITIS

Viral infections can cause arthritis and the spectrum of illness can range from mild arthralgia to chronic arthritis (9). Respiratory viral infections have also been associated with an increased number of cases of rheumatoid arthritis (RA) (10). COVID-19 has also been found to cause reactive arthritis and new-onset infammatory arthritis typically occurring within a month after its diagnosis (11). In a systematic review 37 articles collectively describing the cases of 54 patients were evaluated. The onset of articular symptoms varied considerably, and the majority of cases were described as polyarticular. The classification of inflammatory arthritis in the included studies was reactive (19), post-viral (13), new-onset rheumatoid arthritis (RA) (8), crystal-proven arthropathy flare (4), acute viral (2), new-onset psoriatic arthritis (2), flare of preexisting RA (2), and other (4). Arthritis treatment regimens varied but consisted largely of NSAIDs and corticosteroids with most patients experiencing improvement or resolution of their joint symptoms (12).

Molecular mimicry appears to play a major role in the pathogenesis. Although viral infections are known to potentially induce reactive arthritis, viremia was documented in only 15% of cases of COVID-19. Interleukin 17 A has been involved in the pathogenesis of both reactive arthritis and spondyloarthritis in general and also in the hyper inflammatory state of COVID-19 (13).

3. AUTOIMMUN DISEASES

Lupus patients have a higher incidence of several viral infections, likely due to a combination of immune dysfunction, immunosuppressive therapy, and comorbidities. Data from the COVID-19 Global

Rheumatology Alliance (C19-GRA) has suggested that SLE patients may be at higher risk of hospitalisation from COVID-19 compared to those with other rheumatic diseases (14). COVID-19 infection causes a dysregulated cytokine response with high resultant expression of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-alpha, which in turn could potentially be exacerbated by the shift in Th1 to Th2 response seen in SLE. It is commonly recognised that SLE, antiphospholipid syndrome (APS) and antiphospholipid antibodies (aPL) can be triggered by viral illnesses (4).

Patients who developed sle after covid 19 infection, as well as patients who developed sle after covid 19 vaccination, are also included in the literature (15,16).

In addition, a small number of SLE cases developed after the covid-19 vaccination have also been published (17). The potential pathophysiology of which the mRNA Pfzer/BioNTech vaccine can induce autoimmune disease like SLE could be related to the vaccine adjuvants that trigger the NLR pyrin domain containing 3 (NLRP3) infammasome which plays a major role in innate and adaptive immune system (18). Aşılama sonrasında konnektif doku hastalığı görülmesi çok sık olmasa da yine de rastlanmaktadır. Cole ve ark yayınında bir vakada 70 yaşında bir erkekte aşılamadan 2 hafta sonrasında Diffuse cutaneous systemic sclerosis gelişmiştir (19).

Vasculitis

A series of publications have reported the development of a vasculitis-like illness in COVID-19 patients, with presentations ranging from vasculitis syndromes to histologic findings of vasculitis seen on postmortem examination. Histologic evidence of COVID-19 induced vasculitis has also been reported in several organs including the lung, liver, kidney, or skin (4). Manenti et al. describe pathophysiologic observations in small/medium sized arteries in COVID-19; first is an acute endotheliitis, followed by peri− /pan-arteritis with deposition of polyclonal antigen-antibody immune complexes, suggesting a type III hypersensitive acute vasculitis (20). There is growing recognition that states of heightened innate immune response and a prothrombotic state elicited by innate immune mediators such as that seen in COVID-19 lead to an escalating cascade of inflammatory pathways that promote profound micro and macrovascular *endothelial* cell dysfunction and damage, with impairment of other important functions of the endothelium (21).

The dermatological manifestations of COVID- 19 as acral areas of erythema with vesicles or pustules (pseudo-chilblain) (19%), other vesicular eruptions (9%), urticarial lesions (19%), maculopapular eruptions (47%) and livedo or necrosis (6%) (22). COVID-19 toes/pseudo-chilblains are reported predominantly in children and young adults and appear to be a relatively late feature of COVID-19 (23). Livedo or necrosis, with lesions suggesting occlusive vascular disease usually affects people with more severe COVID-19 with no association with the duration of infection. A French retrospective study suggested that chilblains are common sequelae of COVID-19 and are associated with microthrombi on biopsy (24). Camprodon Gomez ´ et al. (25) and Mayor-Ibarguren et al (26) both described patients with leucocytoclastic vasculitis with positive SARS-CoV-2 PCR in skin biopsies with positive serum IgM and IgG against SARS-CoV-2 but negative oropharyngeal swab PCR. This could indicate that leucocytoclastic vasculitis develops as a late manifestation of COVID-19 infection.

In some of the case reports, patients who developed central vasculitis after covid-19 infection were mentioned. These cases lend support to the suspected mechanism of “endotheliitis” associated with this novel coronavirus. It is presumed that SARS-CoV-2 infects vascular endothelial cells and causes inflammation through angiotensin converting enzyme inhibitor 2 (ACE2) expressed in vascular endothelial cells. The expression of the ACE2 receptor in neurons and cerebral endothelial cells indicates a high level of invasiveness for the SARS-CoV-2 in comparison with other coronaviruses. Activation of the vascular endothelium is likely to lead to development of vasculitis with another insult or injury for other types of vasculitis as well (4).

Inflammation of large vessels have dilemmas. Underlying mechanism has been attributed to infection with SARS-CoV-2 or vasculitis (20,27).

In some of the COVID-19 patients, vasculitis, with or without antibody positivity, were seen. These include granulomatosis with polyangiitis with proteinase 3 antibodies that presented with alveolar haemorrhage, acute tubular injury, ANCA-associated glomerulonephritis, MPO or PR3 positive of pauci immune GN, gout vasculitis due to small vessel vasculitis (4).

During this infection, cases of IgA vasculitis were also encountered. Anti-SARSCoV-2 IgA is the first immunoglobulin to be detected in COVID-19 as early as 2 days after onset (28). In children with chilblain lesions, IgA antibodies to SARS-CoV-2 spike protein S1 domain are observed, suggesting that their immune response represents mucosal protection that lessens the likelihood of triggering an IgG response (29).

Analogous to the cutaneous vasculitis seen in younger patients with COVID-19, coronary artery vasculitis or a Kawasaki-like disease has been reported in predominantly younger patients with COVID-19 infection. Kawasaki-like disease typically occurs as part of a severe hyper inflammatory state, termed multisystem inflammatory syndrome (MIS, or MIS-C) with the latter terminology now being adopted. This syndrome is rarely associated with the coronary artery aneurysms typical of Kawasaki disease, which occur in less than 10% of cases. Older children and even young adults might be affected, and abdominal pain and diarrhoea of unclear cause might occur. Kawasaki-like disease typically occurs in patients without discernible COVID-19 pneumonia or active infection, suggesting that direct viral infection is unlikely to be a factor. The fact that coronary artery aneurysms are uncommon in COVID-19-related Kawasaki-like disease, yet myocarditis is very common. Nevertheless, the mechanisms are unclear, and a link to either vasculitis or cardiac viral myocyte infection remains unproven (30).

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CHAPTER 10

RADIOLOGY IN COVID-19

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INTRODUCTION

Coronavirus disease 2019 (COVID-19), which first appeared in Wuhan, Hubei Province, China, is a highly infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1,2). It causes a range of respiratory tract infections varying from mild complaints to severe respiratory distress syndrome. The most common clinical symptoms are fever and cough, however it may also present with other non-specific symptoms such as fatique, myalgia, sore throat, headache and diarrhea (3). COVID-19 disease is diagnosed basically from epidemiologic factors, clinical manifestations, radiological findings and nucleic acid detection of the virus. However, symptoms and clinical features are not specific in COVID-19 disease. Therefore, special diagnostic methods are needed. The gold standard method for detection of COVID-19 disease is the reverse transcription-polymerase chain reaction (RT-PCR) test. Although RT-PCR is highly specific, its sensitivity can vary widely. This test has a low sensitivity, especially at the beginning of the disease. Also, the RT-PCR test may be negative due to improper sample collection and laboratory analysis. Therefore, radiological imaging, particularly chest CT, plays an important role in the early detection of patients with suspected COVID-19 pneumonia. In addition, these methods are also used to determine the severity of the disease, guide treatment and evaluate treatment response (4-7).

The basic radiologic methods used in the diagnosis of COVID-19 are Chest X-ray and CT. In the following sections, the features of these methods will be stated in subheadings.

CHEST X- RAY

Chest X-ray can be used as a first-line imaging modality in Covid-19 patients due to its advantages such as availability, low cost, less ionizing radiation, portable equipment and easy sterilization of the device. Taking a radiological examination in the same room is a risk for radiology workers and uninfected patients. Performing chest X-ray at specific locations for COVID-19 patients using a mobile system helps reduce transmission of infection. Hence, American College of Radiology recommended that portable chest X-ray may be considered to reduce the risk of disease spread and decrease the heavy burden on the radiology units. However, the most important limitation of chest X-ray is its high false negative rate. The sensitivity has been reported between 33% and 69% in different studies. Therefore, a normal chest X-ray does not exclude the disease. Possible factors of low sensitivity are technical limitations of the mobile X-ray systems, absence or initial phase of lung disease at the time presentation; and the fact that the ground-glass opacities and reticular pattern related to COVID-19 may be difficult to identify on chest X-ray. However, pathological findings on chest x-ray are usually more prominent and common 10-12 days after onset of symptoms. Chest X-ray findings of COVID-19 pneumonia include consolidations or hazy opacities that are usually bilateral, peripheral and multifocal (7-10).

Chest X-ray findings in cases with suspected COVID-19 have been divided into four categories (11-13):

Normal chest X-ray; Chest X-ray may be normal in the initial stages of the disease, therefore a normal x-ray does not exclude the infection.

Typical findings; These findings include a reticular pattern, ground-glass opacities, and consolidations, with rounded configuration and a confluent or patchy multifocal distribution. The distribution is generally peripheral, bilateral, and predominant in the lower lobes. In the differential diagnosis, pathologies such as organizing pneumonia, drug toxicity and other causes of acute lung injury.

Indeterminate findings; These findings are consolidations and ground-glass opacities with a unilateral or nonperipheral distribution. Indeterminate features may present COVID-19 pneumonia or other causes such as alveolar edema and different infections**.**

Atypical findings; It is defined as unusual and unreported findings in COVID-19 pneumonia. These findings are lobar consolidation, lung nodules, miliary pattern, cavitation and pleural effusion.

A correlation between progressive chest X-ray findings and the onset of symptoms has been reported. Initially, the reticular pattern and ground glass density predominate, whereas consolidations are more typical in later stages. In the early stage of the disease and in mild disease x-ray may be negative, So, in suspected patients CT should be considered for early diagnosis of COVID-19 disease (12,14).

CHEST CT

Chest CT is an important modality to identify and investigate patients with COVID-19 disease, especially in the early stage of the disease. CT is considered the first-line imaging modality in suspected patients whose RT-PCR tests and chest x-ray images were negative. Besides, it is also helpful for monitoring imaging changes during treatment and for guiding management in complex settings. Recent studies have shown the sensitivity and specificity of CT in COVID-19 disease as 60%–98% and 25%–53%, respectively. Despite its high sensitivity, there is some disagreement and controversy regarding the use of CT as the initial diagnostic method. Many instutions consider CT as the first-line technique with the limitations of PCR testing. However, The American College of Radiology (ACR) suggests CT as a second-line technique due to some conditions such as the difficulty of sterilization and heavy burden on it in pandemic. As well, the specificity of CT is not as high as its sensitivity. CT findings in Covid pneumonia may overlap with findings other viral pneumonia (H1N1, SARS, and MERS) findings and non-infectious conditions such as organizing pneumonia (10, 15-19).

CT manifestations

Many articles have been published about CT findings in the COVID-19 pandemic. Some typical findings are demonstrated such as bilateral peripheral ground-glass opacity and/or consolidation. However, the CT features of the disease have wide variability, depending on the clinical severity and the time elapsed since the onset of symptoms (11,12, 20-22). The findings, staging and reporting of CT in COVID-19 will be stated in subheadings.

FINDINGS

A. TYPICAL FINDINGS:

Ground glass opacity (GGO)

GGO is defined as a hazy increased attenuation area without obscuration of bronchial and vascular margins and is typically caused by partial filling of the airspaces or interstitial thickening. In patients with COVID-19 pneumonia, unilaterally or bilaterally GGO with peripheral and subpleural distribution are frequently observed (Fig. 1). Also, it is usually the earliest appearance. There are studies presenting GGO as the most common imaging finding with an occurrence rate of up to 98%. It may or may not be accompanied by other manifestations, particularly consolidation and reticulation. Furthermore, GGO may be often accompanied by other manifestations or patterns, including consolidation, interlobular septal thickening and reticulation (10, 22-25).

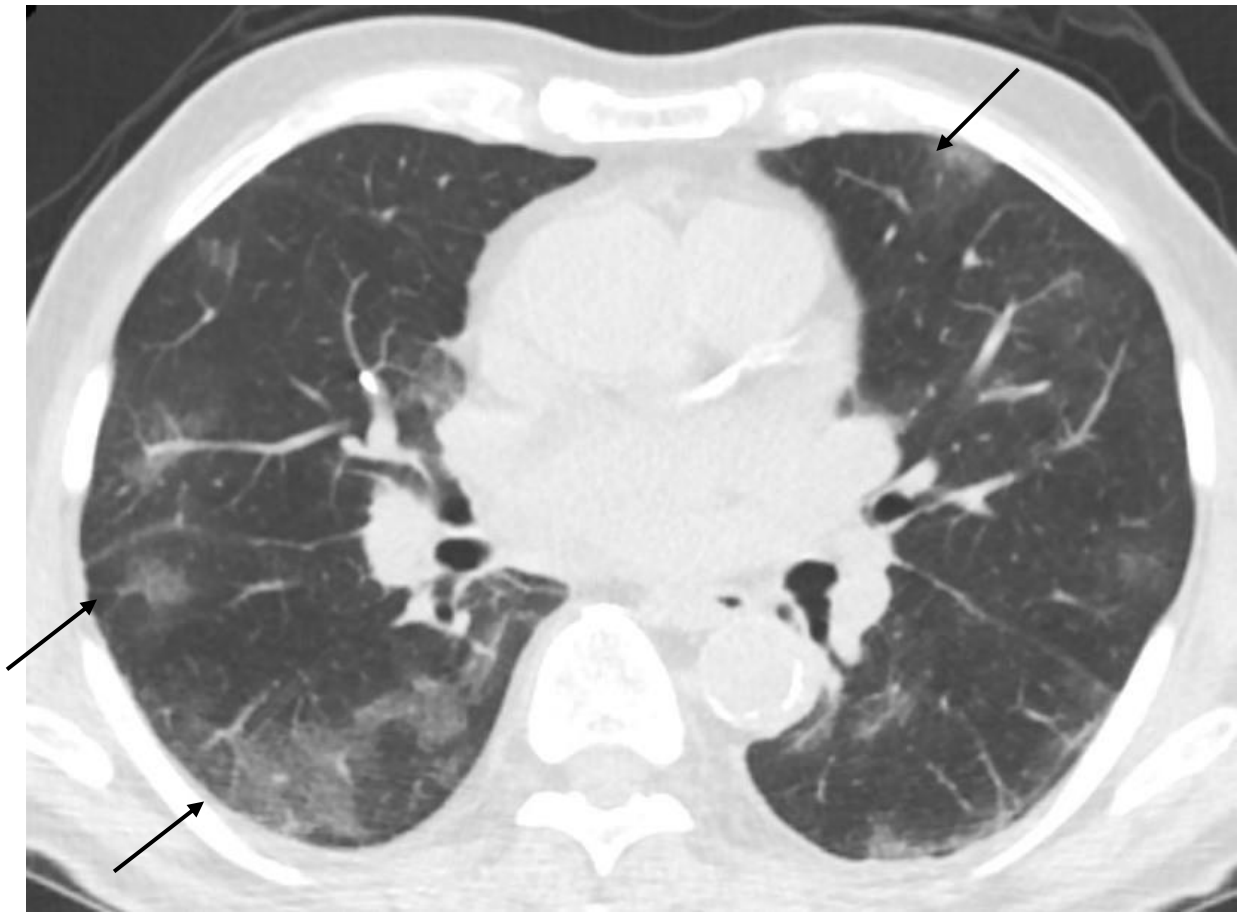

Figure 1. Typical findings in COVID-19 pneumonia on computed tomography (CT). Axial CT image shows bilateral, multifocal and peripheral ground glass-opacities (arrows).

Consolidation

Consolidation is defined as an increased attenuation area with obscuration of underlying vessels and airway walls, and is caused by complete filling of the alveolar airspaces with pathological fluid or tissues. Multifocal, patchy, or segmental consolidations are seen in COVID-19 pneumonia in 2%–69% of patients (Fig. 2). Consolidations can be patchy or round shaped. Round lesions were considered relatively more specific to Covid-19. Peripheral and subpleural lesions are more frequently compared with central peribronchovascular lesions. The consolidation is thought to be related to the accumulation of cellular fibromyxoid exudate in the alveoli. In COVID-19 pneumonia, consolidation is considered as a manifestation of progressive disease (22,26-28).

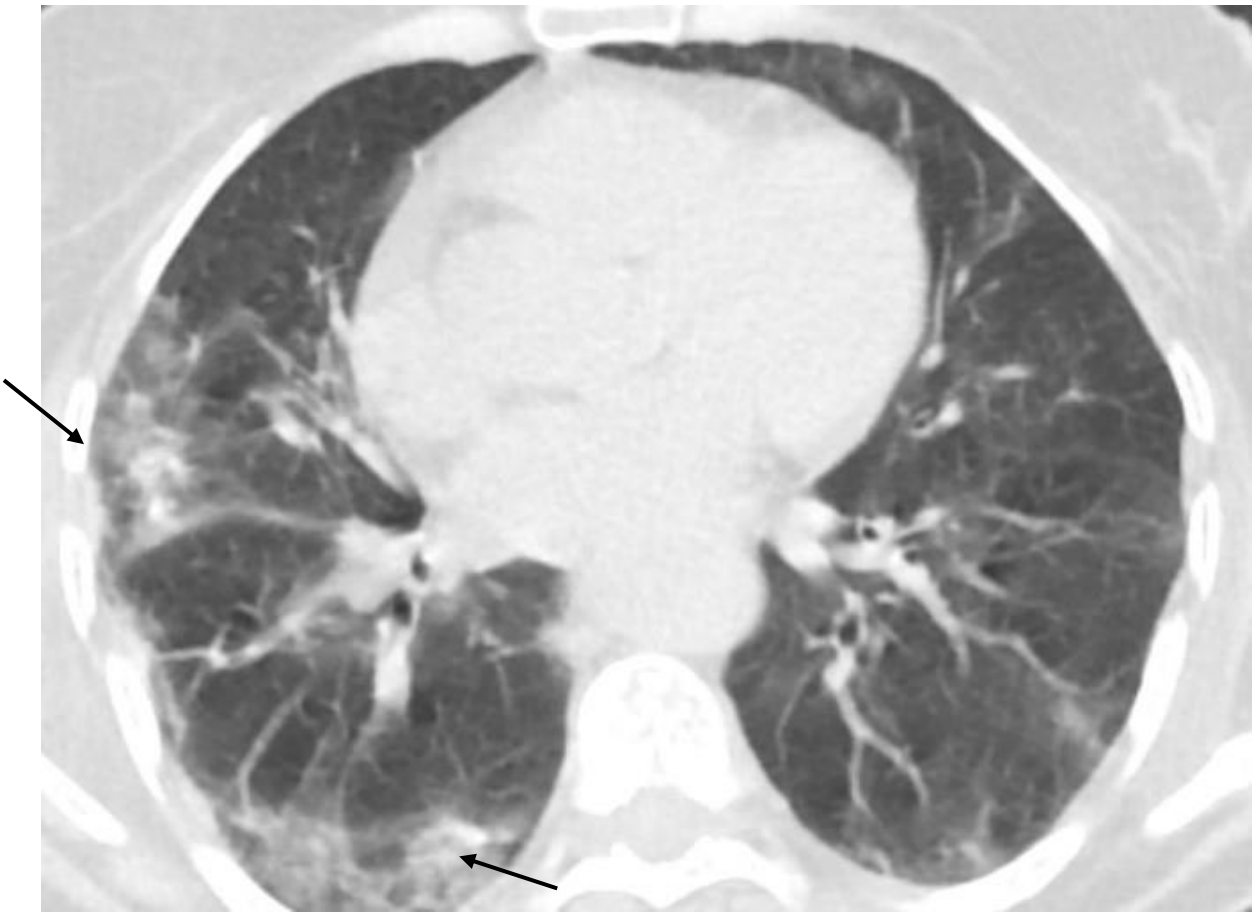

Figure 2. Axial CT image shows peripheral ground-glass opacities and accompanying areas of consolidation (arrows) in the right lung.

Reticulation

Reticulation is defined as thickened interlobular septa and intralobular lines, manifested as small linear opacities on CT images. This pattern is accepted as interstitial lymphocyte infiltration causing interlobular septal thickening. Reticulation has been shown to be the third most common CT feature after GGO and consolidation, with a rate of 48.5%–59%. Compared with GGO and consolidation, reticulation is a relatively late-stage finding of the disease (10, 22, 29).

Crazy-paving pattern

Crazy-paving pattern is defined as thickened interlobular septa or intralobular lines superimposed on GGO backround, resembling irregular paving Stones (Fig. 3). This sign is considered to represent alveolar edema and interstitial inflammation. Crazy-paving pattern was reported in 5%–36% of COVID-19. In combination with diffuse GGO and consolidation crazy paving pattern could be an indication of COVID-19 entering progressive or peak stage (10, 20, 22).

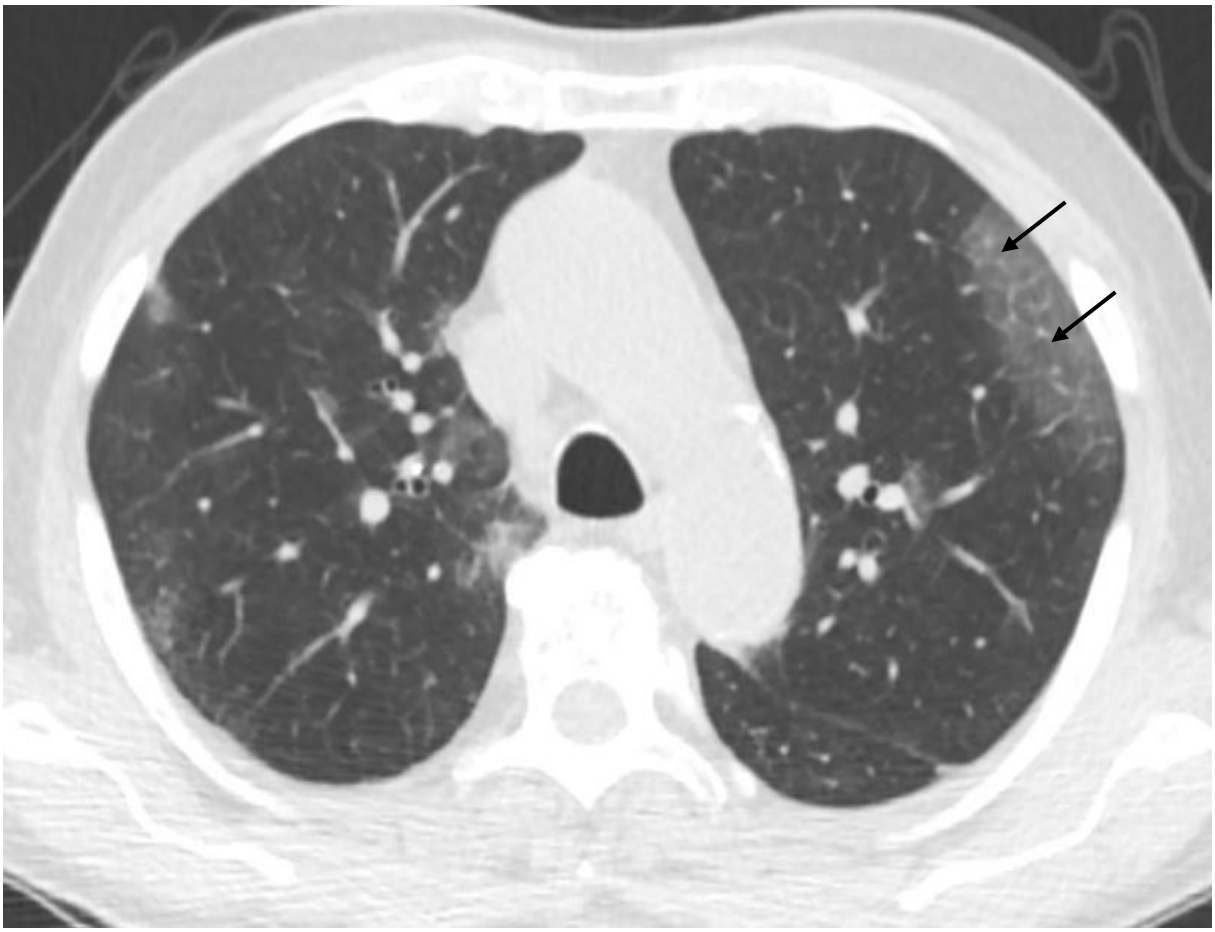

Figure 3. Axial CT image shows thickened interlobular (arrows) and intralobular septa superimposed on a background of ground-glass density showing a crazy paving pattern in the left lung.

Air bronchogram

Air bronchogram defined as visible air-filled bronchial lumina in a hyperattenuated lung area. This appearance is often accompanied by mild bronchiolar dilatation. Air bronchogram was reported to be another CT manifestation of COVID-19 disease in 28%–80% of patients (10, 22, 24).

Reversed halo sign

Reversed halo sign defined as a focal round GGO surrounded by complete or partial ring-like consolidation. Although initially considered pathognomonic for cryptogenic organizing pneumonia, it was later identified in some other diseases. Recent studies have shown that this sign may appear in COVID-19 patients. This appearance is thought to occur longer after symptom onset in patients and reflects absorption within the lesion. Reverse halo sign was reported in 2%–5% of patients with COVID-19 (5, 10, 22, 30).

Vascular enlargement

Vascular enlargement defined as the dilation of the perilesional or intralesional pulmonary vessels due to capillary wall damage and swelling in response to inflammatory factors (Fig. 4). This CT appearance is quite common and has been reported in 71.3%–82.4% of COVID-19 patients (12, 30-31).

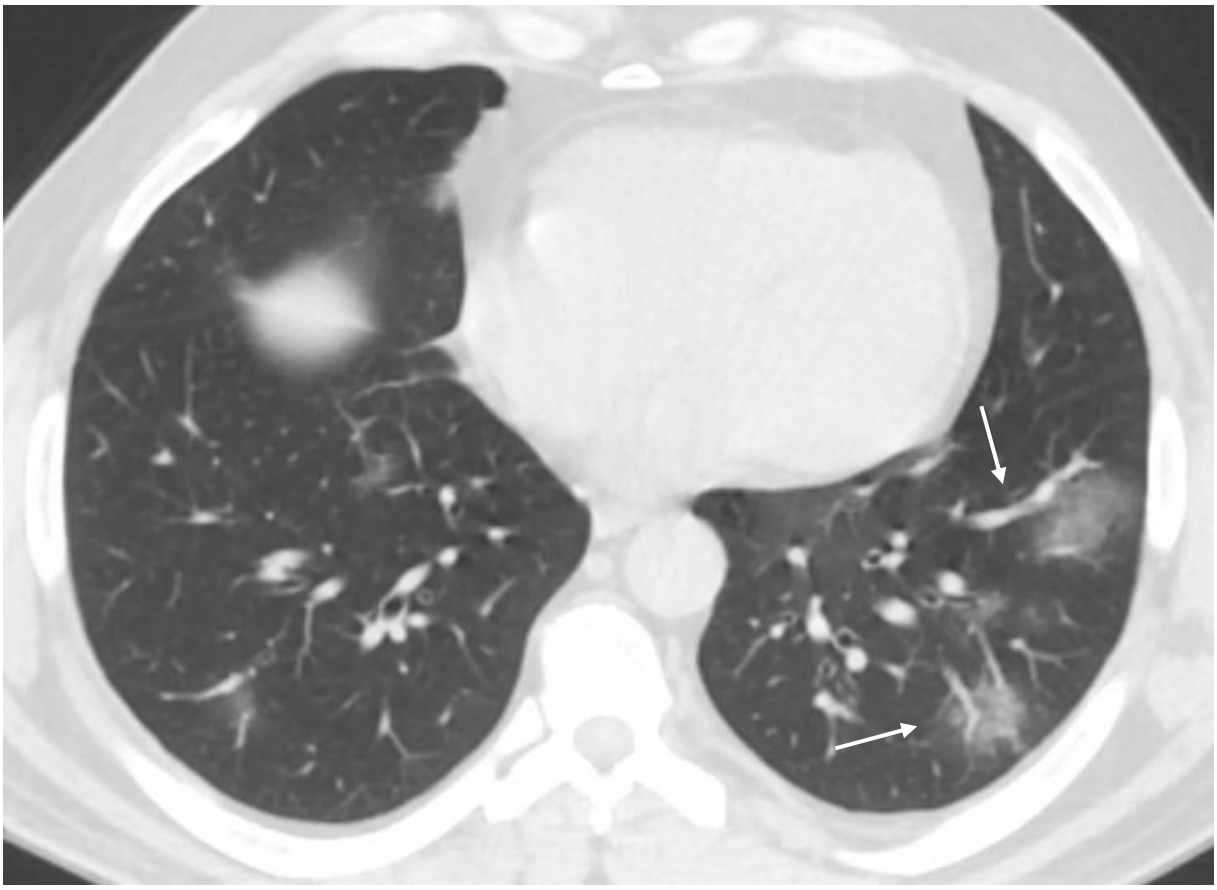

Figure 4. Axial chest CT image shows round-shaped ground-glass opacities and concomitant vascular enlargement (arrows) in the left lung.

Airway changes

Airway changes defined as bronchiectasis and bronchial wall thickening. These changes are considered related to the inflammatory damage of the bronchial wall. Bronchial wall thickening has been reported between 10% and 20% COVID-19 patients. Airway changes may be more common in other viral pneumonia types (22, 32).

Air bubble sign

Air bubble sign defined as a small bubble-like air-containing space in the lung. This sign was also defined as cystic change or cavity, and the incidence rate was reported as 10% in a study. Air bubble sign is considered related to the pathological enlargement of a physiological space or a bronchiolectasis section, or a resorption area within consolidation (21, 22, 30).

Nodules

A nodule defined as a rounded or irregular opacity with well- or poorly defined edges, measuring less than 3 cm in diameter. Multifocal irregularly nodules or nodules with halo sign were reported in 3–13% of patients with COVID-19 pneumonia (22, 23 and 33).

Pleural changes

Pleural abnormalities include pleural thickening and pleural effusion. In a study, these changes were reported as pleural thickening in 32% and pleural effusion in 5% of COVID-19 patients. The occurrence of pleural effusion may indicate poor prognosis in COVID-19 pneumonia (5, 21-22).

Subpleural changes

Subpleural abnormalities include subpleural curvilinear line and subpleural parenchymal band. Subpleural curvilinear line is defined as a thin curvilinear opacity with 1–3 mm thickness, seen close and parallel to the pleural surface (Fig. 5).

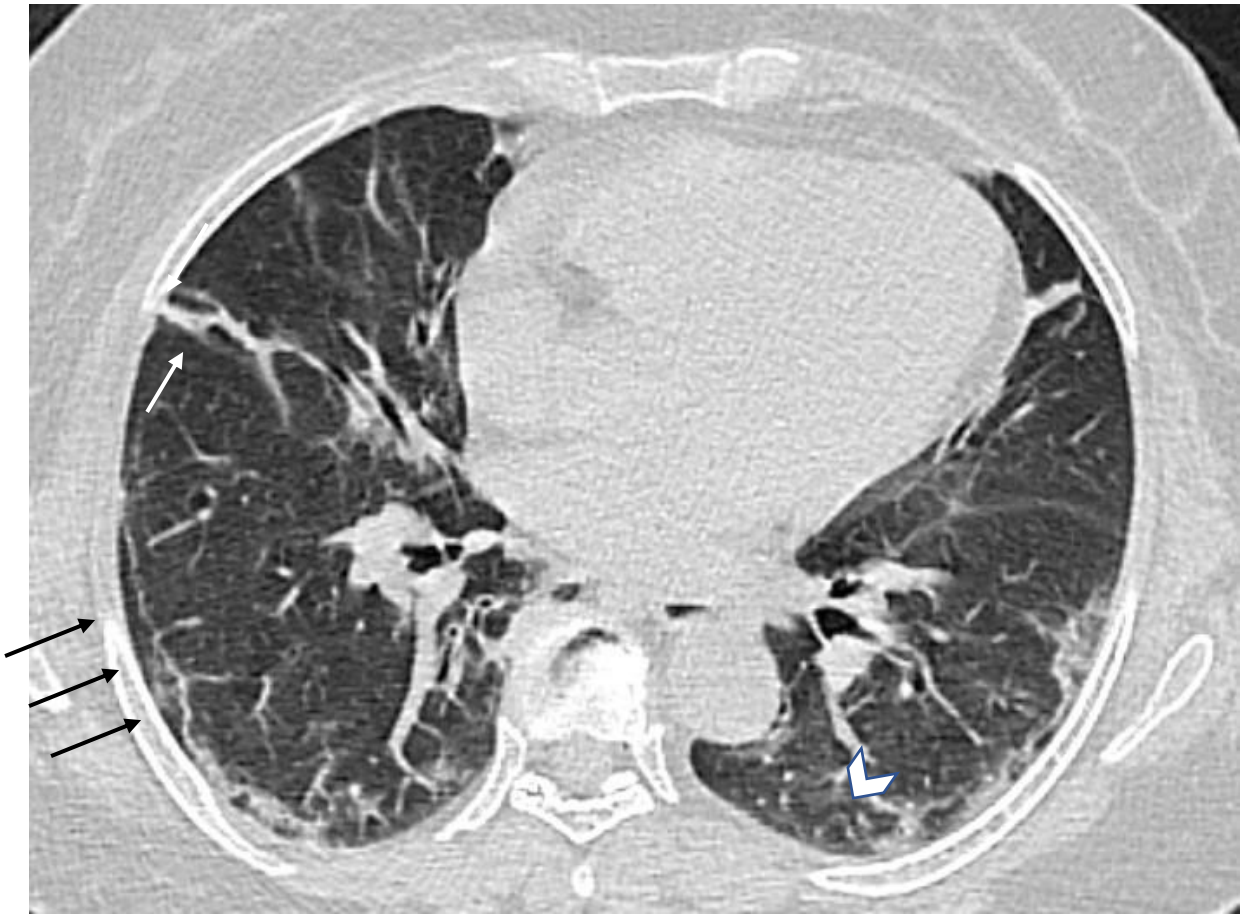

Figure 5. Axial chest CT image shows subpleural curvilinear line (black arrows), subpleural parenchymal bands (arrowhead), bronchial wall thickening and irregular opacities with traction bronchiectasis (white arrows).

It is thought to be due to pulmonary edema or fibrosis and has been reported in 20% of patients with COVID-19 pneumonia. Subpleural parenchymal band is defined as a linear opacity, usually 1-3 mm thick and up to 5 cm long, perpendicular and mostly extending to the visceral pleura. It is often associated with distorted parenchymal architecture (10, 12, 30).

Halo sign

Halo sign is defined as nodule or mass consolidation surrounded by ground glass opacity. It has been described in various diseases such as angioinvasive fungal infections, viral infections organizing pneumonia, and hypervascular metastases. It has been variably reported in 18%–64% of patient with COVID (3,10, 19, 31).

Perilobular pattern

Perilobular pattern, which is typical feature of organizing pneumonia, defined as arcade-like or polygonal curvilinear opacities. It can also be seen as a late finding in the course of the covid pneumonia (10, 34-35).

Pericardial effusion

Pericardial effusion is not a common manifestation in Covid 19 patients, with incidence of about 5%. It may refer progressive ilness and the occurrence of severe inflammation (22, 36).

B. INDETERMINATE FINDINGS:

Indeterminate findings are nonspecific manifestations as they can be seen in both COVID-19 pneumonia and in pneumonia caused by other pathogens (12);

-Non-peripheral, patchy ground-glass densities or consolidations, with a unilateral distribution, more common in the upper lobes,
-Ground-glass fibrosis,
-Lymphadenopathy,
-Pleural effusion

C. ATYPICAL FINDINGS:

These are findings that may consider alternative diagnoses (12, 21);

-Tree-in-bud pattern,
-Segmental-lobar consolidation,
-Cavitation,
-Well-defined nodules- masses,
-Diffuse ground-glass opacities with a peribronchovascular distribution,
-Honeycombing fibrotic densities

CT findings according to the stage of the disease

Some studies have been reported on the radiological course of COVID-19 pneumonia over time. There is a relationship between radiological findings and the time elapsed from the onset of symptoms. Accordingly, four main stages have been reported (10, 12, 37, 38);

Early stage (0-4 days after the onset of symptoms): At this stage, the main CT finding is unilateral or bilateral subpleural ground-glass opacities predominantly in the lower lobes. It can present rounded morphology. CT may also be normal during this period especially in the first two days.

Progression stage (5-8 days): At this stage, rapid progression of ground glass opacities, addition of consolidation and multilobar involvement are observed. Prominent bronchovascular structures and interlobular and intralobular septal thickening (revealing the crazy pattern) may appear

Peak stage (9-13 days): The most severe findings are observed at this stage. Areas of ground-glass opacities transforming into consolidation. Main findings of the stage are consolidation, GGO, crazy-paving pattern and residual parenchymal bands. Reverse halo sign, air bronchograms, and mild pleural effusion can also be seen.

Resolution stage (>14 days): At this stage, gradual regression in lesions is observed. Consolidation and crazy paving areas are markedly absorbed. These areas leave their place to fibrosis and extensive GGO. Subpleural fibrotic parenchymal bands and subpleural curved lines may accompany in the same peripheral localizations. It has been reported that fibrotic bands and stripes may be an sign of the stabilization and healing of the disease.

CT reporting standardization

There are different recommendations for using a standardized language in reporting COVID-19 CT findings. The most commonly used in the current literature are the classifications advised by the British Society of Thoracic Imaging (BSTI) (Table 1) (39) and partnership of Radiological Society of North America (RSNA), Society of Thoracic Radiology (STR), and American College of Radiology (ACR) (Table 2) (40,41).

Table 1. Chest CT findings according to structure by British Society of Thoracic Imaging in COVID-19 pneumonia (39).

Pattern	**Appearance**
Classical COVID-19 (100% compatible)	Peripherally, lower lobe predominant, multiple, bilateral* ground glass opacities (GGOs) ± Crazy paving Peripherally consolidation** Air bronchogram Converse halo sign/perilobullary pattern**
Possible COVID-19 (71–99% compatible)	Peripherally, lower lobe predominant, bronchocentric consolidation Reverse halo sign/perilobullary pattern** Limited GGOs
Indeterminate (<%70 compatible)	Incompatible with the other three radiological groups. Compatible with radiological appearance but has another diagnosis such as ILD, CTD.
COVID-19 exclusion (< %70 compatible other diagnosis)	Lobar pneumonia Cavitation Tree in bud/nodularity Lymphadenopathy Pleural effusion, Advanced pulmonary fibrosis
* >1 lesion can be unilaterally (it is usually bilateral); ** e.g.; Organised pneumonia patterns	

Table 2. Expert consensus statement on reporting chest computed tomography (CT) findings related to COVID-19 (endorsed by ACR, RSNA, and STR)(40,41).

Classification	Rationale	CT Findings	Suggested reported language
Typical	Commonly reported imaging features of greater for specifity for COVID-19 pneumonia	Peripheral, bilateral (multilobar) GGO*, consolidation, or visible intralobular lines	Commonly reported imaging features of COVID-19 pneumonia are present.
Indeterminate	Nonspecific imaging features of COVID-19 pneumonia	Multifocal, perihilar, unilateral GGO or nonrounded or nonperipheral	Imaging features can be seen with COVID-19 pneumonia
Atypical	Uncommonly or not reported features of COVID-19 pneumonia	Isolated lobar or segmental consolidation, discrete small nodules, cavitation or interlobular septal thickening, pleural effusion	Imaging features are atypical or uncommonly reported for COVID-19 pneumonia, alternative diagnosis should be considered
Negative	No features of pneumonia	No CT features to suggest of pneumonia	No CT findings present to indicate pneumonia

CONCLUSION

Radiological examination plays an important role in the diagnosis and follow-up of COVID-19 disease. The initial imaging method is the chest X-ray with advantages such as easy accessibility, low cost, low radiation and portable equipment. However, chest CT is a more sensitive method than chest x-ray and PCR test, especially in the early phase of the disease. It is also widely used in the management of the disease, explaining complex conditions and alternative diagnoses. Although bilateral, peripheral GGO and/or consolidation are the predominant imaging characteristics, CT features may vary according to the stage of the disease. Recognizing the appearance patterns is very important to understand the course of the disease and to make a differential diagnosis.

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CHAPTER 11

STATISTICAL INVESTIGATION OF COVID-19 PANDEMIC

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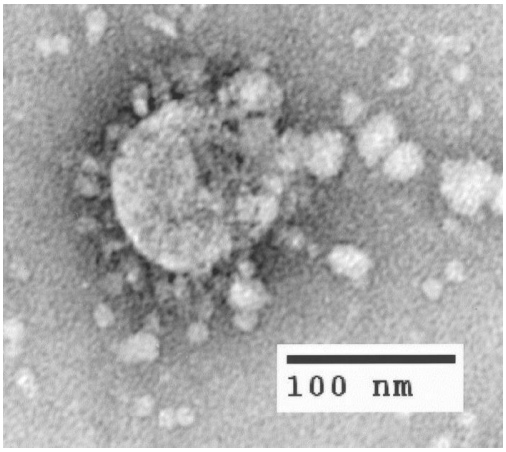
INTRODUCTION

Human coronaviruses (HCoV), which cause respiratory tract infections, first emerged in the 1960s with the discovery of HCoV-229E and HCoV-OC43 in the nasal cavities of human patients with the common cold (1, 2). Human coronaviruses that were discovered SARS-CoV (severe acute respiratory syndrome coronavirus) in 2002, HCoV NL63 in 2004, MERS-CoV (Middle East Respiratory Syndrome) in 2012, and most recently SARS-CoV-2 (COVID-19) in 2019, have caused severe respiratory infections (3, 4).

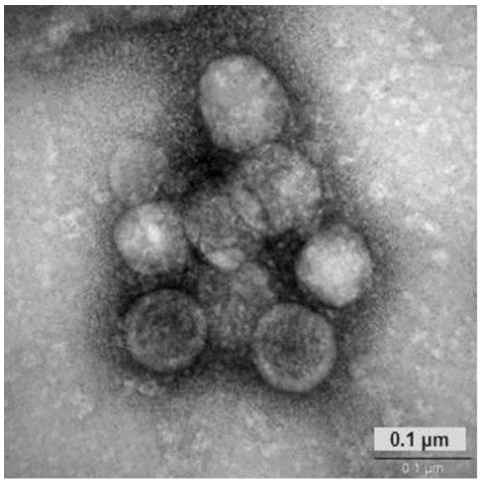
Evaluating a pandemic case statistically can provide significant benefits for understanding the case, diagnosing and developing treatment. Statistics such as the course of the disease, the rate of spread, death rates, survival rates, recorded symptoms can be vital in matters such as the severity of the disease, the detection of similar diseases, and therefore the detection and implementation of emergency temporary treatment methods until permanent treatment is found (5).

The first case of COVID-19 recorded in Wuhan, China in December 2019 (6) was declared a pandemic by the world health organization (WHO) on 11 March 2020, when the first case was seen in Turkey (7). As of 27 April 2022, 511.165.180 cases and 6.250.404 deaths were observed worldwide due to the COVID-19 virus (8). Transmission electron micrograph images of coronaviruses are shown in the figures (Fig. 1) (9-12).

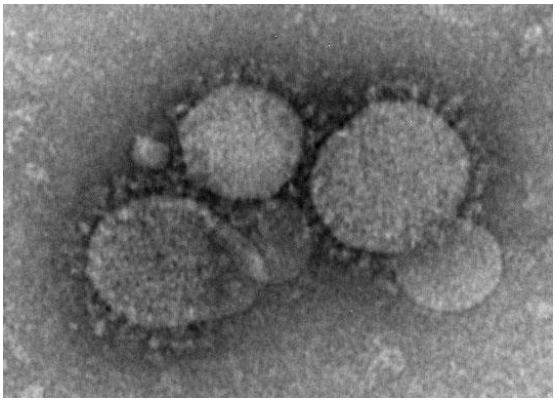
To understand the COVID-19 pandemic, it also would be helpful to study the coronavirus and other pandemics.



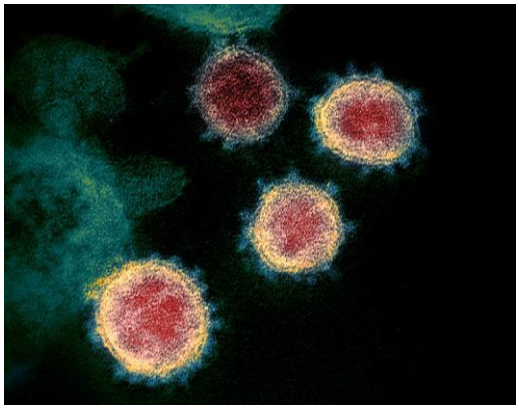
SARS-CoV (9)



HCoV NL63 (10)



MERS (11)



SARS-Cov-2 (12)

Figure 1: Transmission electron micrograph images of coronaviruses (9-12)

1. HCoV PANDEMICS AND BASIC STATISTICS

1.1. SARS PANDEMIC AND BASIC STATISTICS

The SARS epidemic started in Guangdong, China in November 2002 and spread rapidly to a total of 37 countries, mainly Hong Kong, Taiwan, Canada, Singapore, Vietnam and the United States, within 9 months. Worldwide, 8.422 cases were detected and 916 deaths were observed (case fatality rate= %10,9) (13,14). Although the spread of SARS has been completely prevented, it is thought that it is present as a source of disease in some animal species and carries a risk of re-infection to humans (15). No case has been seen in Turkey (14).

While the most common symptom of the disease is fever over 38˚, symptoms such as lethargy, myalgia, sore throat, myalgia, cough, chills and diarrhea have been recorded. In some cases, dyspnea has also been observed (15). Some Russian scientists such as Kolesnikov and Filatov have put forward some theories that the SARS virus may have been produced in the laboratory as a biological weapon (16).

1.2. HCOV-NL63 PANDEMIC AND BASIC STATISTICS

In 2004, a new human coronavirus HCoV-NL63 was discovered in a seven-month-old baby in the Netherlands with respiratory symptoms. 807 HCoV-NL63 cases of the 24.311 patients who applied to five hospitals with respiratory tract virus PCR testing over 27 months were identified. All-cause mortality rates of these patients were 3,1% in all patients and 10,8% in patients under 18 years of age (17). At first, palm civets were thought to be the natural source of the virus, with next phylogenetic studies African trident bats and roundleaf bats (18). According to the Cox proportional hazard regression analysis (17), it has been determined that HCoV-NL63 is mostly observed in children with mild upper respiratory tract symptoms such as fever, cough and runny nose, or more severe lower respiratory tract involvement such as bronchiolitis and croup, and infects people with weakened immune systems. Dutch scientists determined that the virus initiates a cytopathic effect when inoculated into tertiary monkey kidney cells (19). In addition, it has been determined that there is a seasonal relationship in temperate climates.

1.3. MERS PANDEMIC AND BASIC STATISTICS

The epidemic, called MERS, started in the city of Jeddah, Saudi Arabia in April 2012 as the second coronavirus epidemic and went down in history. MERS, declared an epidemic by WHO, caused 1589 cases and 567 deaths in 26 countries (case fatality rate= %35,7). MERS is a viral zoonotic disease with limited human-to-human transmission. Although the source of the disease is not known exactly, it is thought to be dromedary camels (20) and Egyptian cemetery bats (21). The first case in Turkey was observed in October 2014, when a citizen who went to Saudi Arabia to work died shortly after returning to the country (22).

The virus, which spreads in the body within the first 15 days of its infection, causes cough, shortness of breath, fever and sometimes sputum symptoms. It is recommended to consume plenty of fluids against non-lethal types, painkillers and antipyretic drugs are used (22).

1.4. SARS-CoV-2 (COVID-19) PANDEMIC AND BASIC STATISTICS

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus (23). The last coronavirus pandemic, called COVID-19, started in the Chinese city of Wuhan in December 2019. COVID-19, which was declared an epidemic by WHO on March 11, 2020, is a virus that has spread to 188 countries and regions (24) worldwide and continues to spread. It caused 511.165.180 cases and 6.250.404 deaths on April 27, 2022 (case fatality rate= 1,2%), and 464.359.529 improvements were observed (8). While the death rate in the world was 3,4% (25) on March 3, 2020, this rate decreased to 1,2% after 2 years.

It is transmitted from person to person by aerosols produced by coughing, breathing or speaking, usually through respiratory droplets accumulated on mucosal surfaces (26). It has been determined that an infected individual can transmit this virus to others even two days before showing symptoms. It has been observed that they can remain infected for up to 10 days in mild cases and up to 14 days in severe cases. The disease source of COVID-19, which is closely related to the SARS virus (27), is thought to be bats. The first case in Turkey was observed on March 11, 2020 (28).

The virus, which spreads in the body within the first 15 days of its infection, has symptoms of cough, shortness of breath, fever, loss of taste (ageusia) and sense of smell (anosmia). However, no symptoms were observed in one of the 5 infected people (29). Headache, runny and stuffy nose, sore throat, myalgia, eye irritation, diarrhea (30), and bruising/swelling of the toes (31) and difficulty breathing in moderate to severe cases have been reported as rare symptoms (32). Considering that the aerosol and surface stability of SARS-COV-1 and SARS-COV-2 viruses are similar (33), it is suggested that a significant difference between the two outbreaks may be due to silent infections in spread.

2. STATISTICAL ANALYSIS OF COVID-19 DATA

2.1. META ANALYSIS FOR SIGNS AND SYMPTOMS

In a meta-analysis study on COVID-19, it was determined that approximately 40% of COVID-19 cases worldwide are asymptomatic. Quantification of asymptomatic infections of COVID-19 plays an important role in the basic prevention response of the population against the epidemic (34). The US Centers for Disease Control and Prevention guidelines for COVID-19 pandemic prediction offer wide limits for the asymptomatic percentage, ranging from 10% to 70% (35). The media and some reports reporting that the asymptomatic percentage has such a wide range have led to misinterpretation of clinical and epidemiological studies (34).

The most reported symptoms are as follows; cough (63-83%), loss of smell (70,2%), headache (34-70%), nasal congestion (67,8%), asthenia (63,3%), fatigue (63%), myalgia (36-63%), runny nose (60,1%), taste disturbance (54,2%), sore throat (52,9%), and fever (45,4%) (36-37).

A meta-analysis study was conducted reporting data on symptoms of agusia/dysgeusia in patients diagnosed with COVID-19 in the form of case series, case control, and cohort studies. This study includes 33 articles published in PubMed/Medline, Embase, Cochrane and Web of Science databases between January 1st and April 21st, 2020. Random-effects model and DerSimonian-Laird approach were applied in this study. Study heterogeneity was calculated using the I^2 statistic. After excluding unrelated articles from the study, a total of 817 patients were studied in 5 (4 single-national, 1 multinational) articles. Only 3 studies reported the proportion of female patients (60,7; 95% confidence interval (CI): 51,3-70,1%). According to the meta-analysis in this study, the prevalence of ageusia/dysgeusia was found to be 49,8% (95% CI: 8,2-91,5%; I^2=99,6%)(Fig. 2) (38).

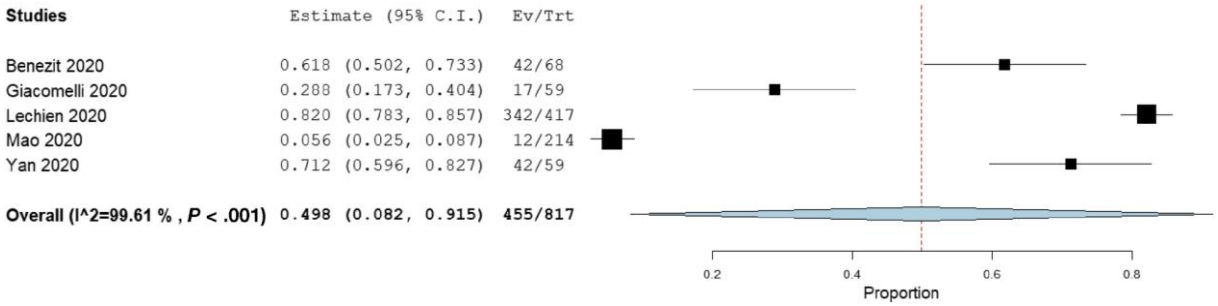
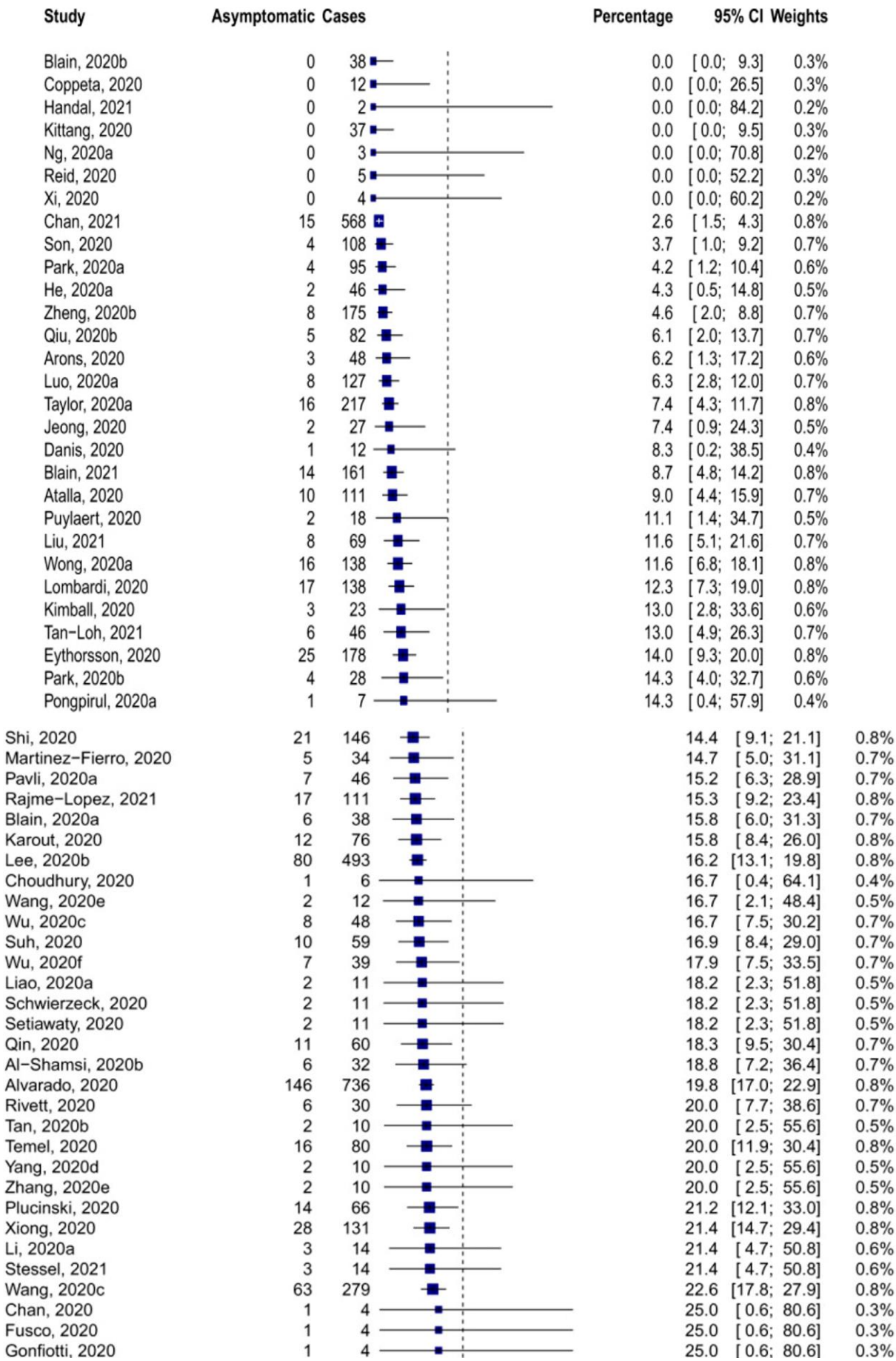
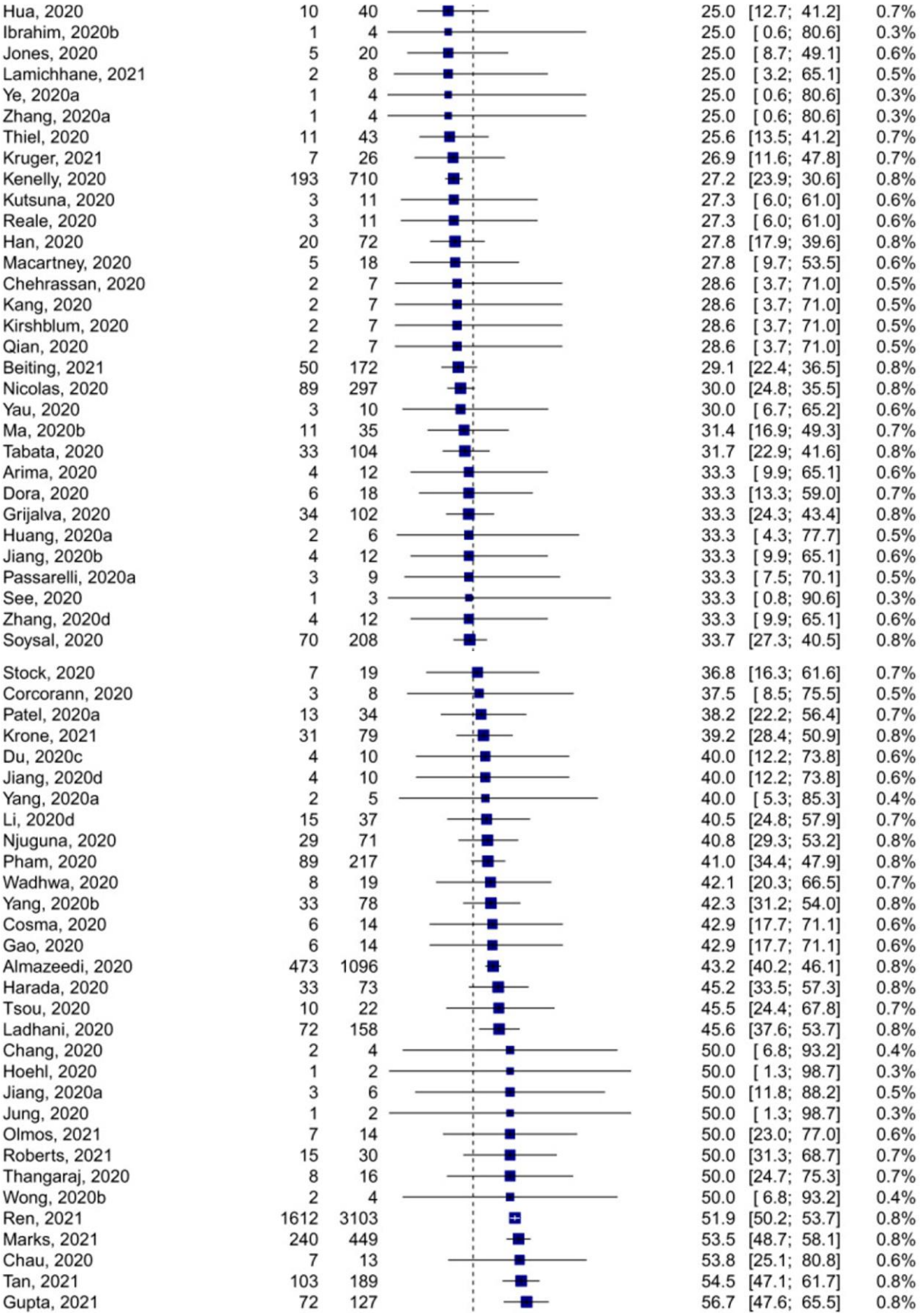


Figure 2. Forrest plot showing the pooled prevalence of ageusia/dysgeusia in patients with COVID-19. C.I., confidence interval; Ev/Trt, patients with symptoms/total patients (38).

According to the results of the analysis, approximately 50% of COVID-19 patients lost their sense of taste. The first symptom is loss of taste, since the virus first infected people from the mouth area. This information shows that it can be used as a distinctive clinical feature in early diagnosis and in regions where access to COVID-19 diagnostic tests is low or impossible. A statistically insignificant prevalence of ageusia/dysgeusia was observed in more severe cases (p=0,24). In this retrospective study, it is thought that the symptom of taste loss is underestimated because there is important missing data on ageusia/dysgeusia symptoms (38). Taste loss appears to be effective but insufficient on its own in predicting COVID-19 mortality.

In a more recent and wider time-lapse study conducted by Sah et al., approximately 350 articles were searched and it was found that elderly patients were more asymptomatic than children, and lower asymptomaticity in cases with comorbidities compared to cases without underlying medical conditions. PubMed, Embase, Web of Science, and the World Health Organization Global Research Database on COVID-19 were searched between January 1, 2020 and April 2, 2021, to identify studies reporting silent infections, whether presymptomatic or asymptomatic at the time of testing. Index cases were excluded from the study to minimize the representative bias that causes overestimation of symptomaticity. The percentage of infections that were truly asymptomatic without developing clinical symptoms was calculated to be 35,1% (95% CI: 30,7-39,9%) (Fig. 3). At the time of testing, 42,8% of cases (95% estimated range: 5,2-91,1%) were asymptomatic and a group of both asymptomatic and presymptomatic infections was mentioned. 19,7% (95% CI: 12,7-29,4%) asymptomaticity in elderly patients was calculated to be significantly lower than 46,7% (95% CI: 32,0-62,0%) asymptomaticity in pediatric patients (p <0,01; Table 1). In addition, cases with comorbidities were found to be significantly less asymptomatic compared to cases without underlying medical conditions. It is thought that the control of the epidemic may take a long time despite vaccination support, without implementing proactive policies such as rapid contact tracing to detect asymptomatic infections (34).


Study
Asymptomatic Cases
Percentage
95% CI
Weights
Blain, 2020b 0 38 0.0 [0.0; 9.3] 0.3%
Coppeta, 2020 0 12 0.0 [0.0; 26.5] 0.3%
Handal, 2021 0 2 0.0 [0.0; 84.2] 0.2%
Kittang, 2020 0 37 0.0 [0.0; 9.5] 0.3%
Ng, 2020a 0 3 0.0 [0.0; 70.8] 0.2%
Reid, 2020 0 5 0.0 [0.0; 52.2] 0.3%
Xi, 2020 0 4 0.0 [0.0; 60.2] 0.2%
Chan, 2021 15 568 2.6 [1.5; 4.3] 0.8%
Son, 2020 4 108 3.7 [1.0; 9.2] 0.7%
Park, 2020a 4 95 4.2 [1.2; 10.4] 0.6%
He, 2020a 2 46 4.3 [0.5; 14.8] 0.5%
Zheng, 2020b 8 175 4.6 [2.0; 8.8] 0.7%
Qiu, 2020b 5 82 6.1 [2.0; 13.7] 0.7%
Arons, 2020 3 48 6.2 [1.3; 17.2] 0.6%
Luo, 2020a 8 127 6.3 [2.8; 12.0] 0.7%
Taylor, 2020a 16 217 7.4 [4.3; 11.7] 0.8%
Jeong, 2020 2 27 7.4 [0.9; 24.3] 0.5%
Danis, 2020 1 12 8.3 [0.2; 38.5] 0.4%
Blain, 2021 14 161 8.7 [4.8; 14.2] 0.8%
Atalla, 2020 10 111 9.0 [4.4; 15.9] 0.7%
Puylaert, 2020 2 18 11.1 [1.4; 34.7] 0.5%
Liu, 2021 8 69 11.6 [5.1; 21.6] 0.7%
Wong, 2020a 16 138 11.6 [6.8; 18.1] 0.8%
Lombardi, 2020 17 138 12.3 [7.3; 19.0] 0.8%
Kimball, 2020 3 23 13.0 [2.8; 33.6] 0.6%
Tan-Loh, 2021 6 46 13.0 [4.9; 26.3] 0.7%
Eythorsson, 2020 25 178 14.0 [9.3; 20.0] 0.8%
Park, 2020b 4 28 14.3 [4.0; 32.7] 0.6%
Pongpirul, 2020a 1 7 14.3 [0.4; 57.9] 0.4%
Shi, 2020 21 146 14.4 [9.1; 21.1] 0.8%
Martinez-Fierro, 2020 5 34 14.7 [5.0; 31.1] 0.7%
Pavli, 2020a 7 46 15.2 [6.3; 28.9] 0.7%
Rajme-Lopez, 2021 17 111 15.3 [9.2; 23.4] 0.8%
Blain, 2020a 6 38 15.8 [6.0; 31.3] 0.7%
Karout, 2020 12 76 15.8 [8.4; 26.0] 0.8%
Lee, 2020b 80 493 16.2 [13.1; 19.8] 0.8%
Choudhury, 2020 1 6 16.7 [0.4; 64.1] 0.4%
Wang, 2020e 2 12 16.7 [2.1; 48.4] 0.5%
Wu, 2020c 8 48 16.7 [7.5; 30.2] 0.7%
Suh, 2020 10 59 16.9 [8.4; 29.0] 0.7%
Wu, 2020f 7 39 17.9 [7.5; 33.5] 0.7%
Liao, 2020a 2 11 18.2 [2.3; 51.8] 0.5%
Schwierzeck, 2020 2 11 18.2 [2.3; 51.8] 0.5%
Setiawaty, 2020 2 11 18.2 [2.3; 51.8] 0.5%
Qin, 2020 11 60 18.3 [9.5; 30.4] 0.7%
Al-Shamsi, 2020b 6 32 18.8 [7.2; 36.4] 0.7%
Alvarado, 2020 146 736 19.8 [17.0; 22.9] 0.8%
Rivett, 2020 6 30 20.0 [7.7; 38.6] 0.7%
Tan, 2020b 2 10 20.0 [2.5; 55.6] 0.5%
Temel, 2020 16 80 20.0 [11.9; 30.4] 0.8%
Yang, 2020d 2 10 20.0 [2.5; 55.6] 0.5%
Zhang, 2020e 2 10 20.0 [2.5; 55.6] 0.5%
Plucinski, 2020 14 66 21.2 [12.1; 33.0] 0.8%
Xiong, 2020 28 131 21.4 [14.7; 29.4] 0.8%
Li, 2020a 3 14 21.4 [4.7; 50.8] 0.6%
Stessel, 2021 3 14 21.4 [4.7; 50.8] 0.6%
Wang, 2020c 63 279 22.6 [17.8; 27.9] 0.8%
Chan, 2020 1 4 25.0 [0.6; 80.6] 0.3%
Fusco, 2020 1 4 25.0 [0.6; 80.6] 0.3%
Gonfiotti, 2020 1 4 25.0 [0.6; 80.6] 0.3%


Hua, 2020 10 40 25.0 [12.7; 41.2] 0.7%
Ibrahim, 2020b 1 4 25.0 [0.6; 80.6] 0.3%
Jones, 2020 5 20 25.0 [8.7; 49.1] 0.6%
Lamichhane, 2021 2 8 25.0 [3.2; 65.1] 0.5%
Ye, 2020a 1 4 25.0 [0.6; 80.6] 0.3%
Zhang, 2020a 1 4 25.0 [0.6; 80.6] 0.3%
Thiel, 2020 11 43 25.6 [13.5; 41.2] 0.7%
Kruger, 2021 7 26 26.9 [11.6; 47.8] 0.7%
Kenelly, 2020 193 710 27.2 [23.9; 30.6] 0.8%
Kutsuna, 2020 3 11 27.3 [6.0; 61.0] 0.6%
Reale, 2020 3 11 27.3 [6.0; 61.0] 0.6%
Han, 2020 20 72 27.8 [17.9; 39.6] 0.8%
Macartney, 2020 5 18 27.8 [9.7; 53.5] 0.6%
Chehrassan, 2020 2 7 28.6 [3.7; 71.0] 0.5%
Kang, 2020 2 7 28.6 [3.7; 71.0] 0.5%
Kirshblum, 2020 2 7 28.6 [3.7; 71.0] 0.5%
Qian, 2020 2 7 28.6 [3.7; 71.0] 0.5%
Beiting, 2021 50 172 29.1 [22.4; 36.5] 0.8%
Nicolas, 2020 89 297 30.0 [24.8; 35.5] 0.8%
Yau, 2020 3 10 30.0 [6.7; 65.2] 0.6%
Ma, 2020b 11 35 31.4 [16.9; 49.3] 0.7%
Tabata, 2020 33 104 31.7 [22.9; 41.6] 0.8%
Arima, 2020 4 12 33.3 [9.9; 65.1] 0.6%
Dora, 2020 6 18 33.3 [13.3; 59.0] 0.7%
Grijalva, 2020 34 102 33.3 [24.3; 43.4] 0.8%
Huang, 2020a 2 6 33.3 [4.3; 77.7] 0.5%
Jiang, 2020b 4 12 33.3 [9.9; 65.1] 0.6%
Passarelli, 2020a 3 9 33.3 [7.5; 70.1] 0.5%
See, 2020 1 3 33.3 [0.8; 90.6] 0.3%
Zhang, 2020d 4 12 33.3 [9.9; 65.1] 0.6%
Soysal, 2020 70 208 33.7 [27.3; 40.5] 0.8%
Stock, 2020 7 19 36.8 [16.3; 61.6] 0.7%
Corcorann, 2020 3 8 37.5 [8.5; 75.5] 0.5%
Patel, 2020a 13 34 38.2 [22.2; 56.4] 0.7%
Krone, 2021 31 79 39.2 [28.4; 50.9] 0.8%
Du, 2020c 4 10 40.0 [12.2; 73.8] 0.6%
Jiang, 2020d 4 10 40.0 [12.2; 73.8] 0.6%
Yang, 2020a 2 5 40.0 [5.3; 85.3] 0.4%
Li, 2020d 15 37 40.5 [24.8; 57.9] 0.7%
Njuguna, 2020 29 71 40.8 [29.3; 53.2] 0.8%
Pham, 2020 89 217 41.0 [34.4; 47.9] 0.8%
Wadhwa, 2020 8 19 42.1 [20.3; 66.5] 0.7%
Yang, 2020b 33 78 42.3 [31.2; 54.0] 0.8%
Cosma, 2020 6 14 42.9 [17.7; 71.1] 0.6%
Gao, 2020 6 14 42.9 [17.7; 71.1] 0.6%
Almazeedi, 2020 473 1096 43.2 [40.2; 46.1] 0.8%
Harada, 2020 33 73 45.2 [33.5; 57.3] 0.8%
Tsou, 2020 10 22 45.5 [24.4; 67.8] 0.7%
Ladhani, 2020 72 158 45.6 [37.6; 53.7] 0.8%
Chang, 2020 2 4 50.0 [6.8; 93.2] 0.4%
Hoehl, 2020 1 2 50.0 [1.3; 98.7] 0.3%
Jiang, 2020a 3 6 50.0 [11.8; 88.2] 0.5%
Jung, 2020 1 2 50.0 [1.3; 98.7] 0.3%
Olmos, 2021 7 14 50.0 [23.0; 77.0] 0.6%
Roberts, 2021 15 30 50.0 [31.3; 68.7] 0.7%
Thangaraj, 2020 8 16 50.0 [24.7; 75.3] 0.7%
Wong, 2020b 2 4 50.0 [6.8; 93.2] 0.4%
Ren, 2021 1612 3103 51.9 [50.2; 53.7] 0.8%
Marks, 2021 240 449 53.5 [48.7; 58.1] 0.8%
Chau, 2020 7 13 53.8 [25.1; 80.8] 0.6%
Tan, 2021 103 189 54.5 [47.1; 61.7] 0.8%
Gupta, 2021 72 127 56.7 [47.6; 65.5] 0.8%

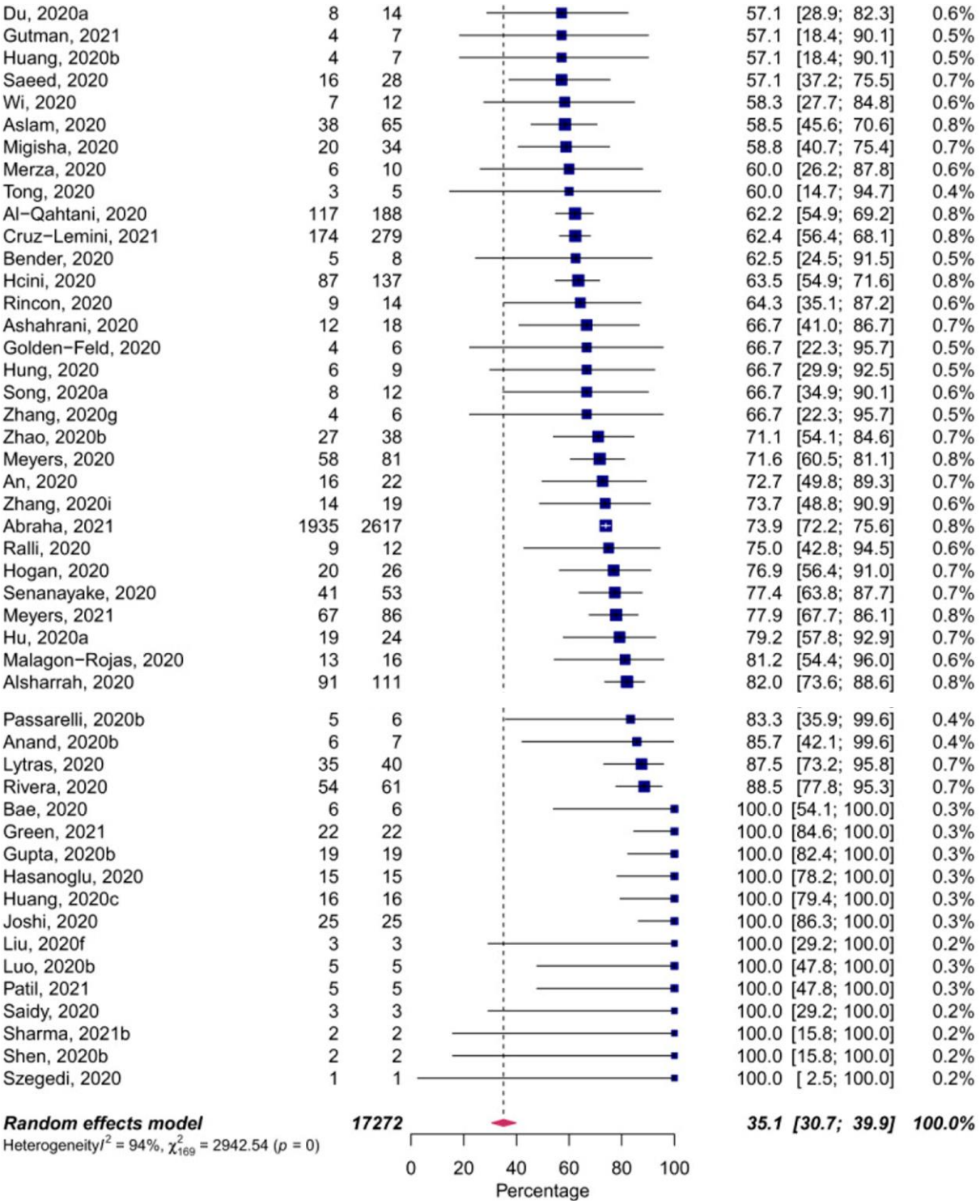


Figure 3. Pooled percentage of laboratory-confirmed COVID-19 cases which remained asymptomatic (34).

Among laboratory-confirmed cases, the percentage of truly asymptomatic cases is 35,1% (95% CI: 30,7-39,9%). Also, due to mislabeling of presymptomatic cases as asymptomatic, a greater percentage of cases showed no symptoms at the time of testing (42,8%; 95% prediction range: 5,2-91,1%). To analyze the extent of mislabeling, a subset of studies reporting symptoms both at the time of testing and at least 7 days later was analyzed. In this study subgroup, 31,8% (95% prediction interval: 5,6-78,7%) of cases that did not show symptoms during testing started to show symptoms. Among these studies, the

percentage of truly asymptomatic cases was close to the 36,9% (95% CI: 31,8% to 42,4%) estimated for all studies reporting asymptomatic infections.

Table 1. Pooled estimates for percentages of all positive cases which remain asymptomatic stratified by age, gender, publication date, symptom follow-up duration, study design, and study setting (34)

	n	Estimate (%)	CI (95%)	p Value
Age Group*				
Children (0-18 years)	18	46,7	32,0-62,0	**<0,01**
Adults (19-59 years)	17	32,1	22,2-43,9	
Elderly (≥60 years)	17	19,7	12,7-29,4	
Study Design				
Population screening	102	38,2	32,0-44,8	0,10
Others	68	30,7	24,8-37,4	
Publication Date				
January–April 2020	27	34,8	23,6-47,9	
May–August 2020	69	29,5	24,2-35,4	0,18
September–December 2020	50	41,1	31,4-51,4	
January–April 2021	24	38,4	25,6-53,1	
Symptom Follow-up Duration				
7-21 days	73	40,6	32,9-48,6	0,07
>21 days	90	32,1	27,0-37,7	
Study Setting*				
Community	39	34,0	25,3-43,8	
Healthcare facility	81	38,5	31,6-45,9	
Household transmission	18	42,5	30,9-54,9	**0,03**
Long-term care facility	15	17,8	9,7-30,3	
Others	17	38,4	23,5-55,9	
Geographic Location				
China	50	33,6	26,1-42,0	
United States	28	33,3	22,6-46,1	0,78
Others	92	36,8	30,4-43,6	

* Stratifications with statistically significant subgroup differences (p<0,05)

According to Table 1, the difference in the percentage of asymptomatics was found to be statistically significant according to age (p<0,01) and the setting in which the study was conducted (p=0,03) (significance threshold was taken as 0,05). In particular, studies of long-term care facilities reported lower asymptomaticity compared to studies of healthcare facilities (p=0,04) and domestic transmission (p=0,04). There was no correlation between the asymptomatic percentage and geographic location, study design, duration of follow-up, or publication date. In addition, gender was unrelated to the asymptomatic face (log incidence rate ratio [IRR]=0,09; 95% CI: −0,07 to 0,25; p=0,27). Cases with comorbidities were found to be less asymptomatic than cases without an underlying medical condition (log IRR=−0,43; 95% CI: −0,82 to −0,04; p=0,03) (34).

Silent infections are laboratory-confirmed cases of COVID-19 that do not show any clinical symptoms at the time of testing, such as upper respiratory tract symptoms, fever, fatigue, pneumonia, myalgia, headache, dehydration, or gastrointestinal dysfunction. Asymptomatic infections are laboratory-confirmed cases of COVID-19 that show no clinical symptoms during at least 7 days of follow-up after testing. Presymptomatic cases are laboratory-confirmed cases of COVID-19 that show clinical symptoms after initial testing. The presymptomatic stage begins with the onset of contagiousness and ends with the onset of symptoms (39).

The logit transformation was applied to the study results to calculate the pooled estimates. A weight was assigned to each study using the inverse variance method, the DerSimonian-Laird estimator was used to evaluate the variance between studies, and the Clopper-Pearson method was used to determine the confidence intervals. Given the heterogeneity in the estimated asymptomatic percentages between studies, a random effects meta-analysis model was used to adjust the test statistics and confidence intervals for the random effect by applying the Hartung and Knapp method. A contour-enriched funnel plot was used for the visual evaluation of small-run effects, and the Egger test was used for statistical evaluation (34).

2.2. SURVIVAL ANALYSIS OF CLINICAL COVID-19 DATA

Advanced statistical methods should be used for the assessment of Intensive Care Unit (ICU) mortality. Incorrect techniques can lead to misinterpretations for clinicians and thus affect treatment. Therefore, it is necessary to have adequate and valid estimates for this important clinical outcome in hospital epidemiology (40).

A. Use of Kaplan Meier Survival Plots

The Kaplan Meier survival plot is one of the best methods used to measure the survival fraction of patients after treatment. In a clinical trial where the effect of a treatment is investigated, the effect is evaluated by detecting cases that survive over a period of time. In this process, some cases can be separated from the study for a reason before the study ends, these separated cases are marked as censored data. This method is also used to compare two drugs or calculate the survival rate of two groups (41).

Using the Kaplan-Meier curve with data from the first wave of the epidemic (February-March), Calabuig and colleagues conducted a study to gain insight into the dynamics of different countries. According to this research, people infected in Spain have a 20% probability of remaining infected after 62 days. According to this curve, a significant increase in the probability of recovery was observed after 10 days (Fig. 4). While the probability of recovery is 4% at the end of the first day, this rate becomes 47% at the end of 10 days. The day the recovery rate was 80% was calculated as 66 in Italy, 62 in France, 29 in China, 32 in South Korea and 26 in Germany (42).

B. Incorrect Use of Kaplan Meier Survival Plots

A fundamental Kaplan Meier assumption for calculating survival curves is that censorship is not informative in the sense that the risk of death remains unchanged when there is a censoring event. Considering discharged patients in such studies as censored leads to artificially reduced survival. The statistical solution for this is to treat discharge as a competing event for death in the ICU, as the cumulative probability of death in the ICU depends not only on the ICU death hazard ratio but also on the rate of discharge (43, 44). Another way to solve this problem is to use landmark models where covariate values are updated at each landmark (45).

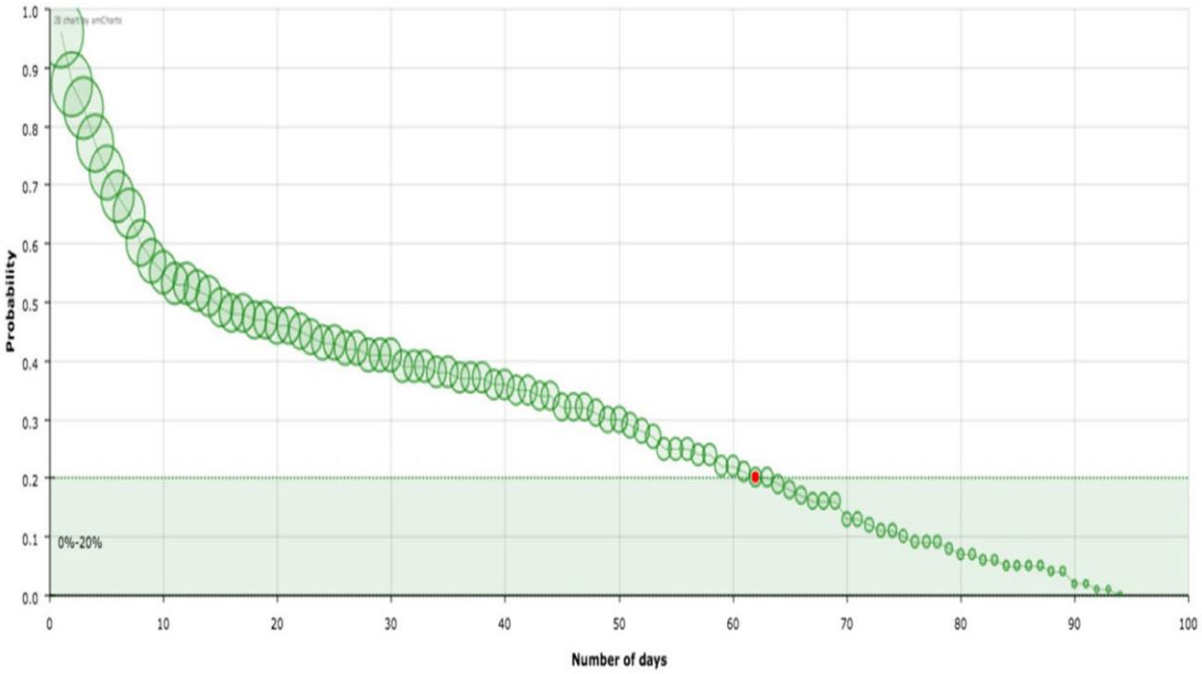


Figure 4. Survival curve (S) of COVID-19 in Spain (42).

Thanks to these results, information can be provided about the efficiency of different health systems (countries) in preventing COVID-19. However, the fact that the data storage and diagnosis standards of each country are not the same prevents making definite comments (Fig.4) (42).

2.3. BENEFITS OF AI APPROACHES FOR COVID-19

Artificial intelligence is the ability of a computer-equipped device to perform various activities in a similar way to intelligent creatures. Artificial intelligence studies often analyze human thinking techniques and focus on developing similar artificial processes (46). Machine learning is the study of computer algorithms that evolve automatically with the use of experience and data. Machine learning algorithms, which are part of artificial intelligence, create a model based on sample data, called training data, to make decisions or obtain predictions. Machine learning algorithms are used in various applications such as medicine, e-mail filtering and computer vision, where it is difficult to develop traditional algorithms to achieve the needed goals. Machine learning is a subfield of computational statistics that focuses on making predictions using computers (47). Image processing is defined as a method for the analysis of pictorial information. Image analysis is to obtain numerical data suitable for statistical analysis from these images (48).

Thanks to this approach, it is possible to detect the case and predict the impact factors of this virus by analyzing all previous data. Health authorities urgently need decision support systems to help them take appropriate decisions in real time to defeat such epidemics and prevent their spread. This technology can be used for appropriate screening, analysis, estimation and monitoring of current patients and potential patients (Fig. 5) (49).

A. Early Detection and Diagnosis of the Pandemic

Due to AI approaches, regular symptoms and other “red flags” can be analyzed quickly and people concerned can be alarmed. It helps to make faster and more cost-effective decisions. Thanks to algorithms, diagnosis and management systems can be developed for pandemics such as COVID-19. AI

can assist in the early diagnosis of infected cases on medical images such as computed tomography (CT) and magnetic resonance imaging (MRI) scanning (49).

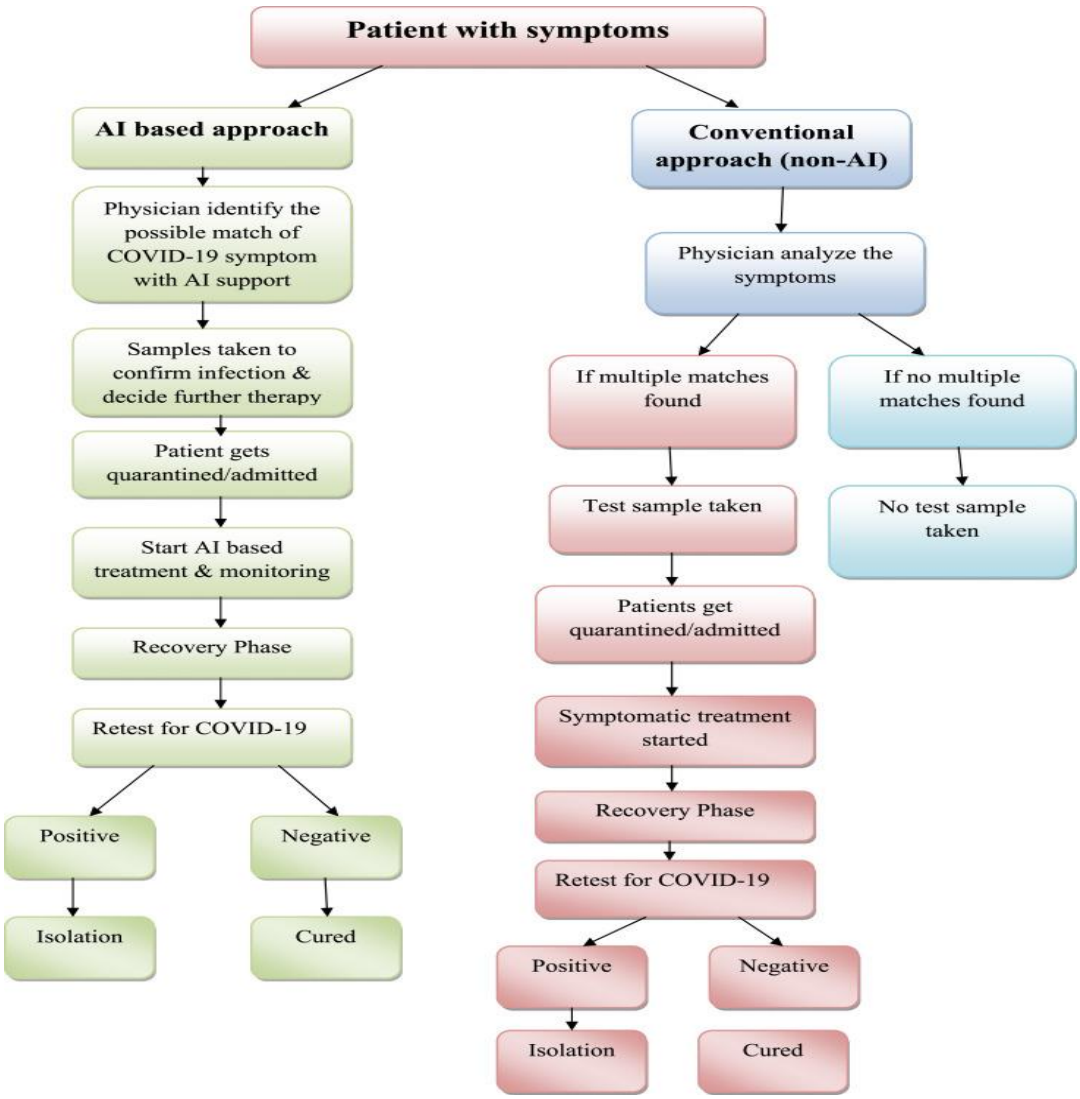


Figure 5. General flow diagram of artificial intelligence (AI) based and conventional approach that help physicians to identify the COVID-19 symptoms (49).

B. Monitoring the Treatment

A smart platform can be created to automatically monitor and predict the spread of the COVID-19 virus with AI methods. A neural network can also be developed to determine the visual features of this disease. With this structure, appropriate monitoring and treatment of patients can be supported (49).

C. Contact Tracing of the Virus

It is possible to analyze the level of spread of this virus by determining the clustering and densities with AI methods and to successfully follow the contact of individuals. The future course of diseases and their possible re-emergence can be predicted (49).

D. Prediction of Cases and Mortality

Via AI methods, the nature of the virus, the risks and potential spread of the infection can be tracked and predictions can be made from available data, social or traditional media. It can make regional estimates for the number of positive cases and deaths. It can help identify the weakest regions, people and countries and take action (49).

E. Development of Vaccines and Drugs

AI can contribute to drug research by analyzing existing data on COVID-19. By identifying priority areas, it can achieve optimum efficiency in drug and vaccine distribution. Because traditional tests take time, artificial intelligence methods can significantly contribute to shorten the duration of the COVID-19 pandemic. It can help identify drugs that may be useful in the treatment of the disease and investigate their effects. It can offer predictive effects and success rates in clinical trials and simulations when developing vaccines and drugs. As a result of these benefits, it can significantly alleviate the workload of healthcare staffs (49).

AI is useful for future virus and disease prevention with COVID-19 data. Our knowledge expands as new data are presented about the very recently emerging COVID-19 pandemic. Thus, our know-how is increasing, which can assist in the analysis of possible future similar pandemics.

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CHAPTER 12

COVID-19 AND INTENSIVE CARE

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INTRODUCTION

At the end of 2019, COVID-19 first broke out in Wuhan, China and quickly spread all over the world, causing a major pandemic. COVID-19 has spread rapidly due to transmission through respiratory droplets. There have been many measures (masks, curfews, quarantines, etc.) implemented all over the world. The whole world has fought against COVID-19, while the guides have been constantly renewed. Treatment protocols have been prepared to take into account publications of the world health organization (WHO), the United States Centers for Disease Control and Prevention, developments and publications in China, publications all over the world and hospitalization in inpatient and outpatient services or intensive care unit hospitalization have been arranged in accordance with the COVID-19 guide developed by the Turkish Ministry of Health (1-3).

The biggest cause of mortality and morbidity of COVID-19 is that it causes acute respiratory failure. Due to the progression of respiratory failure, patients develop acute respiratory distress syndrome (ARDS) where the oxygen needs cannot be met with a nasal cannula, oxygen masks, and oxygen masks with reservoirs, thereby requiring mechanical ventilatory support for patients receiving non-invasive mechanical ventilation or intubation.

As shown by the data obtained at the beginning of the pandemic, the rate of the infected population requiring intensive care was approximately 5-8 %. Hospitalization rates vary with geographical condition and cultural level. Different rates of ICU admission are observed even in different cities of the same country (4-7).

1. CLINICAL FEATURES OF COVID-19 PATIENTS ADMITTED TO INTENSIVE CARE UNIT

- Advanced Age
- Comorbid Diseases (cerebrovascular diseases, diabetes mellitus, chronic lung diseases, chronic liver diseases, malignancies, chronic kidney failure, heart diseases, HIV, neurological diseases, obesity, smoking, immunosuppressive agents, hypertension)(8,9).
- Socioeconomic status and gender (higher in males) (10,11).
- Laboratory Findings: Lymphopenia, elevated lactate dehydrogenase, thrombocytopenia, elevated inflammatory markers (eg, C-reactive protein, ferritin), high D-dimer (>1 mcg/mL) elevated liver enzymes and inflammatory cytokines (eg, tumor necrosis factor-alpha and interleukin 6), high prothrombin time, high troponin, high creatine phosphokinase, acute kidney injury (12-14).
- High Viral Load (15, 16).
- Genetic Factors (17).
- High Fever
- Severe Hypoxia, Hypercapnia
- COVID-19-related Complications

2. COVID-19 COMPLICATIONS

- Acute kidney failure
- Liver failure, gastrointestinal complications
- Delirium, encephalopathy
- Cardiac damage
- Endocrinological complications
- Thrombosis
- Sepsis, septic shock, macrophage activation syndrome (MAS)

- Pneumothorax and barotraumas

3. TREATMENT OF COVID 19 PATIENTS WITH HIGH-FLOW OXYGEN OR NON-INVASIVE MECHANICAL VENTILATION

The choice of non-invasive mechanical ventilation and high flow oxygen in Covid-19 patients is decided according to the patient's comorbidities and the tolerability of the device. Non-invasive mechanical ventilation is mostly preferred in patients with chronic lung diseases, obstructive sleep apnea syndrome, pulmonary edema, and acute hypercapnia. Non-invasive mechanical ventilation or high-flow oxygen is selected according to the patient's comfort and tolerance in the absence of such additional diseases. Non-invasive mechanical ventilation and high flow oxygen therapy can be arranged for the same patient during the day, depending on the patient (36, 37).

High-flow oxygen and non-invasive mechanical ventilation have contributed to decreasing the rate of Level 3 ICU admittance, which has helped reduce mortality in COVID-19 patients. Undoubtedly, this is provided by the use of high-flow oxygen, non-invasive mechanical ventilation and increasing clinical experience (31-35).

Dexamethasone is recommended for patients in addition to high-flow oxygen or non-invasive mechanical ventilation as part of routine COVID-19 treatment. It is recommended to add baricitinib or tocilizumab to the treatment following 24-48 hours of ICU follow-up. Several available studies show that dexamethasone and baricitinib or tocilizumab treatments reduce mortality (18-22).

4. INDICATIONS FOR TRACHEAL INTUBATION AND MECHANICAL VENTILATION IN COVID-19 PATIENTS

- Rapid progression of Covid-19 lung involvement within hours
- Inability to increase saturation above 60% despite receiving
- high-flow oxygen
- Hypercapnia
- Tachypnea (respiratory rate over 30)
- Involvement of accessory respiratory muscles in breathing, abdominal breathing
- No response to non-invasive treatment
- Worsening mental state
- Hemodynamic instability or multi-organ failure
- Severe COVID-19 complications

5. HOW TO APPROACH PATIENTS DURING TREATMENT, INTERVENTION AND INTUBATION

4. Healthcare workers should approach patients with hand hygiene, N-95 mask, visor or protective glasses, protective clothing, and double gloves while providing medical treatment, care, and intervention. A surgical mask should be placed if the patient is receiving high flow oxygen or nebulized therapy. Things to consider in patients scheduled for intubation.
5. Since the risk of transmission of the virus with aerosols is very high when intubating the patient, it is best to choose the person who will do the intubation as the most experienced person in intubation.
6. Rapid sequence intubation techniques should be adopted and the intervention team should be reviewed before the procedure. Rapid sequence intubation techniques help to decrease exposure

time and the spread of the virus through cough droplets or other problems that may prolong the intubation period in cases where the patient is not fully sedated.

7. Before the intubation procedure, any excess material around the patient or in the room should be removed.
8. All drugs and medical equipment required for intubation should be kept ready with labels affixed on the drugs, thereby saving time and decreasing exposure to the virus.
9. It should be ensured that intubation is performed in a negative pressure room with a 3-person intubation team, one of whom is a doctor. An experienced doctor who can perform intubation should wait outside the room in case of difficult intubation and clinician failure. Entry and exit to the room should be restricted.
10. Apart from routine intubation preparations. Hepa filter bag-mask, video laryngoscope, personal disposable aspirator, and intubation tube clamp to prevent air leakage from the intubation tube following intubation should be kept ready.
11. A Hepa filter should be attached to the tip of the intubation tube, the capnograph should be placed, and then the clamp should be opened and intubation should be confirmed. After intubation is confirmed, the patient should be connected to the mechanical ventilator with appropriate mechanical ventilation settings and the intubation tube should be clamped again and connected to the mechanical ventilator circuit with the Hepa filter, then the clamp should be removed, thereby ensuring mechanical ventilation. (38-41).

6. PHARMACOTHERAPY FOR INTUBATED COVID-19 PATIENTS

1. Sedation
2. Analgesia
3. Neuromuscular blockade (if necessary)
4. Stress ulcer prophylaxis
5. Thromboembolism prophylaxis
6. Medical treatment arranged depending on glucose level
7. Empirical antibiotic therapy
8. COVID-19-specific therapies (e.g, Dexamethasone, high-dose steroid therapy, convalescent plasma therapy donated from recovered COVID-19 patients, remdesivir and interleukin-6 inhibitors (tocilizumab), baricitinib therapy, and prospective therapies to be developed) (18-22).
9. Prone (supine) position (24).
10. Pulmonary vasodilators (23).
11. Vasodilator, vasoconstrictor agents
12. Antipyretic treatment
13. Nebulized drugs (should be used with caution)
14. Providing fluid-electrolyte balance
15. Nutrition solutions (25,26).
16. Medical treatment arranged depending on patients' vital signs
17. Physical therapy (respiratory therapy is applied in conscious
18. intensive care patients while routine physical therapy
19. protocols are applied in unconscious patients)
20. Extracorporeal Membrane Oxygenation (ECMO) (27-30).

7. SPECIAL COVID-19 TREATMENT

Hydroxychloroquine sulfate: A dose of 200 mg was applied as 2x1 for 5 days when Covid-19 first broke out. However, it was later found to be ineffective and was removed from the treatment protocol (3).

Favipiravir: It acts on the virus by inhibiting RNA polymerase, thus stopping gene proliferation and preventing the virus from multiplying in the infected cell. During the first outbreak of COVID-19, a loading dose of 2x8 tablets was given on the first day followed by 2x3 tablets continued for 4 days, with a total of 5 days of treatment. Some selected patients whose symptoms worsened afterward underwent a second 5-day treatment. Currently, it is excluded from COVID-19 treatment (3).

Remdesivir: Remdesivir is a novel antiviral drug belonging to the class of nucleotide analogs. Selected patients receive a loading dose of 200 mg on the first day followed by 100 mg once daily for 5-10 days.

Molnupiravir: It is started in PCR-confirmed patients who are at high risk for progressing to severe COVID-19 within 5 days from the onset of symptoms. A daily dose of 2x800 is applied for 5 days (3).

Corticosteroid: If oxygen therapy and mechanical ventilation are required, 6 mg of dexamethasone daily or its equivalent corticosteroid (0.5-1 mg/ kg methylprednisolone, prednisolone, or prednisone) is recommended for a maximum of 10 days. High-dose glucocorticoids (pulse, ≥250 mg/day methylprednisolone) can be administered to patients who require oxygen within 24 hours despite the treatment. High-dose glucocorticoids can be used for up to 3 days. Following the administration of high-dose steroids, treatment should be continued with 6 mg/day dexamethasone or 0.5-1 mg/kg prednisolone or equivalent methylprednisolone. The use of anti-cytokine drugs should be considered in cases with ongoing inflammation or severe clinical MAS findings that progress very rapidly with no response to glucocorticoid therapy for at least 3 days. Administration of corticosteroids is thought to be ineffective within the first 5-7 days (viral) of the disease (3).

Tocilizumab: It is a monoclonal antibody that blocks interleukin-6 (IL-6) and used in COVID-19 patients who develop macrophage activation syndrome (MAS) as well as those who do not respond to corticosteroid therapy. A maximum of 800 mg can be given at a dose of 8 mg/kg. A single dose of 400 mg-800 mg can be given at a time depending on the patient. Following the administration of 400 mg, a repeat dose of 200-400 mg can be given depending on the 24-hour follow-up. After 800 mg is given, care should be taken about re-administering the drug for the second time, and hematology and rheumatology opinion should be obtained. (3).

Anakinra: It is a recombinant intercoquin-1 receptor antogonist. It can be applied at a dose of 2-10 mg/kg through the preferred route of administration (subcutaneous or intravenous) in patients with MAS findings. Depending on the severity of the patient's clinical findings, the dosage can be adjusted from 100 mg subcutaneous injection once or twice a day up to 200 mg intravenous administration 3 times a day in the presence of severe symptoms. The dose can be increased up to 4 x 200 mg in resistant patients. Intradermal administration is recommended in patients with macrophage activation syndrome.(3).

Janus Kinase (JAK) Inhibitors: Ruxolitinib can be used in cases where an anti-cytokine therapy with tofacitinib and baricitinib falls insufficient. The recommended dose of baricitinib is 4 mg once daily for 14 days (3).

Intravenous Immunoglobulin (IVIG) Therapy: It can be applied in cases where anti-cytokine treatment falls insufficient. Although a daily dose of 20 g is recommended for 5 days, it can be administered for 2 days (1 g/kg/day) or 5 days (0.4 g/kg/day) with a total dose of 2 g/kg depending on the patient (3).

COVID-19 Immune Plasma Therapy: Immune plasma therapy exhibits beneficial effects within the indications (over 60 years of age, within a maximum of 7 days from the onset of symptoms, patients under 60 years of age with severe comorbidities, no pneumonia, no intensive care requirement), where

it is performed immediately after diagnosis within 7 days following the onset of symptoms before the requirement of intubation. It is not recommended for patients with advanced pneumonia, increased oxygen need with saturation below 90 under 5 L/min oxygen therapy, patients with MAS, intubated patients, and patients who do not respond to advanced COVID-19 treatment. The IgA level is assessed for suitability (42).

8. EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) AND COVID-19 INTENSIVE CARE

Given that appropriate conditions are provided, ECMO can be applied in case of COVID-19 induced cardiogenic shock in intubated COVID-19 patients followed in the ICU whose oxygen saturation does not increase despite appropriate pharmacological treatment and mechanical ventilation.

Extracorporeal membrane oxygenation (ECMO): It is a life support device that is used to correct a life-threatening cardiac and respiratory problem. There are 2 types, venoarterial and venovenous, while both types provide respiratory support, only the venoarterial can provide cardiac support. The ECMO device replaces the heart and lungs, providing an opportunity for these organs to rest and heal.

Things to consider when choosing a patient who will undergo ECMO from COVID-19 patients:

- Proper use of resources
- ECMO is not preferred in advanced age as elderly COVID-19 patients exhibit poor prognoses.
- ECMO is not preferred in patients with comorbidities.
- ECMO can be rarely used in patients with multiorgan failure.
- ECMO is mostly preferred in young patients whose comorbid condition does not yield an effect on prognosis (43, 44).

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CHAPTER 13

REHABILITATION OF COVID-19 SURVIVORS

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INTRODUCTION

Coronavirus 2019 (COVID-19) infection is a very contagious global pandemic (1) that causes systemic dysfunction (2). Studies have shown that COVID-19 is characterized primarily by lung involvement as well as injury to the heart, immune system, brain and nerves, motor function and mobility, and other organs and systems (3-5). The major manifestation of the disease is interstitial pneumonia (6,7). At the same time, not only respiratory muscle dysfunction, but also peripheral muscle dysfunction, heart failure, deconditioning, fatigue, and psychosocial problems develop in COVID-19 patients, especially who needed intensive care unit (ICU) care (8).

Despite outstanding efforts in vaccination, it remains a threat, especially for some risk groups such as advanced age, sex, elevated body mass, and having co-morbidities, for severe infections requiring hospitalization (9-14). Hypertension, cardiovascular disease, chronic respiratory disease, and diabetes were the most common co-morbidities documented (15-17).

The clinical spectrum of the disease is very wide, ranging from asymptomatic/mild symptoms to acute respiratory distress syndrome (ARDS) and multi-organ failure, and death (1, 18, 19). Patients with co-morbidities are more prone to complications (20, 21). A wide spectrum of the clinical symptoms is classified into 4 groups; 1) asymptomatic patients, 2) patients with mild symptoms, 3) patients followed in the hospital, and 4) symptomatic patients requiring ventilatory support in ICU (1, 22, 23).

The upper respiratory tract infection is presented generally by fever, dry cough, sore throat, congestion, shortness of breath, fatigue, headache, myalgia, and anosmia. The clinical picture may evolve to a hyper-immune phase (24-28) and progression to pneumonia may result in an oxygen de-saturation and lymphopenia (29, 30) which is the probable cause of multiple organ dysfunction and lingering long-term symptoms (29-31). The early data revealed that up to 12% of cases needed ICU care (32). Post-intensive care syndrome (PICS), a deterioration in condition, appears when the patient is followed in the ICU receiving long-term non-invasive mechanical ventilation and persists after discharge (33-35). In addition to systemic dysfunction, prolonged immobilization results in muscle deconditioning, joint stiffness, and pain, leading to general condition impairment (36-38). Furthermore, the isolation and consequent lack of stimuli to prevent the spread of the virus can cause cognitive decline and some psychological problems ranging from depression to anxiety in susceptible individuals (39). Many patients with COVID-19 followed in the ICU show similar symptoms to other patients treated in the ICU with various diseases, such as dyspnea, anxiety, prolonged pain, depression, and physical function impairment, resulting in poor quality of life (QoL) (40-42). To manage the physical, cognitive, and psychological problems that arise, patients should be closely and carefully monitored and supported throughout their rehabilitation processes (43).

COVID-19 causes the most serious morbidity in 3-6 months, but it is observed that some symptoms can be prolonged for 12 months and so (1). In previous epidemics, as in the epidemic that caused severe acute respiratory syndrome (SARS) in 2003, one-third of the patients had persisting symptoms such as chronic dyspnea, headache, anxiety, depression, and myalgia (1, 9) and pulmonary fibrotic changes were shown radiographically 1 year after the initial infection (44). Similarly, several studies have documented prolonged symptoms in patients with COVID-19, the most common symptoms were fatigue and dyspnea (45, 46), followed by perceived cognitive difficulties, sleep disorders, and headache (1, 46). Headache is the most common neurological symptom (1), but others include difficulty in concentrating, decreased attention, memory loss, and delirium (47-51). The prolonged symptoms are also observed in outpatients. In the study conducted by Vanichkachorn et al.(9) 75% of the patients whose complaints continued approximately 3 months after the disease was not hospitalized due to COVİD-19. The most common complaint presented was fatigue, followed by respiratory and neurological symptoms. Patients also suffered from cognitive impairment, mental health symptoms, and sleep disturbance. One-third of patients struggled in basic activities of daily living (9). In a longitudinal prospective cohort study (52), follow-up of patients with laboratory-confirmed COVID-19 demonstrated that 30% of them had 1 or

more symptoms that persisted for 9 months or more; such as generalized fatigue, anosmia, and brain fog (52).

The pandemic has put a sudden strain on healthcare professionals, especially in acute care departments (36, 37). In the management of COVID-19 patients, establishing multidisciplinary and professional rehabilitation strategies is very important, focusing on respiratory and neuromotor functions, and ensuring optimal recovery of these patients, to reduce the length of stay and prevent long-term sequelae as much as possible (19, 53). In this context, PICS patients need prolonged hospitalization and a careful multidisciplinary approach to optimize their functional capabilities and reduce long-term impairment (54). As COVID-19 has a systemic involvement and clinical presentation and pre-existing co-morbid conditions may differ. Therefore, customization of the rehabilitation intervention for the specific situation of the patients is essential (19). For this reason, a patient-specific respiratory and neuromotor rehabilitation plan should be made, taking into account the persistent respiratory problems of the patients admitted and the consequent serious disabilities as a result (55). Furthermore, it seems that the rehabilitation need of about half of the patients will continue after discharge, and predicting 4% will require an in-patient rehabilitation program (56). Therefore, clear planning is needed not only in the acute and post-acute phases of patients recovering from COVID-19, but also in the chronic phase (1). In this context, efforts are made to create rehabilitation guidelines for patients' impairments in the acute, post-acute and chronic periods (1, 9).

GENERAL OVERVIEW OF REHABILITATION

Rehabilitation focusing on respiratory, as well as motor functions plays a very important role in the management of COVID-19 patients, and therefore it is very important to establish rehabilitation strategies that will ensure the recovery of these patients as much as possible (19). Adequate care should be provided to improve patients' health, speed discharge, and avoid readmission, as well as optimize return to work for patients recovering from COVID-19 (31). The current literature mostly covers the acute and subacute periods and consists of case reports and case series (57, 58). Randomized controlled studies on the impact of post-COVID-19 rehabilitation have begun to appear (59, 60), in addition to some guidelines recommended by healthcare professionals based on their experience and expert opinion (1, 61, 62).

COVID-19 infection, causing a multisystem disease, requires a multidisciplinary approach for some cases. Rehabilitation is to be provided from admission to discharge (63), and so after discharge (1). Depending on the severity of the infection, symptomatic and supportive treatment is crucial in the acute phase. Early rehabilitation should be provided for patients hospitalized for COVID-19 infection in the recovery phase after severe respiratory failure or prolonged hospital stay, as well as inpatient/outpatient/home rehabilitation for people with chronic/progressive disabilities who experience functional decline after COVID-19 infection (15). Rehabilitation should begin as soon as possible, even while the patient is in the intensive care unit (ICU), where early mobilization has shown to be practical and safe (64). Progressive rehabilitation programs are recommended to be started within the first 30 days to obtain the best effect on recovery, by The National Institute for Health and Care Excellence (NICE) (63).

For a successful rehabilitation program, it is very important to evaluate the patients before and during rehabilitation, establish a personalized plan, inform the patients about the disease, its sequelae, and the rehabilitation program, and ensure cooperation (43). Evaluation of range of motion, strength, and balance should be performed (43, 65). A 6-minute walk test and a cardiopulmonary exercise test can be used to assess exercise capacity, with continuous oxygen saturation monitoring. Function and disability can be evaluated by the International Physical Activity Questionnaire and activities of daily living (ADLs) by the Barthel Index (66). Rehabilitation should include many methods such as bed positioning, pulmonary techniques, exercise training and mobilization, speech and swallowing therapy, physical

modalities, and occupational therapy, by establishing the treatment at the right time with appropriate methods planned for the patient (1, 3, 67, 68).

1. ACUTE REHABILITATION

1.1.Non-Hospitalized patients with mild COVID-19: Considering the needs of the patients, it is recommended to start an appropriate exercise program to counteract the negative consequences of chronic diseases, reduce the risk of sarcopenia and dementia, and prevent the psychological effects of quarantine (19). These patients are recommended a rehabilitation program that includes moderate-intensity aerobic, resistance, balance, coordination, and movement training exercises 5-7 days a week (15, 69).

1.2.Hospitalized patients with mild/moderate COVID-19: Neuromuscular rehabilitation and pulmonary training are recommended (3, 19, 43). Thus, it is aimed for patients to maintain their full capacity when they are discharged (3). Psychological support had a positive effect on maintaining mental function (43).

1.3.Hospitalized severe stable patients with COVID-19: As soon as the patient is stabilized an early global rehabilitation should be started delivered by a multidisciplinary team (MDT) (19). Neuromuscular and pulmonary interventions including passive and active mobilization, positioning and breathing techniques, swallowing exercises, and psychological support should be provided in the rehabilitation context, as is needed. For patients who are sedated or uncooperative due to cognitive or physical impairment, passive mobilization should be applied through either passive mobilization techniques or passive motion devices and neuromuscular electrical stimulation (35, 43, 70).

1.4.Hospitalized severe unstable COVID-19 patients: Respiratory rehabilitation is not recommended to prevent exacerbation of respiratory distress or unnecessary spread of the virus (43, 66, 71). Early rehabilitation does not seem to be well tolerated as it causes rapid de-saturation. Diaphragmatic breathing, pursed breathing, positive expiratory pressure technique, incentive spirometry, bronchial hygiene, manual mobilization of the thorax, pulmonary muscle training, and aerobic exercise are not recommended in the acute phase (38, 71). If there is increased secretion, postural drainage may be considered cautiously (65)(72). The extubation and weaning phase and transfer to a rehabilitation service for these patients should be gradual and closely monitored. The patients are recommended to be transferred from ICU when they are weaned from the sedative and antipsychotic drugs and bedside active mobilization be performed without a reduction of oxygen saturation (SpO2) below 90% (39).

2. POSTACUTE REHABILITATION

The need for medical treatment generally continues after patients are discharged. Patients' desired medical outcome is not only obtaining a negative test and controlling the pulmonary inflammation but functional recovery and return to society and work (3).

2.1.Mild COVID-19 patients after discharge: Aerobic exercise is recommended to restore the patient's motor skills gradually, and psychological support to encourage social reintegration (19, 43).

2.2.Severe COVID-19 patients after discharge: Direct lung damage and as well as injuries to other organs and systems, and also the co-morbidities should all be considered when establishing a rehabilitation plan for patients recovering from COVID-19 (38, 66). Additional problems may occur in patients followed in the intensive care unit for a long time or during mechanical ventilation. Patients with complications such as posterior reversible encephalopathy syndrome, plantar flexion contractures, pressure ulcers, critical illness myopathy/neuropathy, bladder dysfunction, and deep vein thrombosis also require appropriate rehabilitation (3, 15). An integrated and customized program should be

established including neuromuscular, respiratory, cardiac, swallowing rehabilitation, and strength and balance training (19). A 30-minute motor and respiratory rehabilitation program twice a day is shown to be beneficial for post-acute COVID-19 patients (39), similar to findings reported by Liu et al. (59). Aerobic exercise should be started at low intensity and increased gradually. Patients who get tired quickly are recommended to do intermittent exercise (19, 43, 73). In addition, support should be provided for the psychological rehabilitation of patients (3).

3. LONG-TERM REHABILITATION

Long-term rehabilitation is required to ensure therapeutic continuity for COVID-19 patients with inadequate functional recovery and who have difficulty returning to the work. Up to 50% of hospitalized COVID-19 cases are proposed ongoing care in the UK, looking out for improvement of the long-term outcome goals (56). According to the accumulated data so far, some guidelines have been recommended to highlight the multidisciplinary rehabilitation requirements of post-COVID-19 illness (1, 74). These guidelines underline the rehabilitation needs on an individual basis, for pulmonary, cardiac, sport and exercise medicine, psychological, musculoskeletal, neuro-rehabilitation, and general medicine (1, 75, 76). For an individualized rehabilitation program, patients' co-morbidities that may interfere with the patients' participation and the final goal of the program, should also be taken into consideration (1, 77).

The recently published consensus (1) provides an evidence-based, comprehensive set of recommendations, and possible requirements for post-COVID-19 rehabilitation, and guiding clinical practice. As emphasized in the consensuses; rehabilitation should be planned according to the individual needs of the patients and their co-morbidities should be taken into account. Through the rehabilitation process, patients should continue to be reviewed. The goals of rehabilitation for COVID-19 patients can be listed as relieving dyspnea symptoms and psychological distress; improving physical function, participation in rehabilitation, and QoL; and ensuring long-term compliance with health-promoting behaviors (1, 17) (8). In long-term rehabilitation, ensuring individualized self-management strategies and telerehabilitation can make a significant contribution (19, 78, 79).

4. PULMONARY REHABILITATION

Pulmonary rehabilitation (PR) is currently required and applied in various lung diseases (in pneumonia, interstitial lung disease (ILD), and SARS) and acute or chronic processes of immobility, surgery, systemic, neurological, musculoskeletal system diseases affecting respiration in both the elderly and young population (8, 17, 80-82). Publications on PR used in COVID-19 are also accumulating (58, 59).

Data from the previous outbreaks of CoV SARS suggests that up to one-third of survivors were reported demonstrating abnormal chest radiograph findings, persistent pulmonary function impairment, and persistent reductions in exercise capacity, musculoskeletal performance, as well as the QoL (83-87), for which the picture was similar to the 2009 H1N1 influenza epidemic (88). Additionally, during the acute phase of the SARS disease, oxygen requirement and lymphopenia were associated with radiologic abnormalities (89) and pulmonary function test (PFT) changes (90). In the light of these experiences, patients with COVID-19 with a similar clinical picture should be evaluated radiologically and pulmonary function tests should be performed (1). The lung injury of COVID-19 causes impaired alveolar air exchange and reduced pulmonary ventilation function, resulting in symptoms such as shortness of breath and chest tightness (3). Computed tomography (CT) findings documented are bilateral ground-glass opacities and patchy consolidations (20, 91, 92). Pulmonary fibrosis is associated with prolonged complaints affecting lung function (3). Respiratory complications may occur not only due to impaired function of the respiratory but involvement of other systems and existing comorbidities (1).

PR is crucial to improving respiratory complaints, preserving function, and reducing complications and disability. It also helps to reduce anxiety and depression triggered by shortness of breath (19). PR plan should be made according to the severity of the disease and the needs of the patients in the acute, subacute and chronic phases. According to the clinical classification of the World Health Organization, COVID-19 disease is classified as a mild illness, pneumonia, severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, and septic shock (17). PR is not recommended for patients in the COVID-19 ARDS period or those with progressive exacerbations (17, 43). An initial assessment is recommended when is safe, taking into account the patient's medical, physical and mental condition, and respiratory capacity (1). PR is initiated when the patient is stabilized (fever drop, relieving dyspnea, respiratory rate <30 breaths/minute, SpO2 > 93%) with close monitoring of symptoms, heart rate, oxygen saturation, and blood pressure, especially in patients who require oxygen therapy (17), and should consist of low-intensity exercise (≤3 METs or equivalent) initially(1). Exercise training (ET) forms the basis of almost all international PR programs (93, 94). ET is formed based on the general principles of exercise physiology and is prepared by determining individual thresholds and workloads by exercise testing (8). The intensity of exercise should be increased gradually, with close monitoring of the patient so as not to overload the respiratory system and cause distress (1, 65, 95, 96). Inspiratory muscle training should be included in the post-acute phase of the inspiratory muscles being weak. Depending on the patient's needs, deep-slow breathing, diaphragm breathing, chest expansion with shoulder elevation, respiratory muscle mobilization, positive expiratory pressure devices, and airway clearance techniques can be added (43, 72).

Pulmonary sequelae and prolonged symptoms may require the continuation of pulmonary rehabilitation in patients who recover. It is seen that most of the patients still present with shortness of breath 3 weeks after discharge from the hospital (97). PaO2/FiO2 ratio and body mass index at the time of admission to the emergency department is the strongest independent predictors of persistent respiratory impairment and the need for follow-up in these patients (57). Not only the acute and subacute phase, but the chronic phase results of PR reveal better respiratory outcomes and QoL (59).

5. CARDIAC REHABILITATION

Cardiac complications, especially arrhythmias and myocardial damage, are seen in patients after COVID-19, which may be caused by many factors such as viral myocardial damage, hypoxia, high systemic pro-inflammatory mediators, ACE2-receptor down-regulation, hypotension, or drug toxicity (20, 98, 99). Acute cardiac injury is more likely to be in patients with severe diseases who require mechanical ventilation, have other complications, and have co-morbidities such as cardiovascular diseases, hypertension, diabetes mellitus, and cerebrovascular diseases, resulting in higher mortality rates (98-100). Troponin I elevation, arrhythmia, decreased ejection fraction, heart failure, and severe myocarditis are the most common presentations (99, 101, 102). The presence of cardiac injury and other comorbidities should be considered in the rehabilitation plan (66).

Regardless of the severity, each patient with COVID-19 infection requires evaluation of their symptoms and potential impairment of the cardiovascular system. After the first evaluation, the patient should be re-evaluated by a specialist and further investigations should be performed if necessary (1). An individualized cardiac rehabilitation (CR) program should be provided according to cardiac complications, disorders, and other rehabilitation needs (1).

According to the international guidelines, CR is already recommended for individuals with specific heart diseases such as acute coronary syndrome, coronary revascularization, and heart failure (103, 104). The increasing data reveals that CR improves not only the exercise capacity, but the QoL, and psychological well-being, as well as reduces mortality, morbidity, and hospitalizations (103-105). COVID-19 infection is likely to increase the CR requirement due to both exacerbations of existing cardiovascular disease and new cardiac sequelae (1). Adaptation of traditional CR in the rehabilitation

of these patients (1) will allow more specific CR protocols to be developed in the light of clinical experience.

A rest period, determined according to the severity and duration of symptoms and complications, reduces the risk of heart failure secondary to myocarditis after infection (106). A complete rest period of 3-6 months is required for young and active patients after myocarditis, and for patients returning to sports or professions that require high activity (106). The severity of disease, left ventricular functional capacity, and the extent of inflammation shown on cardiovascular magnetic resonance imaging, play a key role in determining resting time (1). The patients can return to active life when left ventricular function and serum biomarkers of myocardial injury return to normal, and there are no relevant arrhythmias on 24-hour ECG monitoring and exercise testing, (106). But still, they should be re-evaluated periodically, especially during the first 2 years (1).

6. EXERCISE TRAINING RECOMMENDATIONS

Moderate activity helps a healthy immunological response to infection, while reduced activity impairs the immune response to microbial agents (107-109), and increases insulin resistance (109). Exercise may have an important role in influencing the immune response; however, it should be kept in mind that physical activity is not recommended for the treatment of COVID-19 (1) and caution should be taken when prescribing exercise intensity, as a prolonged and strenuous activity may exacerbate symptoms (108).

Given the rapid spread of COVID-19, rest and isolation periods may need to be extended in certain groups (1). It is recommended to start with a muscle strengthening program before cardiovascular work on return to exercise after COVID-19. Patients recovered from mild/moderate COVID-19 disease are initially recommended a low-level stretching and strengthening program for 1 week, then move on to targeted cardiovascular sessions (1). Severe COVID-19 patients requiring oxygen therapy or with acutely manifested lymphopenia should be screened for radiological pulmonary findings and pulmonary function test impairments and enrolled in a pulmonary rehabilitation program. Exercise progression should be performed following the pulmonary rehabilitation approach (1).

COVID-19 patients with severe symptoms such as fatigue, severe sore throat, cough, fever, dyspnea, chest pain, and body aches, should avoid exercise >3 METs or equivalent for 2-3 weeks after these symptoms have resolved (108). A more conservative approach to COVID-19 is recommended (1,110), assuming a 2-3 week period is required to allow time for adequate cytotoxic T-cell response to occur (108).

The activity of the patients with mild symptoms is recommended to be limited to mild activity (≤3 METs or equivalent). The rest periods are limited but are to be increased if the symptoms worsen. Prolonged extensive or high-intensity exercise should be avoided as it may worsen symptoms (108).

7. NEUROLOGICAL REHABILITATION

Symptoms due to both central and peripheral nervous system involvement in COVID-19 patients can be listed as dizziness, headache, impaired consciousness, ataxia, seizures, nerve pain, paresthesia, and taste, smell, and visual disturbances (111-114). Posterior reversible encephalopathy syndrome (headache, confusion, seizure, and vision loss) may present as a potential complication of COVID-19 (115). Cases of viral encephalitis and infectious toxic encephalopathy (116), acute necrotizing encephalopathy (117), and acute transverse myelitis (118) have also been reported. Pathological findings revealed brain tissue edema and partial neuronal degeneration in patients who died (116, 119). While there is no evidence that COVID-19 increases cerebrovascular disease, patients with cerebrovascular disease have a more severe course of COVID-19, an inflammatory response is increased and

hypercoagulability is reported (120, 121). Those have a worse prognosis, with an over nine-fold increase in mortality and a higher probability of requiring rehabilitation (120). Cognitive impairment may be seen as a part of PICS with major risk factors such as sepsis, ARDS, increased age, presence of previous cognitive impairment, and delirium (122, 123), which persist for up to 1 year (1).

It is recommended that all patients with COVID-19 be searched for any neurological symptoms. A cognitive screen should be considered for those under post-critical care or who have cognitive impairment (1). Conversely, since mild neurological signs may begin before the onset of COVID-19 (49), evaluation of patients presenting with neurological symptoms for COVID-19 is important for early diagnosis, management of the disease, and its complications (99)(49)(116).

A neurological rehabilitation plan should be made considering all findings and comorbidities of the patients. Providing education that milder neurological symptoms like headache, dizziness, sensory changes, loss of smell or taste are likely to improve and recover fully with minimal intervention, will help patients relax (1).

Severe symptoms can potentially cause significant lifestyle changes or serious deterioration in the QoL. Therefore, for patients with moderate to severe neurological symptoms inpatient multidisciplinary rehabilitation is recommended to maximize recovery (1).

An adequate assessment and management of oropharyngeal dysphagia (speech-language treatment) is crucial for patients with COVID-19 in the intensive care unit and is found to be effective in improving swallowing (124).

Since some neurological findings tend to be prolonged, physical, cognitive, and functional evaluation should be performed and support should be provided to facilitate return to work according to occupational setting (1).

8. MUSCULOSKELETAL REHABILITATION

It is well known that patients hospitalized in intensive care units are prone to weakness and neuromuscular disorders due to prolonged mechanical ventilation and immobilization that is not directly associated with primary disease processes (123, 125, 126). The disorders include critical illness-related polyneuropathy, myopathy, and neuromyopathy (123, 125, 126).

Patients who have received respiratory care and survived ARDS experience impairments that affect muscle strength and physical activity (123). Muscle atrophy and associated weakness begin within the first days of admission to the ICU and are associated with prolonged ICU stay, multiple organ failure, and sepsis (18, 125, 127). Other musculoskeletal complications include joint stiffness/contractures, heterotopic ossification, prolonged pain, adhesive capsulitis, decubitus ulcers, brachial plexus injuries, and entrapment neuropathies (peroneal and ulnar) leading to impaired functioning concerning mobility, activities of daily life and work (38, 65, 76, 128, 129). Depending on the dose, patients receiving steroid therapy may develop osteonecrosis (130). The osteonecrosis risk is less likely as WHO recommends a careful approach to steroid use for COVID-19 (131).

A functional assessment should be performed to identify the most vulnerable patients and residual musculoskeletal disorders to measure the functional status and independence of COVID-19 patients in the ICU before discharge (1, 39). These patients also have increasing nociceptive, neuropathic, and nociplastic pain (40, 132, 133). Therefore, instead of focusing on musculoskeletal disorders in isolation, there is a need for an MDT evaluation for the management of such a patient's rehabilitation, including pain (1).

Rehabilitation of patients with complex impairments should include physical, psychological, and cognitive rehabilitation (1, 40-42). Exercise-based interventions such as muscle stretching,

strengthening, and range of motion are helpful to prevent post-ICU-related weakness, contractures, and pressure sores. Post-COVID-19 physical rehabilitation can be provided in an inpatient, outpatient, or telerehabilitation setting, depending on the severity of the disease and the needs of the patient (1). Outpatient programs may vary, but they usually last 6-12 weeks after discharge and can be combined with cognitive rehabilitation (40, 41). Pain management should be patient-centered including non-pharmacological and pharmacological interventions (1). Vocational intensive inpatient rehabilitation, determined by addressing occupational goal setting, motivation, and psychology, has been shown to lead to better functional improvements compared to traditional rehabilitation models (134, 135).

9. POST-COVID SYNDROME

Most patients with COVID-19 were not hospitalized. Additionally, due to the high number of COVID-19 cases, patients were promptly discharged as soon as basic functional needs could be met (79). Only patients with severe physical defects were referred to specialist inpatient rehabilitation before discharge. Some of the discharged patients had difficulty returning to work, and their return to their previous occupations is sometimes delayed for months. As the data increased, it is noted that some of the patients who survived Covid-19 had prolonged symptoms, even if the COVID-19 polymerase chain reaction (PCR) testing became negative, which is called Post-Covid Syndrome (PCS) (Long Haul Syndrome) (9).

Similar to other postviral syndromes, PCS appears to be more common in female patients, although the outcome of acute Covid-19 infection is worse in the male sex (9, 13). These patients have lingering symptoms, such as fatigue, dyspnea, joint pain, chest pain, headache, muscle weakness, neurological symptoms, subjective mood disorders, interrupted sleep, and cognitive impairment (45, 136), who still need treatment, which is mostly related to rehabilitation (3, 31). Among these patients, the rate of difficulty in performing functional ADLs is 34%, while the rate of problems with housework, exercise, driving, and completing necessary tasks at work is 84% (9), and are suffering from severe disability (137).

Diseases that cannot be cured completely lead to dysfunction. Since functional recovery is an important indicator of medical efficacy and health, in 2016 WHO recommended the addition of a third clinical outcome indicator "function", in addition to the two clinical outcome indicators, recovery, and death (3). The importance of rehabilitation after COVID-19 has been emphasized according to the framework of the International Classification of Functioning, Disability, and Health (66,138). As long-term dysfunction has a high impact on QoL, self-care activities, and return to work, rehabilitation should be provided to patients in need after COVID-19 (3,137,139). In this respect, all patients should be evaluated for rehabilitation and multidisciplinary rehabilitation should be offered to patients according to identified needs such as dysphagia, cognitive difficulties, psychological problems, balance, or other neuromuscular sequelae (19, 79, 139).

To help these patients; The COVID-19 Activity Rehabilitation Program (CARP) teams began to be established, taking advantage of both the available data on the recovery of COVID-19 patients and the recommendations focused on treatment, prevention, diagnosis, and rehabilitation used for SARS/Middle East respiratory syndrome (MERS) and chronic fatigue syndrome (9). These programs are made available to patients to be performed face-to-face and/or by telerehabilitation or telephone calls depending on the symptoms and severity of the clinical picture. Tele-rehabilitation services may help prevent a gap in service delivery after hospital discharge, as the capacity of inpatient and outpatient rehabilitation services is often insufficient. Making the necessary arrangements for the provision of telerehabilitation services may play a key role in solving these problems experienced by patients with PCS (79).

On the other hand, although most patients with COVID-19 in the community recovered within 2-3 weeks (140, 141), It is found that some of the patients hospitalized with COVID-19 died within the first

two months of discharge, with a higher mortality rate among patients admitted to the intensive care unit (140). A retrospective cohort study revealed that total mortality was 6.4% and the readmission rate was 11.7% after discharge with a median of 80 days (142). Therefore, it is recommended that CARP intervention be initiated at least 4 weeks after a positive COVID-19 PCR test and/or the symptomatic start of confirmed infection (9). Before initiating the program, it is recommended that patients be evaluated for any associated conditions that can cause early recovery phase decompensation (9).

It is recommended to evaluate vital signs (blood pressure, pulse, and oxygen saturation) and perform a 6-minute walk test before physical therapy. The strengthening and endurance program is determined according to the data obtained. To facilitate improvement in function in these patients and to ease getting back to work, an intense psychosocial-focused, physical/occupational therapy process is needed, with a focus on therapy as an individualized-paced program (9, 143). Psychosocial-based therapy uses both physical therapy and occupational therapy to enhance recovery (144). While treatments needed are planned to improve the patient's medical condition; factors such as the patient's education level, occupation, perception of pain, symptom amplification, catastrophizing, and fear of re-injury must be taken into account (145). Instructions for optimizing sleep hygiene, diaphragmatic breathing, and relaxation techniques help reduce muscle tension and reduce the work of breathing, as well as manage stress (144). Occupational therapy has an important role in facilitating efforts to return to full activity and restore self-care activities and help patients concentrate on their daily routine (146, 147). Job-specific tests and simulations are often used to reduce job restrictions (9).

Rehabilitation has valuable beneficial effects in the acute phase, especially in the recovery phase. However, it is very important to avoid arbitrary consequences with a reasonable rehabilitation program provided to patients with PCS (3). Not only the active phase of the disease but also these prolonged symptoms described as PCS (Long Haul Syndrome)(9) seem to keep the medical practice and rehabilitation medicine busy for years (47).

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CHAPTER 14

APPROACH TO COVID 19 DURING PREGNANCY: SYMPTOMS, LABORATORY FINDINGS, DIAGNOSIS, IMAGING METHODS, AND VERTICAL TRANSMISSION

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INTRODUCTION

In December, 2019, several cases of pneumonia with an unknown cause were reported in Wuhan, China and it turned into a pandemic within a few months . On 7 January 2020, investigators identified the etiological agent as a novel coronavirus, initially designated as 2019n-CoV, which was later changed to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). On February 11, 2020, the World Health Organization (WHO) named the disease resulting from this novel viral infection Coronavirus Disease-19 (COVID-19) . As of March 30, 2021, 127,258,173 individuals had been diagnosed, and 2,785,286 deaths had been recorded, according to an epidemiological study conducted at John Hopkins University (1-5).

Coronaviruses are non-segmented, ribonucleic acid (RNA) viruses with a single chain with positive helix structure.They have an envelope and crown-like appearance when examined with an electron microscope (6). They can cause infections in humans and animals like camel, cattle, cat or bat with a wide range of disesases from a simple flu to fatal serious illnesses. The most seen infections caused by coronaviruses in human are mild upper and lower respiratory tract infections.It may be as severe as pneumonia or bronchiolitis. Gastrointestinal hepatic and neurological system symptoms may also be seen with this infection. The clinical significance of coronaviruses was unknown until the 21st century (7). Three new coronaviruses have emerged since 2002: SARS-CoV was detected in 2002, middle east respiratory syndrome coronavirus (MERS-CoV) was searched in 2012, and the last one SARS-CoV-2 was found in 2019. SARS-CoV is the cause of severe acute respiratory syndrome (SARS) and MERS-CoV causes Middle East respiratory syndrome (MERS). The SARS-CoV-2 genome shares approximately 80% and 50% homology with SARS-CoV and MERS-CoV, respectively (8-10).

The main target tissue of SARS-CoV appears to be ciliated epithelial cells and it connects to the ACE2 receptor of these cells. ACE2 functions in the cardiovascular system, intestinal and adipose tissue, placenta, lungs, kidneys, fetal tissue, and respiatory system of the newborn (9-11). SARS-CoV-2 also binds to ACE2 to enter cells (11-13). We have a group of enzymes that help the Spike protein (S protein) to get into the cell by attaching to the ACE2 receptors in our cells. This group of enzyme that affect SARS COV- 2 is the Serine protease TMPRSS2. TMPRSS2 breaks down the viral S protein and allows the virus and host cell membrane to fuse. Clinical presentation of coronaviruses probably depends on both direct cell damage and host response.

Since the first case of covid 19 disease was seen in the Wuhan seafood market, it was assumed that it was transmitted from animal to human. However, when the later cases were evaluated, it was concluded that the virus was transmitted from person to person and symptomatic people were the main source of the spread of Covid 19 (14). The disease is typically transmitted by droplets. In addition, droplets released by sick individuals through coughing and sneezing can be touched by healty people's hands and then virus will be transmitted by contacting the mouth, eye or nose mucosa. As if the virus is found in the respiratory tract of asymptomatic people, they can be contagious (15).

In general, the incubation period varies between 2-14 days. The contagious period of COVID-19 is not known certainly. It is thought to begin 1-2 days before the symptomatic period and end with the disappearance of symptoms (1).

Common signs of infection are respiratory symptoms, fever, dry cough and dyspnea. Symptoms such as headache, sore throat, runny nose, muscle and joint pain, extreme weakness, new loss of sense of smell and taste, diarrhea can also be seen. Although the disease can be asymptomatic, in severe cases, pneumonia, severe acute respiratory tract infection, kidney failure, sepsis, septic shock and multi-organ failures even death may develop (16). In suspicious cases, the diagnosis is made by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of samples taken from the lower (more sensitive) and/or upper respiratory tract. Concerns about the prevention and management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in pregnant women arose with the onset

of the pandemic. Pregnancy is a period that makes women vulnerable to viral infections and causes partial suppression of the immune system. It is very important to prevent and control infection among pregnant women. Elevation of diaphragm , increased oxygen consumption and edema in the respiratory tract mucosa make pregnant women intolerant to hypoxia. Wong et al. reported in their article that approximately 50% of the pregnant women who developed SARS were treated in the intensive care unit, 33% of them required mechanical ventilation and the mortality rate increased up to 25% among them (17).

During pregnancy, Covid 19 can cause high mortality and morbidity in both the baby and the mother. Hypertansive disorders like preeclampsia, intensive care unit admission, fetal infections, preterm birth and low birth weight are the most seen complications in this group.The pregnants with pre-existing co-morbidities, such as overweight, diabetes, hypertension or cardiac and chronic respiratory diseases are the most risky part. Furthermore , detection of viral RNA in the placenta or in foetal membranes indicates that vertical transmission of SARS-CoV-2 from mother to foetus is rare but possible (18-21).

1. SYMPTOMS OF THE DISEASE IN PREGNANTS

All pregnant patients should be followed up for signs and symptoms of COVID-19, especially if they have a history of contact with patients with a definite or suspected diagnosis (Table 1).

Table 1:Symptoms of Covid 19 during pregnancy. [26]

Cough	% 50,3	Loss of taste/smell	% 21,5
Headache	% 42,7	Nausea/vomiting	
Myalgia	% 36,7	Fatigue	
Fever	% 32	Nasal congestion	
Sore throat	% 28,4	Diarrhea	
Dyspnea	% 25,9	Chest pain	

The most common symptoms in pregnant women: Cough (50.3%), headache (42.7%), myalgia (36.7%), fever (32%), sore throat (28.4%), dyspnea (25.9%), loss of taste or smell (21.5%) (22). Some of the clinical manifestations of COVID-19 overlap with normal pregnancy symptoms (eg, fatigue, dsypnea, anorexia, nasal congestion, nausea/vomiting). Therefore, a symptomatic pregnant woman without fever should be carefully evaluated for COVID-19.

2. DIAGNOSIS

The gold standard for diagnosis is a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test.. Chest x-ray (CXR) and chest computed tomography (CT) can be used to determine the extent of the disease and to follow the course of COVID-19. CXR can be performed quickly and easily at the bedside, while chest CT is more sensitive in the early stage of infection. However, concerns about the potential teratogenic effects of radiation exposure on the fetus are unavoidable. The accepted cumulative dose of ionizing radiation during pregnancy is 5 rad, and no single diagnostic study can exceed this dose. Fetal exposure from a two-image CXR of the mother is only 0.00007 rad, and 10 chest CT scans result in <0.1 rad exposure.Therefore, in pregnant women with suspected COVID-19, CXR and CT can be considered and safely performed if indicated. An abdominal irradiation shield over the

pregnant uterus can also be used for fetal protection. Lung ultrasound has also been suggested for rapid diagnosis of pneumonia in pregnant women (23-24).

3. LABORATORY FINDINGS AND IMAGING METHODS

Laboratory and imaging findings in pregnant women with COVID-19 are generally similar to non-pregnant women (Table 2). Increased C-reactive protein level (49%), lymphopenia (33%), leukocytosis (26%), increased procalcitonin level (23%) , abnormal liver enzymes (15.4%), thrombocytopenia (6.6%)(25).Leukocytosis can also be normal during pregnancy. Some of the other laboratory findings may be seen in pregnancy-related disorders (eg, thrombocytopenia and increased liver enzyme levels in severe preeclampsia).

Table 2: Laboratory findings associated with serious patients of COVID 19 (25).

Laboratory parameters	Threshold value
D-Dimer	>1000 ng/mL(normal cut-off:<500 ng/mL)
CRP	>100 mg/ L (cut-off:<8 mg/L)
LDH	>245 units/L (cut-off: 110-210 units/L)
Troponin	> twice the upper limit of normal(cut-off in women: 0-9 ng/L)
Ferritin	>500 mcg/L (cut-off in women:10-200 mcg/L)
CPK	> twice the upper limit of normal (Norm 40-150 units/L)
lymphocyte count	< 800/microL (cut-off: ≥ 21 years: 1800-7700/ microL)

CRP: C reaktive protein; LDH: lactat dehydrogenase; CPK: creatine phosphokinase.

Chest X-Rays may be normal in early or mild disease. The most common pulmonary findings in thorax computed tomography (CT); ground glass opacities (77%), posterior lung involvement (73%), multilobar involvement (72%), bilateral lung involvement (69%), peripheral distribution (68%), and consolidation (41%) (26).

4. VERTICAL TRANSMISSION AND PLACENTAL INVOLVEMENT

At this time, it is unclear whether SARS-CoV-2 can be vertically transmitted. Most case reports of SARS-CoV-2 positive pregnancies document a negative polymerase chain reaction in newborns, placenta, cord blood and vaginal secretions (27-32). Most of the neonates in these cases had uneventful hospitalizations. However, there are cases of newborns with positive SARS-CoV-2 test after birth (33,34) and a few newborns with positive IgM antibodies against SARS-CoV-2 (35,36). IgM does not cross the placenta. SARS-CoV-2 IgM may appear a few days after infection, but peaks around in 2 weeks (37,38).Therefore, the presence of IgM in the newborn after birth may indicate congenital infection. In a case report from Italy, it was stated that 2 newborns and their placentas were PCR + and its pathology was reported as chronic villitis (35).

Kirtsman et al. report a case of SARS-CoV-2 infection in a Canadian woman., who had urgent cesarean section for coagulopathy at 35 5/7 weeks of gestation. PCR of maternal and fetal placenta,

vaginal swab, breastmilk, neonatal blood, and neonatal nasopharynx were positive for SARS-CoV-2. All placental sections sampled had diffuse inflammation and early infarction (33). Infection causes inflammatory and vascular changes in the placenta (Table 3).

Table 3: Pathologic and infectious findings of placentas in 45 studies (Baud et al, Chen et al, Shanes et al, Patanè et al, Kirstman et al, Baergen et al, Schoenmakers et al, Blauvelt et al, and Hosier et al)(9).

Placental Pathology or Infection	Number of Specimens
Maternal vascular malperfusion	17
Fetal vascular malperfusion	21
Inflammatory Infiltrates	4
Increased fibrin	15
Thrombi	9
Villitis	6
Villous edema	5
Deciduitis	1
Intervillous inflammation	6
Infarction	1
Chorioamnionitis	3
Funinitis	3
Umbilical arteritis	1

5. EFFECT OF SARS-CoV-2 ON PREGNANCY PROGRESS

Pregnancy is considered a risk factor for serious disease from COVID-19 caused by SARS-CoV-2 infection. Additionally, pregnant women with COVID-19 may be at increased risk for other adverse outcomes such as preterm birth and abortion (39-43). Studies have shown increasing evidence of a relationship between abortion and COVID-19. Virus transmission from infected mother to fetus has been discussed since the pandemic began. Because fetal organs develop during the first trimester of pregnancy, maternal infections at this stage may be more severe than later in gestation. During the first 24 weeks, pregnancy loss is defined as abortion or miscarriage, counting most cases of pregnancy loss . First trimester miscarriage occurs in an estimated 10% to 15% of clinically diagnosed pregnancies. Risk factors and etiologies for first trimester abortion are genetic, environmental, or multifactorial . Mid-trimester pregnancy loss (MTL) occurs between weeks 12 and 24 of pregnancy, with an estimated incidence rate of 1% to 2% of pregnancies. The cause of MTI is often heterogeneous and more affected by maternal conditions than by first trimester abortion (44-46). It is concluded that an important factor associated with abortion in mothers with COVID-19 is inflammation and placental insufficiency due to the direct effect of the virus on the placenta (19, 47). Therefore, fetal death may be a consequence of COVID-19 in pregnancy (48).

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CHAPTER 15

COVID-19 AND MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN

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INTRODUCTION

1. COVID-19 IN CHILDREN

1.1.Etiology and Epidemiology

Coronaviruses are positive sense, *single-stranded* enveloped *RNA viruses*. Coronaviridae family divided into four genera; *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus*. The four human coronaviruses (229E, OC43, NL63, and HKU1) have long been known as a common causative agent of upper respiratory tract infection. They essentially create a mild clinical appearance. But, recently three coronaviruses which are identified leading to serious diseases and outbreaks; the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and finally the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1).

Coronavirus Disease 2019 (COVID-19) is an infectious *disease caused* by SARS-CoV-2, that was first emerged from Wuhan, China in December 2019. The virus is from the beta coronavirus family and has been named SARS-CoV-2 because of genetic similarity to SARS-CoV (2).

Many studies stated that older adults and patient with comorbidities (hypertension, diabetes, cardiovascular disease) are at a significantly increased *risk* of severe *disease (3).* Children of all ages can get the COVID-19 and incidence increases with age. Because of high proportion of mild and asymptomatic cases in children, reported laboratory-confirmed COVID-19 cases were less than adults. Actually, it is difficult to determine the true incidence (4).

1.2.Pathogenesis

The virus has four structural proteins: nucleocapsid (N) protein, transmembrane (M) protein, envelope (E) protein and spike (S) protein. The S, E, and M proteins together form the viral envelope. The virus enters the host cell by binding to the Angiotensin converting enzyme-2 (ACE-2) with the S protein. This complex required Type II transmembrane serine protease (TMPRSS2) for initiate membrane fusion (5). SARS-CoV-2 viruses can cause complication in multiple organs by ACE-2 receptors, including lung cells, enterocytes, hepatocytes, heart, blood vessels and kidneys (6).SARS-CoV-2 virus which is recognized by antigen presenting cells stimulate lympocytes and cause of releasing of cytokines including interferon-γ (IFN-γ), interleukins (IL-1 and IL-6), tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF). Cytokine storm leads to acute systemic inflammatory syndrome with fever and multi-organ involvement. (6). Mild clinical course of COVID-19 in children than adults were attributed to; children have innate immunity, difference in ACE-2 receptor expression, and previous infections with coronaviruses (7).

1.3.Transmission of Infection

SARS-CoV-2 is primarily transmitted via respiratory droplets, aerosols and contact routes. The virus can be transmitted from contaminated surfaces to the mucosa (eye, mouth, nose). Additionally fecal-oral transmission has also been reported in children with or without gastrointestinal symptoms.The median incubation time for COVID-19 4-5 days, but may extend to 14 days (8).Transmission of SARS-Cov-2 is possible during antenatal, intrapartum and postpartum period. Whereas, rates of mother-to-child transmission is low (<2%). Postnatal infection of newborn relate with contact with maternal repiratory secretions, healthcare providers and, contaminated objects. Breastmilk is considered possibly for transmission but as of yet unconfirmed (9).The most COVID-19 cases in *children* resulted

from contact with *household* exposure or other children at social activities. Health care and school associated outbreaks have also been reported (10-11).

1.4.Clinical Features

Clinical severity of patient with SARS-CoV-2 infection can change from no symptoms to critical illness. SARS-CoV-2 infection can be grouped as asymptomatic infection, mild, moderate, severe, or critical disease according to clinical features, laboratory testing and, radiograph imaging (12).

- Asymptomatic infection: Patient with only SARS-CoV-2 nucleic acid test posivity without clinical and radiological signs.
- Mild: Patients who have any of the various signs and symptoms of COVID-19 (fever, upper tract infection, acute gastroenteritis, muscle pain, loss of taste and smell) without pneumonia.
- Moderate: Patients with pneumonia without hipoxemia.
- Severe: Pneumonia with hypoxemia (SpO2 <92%)
- Critical: Patient with acute respiratory distress syndrome along with heart failure, coagulation defects, encephalopathy, and acute kidney injury

Children with COVID-19 tend to have a milder clinical course or asymptomatic. Critical illness develop rarely. Although the frequency varies according to age, the most common symptoms are; fever, cough, rhinorrhea, sore throat, myalgia, abdominal pain, diarrhea, vomiting, headache, loss of smell or taste. Symptoms usually resolve within 1-2 weeks. Clinical presentation can overlap with pneumonia, bronchiolitis, croup, gastroenteritis (13).

Cardiac complications such as arrhythmias, myocarditis, pericarditis, pulmonary embolism associated with COVID-19 has been reported with low incidence (<0.15 percent) (14). Children can present with gastrointestinal symtoms without respiratory symptoms. Although common semptoms are diarrhea, vomiting, and abdominal pain; acute cholestasis, pancreatitis, and hepatitis have been reported. Neurological manifestations like headache, acute encephalopathy, seizures, anosmia, ageusia are common in children hospitalized for COVID-19 (15-16). Also encephalopathy, central nervous system infection, stroke, Guillain-Barré syndrome, cerebral edema have been reported. Children with COVID 19 may present with different rashes include maculopapular, urticarial, vesicular and vasculitic eruptions. In infants, feeding difficulty and fever without an obvious source are commonly seen (17).

Although SARS-CoV-2-associated bronchiolitis has also been reported, respiratory involvement not common in infants. Although children are less susceptible to COVID-19 than adults, underlying medical problems (genetic, neurological, or metabolic conditions; congenital heart diseases; asthma; malignancies; obesity; immunosuppression) and very young age are considered risk factors for the severe COVID-19 and mortality (18).

1.5.Diagnosis

COVID-19 should be considered in patient with new-onset symtoms such as fever and/or respiratuary symtoms, severe lower respiratory tract illness, sore throat, rhinorrhea, nasal congestions. COVID-19 is clinically similar to other viral respiratory infections. Also, patient can present with smell or taste disturbances, and acute gastroenteritis without respiratuary sypmtoms (19).

COVID-19 testing is suggested in children who have symptoms consistent with COVID-19, multisystem inflammatory syndrome, close contact with laboratory-confirmed case of COVID-19 within the previous 14 days, and before scheduled procedures. It is necessary to emphasize that the detection of other respiratory viruses in nasopharyngeal specimens does **not** exclude COVID-19 (20). Although viral culture is required for definitive diagnosis, is not possible in routine practice. SARS-CoV-2 polymerase chain reaction (PCR) test is widely used for the diagnosis of acute COVID-19. The

genetic material of the virus is detected by PCR test, but dead or live virus cannot be distinguished. However, it is an available in many centers and results can be obtained quickly. Samples are collected from nasopharyngeal and oropharyngeal swab, and lower respiratuary samples can be collected if an upper respiratory tract specimen tests negative. If the first test is negative and clinical suspicion remains, a second test may be performed 24 to 48 hours after the first test. Characteristic laboratory or imaging findings can further support the diagnosis of COVID-19 in some patients even if RT-PCR is negative (12-21).

Antigen tests are rapid test but less sensitive and additional test may require if symptoms compatible with COVID-19. Serology tests provides diagnosis of prior infection. Specific antibodies reachs detectable levels in a few days to a few weeks. Detectable generally take several days to weeks to develop. However, it remains unclear whether a positive antibody test indicates immunity to future infection (21-22).

Laboratory findings are variable. The most common hemotological finding were lymphopenia, leukopenia, thrombocytosis, and neutropenia in children with milder clinical course of COVID-19. Increased levels of C-reactive protein, ferritin, lactate dehydrogenase, D-dimers and serum aminotransferases and, lymphocytopenia or lymphocytosis may be occur. Kidney dysfunction was reported in critically ill children (12,23).

Radiogical features are variable. Chest X-ray generally normal or may show nonspecific findings similar to other viral infection (hyperinflation, peribronchial markings). As the infection progress alveolar opacities, consolidation or pneumonic infiltrates, pleural effusion are observed. Thorax tomography is sensitive for diagnosing pneumoniae especially in initial stage of illness (24). Chest imaging is indicated in children with suspicion of lower respiratory involvement and complications (25).

1.6. Management

Children with SARS-CoV-2 infection generally exhibit benign clinical course in most cases, therefor supportive treatment is sufficient (oxygen supplement, hydration). Safety or efficacy of medical therapy is controversial and recommendations are based on adult experience. Specific treatment is not recommended for children with mild or moderate cases who do not progress to serious illness (25).

Remdesivir is the only anti-viral drug approved by the Food and Drug Administration (FDA) for the treatment of COVID-19. only recommended for children over 12 years of age with COVID-19 who are ill enough to require hospitalization and have an urgent or increased need for supplemental oxygen (26).Use of dexamethasone recommended for children with severe SARS-CoV-2 who is required high oxygen support (0.15 mg/kg/dose, maximum dose 6 mg, up to 10 days) (26). There is limited data about safety and efficacy about Anti-SARS-CoV-2 monoclonal antibodies in children (26) Convalescent plasma is not recommended for children who are mechanically ventilated or not. Although sarilumab is not recommended, tocilizumab is controversial for COVID-19 or multisystem inflammatory syndrome in children (MIS-C) (26).

2. MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN

2.1. *Etiology and Epidemiology*

Although clinical course of COVID-19 in children usually mild, uncommon complications may develop, unlike adults. In April of 2020, firstly from United Kingdom, then different countries of the world, children with clinical features suggestive of toxic shock syndrome or Kawasaki disease were reported (27). The new clinic condition related to COVID-19 termed multisystem inflammatory syndrome in children (MIS-C). The estimated incidence of MIS-C was 2 per 100,000 (28).

While COVID-19 may severe in children with underlying health problems, MIS-C is usually observed in children who were previously healthy. Unlike classical KD most MIS-C cases are older children and Black, Hispanic children are most affected than Asian (29-30). The fact that the MIS-C spike is three-to four weeks after the COVID-19 peak supports; MIS-C is a post-infectious complication of COVID-19 (31). Underlying pathophysiology of MIS-C is unclear. Positive anti-SARS-CoV-2 antibody with low SARS-CoV-2 viral loads in patients suggest that MIS-C a post-acute phase of COVID-19 infection and autoimmune involvement (32).After acute COVID-19 infection SARS-CoV-2 epitopes activate T-cells, leading to release of autoantibody production, inflammatory cytokines, and tissue damage. T-cell, B-cell activation, cytokine release is considered play role in pathogenesis of MIS-C. MIS-C cases have higher levels of IL-6, IL-10, and tumor necrosis factor α (TNF-α) relative to severe pediatric COVID-19. Cytokines is positively associated with disease severity of MIS-C (32).

2.2.Clinical presentation

MIS-C cases may present with different clinical manifestations. Children usually present three clinical features: i) Cases with clinical findings similar to KD ii) Cases with clinical findings similar to severe acute COVID-19 (respiratory symptoms and SARS–CoV-2 RT-PCR posivity) iii) Cases with mainly cardiovascular and gastrointestinal involvement without acute COVID-19 or KD. Clinical severity of MIS-C was defined as mild, moderate and severe, due to organ damage and inotropic support requirement (33).

Fever, mucocutaneous involvement (conjunctivitis, strawberry tongue, rash), gastrointestinal symptoms (diarrhea, abdominal pain) are common in MIS-C patients. Neurological and respiratory symptoms, myalgia, lymphadenopathy may be observed less often (34). Children may present different clinical presentations; shock, with complete KD features, myocarditis, arrhythmia, acute respiratory failure, acute kidney injury, pleural, pericardial, and ascitic effusions, hepatitis, hepatomegaly, meningoencephalitis, pulmonary embolism, etc. (30).Cardiac manifestation is common in MIS-C. Although, ventricular dysfunction is most commonly seen cardiac involvement, echocardiographic findings may include; coronary artery abnormalities, valve insufficiency, pericardial effusion (34). Laboratory abnormalities may include; lymphocytopenia, neutrophilia, thrombocytopenia; elevated inflammatory markers (C-reactive protein, procalcitonin, sedimentation, IL-6, fibrinogen, D-dimer, ferritin), elevated cardiac markers, liver enzymes and lactate dehydrogenase (35).

Children usually have normal chest radiographs. Abnormal findings include consolidations, atelectasis, pleural effusions and ground-glass opacification. Thorax thomograpy findings are similar to chest radiograph (30). Abdominal ultrasound may show ascites, mesenteric inflammation, terminal ileitis, pericholecystic edema, mesenteric adenitis (36).

2.3.Diagnosis

MIS-C diagnostic criteria according to The Centres for Disease Control and Prevention and World Health Organization (WHO) are given in the table (37) **(Table 1)**. SARS-CoV-2 test including serology and RT-PCR should be tested in patients with suspected MIS-C. Patients may have positive serology with negative RT-PCR (60%) or positive on both tests (30-35%). A minority of MIS-C patients have negative on both test with an epidemiologic link to SARS-CoV-2 (38-39). Differential diagnosis include other infectious and inflammatory conditions: KD, severe COVID-19 infection, **toxic shock syndrome,** other viral infections, **vasculitis,** acute **appendicitis, systemic lupus erythematosus.** Blood, urine, throat, stool cultures, respiratory viral panel, Epstein-Barr virus, cytomegalovirus, enterovirus, adenovirus serology and PCR should be done according to the clinical findings of the patients (40).

2.4.Management

Children with MIS-C may present similar clinic to toxic shock syndrome or septic shock. These patients should be given broad-spectrum antibiotics after their cultures have been taken. Empiric antibiotic regimen include ceftriaxone plus vancomycin. Clindamycin should be added if toxin-mediated illness is considered. Antibiotic therapy should be discontinued once bacterial infection has been excluded. Efficiency of antiviral therapies (remdesivir) in MIS-C is unclear. Rarely cases was received antiviral therapies who have clinical features of COVID-19 in literature (30).

Intravenous immune globulin (IVIG; 2 grams/kilogram) is recommended for all patients diagnosed with MIS-C. IVIG can be administered in a single dose over 12 hours, with a maximum dose limit of 100 grams. If the patient cannot tolerate the volume in a single infusion, IVIG can be given in divided doses over two days (41). There are different approaches to glucocorticoid therapy in MIS-C. In addition to IVIG, glucocorticoid therapy is recommended in the following patients; moderate or severe manifestations (shock, ventricular dysfunction, myocarditis), persistent fever and elevated inflammatory markers. Some authors recommend that all patients receive a minimum of 2 mg/kg methylprednisolone with IVIG. Dosages can be adjusted according to clinical severity. In mild, moderate and severe cases, the initial steroid dose is recommended as 2 mg/kg/day, 10 mg/kg/day, and 30 mg/kg/day, respectively. The dose was then continued at 2 mg/kg/day for 7-14 days, followed by gradual tapering over a period of 3-6 weeks. Many studies have shown that IVIG and steroid combination therapy was associated with reduced need for immunomodulatory therapy, hemodynamic support, and a lower likelihood of having persistent fever (41-42).

Anakinra is an immunomodulatory treatment option for MIS-C patients refractory to initial therapy with IVIG and glucocorticoids; in patients with macrophage-activating syndrome (MAS) or with contraindications to long-term use of glucocorticoids. Infliximab can be considered as an alternative biologic to anakinra, but should not be used to treat MIS-C patients with MAS (43).

Patients with MIS-C are at risk for thrombotic complications. Low-dose aspirin (3 to 5 mg/kg/day) is recommended for all cases of MIS-C unless contraindicated (risk of bleeding and/or platelet count ≤80,000/μL). If coronary arteries are normal on echocardiography ≥4 weeks after diagnosis, they can be stopped (43). Intensive Care Unit admission, >12 years of age, high D-dimer levels (greater than 5 times the upper limit of normal), severe left ventricular dysfunction are independent risk factors for thrombosis in MIS-C. Therapeutic or prophylactic anticoagulation with enoxaparin is recommended in cases of MIS-C. Each patient should be evaluated for antithrombotic therapy according to bleeding and thrombosis risk factors (43).

Patients may present with unstable vital signs (hypotension, cardiac dysfunction, encephalopathy) or worsen during follow-up. Approximately 65% of patients require critical care requiring close monitoring, fluid resuscitation, inotropic support and mechanical ventilation. Fortunately, children tend to respond well to treatment. As of October 2021, the CDC reported that the overall mortality rate had decreased over time (44).

3. CONCLUSION

COVID-19 is usually mild in children. Early diagnosis of COVID-19 is important to prevent the potential spread of infection. Although acute COVID-19 are silent in children, complications from the accelerated immune response seen in MIS-C can result in mortality and morbidity. Therefore, early diagnosis and treatment of MIS-C is important for preventıon of complications.

Table 1: MIS-C diagnostic criteria according to The Centres for Disease Control and Prevention and World Health Organization (WHO) (37).

Diagnostic Criteria	The Center for Disease Control and Prevention	World Health Organization
Age	<21 years	0 to 19 years
Fever	∗ Documented fever >38.0°C (100.4°F) for ≥24 hours **or** ∗ Report of subjective fever lasting ≥24 hours	≥3 days
Clinical signs of multisystem involvement (at least 2 organ systems**)**	∗ Cardiovascular (eg, shock, elevated troponin, elevated BNP, abnormal echocardiogram, arrhythmia) ∗ Respiratory (eg, pneumonia, ARDS, pulmonary embolism) ∗ Renal (eg, AKI, kidney failure) ∗ Neurologic (eg, seizure, stroke, aseptic meningitis) ∗ Hematologic (eg, coagulopathy) ∗ Gastrointestinal (eg, abdominal pain, vomiting, diarrhea, elevated liver enzymes, ileus, gastrointestinal bleeding) ∗ Dermatologic (eg, erythroderma, mucositis, other rash) System involvements must be **Severe illness** requiring hospitalization	∗ Rash, bilateral nonpurulent conjunctivitis, or mucocutaneous inflammation signs (oral, hands, or feet) ∗ Hypotension or shock ∗ Cardiac dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiographic findings or elevated troponin/BNP) ∗ Evidence of coagulopathy (prolonged PT or PTT; elevated D-dimer) ∗ Acute gastrointestinal symptoms (diarrhea, vomiting, or abdominal pain)
Laboratory evidence of inflammation	*Including, but not limited to, **any** of the following:* ∗ Elevated CRP ∗ Elevated ESR ∗ Elevated fibrinogen ∗ Elevated procalcitonin ∗ Elevated D-dimer ∗ Elevated ferritin ∗ Elevated LDH ∗ Elevated IL-6 level ∗ Neutrophilia ∗ Lymphocytopenia ∗ Hypoalbuminemia	∗ Elevated CRP **and/or** ∗ Elevated ESR **and/or** ∗ Elevated procalcitonin, etc.

6. Evidence of SARS-CoV-2 infection	▪ ***Any*** *of the following:* ∗ Positive SARS-CoV-2 RT-PCR ∗ Positive serology ∗ Positive antigen test ∗ COVID-19 exposure within the 4 weeks prior to the onset of symptoms	*Any of the following:* ∗ Positive SARS-CoV-2 RT-PCR ∗ Positive serology ∗ Positive antigen test ∗ Contact with an individual with COVID-19
3. Exclusion	No alternative plausible diagnoses	No other obvious microbial cause of inflammation, including bacterial sepsis and staphylococcal/streptococcal toxic shock syndromes

ARDS: acute respiratory distress syndrome; AKI: acute kidney injury; BNP: brain natriuretic peptide; COVID-19: coronavirus disease 2019; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; IL-6: interleukin 6; LDH: lactate dehydrogenase; MIS-C: multisystem inflammatory syndrome in children; PT: prothrombin time; PTT: partial prothrombin time. RT-PCR: real-time polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

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CHAPTER 16

COVID-19 DRUGS

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INTRODUCTION

New respiratory infection with clinical symptoms of fever, cough, pneumonia, rhinorrhea, dyspnea, fatigue, and myalgia was first reported in Wuhan city, China in December 2019 (1).The rapid spread of COVID-19 was identified as a pandemic and public health emergency of international concern (PHEIC) by the World Health Organization (WHO) on March 11, 2020 (2). As of January 3, 2021, around 82 356 727 COVID-19 patients and over 1 815 433 deaths worldwidehave been confirmed (3). The genetic sequence of COVID-19 classified as a beta coronavirus has similarities to other epidemic-causing viruses of this group, with more than 80% similarity to severe acute respiratory syndrome-related coronavirus (SARS-CoV) and 50% similarity to the Middle East respiratory syndrome-related coronavirus (MERS-CoV) (4). Coronaviruses belong to the large family of Coronaviridae and their genome size ranges from 26 to 32 kb.These virusespossess positive-sense single-stranded RNA (+ssRNA) astheir genome (5). COVID-19 initiates by binding to angiotensinconverting enzyme 2 (ACE2) receptor on respiratory epithelial cells. Following the attachment and cell invasion, heart damage, kidney damage, blood infection (RNAemia), and lung disease (acute respiratory distress syndrome [ARDS] and pneumonia) may occur (6). There is a pressing demand to provide efficient drugs for this pandemic disaster to decrease the global vulnerability to a highly infectious coronavirus (6).

Since there is no known effective treatment for this disease, has created a sense of urgency towards exploring novel drug discovery approaches for its treatment. However, most of the immediate endeavors were centered towards repurposing of known clinically approved drugs, and virtual screened molecules from large chemical databases have shown minimal effects (7). Antibody development and small molecular development are two known approaches for drug discovery. In antibody development, antibodies bind to the virus surface protein and stop binding to a host cell receptor. In small molecular development, the novel molecules are designed by employing computational techniques that act as a ligand to inhibit the target protein (8).

1. CHEMICAL STRUCTURE

Coronaviruses encode four main structural proteins, namely Envelope (E), Membrane (M), Nucleocapsid (N), and Spike (S), which are defined in feature as below.

1.1. Membrane (M) [M protein]: The Membrane (M) (M protein) is highly varied concerning amino acid contents. It is the most bountiful viral protein present in the virion structure, giving the viral envelope a distinct form. It binds to nucleocapsid and acts as the central organizer of coronavirus assembly (9). The M protein has three transmembrane domains, flanked by a long carboxy-terminal inside the virion short amino-terminal outside the virion (10). Generally, the viral framework is conserved by M-M interaction. In the study made by Wu *et al.*, the M protein of COVID-19 does not have any amino acid substitution compared to the SARS-CoV (11).

1.2. Envelope (E) [E protein]: The role of protein E in pathogenesis and virus release is multifunctional. It is the most mysterious and lowest among the main structural proteins [11]. It is a small integral polypeptide membrane that functions as an ion-channel. The E protein contains three domains: an excellent C terminal domain, a large hydrophobic transmembrane domain, and a short hydrophilic amino-terminal domain. The E protein of COVID-19 exposes a similar amino acid composition without any change (12).

1.3. Nucleocapsid (N) [N protein]: There are multipurpose of the N protein of coronavirus. It supports the formation of the complex with the genome of viral, enables the interaction of M protein through virion assemblage, in addition to increases transcription efficiency of the virus (11). There are three extremely distinct and conserved domains, namely a linker region (LKR), an N-terminal domain (NTD), and a C-terminal domain (CTD) [13]. The charged LKR is rich with arginine and serine and is also recognized as SR (Serine and Arginine) domain (14). The LKR region is responsible for the cell's signaling and is also able to interact with *in vitro* RNA directly (15). The NTD binds with the 3' end of the viral genome, probably by its highly splay in the sequence and length and electrostatic interactions

(15). Compared to SARS-CoV, the N protein of COVID-19 has five amino acid mutations, wherever the two mutations are in the intestinally distributed region, one in the 103, 217, and 334 positions of NTD, LKR, and CTD, respectively (12).

1.4. Spike (S) [S protein]: The coronavirus spike (S) or [S protein] is a multifunctional class I transmembrane protein. This rests on the top of the virion like a trimmer, giving the virion a 'corona' or crown-like outer look. Operationally, interaction with cellular receptors of various kinds is necessary for the entry of infectious virion components within the cell (11). Moreover, it works as a significant feature for tissue tropism. Particularly, S protein is a single vital immune dominant protein of coronaviruses that prompt the host immune response. The S protein of all coronaviruses is divided into two domains (16). Initially, S1, facilitates the binding of host receptors while the other, S2, is responsible for the fusion. Already, the structural study of COVID-19's S-proteins exposed 27 amino acid substitutions over a period of 1273 amino acid stretches (11). Inter the six substitutions, placed in the receptor-binding domain (RBD) (aa 357-528) while four substitutions in the receptor-binding motif (RBM) at the CTD of the S1 domain. There is no shift in amino acid in the RBM that binds directly to the angiotensin-converting enzyme-2 (ACE2) receptor in SARS-CoV (17). It is currently important to identify how many changes will be essential to alter the host's tropism. The evaluation of sequence exposed 17 non-synonymous variations in the primary sequence of SARS261 CoV-2 than the later isolates of SARS-CoV, as shown in Figure 1(A-C). The modifications were located distributed in the open reading frame (ORF) 1ab, ORF8 (4 substitutions), spike gene (3 substitutions), and ORF7a (single substitution) over the genome of a virus with 9 substitutions (18). Particularly, the same non-synonymous modifications were detected in a familial group showing that the viral evolvement might have happened throughout person-to-person transportation (11). Such adaptive evolvement is public and constitutes a constantly ongoing process once the spread of virus between new hosts. Despite this fact, no changes in the functional occur in the virus associated with this adaptive development. Observing the viral mutations that happen throughout subsequent human-to-human transmission is verified (19, 20).

2. COVID-19 TREATMENT

In general, there are different approaches used together in CoVID-19 treatment, and in these approaches, drug compounds can be selected according to virus-based and host-based treatment (11). The first process offers broad-spectrum antiviral drugs, previously used to treat viral infections by typical assays. These drugs' effects on pseudo coronaviruses, cytopathic, viral production, and living cells plaque formation can be measured in these approaches. This method contains interferon I and interferon II (21, 22). These drugs have pharmacodynamic and pharmacokinetic features with their drug regimens and side effects. On the other hand, the anti-COVID-19 virus has no specific effects and can be related to extreme opposite reactions. The second process includes improving exact novel drugs created on the biophysical and genome understanding of the COVID-19 virus patients. The method includes an inhibitor of the host cell endocytosis virus, inhibitors targeting specific viral enzymes found in a viral replication cycle, inhibitors of the host cell's protease enzyme, targeting human-derived or humanized monoclonal antibodies (mAb) S1 RBD, and antiviral targeting peptide S2. On the other hand, the improvement of these drugs may permit drugs to become beneficial treatment choices, but it will take a long time to supply dependable drugs for COVID-19 virus patients (11). The chief problem of this method is that though most of the recognized drugs display *in vitro* anti-coronavirus activity, the greatest of them are not beneficial in clinical treatment because they have half the EC50 value of the anti-coronavirus, which is considerably higher than the concentration of peak serum that can be performed at the treatment dose or associated with immunosuppression (23, 24). The third process includes the publication of a chemical library with various compounds or databases containing knowledge about the properties of transcription for different cell lines (25). This process may provide fast and high yields for many simply obtainable compounds and then estimate them with an antiviral test. Several drugs with significant immunological and physiological effects such as kinase signal transduction, an estrogen receptor, DNA synthesis or repair, lipid, and protein metabolism have been recognized in these drug reuse programs (26, 27). The fourth process also includes treatment methods used by Acupuncturists with a meaningful result in the management of patients with COVID-19. One

hypothetically effective method is a synthetic form of quinine, called hydroxyl chloroquine (28, 29). Presently, malaria is treated with natural and synthetic forms of the Chinese herbal medicine Qing Hao. On the other side, Cortegiani *et al.* found that chloroquine appears to be effective in reduced the replication of COVID-19 *in vitro* [29]. Furthermore, Gao *et al.* showed that chloroquine is significantly effective compared to a control group in 100 patients for symptoms duration, decreasing pneumonia exacerbations, and viral clearance postpone. Also, this study displayed that chloroquine might decrease the length of hospital stays (30). Chloroquine and oxygen treatment is recommended by the Infectious and Tropical Disease Society and the Dutch Centre for Disease Control in Italy, with separate dosage guidelines compared with the Chinese Protocol. Colson *et al.* conclude that sufficient preclinical indication is available for the chloroquine used in COVID-19 treatment (11).

3. SOME DRUGS USED IN THE TREATMENT AND INTERACTIONS

General drugs used in the treatment of Covid-19 and drug-drug interactions are given below (31,32).

3.1. **Lopinavir+ritonavir** may increaseor decrease the concentrationsof some drugs with concomitant use. In addition,**lopinavir+ritonavir** concentrations might be also decreasedor increased by some drugs with concomitant use.Dose adjustment, alternating drug use or monitoring should beconsidered case by case according to the severity and clinicalsignificance of the interaction.

3.2. Due to risk of QT prolongation with **lopinavir+ritonavir, hydroxychloroquine and azithromycin,** when they used in combination with each other or concomitant use with other drugs that prolong QT interval (such as quinolones, macrolides, ondansetron, antiarrhythmic agents, antidepressants and antipsychotics), clinicians should be aware of the increased risk of this adverse drug reaction. Therefore, electrocardiography monitoring and/or discontinuation of one of the drugs should be considered.

3.3. **Hydroxychloroquine** concentrations might be decreasedwith inducers such as carbamazepine and rifampicin withconcomitant use. Antacids may decrease the bioavailabilityof hydroxychloroquine with concomitant use. Therefore,hydroxychloroquine should be used 1-2 hours before or 4hours after antacids.

3.4. **Hydroxychloroquine** may increase the concentrations ofamiodaron, dabigatran, edoxaban and immunosuppressants(such as cyclosporine, sirolimus, tacrolimus) with concomitantuse. Dose adjustment of these drugs need to be consideredaccording to clinical response or drug levels.

3.5. Concomitant use of **hydroxychloroquine** with metronidazole, isoniazid or ethambutol may increase the risk of peripheral neuropathy especially in elderly (≥60 years) or diabetic patients. Patient monitoring is needed.

3.6. Even though **azithromycin** has a low risk of drug interactionpotential, concentrations of some narrow therapeutic indexdugs such as digoxin, theophylline and warfarin might beelevated with concomitant use. Therapeutic drug monitoringof these drugs is recommended.

3.7. Antacids may decrease the bioavailability of oral **azithromycin** with concomitant use. Therefore, oral azithromycin should beused 1-2 hours before or 4 hours after antacids.

3.8. Concomitant use of **azithromycin** with atorvastatin orsimvastatin may increase the risk of rhabdomyolysis. Patientmonitoring or alternating with rosuvastatin or pravastatinshould be considered.

3.9. **Favipiravir** has low risk of drug interaction potential withconcomitant use of theophylline and paracetamol.

3.10. **Favipiravir** may increase the concentrations of pioglitazoneor repaglinide with concomitant use that leads to risk of hypoglycemia. Blood glucose monitoring should be consideredwith concurrent use.

3.11. **Oseltamivir** has low risk of drug interaction potential.

3.12. **Tocilizumab** may increase the immunosuppressive effectsof Anti-Tumor Necrosis Factor (Anti-TNF) agents, Biologic Disease-Modifying Antirheumatic Drugs (DMARDs),cladribine, infliximab, natalizumab, tacrolimus. Therefore, the concomitant use of tocilizumab with any of these medications should be avoided.

3.13. **Tocilizumab** may also decrease the serum concentration of CYP3A4 substrates, therefore, monitor therapy is needed withconcomitant use.

3.14. Drug-drug interactions can cause negative outcomes in patient treatment and triggers adverse drug effects, however they are preventable. The severity, mechanisms, onset of action and clinical significance of the drug-drug interactions may vary. Therefore, management of drug interaction is important as well as detection.

4. DRUGS

4.1. MOLNUPIRAVIR

One of the helpful drugs for the treatment is molnupiravirwhich is an anti-viral drug with commercial cod (MK-4482 and EIDD-2801), and is administered to treat influenza. This prodrug has a nucleoside scaffold of N4- hydroxycytidine (33, 34), which was first produced at Emory university with the cooperation of the university's drug innovation company and is developing by Merck company as a novel oral anti-viral drug for the treatment of COVID-19 (35). The application of this drug on animal species demonstrated the success of molnupiravir in prevention of viral transmission and inhibition of SARS-CoV- 22 (36). Based on an oral anti-viral ribonucleoside analog, this drug is regarded as 5′-isobutyrate prodrugs of directacting anti-viral ribonucleoside analog, EIDD-1931 or β-DN4-hydroxycytidine. In the plasma, molnupiravir is cleaved to release EIDD-1931. Intracellularly EIDD-1931 is phosphorylatedby host kinases to its corresponding 5′-triphosphate,the active anti-viral agent (37). In animal models of various coronaviruses, influenza, Ebola virüs infection demonstrated that EIDD-1931 inhibits replication of multiple RNA viruses successfully (37). This oral drug with high potency toward SARS-CoV-2 infectious showed desirable safety and acceptable profile (38).Phase 1 clinical trial demonstrated that molnupiravir as anovel oral anti-viral drug has been very good tolerated and safe for some healthy volunteers (39). Patients with mild to moderate COVID-19 were administered twice a day for five days in Phase 2 trial, namely placebo-controlled, doubleblind, randomized, multicenter trial indicating that the drug reduces the transcription of SARS-CoV-2 and the rate of clearance of infectious virus and prevents the progression of COVID-19 and replication of SARS-CoV-2 successfully (37).The results of the phase 2/3 trial were presented atthe European congress of clinical microbiology and infection disease [ECCMID]. Phase 2/3 clinical trial showed a promising drug for non-hospitalized patients with COVID-19.

4.1. Metabolism: Molnupiravir is an oral prodrug of N6-hydroxycytidine which was planned against influenza in 2019. With the advent of SARS-CoV-2, molnupiravir has shown strong anti-SARS-CoV-2 activity (in animal models and in vitro) (36). Potent and elective antiviral inhibitors of coronaviruses containing SARS-CoV-2 are known among nucleosides and analog nucleotides with a wide range of antiviral activity, some of which have rapidly advanced in clinical trials for the treatment of COVID-19 (40). Unlike other drugs of COVID-19 with emergency use authorization (EUA), molnupiravir can be produced at a bigger scale. This drug doesn't need in-hospital settings and cold transportation for administration. The evidence has shown that molnupiravir is better tolerated and safer in phase 1, 2, and 3 clinical trials, at least in the short term, without any significant side effects (41).

4.2. Mechanism of action: Since the outbreak of the Coronavirus epidemic, several scientific projects have been launched to investigate measures against the new virus. Researchers has been developing different vaccines and drugs with various degrees of success. Molnupiravir was developed to treat influenza and was recognized as another candidate for antiviral drugs. Understandingthe mechanism of molnupiravir at the molecularlevel is critical to the further development of antiviral drugs.The drug is activated through metabolism in the body. Onceinside the cell, it becomes an RNA-like component. In thefirst step, RNA polymerase (the viral copy machine) incorporatesthese components into the RNA genome of the virus.In the next step, RNA-like components are paired with viralgenetic material components. Viral RNA contains severalmutations when it multiplies to produce new viruses, preventingthe reproduction of pathogen. This viral drug causesmutations in other RNA viruses and prevents them fromexpansion (42). Molnupiravir, a promising drug, is in the thirdphase of studies. When molnupiravir enters the cell, the activemolnupiravir forms N-hydroxycytidine hydrate (NHC triphosphate(MTP)), which can be replaced by cytidine triphosphate(CTP) or uridine triphosphate (UTP) by RNAdependentRNA polymerase (RdRp) of SARS-CoV-2. Initially, when RdRp uses positive-strand genomic RNAfor synthesis sub-genomic RNA and negative-strand genomicRNA as a template, it regularly substitutes M for U or C. Inthe next step, +gRNA or +sgmRNA (positive-strand subgenomicmRNA) can be used from RNA including M as atemplate. Then mutations are formed in positive-strandedgenomic RNA products due to the presence of M in negativestrand genomic RNA, and these products preventthe formation of healthy new viruses (34, 36). At the end ofthis two-step mechanism, the mechanism of molnupiravir andits activated type were shown resulting in RNA mutationsthrough polymerases of other viruses (42, 43). According to previous studies, molnupiravir-inducedlethal mutagenesis was determined by a relatively highselectivity of MTP for incorporation as a CTP analog andthe indiscriminate incorporation of either adenosine triphosphate(ATP) or GTP when MNP (incorporation ofmolnupiravir as the monophosphorylated MNP) is centralizedin the template strand (at least a two-step mechanism).Downstream of C-to-U mutations generated by theerroneously incorporated AMP could subsequently templateUTP incorporation. The replication fidelity required forviability is demarcated by mutations' agglomeration thatpushes viral replication over the "error threshold". In theend, molnupiravir has excellent pharmacokinetic properties,including oral administration (44).

4.3. Desirable dose and safety in infected patients: AGILE is an Ib/IIa phase substrate for rapid evaluation of COVID-19 therapies. In the experiment (NCT04746183), Khoo, Saye H., et al. (37) appraised the safety and desirable dose of molnupiravir in contribution with primary signaling infection. Patients (in groups of 6) were randomly assigned to 300, 600, and 800 mg of oral molnupiravir administered twice a day for five days. If the probability of 30% or more of doselimiting toxicity (initial result) is more than 25% or more, it is unsafe. Secondary results represented clinical improvement, safety, virological reactions, and pharmacokinetics. Between July 17th and October 30th, 2020, eighteen participants (out of 103 participants screened) were registered, and it was shown that molnupiravir was well resisted at doses of 300, 600, and 800mg without severe side effects. Overall, molnupiravir was safe and well resisted in the second phase of assessment so it was recommended administrating a dose of 800mg twice a day for five days.

4.4. Consequently: Molnupiravir as an oral antiviral agentdisplayed a promising compound for non-hospitalizedpatients with COVID-19. Phases 1, 2, and 3 clinical trialsresulted molnupiravir remarkably reduced the risk of hospitalizationor death in adults experiencing mild or moderateCOVID-19. According to emerging findings,molnupiravir may be proved to be a global game-changerin the fight against SARS-CoV-2. In conclusion, the preparationof this potent antiviral drug has attracted attentionall over the world.

5. FLUOXETİNE

Antiviral properties in vitro have been known for many but not allantidepressant drugs in the past years and could be confirmed inmany cases for SARS-CoV-2 with effective concentrations in themicromolar range (45). Fluoxetine turned out to be one of themore potent molecules but its half-maximal inhibitory concentrationwas never lower than about 1 to 2 μmol/L. As lipophilic moleculeswith

a cationic structure (secondary or tertiary amine), thesecompounds are easily taken up into acidic cell organelles (e.g. lysosomes)(lysosomotropic properties). Once inside the lysosome,they are trapped and can interfere with the release of the virus fromthe lysosome probably by reducing the concentration of ceramide (46, 47).

For some antidepressants, antiviral properties could be linked to the enzyme acid sphingomyelinase (ASM) which is widely expressed in lysosomes (48). ASM releases ceramide from sphingomyelin. Its inhibition by fluoxetine and a few other antidepressants including amitriptyline has been shown to reduce ceramide levels in the brain causing antidepressant-like effects at the behavior and biochemical level (48). Because of the important role of ceramide in the processing of different virus species in the lysosome the antivirus activity of fluoxetine has been explained by its inhibitory effect on ASM and the reduction of ceramide (45). The antiviral activity of fluoxetine has recently been shown to be additive to theantiviral effects of the RNA-dependent RMA polymerase inhibitorremdesivir (49).

While these data appear quite interesting it should be realized that fluoxetine was always active in the low micromolar range only with IC50 values at best around 1 μmol/l (50). Considering the recommended therapeutic plasma levels for the treatment of depression (about 0.5 to 1.0 μmol/l) (51) further studies are needed to show if these concentrations are sufficient to achieve antiviral effects in patients. One double-blind study examining the effects of fluoxetine on COVID-19 patients is currently in progress using time of intubation and/or death as endpoints. Hoertel et al. (52) reported that the concomitant use of fluoxetine in COVID-19 patients was associated with a reduced risk of death or intubation. Still, similar effects were reported in the same study for other SSRIs not showing specific antiviral properties and also for many other antidepressant drugs independent of inhibitory effects on ASM (52).Besides fluoxetine, several other antidepressants, but also some antipsychotics, and cardiovascular drugs were included.

6. PİRFENİDONE

Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone), is a novel antifibrotic agent with trivial adverse effects. Pirfenidone is approved for the treatment of Idiopathic Pulmonary Fibrosis (IPF) in humans for patients with mild to moderate disease (53). Diverse action mechanisms have been suggested for pirfenidone, among them are downregulating effects on a series of cytokines, including transforming growth factor (TGF)- β1, connective tissue growth factor (CTGF), platelet-derived growth factors (PDGF), and tumor necrosis factor (TNF)- α (54, 55). Additionally, pirfenidone is a reactive oxygen species (ROS) scavenger, and last but not the list, pirfenidone downregulates the expression of ACE receptor, the major cellular receptor for COVID-19 (53). Additioally, some other characteristics of pirfenidone makes it an appropriate treatment for COVID-19, among them are anti-apoptotic and anti-fibrotic effects of pirfenidone. The details of the hypothesis have been discussed below. Based on known pirfenidone mechanism of action and the pathophysiology of COVID19, I believe that pirfenidone has the potential for the treatment of COVID-19 patients.

6.1. Anti-inflammatory effects of pirfenidone: The anti-inflammatory effects of pirfenidone have been shown in several experimental studies. It has been shown that pirfenidone inhibits TNF-α secretion and decrease a large number of other inflammatory cytokines as well (56). Additionally, Li et al. in a recent study shown that pirfenidone ameliorates lipopolysaccharideinduced pulmonary inflammation and fibrosis by blocking NLRP3 inflammasome activation (57, 58).

6.2. Anti-fibrotic effects of pirfenidone: It has been shown in several studies that pirfenidone significantly inhibits TGF- β 1-induced fibronectin synthesis [59, 60]. Down-regulating of profibrotic gene expression and collagen secretion has been shown in humans and animal models treated with pirfenidone . Reduction of overexpression of TGF- β in inflammatory conditions plays a key role in the antifibrotic activity of pirfenidone (56). Pirfenidone inhibits collagen I fibril formation and causes a reduction in collagen fibril bundles (56). It has been shown that pirfenidone has pleiotropic actions on both the immune system and extracellular matrix (ECM), such as hyaluronan, a major component of the ECM that regulates tissue injury and repair (61). Recently, the upregulation of RGS2 has been suggested as a novel mechanism of amelioration of pulmonary fibrosis with pirfenidone treatment (62).

6.3. Down regulation of ACE receptor expression: ACE receptors are the major COVID-19-SARS virus receptor in humans. Trials that targeted the inhibition of these receptors with antibodies are under investigation (63). Surprisingly, it has been shown that pirfenidone inhibits the AT1R/p38 MAPK pathway, decreased angiotensin-converting enzyme (ACE), angiotensin II, and angiotensin II type 1 receptor expression, and strongly enhanced liver X receptor-α expression (64). This will not only protect cells from developing fibrosis (LXR-α) also by decreasing the ACE receptor expression decrease entrance of the COVID-19-SARS virus into cells. With respect to the known characteristics of pirfenidone (anti-inflammatory, anti-fibrotic, antioxidant) and our current understanding of severe COVID-19 pathophysiology (cytokine storm, inflammation, probable fibrosis, hyper-immunity and as a result oxidative stress, it is rational to suggest pirfenidone application in the treatment of patients with moderate to severe COVID-19-SARS (56).

6.4. Protection against oxidative stress and lipid peroxidation: The followings are probable endpoints of an overactive inflammatory response and WBC free radical formation in Microsome (via microsomal NADPH cytochrome c reductase) and Mitochondria (NADH-quinone oxidoreductase of the inner/outer membranes): excitotoxicity, damage to lipids and proteins, apoptosis, ADP-ribosylation, injury to mitochondrial DNA, and impaired NO activity (56). Cytoskeletal damage and lipid peroxidation are the other destructive effects of inflammation and severe oxidative stress due to cytokine storm (65, 66, 67). Hence, the antioxidant character of pirfenidone makes it potent for the treatment of hyperimmune response (56). Lipid peroxidation, which is initiated by generated superoxide in the cyclic reduction–oxidation is one of the mechanisms of cytokine storminflammation-oxidative stress end-organ-damage and pulmonary toxicity. It has been shown that pirfenidone could inhibit NADPH dependent lipid peroxidation (66, 68).

6.5. Conclusion: New therapeutic strategies are being considered in the treatment of COVID-19. However, pirfenidone has not yet been tried. As discussed above, pirfenidone is considered to be a safe addition to existing COVID-19 treatment protocols, with minor side effects and many potential benefits (56).

7. NAFAMOSTAT AND CAMOSTAT

Nafamostat mesylate and Camostat mesylate belong to synthetic serine protease inhibitors; Nafamostat mesylate, also named FUT-175 and 6′ -amidino-2-naphthyl-4 -guanidinobenzoate dihydrochloride. At the first phase of infection with SARS-CoV-2 Virus, Nafamostat can inhibit the S-mediated membrane fusion. This drug is used for acute pancreatitis, and intracellular coagulation has been revealed to be useful in the first phase of infection with the SARS-CoV-2 virus. Nafamostat prevents cell entry of virus by inhibiting enzyme transmembrane protease serine 2 (TMPRSS2), blocking the S-protein mediated membrane fusion. These inhibitors form a close interaction to Asp435 in the S1 pocket and close contact with catalytic serine in TMPRSS2 and produce a reactive complex, resulting in enzyme hampering (69). A German group introduced Nafamostat as an inhibitor of SARS-CoV2 infection. They stated that Nafmostat is more effective than Camostat to prevent membrane fusion and host cell entry (70). In a comparative analysis, covid-19 antiviral drugs' efficacy was determined in human lung cells and revealed Nafamostat is the most potent drug for blocking virus entry (71). Moreover, a case report on three old patients with COVID-19 pneumonia showed that Nafamostat's use could improve clinical symptoms. Disseminated intravascular coagulation (DIC) dose (0.06–0.2 mg/kg/hour) was used for these patients. However, harmful incidents such as bleeding should be considered after using this drug (69).

8. REMDESIVIR

Remdesivir is an adenosine analogue with broad-spectrum antiviral activity against several single-stranded RNA viruses. It was originally developed for treating patients with Ebola virus infection (72). After recording the potential benefts of remdesivir against SARS-CoV-2 in in vitro, pre-clinical, and human cell line studies, its efcacy was evaluated in patients with COVID-19 (73). On 1 May 2020,

remdesivir received the Emergency Use Authorisation (EUA) status based on a preliminary report from an interim analysis of an ongoing double-blind randomised controlled trial by the United States Food Drug Administration (US FDA) (74). On 21 June 2020, the Central Drugs Standard Control Organisation (CDSCO) approved its restricted emergency use for treating patients with severe COVID-19 infection in India; the indication was later expanded to moderate and severe disease. However, the CDSCO approved remdesivir with a condition to provide data from an active surveillance programme on a monthly basis by the pharmaceutical manufacturers (73). Given the global emergency and the unmet medical need with respect to COVID-19 treatment, the Drugs Controller General of India (DCGI) also provided a clinical trial waiver for remdesivir use in India (74). At the time of writing this paper, clinical evidence for its safety and efcacy in COVID-19 pertains mainly to randomised trials, and only few observational data are available that show its safety in real practice. In this paper, we present a retrospective analysis of data from an active surveillance programme conducted for remdesivir use in patients with COVID-19 in India.

Conclusion: Te retrospective analysis of data from an active surveillance programme of remdesivir therapy in patients with COVID-19 showed that remdesivir was well tolerated and had an acceptable safety profle. Te clinical outcome of cure and improvement rate was 84%, with greater improvement in patients with age < 60 years and receiving standard low-fow oxygen and mortality rate was 6.77% (73).

9. DARUNAVIR

Darunavir is a nonpeptidyl HIV-1 protease inhibitor with a bimodal mechanism of action including, inhibition of HIV protease dimerization and protease enzymatic activity. It selectively inhibits Gag-Pol polyprotein cleavage leading to immature and non-infectious viral particles (75, 76). One of the best targets for SARS-COV-2 is its main protease, so its inhibition may block the virus. Several in silico studies have introduced Darunavir with a high score for binding to SARS-COV-2 protease, which may be useful in the battle with the COVID-19 disease after further testing (69). Although Darunavir was thought to be an effective candidate, De Meyer et al. showed this drug does not have an antiviral effect for treatment of covid-19 (77). Reportedly, Darunavir's co-administration with other antivirals has had positive effects on SARS-CoV-2 patients (69). Darunavir has also been used for a married couple in which the wife was partially immunocompromised because of starting chemotherapy. They received 200 mg Darunavir/Cobicistat and Hydroxychloroquine along with antiviral therapy, twice daily. Both patients were recovered (78). There is a report of HIV positive patients who received Darunavir-based antiretroviral treatment (800 mg), which were also admitted as SARS-CoV-2 positive. This study suggests that despite Darunavir's potential effectiveness, it did not protect people living with HIV from SARS-CoV-2 infection, at least 800 mg, the currently given dosage (79).

10. BARICITINIB

Baricitinib (C16H17N7O2S, formerly LY3009104) is a small molecule reversible Janus-associated kinase (JAK)-inhibitor approved in over 65 countries for the treatment of adults with moderate to severe rheumatoid arthritis (RA) (80). The JAK/signal transducers and activators of transcription (STAT)-pathway mediates the signaling of multiple cytokines and interrupting this pathway may therefore be an attractive strategy to modulate the immunopathology seen with SARS-CoV-2 infection (80). Furthermore, many drugs within this class exhibit antiviral effects, albeit often at supra-therapeutic concentrations, by targeting host factors that viruses usurp for cell entry (81, 82). Baricitinib has the advantage of providing in vitro antiviral activity at concentrations achieved with approved dosing (83, 84).

10.1. Pharmacology and pharmacodynamics: Basic and translational science have identified a wide array of subcellular pathways that regulate normal and aberrant immune responses (80). One of these is the JAK / STAT pathway (85, 86). The JAK/STAT pathway mediates signal transduction from extracellular stimuli, including cytokines, growth factors and hormones, to the nuclei of cells (85 , 86). Baricitinib exerts its anti-inflammatory effects through reversible JAK inhibition.Signaling is initiated when cytokines bind to their receptor on the cell membrane (86). This results in conformational changes that trigger activation of associated JAK complexes. JAK activation in turn leads to autophosphorylation and subsequent increased JAK kinase activity as well as phosphorylation of the intracellular portion of their cognate receptors (86). Receptor phosphorylation creates a docking site for signaling molecules especially members of the STAT family (80). Once docked to the receptor, STAT molecules are also phosphorylated by JAKs. The phosphorylated STATs are then released from the receptor, form homo- or hetero-dimers through reciprocal interactions with their newly phosphorylated tyrosine domains, and translocate to the cell nucleus where they bind to specific DNA sequences to activate target gene transcription (86).

10.2. Antiviral activity: Baricitinib may also have antiviral activity. It's potential antiviral activity was identified by searching a large repository of structured medical and drug information extracted using machine learning (80). Nearly 50 currently approved drugs for variety of indications from oncology to auto-immune disorders were identified by this approach as inhibitors of host enzymes involved in regulating intracellular viral trafficking. Only baricitinib however showed inhibitory activity at clinically achievable serum concentrations.

10.3. Pharmacokinetics: After oral administration baricitinib is rapidly absorbed reaching peak plasma concentrations within 60 minutes.The absolute bioavailability is 79% and food has minimal impact on PK parameters (87, 88) Baricitinib exhibits linear dose proportional PK following single oral doses between 1 mg and 20 mg with minimal accumulation for up to 28 days.3, 32 Both Cmax and area under the concentration time curve over 24 hours (AUC24) values increase approximately 60% and 75% in patients with RA compared to healthy subjects, respectively and inter-individual variability is higher in RA patients (87, 88). Exposure is also increased greater than 2-fold in those with moderate to severe renal impairment and end stage renal disease (ESRD). Exposure in patients with COVID-19 or other acute viral infections has not been reported at this time (acute infection at baseline was a contraindication for all RA clinical trials) (89) Additionally, PK modeling of 4mg once daily dosing showed that there is a 12 hour window when baricitinib serum levels fall below IC50 values for JAK complexes (87). The clinical implications of this in the setting of COVID-19-related cytokine storm are unclear.

10.4. Safety: Pooled data from 3492 baricitinib exposed patients (7860 patient-years) enrolled in Phase 2 and 3 339 RA clinical trials together with long-term extensions of these studies in the baricitinib development program has been used to characterize baricitinib's safety profile (80).

11. ANAKINRA

Anakinra is a 17 kD biological recombinant, non-glycosylated human interleukin-1 receptor antagonist with a short half-life of approximately 3–4 h and an acceptable safety profile to neutralize hyperinflammatoryrelated to COVID-19 with the severe respiratory syndrome. IL-1 plays a significant role in stimulating the production of inflammatory cytokines and TNFα. Anakinra blocks the action of IL-1, which leads to inhibit the inflammatory responses (90). A cohort study evaluated the effect of Anakinra on the severe respiratory syndrome of COVID-19. Patients received a dose of 100 mg subcutaneously twice daily for 72 h, followed by 100 mg once daily for seven days along with a standard treatment regimen consist of oral agents (10 days course of Hydroxychloroquine 600 mg/day, five days course of Azithromycin 250 mg/day), and intravenous antibiotics (Ceftriaxone 1 g/day or Amoxicillin 3 g/day) for seven days. Prophylaxis of thromboembolism was considered for all cases. Some patients were a candidate for an intravenous bolus 500 mg dose of methylprednisolone. This study determined a notable decrease in the demand for admission to the Intensive Care Unit, invasive mechanical ventilation, and mortality compared with standard of care. More patients experienced elevated liver enzymes in the Anakinra group than the control group (91). A previous study exhibited improved respiratory system and reduced serum C-reactive protein in 72% of patients with a high-dose of Anakinra (IV) in severe COVID-19, ARDS, and hyper inflammation (92). An open-label study recruited

nine patients with moderate to severe pneumonia results from COVID-19. Only an old patient exhibited an acute respiratory failure after Anakinra that led to discontinuing the treatment and ICU admission. Other patients revealed good clinical and biological outcomes, in which C reactive protein (CRP) levels reduced at day 6 in all cases and controlled in 5 at day 11. Chest CT scan showed cessation of lesions development. Patients who received Anakinra were alive at the latest follow-up (69).

12. OTHER NEUROPSYCHIATRIC DRUGS

Within the large screening programs initiated at the beginning of the COVID-19 pandemic many other psychotropic drugs have been identified with antiviral activity in vitro (inhibition of the cellular uptake of SARS-CoV-2 or its replication), but in most cases, the concentrations needed for the antiviral effects were too high for considering a possible therapeutic effect (45). Therefore, only a few other psychotropic drugs have been investigated in COVID- 19 patients.

12.1. Lithium: Antiviral effects of lithium against several classes of viruses including some coronavirus species are known for several years but require rather high concentrations in vitro (93). Specific effects on SARS-CoV-2 have not yet been reported. Spuch et al.,reported of 6 patients who possibly responded to lithium during COVID- 19 and proposed lithium as a possible candidate for COVID-19 treatment. A similar case report was published recently by Sönmez and Hocaogu [94]. However, other data except these 7 case reports have not yet been published. Moreover, there is some concern about the use of lithium in COVID-19 patients because of its significant side effects and toxicity (45).

12.2. Cannabis: Cannabis and/or cannabidiol show several properties making it a potential candidate for the treatment of COVID-19 (95). Cannabis and cannabidiol possess rather potent antiviral activity against SARS-CoV-2 in vitro with IC50 values around 1–2 μmol/L and also potent anti-inflammatory properties. Cannabis users seem to have a lower risk for COVID-19 relative to non-users [96]. Thus, several authors proposed cannabis and/or cannabidiol as a candidate for COVID-19 treatment as an antiviral drug but also against different psychiatric symptoms like depression, anxiety, or psychosis related to a SARS-CoV-2 infection (45). Still, clinical data about the possible use of cannabis and/or cannabidiol to treat COVID-19 patients is not available. However, several studies have been registered so far to investigate the possible effects of cannabidiol on inflammation during the course of COVID-19.

12.3. Memantine and amantadine: Amantadine has been introduced many years ago as an antiviral drug to treat influenza A2 but has not been widely used. Besides the antiviral activity, it also possesses a broad spectrum of pharmacological properties including anti-inflammatory effects probably related to its activity as a sigma-1 receptor agonist (97). The structurally related compound memantine is predominantly an NMDA-receptor antagonist and is used clinically as an antidementia drug . Both compounds show in vitro activity against SARS-CoV-2 and have anti-inflammatory properties [45]. A few case reports suggest a possible beneficial effect of amantadine on the clinical course of COVID-19. An observational study using a national Korean database found no effect of memantine on the course of COVID-19 disease (45). Further clinical data for either drug in COVID-19 patients is not available.

13. CORTICOSTEROIDS

Corticosteroids, including glucocorticoids and mineralocorticoids, are produced by the adrenal cortex. They have been proved as immunosuppressive and anti-inflammatory drugs for the treatment of conditions such as asthma, allergy, septic shock, multiple sclerosis, and lung tissue disorders. Corticosteroids alter gene transcription through binding to a particular receptor in target cells. However, their use is limited by their massive probable side effects as hyperglycemia, hypertension, infection, osteoporosis, growth retardation, skin atrophy, glaucoma, and cataract (69). Systemic inflammation is an adverse outcome caused by coronaviruses, which persists after viral clearance. So, theoretically,

corticosteroids can be potential candidates for suppressing lung inflammations. There are some reviews summarizing reports on SARS and MERS, revealing no benefits of corticosteroids. In general, the studies suggest associations between corticosteroid administrations and disease deterioration (worsening pulmonary conditions) and mechanical ventilation requirements, delayed viral clearance, avascular necrosis, and diabetes. They have called it a double-edged sword (98, 99). Since the outbreak of COVID-19, new studies have been designed on Corticosteroids. In an in vitro study on VeroE6 cells, Ciclesonide has been introduced as a safe corticosteroid to reduce viral replication and host inflammation by EC90 = 6.3 μM. A clinical study reviewed 46 patients with severe COVID-19, in which 26 patients received 1–2 mg/kg/d methylprednisolone intravenously for 5–7 days. Results revealed faster improvement of oxygen saturation, better absorption degree of the focus in chest CT, and shorter time to overcome hyperthermia (100). Nevertheless, a report on 31 patients with 11 administrated corticosteroids indicated no statistically significant differences in treated patients and the non-treated. They investigated the virus clearance time, hospital length of stay, and duration of symptoms, and there was no improvement compared with the control patients (69). Moreover, an open-labeled, randomized controlled trial enrolled 48 cases from Chongqing Public Health Medical Center, China. The subjects are assigned in two groups, the intervention group, which receives an intravenous injection of 1–2 mg/kg/day methylprednisolone for three days, and the control group. The study is examined the timing of clinical improvement, duration of mechanical ventilation and hospitalization, rate of adverse effects, and mortality. The results have not yet been revealed (69). As of now, the use of corticosteroids in patients with COVID-19 is controversial since the WHO and the Centers for Disease Control and Prevention (CDC) generally recommend that glucocorticoids not be used in COVID-19 pneumonia unless in specific comorbid clinical conditions, e.g., exacerbation of chronic obstructive pulmonary disease (101).

CONCLUSIONS

At present, with the COVID-19 outbreak in full swing, the number ofinfected people is growing and challenging to control. Any progress in scientific research requires the accumulation of time. Even thoughscientific researchers around the world are dedicated to studying the novel coronavirus infection, the progress and outcome of COVID-19, and related treatment drugs, less than 2 years after the outbreak, there are still many research gaps and many mechanisms. The problem is difficult to explain clearly.Most importantly, drugs for the treatment of COVID-19 are still being explored and discussed, and we have not found the specific drugs we hoped (102).

Scientists are working hard to determine the new coronavirus's characterization and develop antivirus therapies and vaccines. However, the virus's pathogenesis is still not fully known, and new studies are needed in this regard. Currently, the only way to prevent the spread of Covid-19 is an effective infection control method (11). The most appropriate treatment for patients under observation diagnosed with Covid-19 is still unknown. Therefore, treatment protocols should be followed within the framework of existing health rules. As a result, there are three main ways to end pandemics: (i) forming protective antibodies by encountering the disease of the majority of the society, (ii) reducing the disease-prone population by using a vaccine or preventive drugs, and (iii) reducing the infectiousness and pathogenicity (disease-causing) of the agent. There is no sign of 3rd way regarding the Covid-19 pandemic yet. The 2nd way some vaccines have been developed, and the prophylactic drug has not yet beendeveloped, although intensive trials are ongoing for both. There remains only the 1st way. But in that method, too, the death rate is very high (11). Turkey is one of the countries taking the earliest precautions in the world regarding COVID-19. In this context, Turkey: (i) for the source of the disease (finding the source, reporting the disease, definitive diagnosis, treatment of patients, isolation, search for carriers, surveillance of suspects, health education), (ii) for the infection way (correction of environmental conditions, control of food and beverages, health education, use of personal cleaning and protective equipment, restricting population movements) and (iii) oriented for healthy person safeguards (quarantine, observation) measures have been taken and continue to be taken. Although some vaccines have been developed for the COVID-19 coronavirus, intensive work is still being done to develop specific drugs or vaccines.

The emergence of novel viruses during the last two decades and their pandemic has called for a need for massive experiments in a short time. As an essential step, drugs can be developed through three strategies:

- Directly developing a new viral-specific drug based on the genomic and pathological information. Theoretically, these drugs would exhibit targeted effects, but the procedure may last several years, which is not appropriate for a pandemic.
- Screening databases for potential molecules with therapeutic effects that introduce good candidates for virtual functions for further investigations.
- Using pre-existing components. That would be the fastest way with known safety and side effects, the dosage used, absorption, and metabolic characteristics (69). The novel coronavirus, SARS-CoV-2, is the latest outbreak with a serious threat to the global public, and to date, there is no approved therapeutic drug or vaccine against it. Many investigations should designed on broad-spectrum inhibitors, in vitro, in vivo, and clinical.

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CHAPTER 17

ANTENATAL CARE, VACCINATION IN PREGNANCY AND LABOUR DURING COVID-19 PANDEMIC

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INTRODUCTION

Pregnancy generally affects the body's immune system and can alter the immune response to viral infections and sometimes it can cause very severe symptoms. Although pregnant women with COVID-19 have more asymptomatic infection compared with non-pregnant women, intensive care unit (ICU) admission, ventilation and extracorporeal membrane oxygenation (ECMO) is more common in pregnant women (1-5). COVID-19 infection seems to be more common and hazardous in third trimester pregnancy than early pregnancy (6).

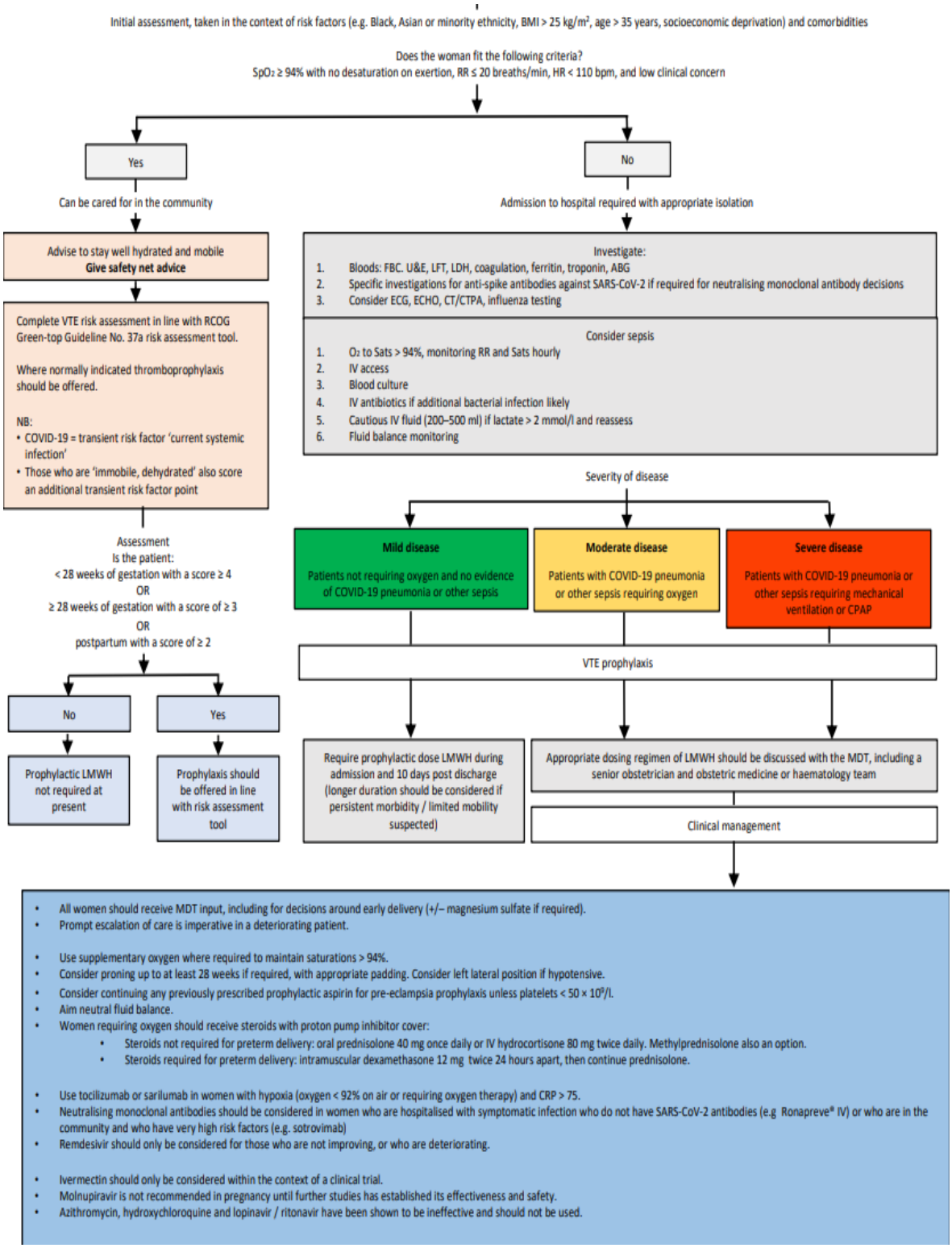
ANTENATAL CARE DURING COVID-19 PANDEMIC

It is recommended that pregnant women should continue to their routine antenatal expert visits and wear masks (7). Studies reported that there was no relation between women receiving antenatal care and risk of developing COVID-19 in pregnancy if nosocomial transmission is reduced by social distancing, measures preventing and mask wearing (8). Healthcare workers who care for pregnant should use the necessary personal protective equipment. Hospitals should have escalation guidelines for antenatal and postnatal care and provide a maternity care checklist (7). Pregnant women who don't attend antenatal care units are at increased risk of maternal death, stillbirth and other adverse perinatal outcomes (9,10).

If there is someone with suspected or confirmed COVID-19 infection from pregnant or households, pregnant must attend to the pandemic hospitals by ambulance if hospital care thought to be necessary (11). It should be evaluated for miscarriage in early week pregnancies although no increase is reported in the risk of early trimester pregnancy loss (12,13), and fetal well-being should be evaluated in late weeks pregnant. Previously planned vaginal delivery or cesarean section births should be delayed until infection regress if there is no obstetric indication. COVID-19 infection alone is not an indication for delivery (7).

There is no difference in antenatal care of pregnant women after COVID-19 infection. Unwell pregnant women due to severe infection should be offered a fetal ultrasound scan within 14 days after the disease recover to assess fetal well-being (7). COVID-19 infection may be associated with severe placental disease and dysfunction including fetomaternal vascular changes, chorioamnionitis, increased perivillous/intervillous fibrin and thrombosis, that can cause stillbirth, fetal growth restriction (FGR) and low birth weight (14-19). Factors that increase the risk of hospitalization in pregnant women with COVID-19 infection (1,7):

1. Overweight or obese / BMI ≥25 kg/m2
2. Unvaccinated
3. Existing co-morbidity such as diabet, hypertension, asthma, chronic heart disease, chronic lung disease
4. Over 35 years age at pregnancy
5. Healtcare workers



BMI, body–mass index; SpO2, oxygen saturation; RR, respiratory rate; HR, heart rate; FBC, full blood count; U&E, urea and electrolytes; LFT, liver function test; LDH, lactate dehydrogenase; ABG, arterial blood gases; ECG, electrocardiogram; ECHO, echocardiogram; CTPA, computed tomography (CT) pulmonary angiogram; IV, intravenous; CPAP, continuous positive airway pressure; VTE, venous thromboembolism; LMWH, low-molecular weight heparin; MDT, multidisciplinary team; CRP, C-reactive protein

Figure 1. Maternity care checklist of acute COVID-19 (7)

Some studies reported that Vitamin D deficiency increases the risk of acute respiratory distress syndrome in COVID-19 infection (20, 21). Folic acid and vitamin D supplementation should be continued in line with recommendations (7).

Pregnant women with additional disease are more susceptible to COVID-19 infection. Screening gestational diabetes during antenatal visits is recommended by National Institute for Health and Care Excellence (NICE) to reduce the risk of being infected with COVID-19 in pregnancy (22). An increased risk of developing hypertension and pre-eclampsia is observed in pregnant women with severe COVID-19 infection (23-26). On the contrary, asymptomatic COVID-19 infection seems not to be a risk factor for hypertensive disease in pregnancy that is why routine antenatal follow-up is sufficient for this group (23, 27).

Despite the lack of accurate information between smoking and COVID-19 infection, World Health Organization (WHO) reported that smoking increases the severity of infection and the risk of death in hospitalized infected patients (28). Increased rates of maternal mental disorders as anxiety, depression and maternal deaths by suicide have been reported during the pandemic (29, 30).

Additionally, pregnancy with influenza is a risk factor for hospitalization so pregnant women should be advised to vaccination against influenza. Influenza vaccination is safe and effective for all pregnancy trimesters and protects from severe disease adverse effects during pregnancy (31). COVID-19 and influenza coexistence has an unknown impact on pregnancy (32).

VENOUS THROMBOEMBOLISM (VTE) PREVENTION

Pregnancy is a hypercoagulable condition and COVID-19 infection is considered as a transient risk factor for VTE (33, 34). Decreased mobility due to self-isolation that causes VTE is another result of COVID-19 infection. Consequently, VTE risk in pregnant women with COVID-19 is multifactorial and it is difficult to detect the cumulative risk. Therefore, VTE should be reassessed in all pregnant women with suspected or confirmed COVID-19 and appropriate precautions should be taken. Sufficient fluid intake and mobility should be offered to home self-isolating pregnant women. Low molecular weight heparin (LMWH) should be supplied for all pregnant women with COVID-19 suspected or positive for if delivery is not expected within 12 hours and there is no risk of serious bleeding (7).

LMWH dose must be prescribed according to the pregnant women's ongoing morbidity (23). If no oxygen requirement, normal prophylaxis must be offered, oxygen dependent illness requires high dose LMWH prophylaxis (e.g. 40 mg BD) balancing with bleeding/delivery risk. Pregnant women with limited mobility who are self-isolating should be received thromboprophylaxis 7-14 days with LMWH until the acute illness regressed. All COVID-19 confirmed and hospitalised pregnant women at any gestation and all women admitted to hospital with COVID-19 within 6 weeks postpartum must be offered LMWH for minumum 10 days and should be considered longer duration of LMWH prophylaxis if persistent morbidity or immobility existance (7). It is appropriate for women who treated for thromboembolism during pregnancy or puerperium due to COVID-19 infection must receive LMWH prophylaxis until 6 weeks postpartum (11).

Thrombocytopenia can occur as a result of COVID-19 infection, although the platelet count is normal or increased in most patient (35). If platelet count falls below 50×10^{3}/l, it is suggested to stop LMWH prophylaxis. Also aspirin sould be discontinue if it has been prescribed for prophylaxis of hypertensive diseases of pregnancy because of the high risk of bleeding in women with thrombocytopenia (36).

COVID-19 VACCINATION IN PREGNANCY

Pregnant women could be more susceptible to COVID-19 infection due to suppressed immune systems and physiological changes. Hence, COVID-19 vaccination is strongly recommended in pregnancy as a priority group (7). Joint Committee on Vaccination and Immunization (JCVI) specifies the eligibility criteria (37). It is not appropriate to include pregnant women in the clinical phases of drug and vaccine trials to determine the safety and adverse effect. As a result of the acceleration of the vaccine development process and the reports of the phase 3 study results, WHO approved the emergency use of 6 vaccines after 3 June 2021 (38). Published data reports that more than 347150 pregnant women have received COVID-19 vaccine in the UK and USA, with no evidence of safety (39-41).

Table 1. Vaccines for Covid 19 (42)

TECHNOLOGY	**VACCİNE COMPANY**
Live attenuated virus	Codegenix
Inactivated virus vaccine	Sinovac * Sinopharm Bharatbiotec
mRNA vaccines	Moderna * Pfizer-Biontech * CureVAc
DNA vaccines	Osaka University CadilaHealthcare GenexineConsortium
Viral vector vaccines	Astra Zeneca Oxford-Nonreplikan* Johnson&Johnson-Nonreplikan* Sputnik-GamelayaResearchinstitute- Nonreplikan Vaxart-Nonreplikan Merck&Co- Replikan IsraelInstitute-Replikan
Protein subunit	Novavax Kentucky Bioprocessing Sanofi-Pasteur Cloverbiopharm
Viral Vector+APC	Shenzhen -Replikan –Nonreplikan

* Vaccines that have been approved for emergency use and are currently available

The use of mRNA technology for COVID-19 is new, it was first introduced against Zika virus in pregnant rats in 2017 by Richner et al. and as a result of the study, it has been stated that the mRNA Zika Virus vaccine is safe in pregnant women and prevents vertical transmission (43). In a recent study, antibodies produced against spike protein following maternal vaccination for COVID-19 infection has been shown to cross the placenta, so, mRNA vaccine is thought to protect both the mother and the newborn baby (44). In an animal trial with mRNA-123 (Moderna, Pfizer-Biontech) vaccine in pregnant rats, malformation or embryotoxicity is not proven (45). As of November 2021, 177,000 pregnant

women were vaccinated with mRNA vaccines in the USA and adverse effects have been studied using V-Safe (post-vaccine effect system) and VAERS (Vaccine adverse Effect Reporting System). It was found that injection site pain in pregnant women is more common than non-pregnant, but systemic adverse effects such as headache, fatigue, muscle pain are found to be less common (40,46). In another cohort study evaluating the results of COVID-19 mRNA vaccines during pregnancy, similar adverse effects were observed in pregnant women compared with the non-pregnant population (47). Theoretically it should be known that fever, which is one of the most common post-vaccine adverse effect, may be considered suspicious in terms of the risk of neural tube and other congenital defects developing, if COVID-19 vaccination performs in the first trimester of pregnancy (48). However, no increase in adverse obstetric outcomes associated with mRNA vaccine has been demonstrated in pregnant women according to the collected data (46).

Although the use of inactivated virus vaccines (Sinovac) in pregnancy is considered safe, the adjuvant Aluminum Hydroxide used in these vaccines does not have an established FDA safety category due to lack of data (49). Data on the use of viral vector-based Covid-19 vaccines (Vaxzevria (AstraZeneca Oxford), Johnson&Johnson) in pregnant women is limited similar with mRNA vaccines. EMA (European Medicines Agency) safety committee reported serious thrombotic/thromboembolic events in 269 people vaccinated with Vaxzevria (AstraZeneca) (50,51) and it has also been reported after the Janssen vaccine (52).

In general, the COVID-19 vaccines should be safely received and recommended at any time in pregnancy and lactation (47,52). 96% of pregnant women applied to hospital with symptomatic COVID-19 and 98% of pregnant women had intensive care need were unvaccinated (52). American College of Obstetrics and Gynecology (ACOG) has recommended that the risk of possible complications of Covid-19 infection during pregnancy should be considered and all pregnant women should be informed, questioned, and encouraged about vaccination at each visit (53). ACOG has been also reported that there is no harm in administering Anti-D Ig in pregnant women who have been vaccinated or planned to be vaccinated, and it is safe to use acetaminophen in pregnant women with fever after vaccination (53).

LABOUR AND BIRTH DURING COVID-19 PANDEMIC

It is appropriate for pregnant with COVID-19 infection to give birth in pandemic hospitals under the supervision of a Perinatologist, Neonatologist, and pulmonologist. Hospitalization should be given in an isolated negative pressure room. All maternal and fetal evaluations should be made. Routine assesments shold be done as maternal blood pressure, pulse, oxygen saturation, fever, urine and blood testing and fetal growth ultrasound scans and fetal monitoring (7). Bloods to be taken on first day are; SARS Spike antibody, FBC/Renal 3/ LFTs, Procalcitonin, D-dimer, LDH, CK, Troponin, BNP, Ferritin, AST.

COVID-19 diagnosis alone should not be accepted as an indication for delivery. Timing and type of delivery should be “individualized” based on the clinical condition of the pregnant woman, the gestational week, and the condition of the baby (54). Besides obstetrics contraindications, unless COVID-19 causes low oxygen saturation, fetal hypoxia, and fetal distress or inhibition on pushing, vaginal birth is not contraindicated (55). Planning birth when the RT-PCR test became negative may reduce the risk of possible neonatal transmission. Although early timing of birth in mild cases is not recommended, the delivery decision can be reviewed after 32 weeks of gestation in severe cases (56).

In the first published series high cesarean delivery rates (>90%) have been reported because fetal and maternal outcomes are not clearly known and it was usual for deliveries immediately by cesarean section after diagnosis of COVID-19 in the third trimester (56). The widest cohort WAPM (World Association of Perinatal Medicine), many other small cohorts, and cases reported that immediate cesarean delivery of a pregnant, following diagnosis of COVID-19 infection without any cesarean indication, will both increase the risk of neonatal complications by increasing the preterm birth and maternal complications (56-59).

Fetal monitoring is not recommended in asymptomatic pregnant women of low obstetric risk with a positive COVID-19 test (60). In accordance with routine care, women with mild symptoms of COVID-19 may be encouraged to stay at home in early (latent phase) labor, unless there is a suspicious obstetric condition for mother and baby. Delayed cord clamping and skin-to-skin contact between women with a positive test for COVID-19 and baby should be offered in line with usual practice because it has been shown not to increase the transmission of COVID-19 to the newborn (61,62). Maternal and fetal assessment should be performed for symptomatic women with suspected or confirmed COVID-19, including the severity of maternal symptoms; temperature, respiratory rate, and oxygen saturation, and continuous electronic fetal monitoring during labor and birth (63). Maternal observations and onset of labour should be discussed with the infection control team, consultant anesthetist, and consultant neonatologist (64). Symptomatic women should be advised to give birth in an emergency care accessible obstetric unit because of the increased risk of cesarean delivery due to COVID-19 (65). The timing of delivery should be discussed by health professionals by informing the mother, depending on the well-being of the mother and the fetus.

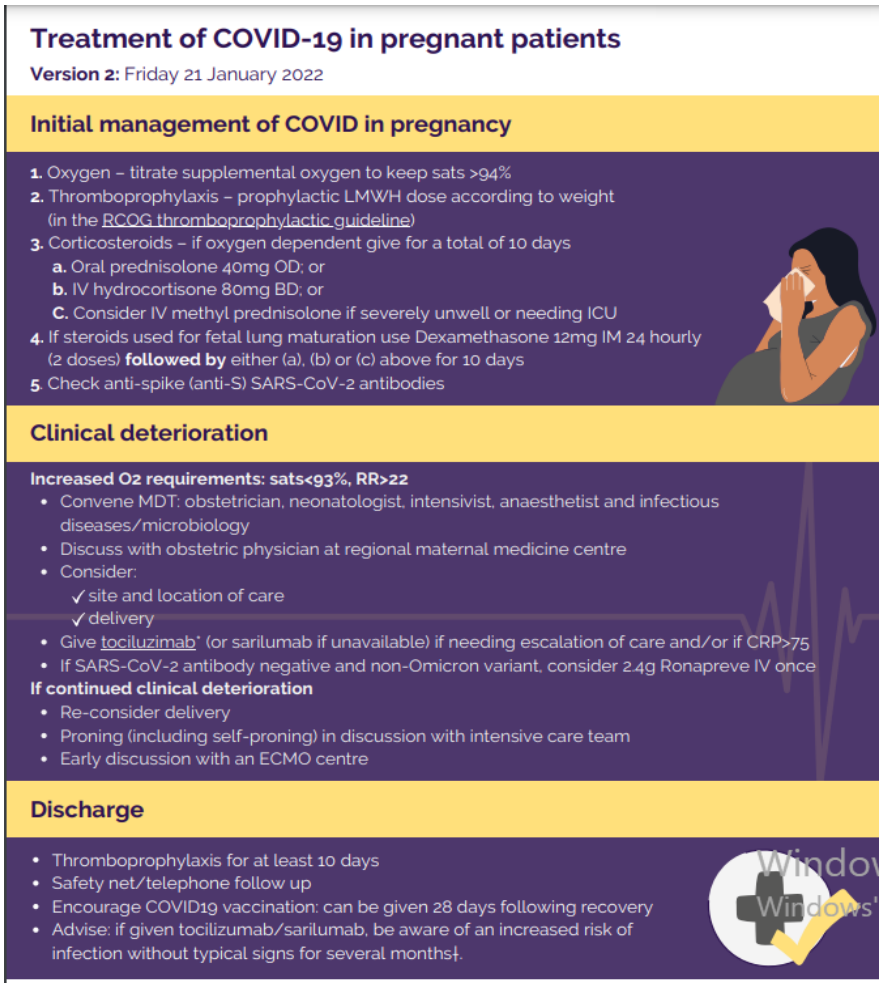


Figure 2. Treatment of COVID-19 in Pregnancy (7)

There is no definitive evidence for the type of delivery in women with COVID-19 (66). Pregnant women in the third trimester should be assess to decide whether emergency caesarean birth or induction of labour should be performed according to maternal and fetal well-being. If maternal stabilization is needed before delivery, this must be the priority, if delivery is indicated due to fetal reasons, birth must be planned as for usual obstetric necessaries. The neonatal COVID-19 infection rate is not higher at vaginal delivery, with breastfeeding, or when given to mothers after delivery (67). Water birth is not recommended to women with COVID-19 symptoms as fever, cough, difficult in breathing to enable closely monitoring (68). Spinal or epidural analgesia or anaesthesia is not contraindicated for labour but

intubation for general anesthesia significantly increases the risk of COVID-19 transmission to attending staff (69).

Iatrogenic preterm birth rate seems to be higher in symptomatic COVID-19 pregnancies (1,14,23,70). The most important reason increasing neonatal morbidity and mortality is preterm birth (71,72). It is seen that mortality and morbidity in babies born due to COVID-19 are related to these preterm births rather than COVID-19 infection itself (14). If iatrogenic preterm birth is required due to maternal or obstetric reasons, antenatal corticosteroids must be considered for fetal lung development and magnesium sulfate should be offered from 30 weeks to 34 weeks of gestation for neuroprotection of the baby (72).

International organizations such as ACOG, WHO, ISUOG and RCOG and national associations clearly state that mother-infant contact and breastfeeding should be ensured by taking necessary precautions (7).

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CHAPTER 18

THE ROLE OF VITAMINS IN THE COVID-19 PANDEMIC

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INTRODUCTION

Vitamins are undoubtedly essential elements for maintaining a healthy life. However, moving to urban life, increasing work indoors, nutrition with relatively hormonal foods, and unbalanced/insufficient nutrition have caused vitamin deficiencies in people. Thus, the importance of vitamins has begun to be noticed in societies. People were pumped into extreme fear of death at the beginning of the COVID-19 (Cov19) outbreak process. As a result of the inability to find a specific treatment for the disease, society has directed supplements that will strengthen the immune system, including vitamins. In this article, the effects of vitamins D, C, and E, which are the most using/ researched/ worth researching among vitamins during the Cov19 pandemic process, on the Cov19 disease process were investigated.

Vitamin D

Vitamin D (D^{Vit}) was previously known to be effective only on calcium and phosphorus metabolism. However today, there have been studies about D^{Vit}, from reducing blood cholesterol levels (1) to strengthening our immune system, and even prevent some types of cancer (2). In addition, it has been reported that it is necessary to increase our serum D^{Vit} [25(OH)D] level (50-80 ng/ml) to prevent cardiovascular diseases and reduce mortality (3).

The direct or indirect effect of D^{Vit} on more than 2000 gene regions on DNA (3) has led researchers to research the D^{Vit}&Cov19 relationship during the Cov19 pandemic. In addition, the Cov19 outbreak occurred during the winter months when blood D^{Vit} levels were lowest. Addition of curfews during the epidemic, the inability of people to synthesize sufficient amounts of D^{Vit} with sunlight has increased the D^{Vit} deficiency. For all these reasons, it brings to the minds of researchers that D^{Vit} may also have a role in reducing the epidemic (4).

In a cohort study in Switzerland, D^{Vit} levels were evaluated retrospectively, and it was reported that blood D^{Vit} levels were lower in Cov19-positive patients than in Cov19-negative patients (median: 24.6 ng/mL and 11.1 ng/mL; respectively) (5). As a result of the meta-analysis/review, in which the prevalence of D^{Vit} deficiency was examined in cases with a confirmed diagnosis of Cov19, the D^{Vit} levels of those with severe disease were found to be lower than those with moderate disease. They also reported a positive correlation between D^{Vit} deficiency and Cov19 severity (6,7). In a study investigating the relationship between Cov19-related deaths and D^{Vit} level, significant inverse correlations were reported between mean serum 25(OH)D values and Cov19 cases and mortality in 20 European countries (8). Yılmaz et al. compared D^{Vit} levels in 40 hospitalized children with Cov19 and 45 healthy children. D^{Vit} level was found to be significantly lower in children infected with Cov19. In addition, a negative correlation was observed between fever and D^{Vit} level in children infected with Cov19 (9). There are also reviews reporting that optimal vitamin supplementation should be taken to survive the Cov19 pandemic with minimal damage (10–14).

A good many randomized controlled clinical trials have been conducted investigating the protection of D^{Vit} supplementation against the risk of acute respiratory tract infection. In a meta-analysis of 25 randomized controlled clinical trials, it was determined that D^{Vit} supplementation reduces the risk of acute respiratory tract infections. It has been reported that the protective effect was evident in those who received daily or weekly D^{Vit} supplementation and that there was no significant effect in terms of protection in those who used bolus doses. It has also been reported that individuals with baseline 25(OH)D levels above 10.2 ng/ml have greater protection than those with less (15). Some recommendations have been presented regarding the optimal levels of D^{Vit} and its use as supportive treatment, and it has been reported that when circulating 25(OH)D levels are in the range of 40-60 ng/ml, it can both reduce acute respiratory distress syndrome and prevent the risk of infection. Although the primary goal is to draw up a support treatment plan according to the serum concentrations of individuals,

it has been recommended to take D^{Vit} as 10,000 IU/day for a few weeks and then continue with 5000 IU/day to rapidly increase serum levels in individuals at risk of influenza or Cov19. It has also been reported that higher doses can be used in the treatment of Cov19 (4). According to the clinical study to examine the effects of different doses of D^{Vit} supplementation on Cov19 treatment and the risk of Cov19-related mortality in high-risk elderly patients (NCT04344041); It has been reported that high-dose D^{Vit} supplementation may be an effective, well-tolerated, easy and immediately available treatment for Cov19, whose incidence has increased dramatically and currently has no scientifically approved treatment (16). In a cohort study, it was reported that among elderly patients with Cov19, those using a combination of magnesium, D^{Vit}, and vitamin B12 needed less oxygen support and/or intensive care compared to non-users (17). However, using the UK Biobank data, it was reported that there was no relationship between D^{Vit} levels and the risk of catching Cov19 in a study with 8297 participants followed up for about three months, but regular D^{Vit} intake may have a milder course of Cov19 symptoms (18). Again, in a study with UK Biobank data, it was reported that no evidence supports the possible role of Cov19 risk and blood D^{Vit} levels in explaining differences in the same ethnic group and/or between ethnic groups, in explaining susceptibility to Cov19 infection (19).

The main function of D^{Vit} is to play a role in calcium metabolism. Calcium plays a particularly important role in virus entry and gene expression in the infected person, while hypocalcemia is commonly observed as a common biochemical abnormality in patients with severe Cov19 symptoms. Therefore, D^{Vit}, which controls the calcium homeostasis of the whole body, can provide more benefits to Cov19 patients by maintaining calcium balance and thus reducing the severity of Cov19 (20).

The active form of D^{Vit}, $1,25(OH)_2D$, has vital importance on the immune response with its modulation effects such as lymphocyte activation and proliferation, production of tissue-specific lymphocyte and antibody isotypes, and regulation of the immune response. Effects of D^{Vit} on the immune system; It occurs by increasing anti-inflammatory cytokine levels (IL-4, IL-5, IL-10, TGF-β) by stimulating T helper (Th)2 cells and decreasing the production of pro-inflammatory cytokines (IL-2, IFN-γ) by inhibiting Th1 and Th17 cells (21,22). The cytokine storm, which occurs with excessive secretion of these cytokines, is associated with the severity of Cov19 and is reported as an important cause of Cov19-related mortality (23,24). It has been reported that D^{Vit} can prevent cytokine storm and consequent acute respiratory distress syndrome with its regulatory effect on the immune system (4,25). It has also been suggested that D^{Vit} may reduce the severity of Cov19 by increasing the expression of angiotensin-converting enzyme 2 and reducing pulmonary vasoconstriction (25).

As can be understood from the data/studies (review, retrospective, meta-analysis, clinical research) so far, the ideal level of blood D^{Vit} in patients with positive Cov19 test; It can be concluded that it reduces hospitalization, Cov19 symptoms are milder in hospitalized patients, it reduces cytokine storm in patients treated with D^{Vit}, and it achieves these effects by suppressing pro-inflammatory cytokines and increasing anti-inflammatory cytokines. It is important to have the ideal D^{Vit} level to take regular optimal D^{Vit} supplements and/or to sunbathe at appropriate times, to have a healthy life/aging, and to be protected from other diseases, including the Cov19 pandemic process.

Vitamin C

Vitamin C (C^{Vit}) is a water-soluble vitamin and is found in fresh fruits and vegetables. It is necessary to take it (especially in humans) to maintain normal metabolic activities healthily. In addition, C^{Vit} is not stored in the body like fat-soluble vitamins, so it should be taken daily and is also known as ascorbic acid. Ascorbic acid is an effective antioxidant with high electron donor and active reducing power. There is compelling evidence that ascorbic acid acts as an important antioxidant in many body tissues. The recommended daily intake for C^{Vit} is 9 - 110 mg/day, depending on age, physiological status, and gender, and the upper tolerable intake is 2 g/day. It is an important vitamin for the innate and adaptive immune system, with the role of a potent antioxidant and a cofactor for the C^{Vit} family of gene regulatory enzymes (26).

C^{Vit} is abundant in phagocytic cells (such as neutrophils). It can increase the formation of chemotaxis, phagocytosis, and reactive oxygen species, especially microbial defense in these cells. C^{Vit} ensures that the neutrophils that have fulfilled their function in the infection areas are removed from the environment, thus, it plays a role in reducing tissue damage. In addition, C^{Vit} can provide immune activation by activating NK and monocytes, and differentiation and proliferation of B and T lymphocyte cells. C^{Vit} deficiency causes impaired immune activation and higher susceptibility to infections. C^{Vit} supplementation can both prevent and treat respiratory and systemic infections [27]. However, there are also statements reporting that the therapeutic role of C^{Vit} supplementation in the treatment of cold infections is not clear. In one study, 6-8 g/day C^{Vit} supplementation was effective in the treatment of common cold symptoms, but low doses (3-4 g/day C^{Vit}) may have been given in studies reporting that it had no effect. (27). In addition, it has been reported that it can also be protective against viral infections via T cells (28).

In a study on the use of C^{Vit} in the treatment of Cov19, it has been reported that C^{Vit} reduces oxidative stress, and inflammation increases vasopressor synthesis, increases immune cell function, increases endovascular function, and provides epigenetic immunological modifications with its antioxidant activity (29). A meta-regression analysis (8 studies - n: 685) found that C^{Vit} shortened the length of mechanical ventilation by an average of 14%. In addition, according to the results of the study requiring more than 10 hours of ventilation (5 studies: 471), it was determined that 1-6 g/day C^{Vit} supplementation shortened the ventilation time by 25% on average (30). However, high-dose C^{Vit} supplementation may create some physio pathological conditions. Although immune effector cells are dependent on glycolysis for their bioenergetic functions, lung epithelial cells use mitochondrial oxidative phosphorylation to produce ATP. Therefore, intravenous (IV) high-dose C^{Vit} therapy may produce a prooxidant effect for immune cells, while an antioxidant effect for lung epithelial cells. Patients diagnosed with Cov19 and hospitalized with respiratory distress and abnormal inflammatory biomarkers can be given high-dose IV C^{Vit} therapy for a short time in the early stages of the disease. However, IV glucocorticoid therapy should be added to reduce possible prooxidant complications of IV high-dose C^{Vit} therapy (31).

Milani et al. (2021) reviewed many studies in their meta-analysis, from case reports to clinical studies. As a result of these examinations, they reported that the necessary data to reach a healthy result is missing, the results in clinical studies are not yet complete, and there has been no effective study on the prevention of Cov19 with C^{Vit} supplementation. Researchers have reported improvement in the medical condition of Cov19 patients treated with C^{Vit} in some clinical observations, but data from controlled studies are scarce and inconclusive. However, based on the theoretical background presented in one study and some preliminary encouraging studies, it has been reported that the role of C^{Vit} in the treatment of patients with Cov19 should be investigated (32). Rawat et al. (2021), in their meta-analysis (6 studies, n: 572), evaluated C^{Vit} supplementation in terms of mortality, intensive care/hospital stays, and need for ventilation, and reported that there was no difference between the groups in terms of C^{Vit} supplementation (33). Some studies do not recommend routine use of C^{Vit} due to conflicting results (34).

Vitamins support human health by inducing immune activation at an optimum level. An optimum intake of vitamins with diet, enriched foods, or as supplements shows immunomodulatory effects on various immune cell types, including monocytes, dendritic cells, lymphocytes, and natural killer (NK) cells, which provide immunity against factors that threaten the organism (35). It is known that C^{Vit} is undoubtedly necessary for maintaining a healthy life, but when the studies have done so far are evaluated, it is early to evaluate that C^{Vit} supplementation may be beneficial in the treatment of Cov19 disease (22). In addition, attention should be paid to whether the administered dose will be toxic.

Vitamin E

Vitamin E (E^{Vit}) is a general term describing structurally related tocopherols and tocotrienols, each called alpha, beta, gamma, and delta. Alpha-tocopherol has the highest bioavailability in the human body and meets the E^{Vit} requirement. It is represented in varying proportions in fat-rich foods such as dietary oils and seeds or found mainly as α-tocopherol in fortified foods (36).

E^{Vit} is a fat-soluble antioxidant that can protect polyunsaturated fatty acids in the cell membrane from oxidation, regulate the production of reactive oxygen species and reactive nitrogen species, and modulate signal transduction. E^{Vit} has been shown to enhance immune responses and protect against a variety of infectious diseases in animal and human models. Suggested mechanisms for these changes; It is reported that inhibition of COX2 activity and reduction of prostaglandin E2 production as a result of decreased nitric oxide production, initiation of cell activation signals, increasing the efficiency of immune synapse formation in naive T cells, and modulation of Th1/Th2 balance. It has been reported that with E^{Vit}, changes such as higher NK activity and lower IL-12 production and migration in dendritic function (28). In addition, different forms of E^{Vit} exert different effects on immune cells (37). In experimental animal models, it is reported that when E^{Vit} is applied together with the existing treatment (erythrocyte, heart, and liver tissue) it protects from oxidative damage, that is, it strengthens the treatment (38,39).

Agler AH et al. (2011) found in a randomized controlled trial (approximately 36,000 participants) that supplementation of 600 IU of E^{Vit} every other day resulted in a 10% reduction in the risk of chronic lung disease in women (40). In addition, C^{Vit} and E^{Vit} supplementation before the surgical procedure resulted in a reduction in the incidence of acute respiratory distress syndrome and pneumonia in critical surgery patients and was associated with less hospitalization, mechanical ventilation, mortality rates, and reduction in proinflammatory cytokine levels (41). Again, studies are reporting that E^{Vit} support reduces the risk of respiratory tract infection (42), and there are data with opposite results (43).

Shakoor H et al (2021) examined the relationship between Cov19&vitamins (211 studies) in their review article. Researchers wanted to determine the effective dose of C^{Vit}, D^{Vit}, E^{Vit}, zinc, and omega-3 fatty acids to protect individuals against Cov19 or alleviate symptoms. They have reported that further research is needed for this (44). In addition, there are other studies with similar results (22).

Although it is thought that E^{Vit} has anti-inflammatory properties, activates Type I IFN, and exerts an antiviral effect (22), and that long-term use at certain doses protect against pneumonia (45), there are inconsistent results in this regard. Further clinical studies are needed to determine whether it can be used in addition to healthier and clearer results and/or existing treatment.

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CHAPTER 19

COVID-19 AND THE ENDOCRINE SYSTEM

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INTRODUCTION

The coronavirus illness 2019 (COVID-19) pandemic continues to exert a big impact on international health care systems, inflicting devastating mortality and morbidity. SARS-Cov-2 infection affects a variety of organs and biological systems, either directly or indirectly through virus-induced damage that can have systemic consequences (1).

As we learn more about this new respiratory virus, it becomes obvious that its impacts go beyond the respiratory system. The angiotensin-converting enzyme 2 (ACE2) receptor allows the COVID-19 coronavirus, severe acute respiratory syndrome coronavirus 2, to gain cellular access. The presence of the ACE2 (Angiotensin-converting enzyme 2) receptor in various organs, which is thought to be at the heart of COVID-19 pathophysiology, is ascribed to these broad symptoms (2). Similarly, expression of the ACE2 receptor has additionally been rumored in numerous endocrine tissues as well as the neural structure, pituitary, thyroid, gonads, and pancreatic islets (3). Therefore, it's imperative to grasp the manner COVID-19 will alter the operation of those tissues and cause pathology, particularly considering the shut interaction between varied endocrine systems as a part of the RAAS (renin-angiotensin-aldosterone system) pathway and therefore the central role of ACE2 during this pathway (4).

After entering an endocrine gland and engaging the ACE2 receptor, the SARS-CoV-2 virus may cause harm through thrombosis and hypoxia, both of which are common in highly vascularized organs like the pituitary. Immune response to the infection and cytokine storm are two further pathways that may be involved (3). Endocrine gland illnesses associated with SARS-CoV-2 infection are primarily produced by three mechanisms: direct virus infection of the endocrine gland, inflammation-mediated activation of the hypothalamo-pituitary axis, and immune-mediated cell lesions (5).

Two years into the COVID-19 pandemic, it is clear that it is a developing clinical scenario in which endocrinologists have found themselves heavily involved not only in fighting the pandemic on the front lines but additionally in describing endocrine and metabolic phenotypes of the illness together with potential endocrine consequences of vaccination efforts (6,7).

COVID-19 can affect the operation of many endocrine glands and metabolic processes, putting individuals at risk of endocrine or metabolic dysfunction, whether acute or late-onset. A lot of fresh data has arisen in the previous two years with the goal of improving our understanding of COVID-19, its symptoms, and management. COVID-19 endocrine manifestations: current knowledge several endocrinopathies have been documented in COVID-19 infected patients. Hypopituitarism, SIADH, central diabetes insipidus, thyroiditis, thyrotoxicosis, hypothyroidism, low T3 syndrome, hyperglycemia, adrenal insufficiency, orchitis, and sperm morphological changes are just a few of them. The majority of the data comes from single case reports and case series. Pre-existing endocrine problems or metabolic processes can further raise the risk of developing COVID-19 or having a more severe clinical presentation and outcome (Figure 1)(8).

Several clinical inquiries are required since the endocrine system is a concern during the COVID-19 pandemic. It should be determined whether (1) COVID-19 patients are at a higher risk of developing acute or late-onset endocrine diseases or dysfunction (2); underlying endocrine diseases or dysfunctions are risk factors for poor prognosis once the infection has occurred (3); pandemic-related community and healthcare service restrictions and reorganization may contribute to changing the epidemiology of endocrine diseases or dysfunctions or affecting their management (1-3,9).

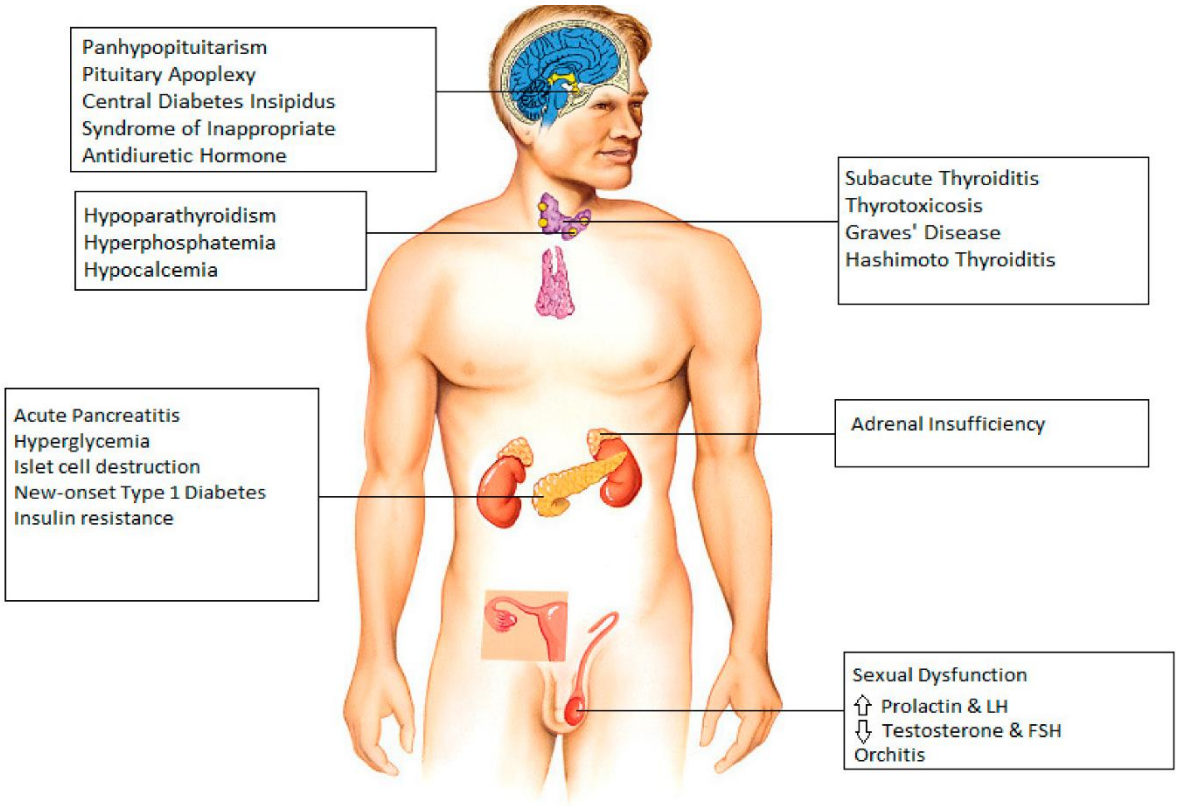

Figure 1: Endocrine manifestations are seen after infection with COVID-19 (8).

Obviously, these effects occur often, particularly at the thyroid level, more than a year and a half after the epidemic began. They have the potential to affect the outcome of SARS-CoV-2 infection. As a result, it's critical to understand their pathophysiology and evolution. even supposing these complications seldom need specific treatment, endocrinologists have to be compelled to be able to acknowledge them further on support the physicians concerned within the front-line care of COVID-19 patients, or within the detection and management of those complications, particularly during the acute phase of the disease (9).

While early case reports first indicated a potential clinical impact on the endocrine system, a larger body of research now describes the effects of COVID-19 on pituitary, thyroid, adrenal, gonadal, and pancreatic endocrine function. However, the contribution of endocrine dysfunction to the symptoms experienced by patients with COVID-19 remains to be fully elucidated. Endocrine disorders are eminently treatable, and their diagnosis and management can result in significant improvements in health and quality of life. Thus, in this review, we appraise the available data investigating the impact of COVID-19 on the endocrine system to aid clinicians in instituting appropriate investigation and management of affected patients (4). We are dedicating this chapter to outline these new discoveries, based on the ubiquitous involvement of the endocrine system in numerous forms of COVID-19, as well as the knowledge collected over the previous two years.

Obviously, these effects occur often, particularly at the thyroid level, more than a year and a half after the epidemic began (10). Obesity, cardiometabolic dysfunction, and type 2 diabetes are now well recognized as significant risk factors for poor SARS-CoV-2 infection outcomes (extended hospital stays, mechanical ventilation, and death). While the focus has been on overt obesity (as measured by BMI), the metabolically unhealthy phenotype (characterized by dystopic fat deposition outside of the normal storage space in subcutaneous adipose tissue, as well as dysregulated adipose tissue distribution intra-abdominally and in organs such as the liver, resulting in NAFLD and abnormal metabolic markers) appears to contribute to poor COVID-19 outcomes, regardless of marked obesity. Although the exact mechanisms linking metabolic dysfunction to a poor clinical course are unknown, it

is likely that low-grade tissue and systemic inflammation contributes to the viral-induced inflammatory response, resulting in cytokine storm, hypercoagulability, and multi-system dysfunction. In addition to promoting vaccination and strategies to reduce SARS-CoV-2 exposure, close and proactive clinical management with regard to proper nutrition, weight management, pharmacotherapy, and psychosocial issues is required, with the potential to significantly reduce SARS-CoV-2 morbidity and mortality (11). For these reason in the first chapter, thyroid, diabetes and obesity will be summirized.

1. THE EFFECTS OF COVİD-19 ON THYROİD GLAND

1.1.General information :

Thyroid glands have been found to be a target organ of COVID-19 in preclinical and clinical trials. It is now obvious that these effects occur often, particularly at the thyroid level, more than a year and a half after the epidemic began. They have the potential to affect the outcome of SARS-CoV-2 infection. As a result, it's critical to understand their pathophysiology and evolution. Despite the fact that these complications rarely require treatment, endocrinologists must be able to recognize them and support physicians who are involved in the front-line care of COVID-19 patients, or in the detection and management of these complications, particularly during the acute phase of the disease (10).

Thyroid problems and hormonal alterations result from COVID-19's connection with the thyroid gland (and HPT axis). COVID-19 can cause subacute thyroiditis, autoimmune thyroiditis, and an unusual form of thyroiditis. Thyroid hormone deficiency has an impact on the outcome by increasing mortality in critical conditions such as acute respiratory distress syndrome (ARDS), which is a common consequence of COVID-19 (Table 1) (12,13).

Based on the results of the SARS-CoV-2 pandemic, more attention should be paid to individuals with undiagnosed thyroid disorders as well as COVID-19-treated thyroid patients. Low T3 syndrome, often known as "non-thyroidal sickness syndrome" (NTIS), and thyroiditis are the most common thyroid illnesses seen in COVID-19 patients (12,14).

Sick euthyroidism is the most common thyroid-related issue in COVID-19 care, especially in hospitalized patients and intensive care units. In critically unwell patients or individuals with a severe nutritional deficit, NTIS is characterized by a drop in circulating free T3 (in the most severe cases, TSH and free T4 are also lowered), but thyroid function remains normal. Changes in deiodinase function, changes in thyroid hormone transport proteins, and TSH release from the anterior pituitary are all part of the pathophysiology of NTIS, which results in low free T3. In patients with NTIS, supplementation with levothyroxine or T3 therapy shows little benefit (15).NTIS can be found during SARS-CoV-2 infection, as previously stated. Low circulating free T3 is linked to illness severity and a worse prognosis, as well as an increase in IL-6 concentration (16,17).

Table 1. Summary of findings regarding the relationship between thyroid and COVID-19 (13).

- The thyroid has higher amounts of ACE2 and TMPRSS2 expression than the lungs.
- Thyroid gland inflammation may be caused by abnormal immune responses and a cytokine storm linked to COVID-19.
- The changes in the thyroid gland and HPT axis could be explained by two processes (indirect and direct).
- Thyroid illnesses linked to COVID-19 include thyrotoxicosis, hypothyroidism, and nonthyroidal sickness syndrome.
- COVID-19-related SAT is similar to classical SAT and can occur before or after COVID-19.
- In individuals hospitalized for COVID-19, thyrotoxicosis without neck pain is common.

- Low TSH and T3 levels, as well as thyrotoxicosis, appear to be predictive of poor COVID-19 patient outcomes.
- Thyroid cancer treatment plans are shifting toward more teleconsultations and less diagnostic and therapeutic procedures.
- More research is needed to investigate the effect of limiting scheduled clinical activities on the outcomes of thyroid cancer patients, as well as whether thyroid cancer (or treatment-specific factors) enhance vulnerability to COVID-19.
- ACE2, Angiotensin-converting–enzyme 2; TMPRSS2, transmembrane protease serine 2; HPT, hypothalamic–pituitary–thyroid; SAT, subacute thyroiditis

Destructive" or inflammatory thyroiditis arises early in the course of COVID-19 infection, according to documented cases of thyroiditis. It is clinically asymptomatic, although lymphopenia is present, and it corresponds with infection severity. Subacute thyroiditis develops later in the infection and is characterized by pain in the anterior cervical region and a total blood count finding of hyperleukocytosis. Patients' thyroid ultrasounds frequently revealed an enlarged, heterogeneous, hypoechoic thyroid with decreased vascularization. These patients' thyroid radio-iodine scans revealed decreased thyroid uptake, indicating subacute thyroiditis.

Thyrotoxicosis was mostly treated symptomatically with beta-blockers, although 70% of patients also received low-dose steroids for a few weeks, while some were given non-steroidal anti-inflammatory medicines (18).

Following COVID-19 infection, a few patients with primary or recurrent Graves' disease have been described (19). Autoimmune hypothyroidism, often known as Hashimoto's thyroiditis, can develop months or years after a subacute viral thyroiditis episode. It would be interesting to monitor thyroid function in these patients in the future (17).

The degree of COVID-19 appears to be the most important factor of the type of thyroid injury that occurs. While destructive thyrotoxicosis with neck pain (classical subacute thyroiditis) is most common during or shortly after mild COVID-19, thyrotoxicosis without neck pain (possibly in the context of the nonthyroidal illness syndrome) may characterize more severe and critical cases of COVID-19 pneumonia. Some signals of hormonal alterations (i.e. low T3 and TSH concentrations) and overt thyrotoxicosis to be regarded as predictors of poor COVID-19 outcome (i.e. longer length of hospital stay and higher death) are already emerging outside the COVID-19 scenario (Table 2) (20, 21).

In an endocrinology outpatient clinic, patients with thyroid issues are regularly seen. Although there is no indication that people with uncontrolled hyperthyroidism are more prone to catch a viral infection in general, it is probable that patients with uncontrolled hyperthyroidism are at a higher risk of consequences (such as thyroid storm) caused by any virus.

In summary, thyroid function may be disrupted acutely in a proportion of COVID-19 patients, either by subacute thyroiditis (which may present atypically, lacking the typical neck pain and lymphocytosis), NTIS or even by triggering the autoimmune disease, despite the fact that the majority of patients are euthyroid. Large long-term studies are limited; nevertheless, current evidence suggests that with cautious care, thyroid function returns to normal. The clinical significance of such thyroid function abnormalities may thus be primarily related to their reflection of more severe disease and a worse prognosis after acute COVID-19 presentation (4).

Table 2. Summary for managing the patients with thyroid disorders (21).

• Patients with hypothyroidism or hyperthyroidism should continue to take their medications as prescribed. The standard recommendation of increasing levothyroxine dosage during pregnancy should be followed (21).
• Thyroid function tests are routinely monitored on a regular basis in the treatment of hyperthyroidism. If biochemical monitoring is not possible owing to unforeseen circumstances, a block-and-replace regimen would be the best option for newly diagnosed hyperthyroidism patients (21,22).
• Anti-thyroid medications (ATDs) should be mentioned separately. Agranulocytosis has been linked to the use of ATDs, while this is a rare occurrence; agranulocytosis may increase the chance of COVID-19 development (21,22).
• Agranulocytosis symptoms (fever, sore throat, oral ulcers) frequently coincide with mild COVID-19 symptoms (fever, cough, headache), making clinical differentiation between the two challenging (21, 22).
• If a patient on ATD develops symptoms suggestive of agranulocytosis, the ATD should be stopped promptly and a full blood count obtained as soon as possible. If a blood count is not possible due to a severe lack of healthcare resources, the ATD might be stopped and reintroduced after one week if the symptoms improve (21, 23)
• Urgent surgery or radioactive iodine ablation may be used in certain cases of uncontrolled thyrotoxicosis that does not respond to medicinal treatment (21).
• Furthermore, patients with COVID-19 presenting with conjunctivitis may pose diagnostic challenges in those with new-onset or established thyroid-associated orbitopathy (TAO) (21, 22). Patients with TAO who are taking glucocorticoids and/or mycophenolate mofetil, on the other hand, are likely immunocompromised and should take particular measures against COVID-19 (21).
• Patients with metastatic thyroid cancer who have lung metastases may be at a higher risk of viral infection or consequences, and should be more vigilant (21,24).

1.2.Recommendations Based on Available Evidence

I. Patients with COVID-19 with normal thyroid function assessed during the acute phase of the disease do not need a routine reestimation of thyroid function on follow-up (25).
II. Patients with COVID-19 with biochemically documented euthyroid sick syndrome during the acute phase of the disease may undergo a TFT performed at 6 weeks after discharge (25).
III. Patients with COVID-19 in whom thyroid function was not estimated during the acute phase of the disease do not require a routine assessment of thyroid function on follow-up, even in those complaining of persistent fatigue (25).
IV. Patients with COVID-19 with biochemically documented subclinical hypothyroidism during the acute phase of the disease should undergo a TFT 3 months after discharge. An anti-TPO antibody assay should be performed if not found to be positive during the acute phase of COVID-19 (25).
V. Patients with COVID-19 with biochemically documented hyperthyroidism/subclinical hyperthyroidism/SAT during the acute phase of the disease should undergo a TFT performed at 6 weeks after discharge. An anti-TPO antibody assay should be performed if not found to be positive during the acute phase of COVID-19. Even if the thyroid function at 6 weeks is normal, a repeat test should be performed at 12 weeks to rule out the possibility of postthyroiditis hypothyroidism (25).
VI. Patients with COVID-19 with biochemically documented overt hypothyroidism during the acute phase of the disease should undergo a TFT performed at 6 weeks after discharge while on levothyroxine supplementation. An anti-TPO antibody assay should be performed if not found to be positive during the acute phase of COVID-19 (25).
VII. Patients who have recovered from COVID-19 complaining of neck pain, weight loss, resurgence of fever, and/or palpitations should be suspected of having SAT. A TFT should be immediately performed, and if suggestive of thyrotoxicosis, a radionuclide thyroid uptake scan using technetium-99m may be ordered (if facilities are available). A combination of high erythrocyte sedimentation rate (and/or C-reactive protein levels) and poor radionuclide uptake by the thyroid gland is diagnostic of SAT. A thyroid-stimulating immunoglobulin assay may be ordered when Graves' disease is suspected (25).

2. EFFECTS OF COVID-19 ON PANCREAS AND DIABETES MELLITUS

2.1.General information :

SARS-CoV-2 infection is linked to pancreas impairment, according to evidence. The processes implicated in this include, but are not limited to, SARS-CoV-2 replication's direct cytopathic effect and systemic and local inflammatory responses (26).At this time, it is certain that the virus targets the endocrine component of the pancreas, as well as the exocrine portion to a much lesser amount. COVID-19 and diabetes have been proven to have a bidirectional association; indeed, diabetes is linked to COVID-19 severity and death, but patients with COVID-19 have also demonstrated new-onset diabetes (27). The SARS-CoV-2 virus not only impacts glycemic levels directly but also exacerbates hyperglycemia that already exists due to its unfavorable impact on the functional competence of the islets of Langerhans. It is impossible to rule out the possibility that the bad side effects of the drugs used to treat the infection are the true cause of exocrine dysfunction in this gland. As the epidemic worsens, extra emphasis should be paid to the examination of chronic and acute pancreatic disorders, including pancreatic cancer, so that treatment can be started sooner (28).

Diabetes mellitus (DM) is one of the most common chronic diseases in the world, affecting around 9.3% of the worldwide population and is predicted to rise in the next years. Diabetes is significant comorbidity to consider during the COVID-19 pandemic due to its high prevalence in the general population. Diabetes has been linked to an increase in infection susceptibility, particularly in the respiratory tract. Prior coronavirus epidemics with the severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory sickness (MERS) demonstrated this (MERS-CoV)(29, 30). There is also

evidence of an increased incidence of COVID-19 in diabetic patients. Primary prevention of COVID-19 infection requires proper blood glucose and blood pressure management. Hyperglycemia impairs innate immunity, causing phagocytosis, cell-mediated immunity, and neutrophil chemotaxis to malfunction. ACE2 expression, which is the COVID-19 viral binding site for host cell entrance, is likewise affected by high blood glucose levels. The increased incidence of COVID-19 infection in diabetic patients is likely to be due to this (31-34).

Coronavirus infections have been shown to have a significant impact on the management of diabetes mellitus because they exacerbate inflammation and modify immune system responses, making glycemic control challenging. In patients with diabetes mellitus, SARS-CoV-2 infection increases the risk of thromboembolism and is more likely to cause cardiorespiratory failure than in people without diabetes mellitus. All of these pathways are now thought to play a role in the poor prognosis of diabetic patients with COVID-19. For individuals with diabetes mellitus, strict glycemic control and management of cardiovascular risk factors are critical during the COVID-19 pandemic. For patients at high risk of SARS-CoV-2 infection, medications for diabetes and cardiovascular disease should be changed accordingly (35).

There is a clear link between diabetes and COVID-19. On the one hand, persons with diabetes have worse outcomes due to a slew of other illnesses that increase their risk. SARS-CoV-2, on the other hand, may cause new-onset diabetes or maintain hyperglycemia throughout hospitalization due to its -cell tropism. -cell dysfunction, along with an inflammatory cytokine storm and counter-regulatory hormonal responses, can lead to more severe metabolic problems (DKA or HHS). COVID-19 results can be exacerbated by new-onset diabetes, hyperglycemia during admission, and abrupt metabolic decline (36) The innate immune system is predicted to be strengthened by good glucose control, which will help ward off problems (37).

In the critical care situation, hyperglycemia is related with a longer ICU stay, a higher demand for mechanical ventilation, and a higher risk of mortality.) People with COVID-19 diabetes have a worse prognosis and mortality. Given the significant incidence of diabetes around the world, these people make up a sizable portion of the COVID-19 population. Hyperglycemia, older age, comorbidities, and in particular hypertension, obesity, and cardiovascular disease all contribute to a poorer prognosis in people with diabetes (figure). However, the picture becomes more difficult when societal issues such as deprivation and ethnicity are taken into account, as well as those that become significant when a patient with severe COVID-19 needs to be handled. A physician must combine carefully glucose-lowering medications with specialized treatments for the viral infection, taking into account not just the health status of the diabetic patient, but also the patient's overall health (36).

When compared to individuals without diabetes, those with diabetes may be more susceptible to SARS-CoV-2 infection and are more likely to develop a severe or critical illness course. As a result, vigilant vigilance and testing in outpatient endocrine clinics, as well as early hospitalization for COVID-19, are indicated in these individuals (31,38). Outpatient medical therapy should be tailored to achieve a plasma glucose goal of 72 to 144 mg/dL (90-144 mg/dL in the fragile or elderly) and a glycated hemoglobin A1c (HbA1c) level of less than 7% in order to prevent infections (39). Also, patients with COVID-19 have worse outcomes when their glycemic control is inadequate at the time of admission and during their hospital stay. Glycemic control appears to be linked to outcomes, according to new research. Thus, prior to the introduction of COVID-19, the final A1c level was reported to have a linear connection with outcomes, notably death, when A1c was > 10% (40).

Furthermore, SARS-CoV-2 has a direct effect on -cell function and survival, resulting in a quick and severe loss of metabolic control in people with pre-existing diabetes or the development of new-onset diabetes in those without diabetes. Glycaemic control should be ensured in persons with hyperglycemia to lower the risk of life-threatening metabolic complications, and this should include all treatment maneuvers implemented to reduce the risk of severe outcomes and mortality. Finally, the usage of

various glucose-lowering medications in the setting of COVID-19 should be considered for achieving and maintaining glycaemic control (36).

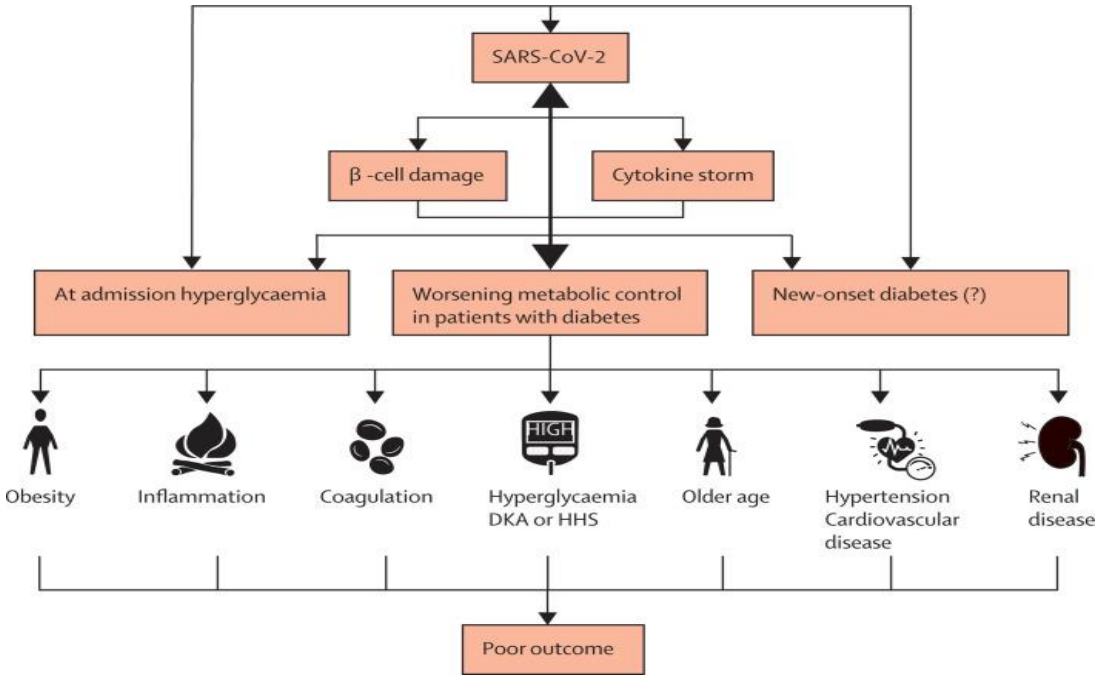

Figure 2. Synopsis of the reciprocal effects of diabetes and COVID-19 (36).

COVID-19-positive diabetics have a poorer prognosis and death rate. These people make for a substantial susceptible portion of the COVID-19 population, given the increasing incidence of diabetes over the world. Hyperglycemia, older age, comorbidities, and in particular hypertension, obesity, and cardiovascular disease all contribute to a poorer prognosis in people with diabetes (Figure 2). However, the picture becomes more difficult when socioeconomic issues like deprivation and ethnicity are taken into account, as well as characteristics that become significant when a patient with severe COVID-19 needs to be handled. A physician must combine carefully glucose-lowering medications with specialized treatments for the viral infection, taking into consideration not just the person's health but also the person's health state (36). Diabetes care in COVID-19 patients is a significant clinical challenge, one that necessitates a well-coordinated team approach, since this is an essential strategy for minimizing the risk of medical complications and mortality. Careful evaluation of the multiple factors that contribute to poor prognosis in diabetic individuals with COVID-19 may be the best, if not the only, approach to solve the current predicament and enable our health systems to respond quickly and effectively to any future issues (Figure 2) (36).

Finally, the link between diabetes and COVID-19 should prompt more research into the extent to which the virus's specific mechanisms (e.g., its pancreatic-cell tropism) may contribute to worsening glycemic control and, in some cases, the emergence of diabetic ketoacidosis or hyperglycaemic hyperosmolar syndrome, as well as the emergence of new-onset diabetes (36).

However, as a result of the ongoing pandemic, physical activity restrictions, dietary changes, limited access to anti-diabetic drugs, and a lack of in-clinic follow-ups are predicted to have a negative impact on glycemic control. A lot of the issues may be overcome with the right diabetes self-management education (DSME). Physicians can deliver DSME to homebound diabetes patients via online media and emphasize the need of a nutritious diet and an active lifestyle (41). Insulin may be a useful option for individuals with poor glycemic control, but it would be difficult to educate them about insulin injection techniques under the current circumstances. Capillary blood glucose self-monitoring should be

continued at home, especially for insulin-dependent patients, and appropriate teleconsultations should be sought during recurring episodes of hyperglycemia or hypoglycemia (42). Patients with type 1 diabetes should be well informed about sick-day guidelines and the fact that failing to take insulin can be lethal. The psychological well-being of diabetic patients is frequently overlooked, and it is likely to be negatively impacted in the current day. Meditation, teleconsultations with psychiatrists, and avoiding undue stress by watching, reading, or listening to news regarding COVID-19 could all be beneficial (21).

If the patient eats and drinks adequately and a more frequent blood glucose-monitoring regimen is instituted in the presence of mild COVID-19 in an out-patient setting, normal glucose-lowering therapy for diabetic individuals could be resumed (39). Patients hospitalized to the hospital with severe COVID-19 may require changes to their diabetic care, such as stopping current medications and starting insulin therapy. The severity of COVID-19, nutritional state, actual glycaemic control, risk of hypoglycemia, renal function, and pharmacological interactions should all be considered when making such a decision (36).

2.2.Glycemic targets in hospitalized patients with COVID-19

In hospitalized patients, regardless of the reason for admission, the ADA advises a blood glucose goal range of 140–180 mg/dL (7.8–10 mmol/L) to avoid both hyperglycemia and hypoglycemia. For senior adults with a high risk of hypoglycemia, it has often been thought that more relaxed control targets should be followed. In the inpatient care context, a BG of 180 mg/dL (10 mmol/L) may be acceptable, especially if frequent glucose monitoring and intensive nurse observation are not possible (43).

2.3.Therapeutic strategies in hospitalized patients with T2D or no previous diabetes

Insulin is the first-line medication for glycemic management because of its potency and ability to titrate quickly. For non-critically ill hospitalized patients, an aggressive insulin regimen including subcutaneous basal and prandial insulin is the best option (43). To avoid both hypoglycemia and hyperglycemia, an initial total daily dosage of insulin of 0.4–0.6 IU/kg of body weight is advised (44). However, in patients over 70 years old or with an estimated creatinine clearance < 60 mL/min, the dose may be reduced to 0.2–0.3 IU/Kg of body weight. The entire daily dose of insulin should be evenly distributed before each of the three main meals, with 50% of basal insulin and 50% of short-or-rapid insülin (45). Notably, insulin needs differ from person to person, and individuals using GCs may require greater dosages of 1.2 to 1.5 IU/kg each day (38). In noncritically ill hospitalized patients with inadequate oral intake, basal insulin only or basal plus bolus regimes are preferable (43). The basal insulin should not be withheld even if the patient is fasting. A full table for adjusting prandial insulin doses based on blood glucose levels can be found elsewhere (45). Sliding –scale insulin is not suggested as a sole regimen since it is a reactive approach that only supplies insulin once hyperglycemia has occurred (43).

2.4.Therapeutic strategies in hospitalized patients with T1D

Insulin should be given to all T1D patients. Those who have been getting mixed insulin at home should switch to a basal bolus regimen, which minimizes the risk of hypoglycemia. Hypoglycemia risk is reduced by reducing the typical at-home fast insulin dose by 20–30%, especially in those with low appetite. The initial total daily dose of insulin for insulin-naive patients should be between 0.3 and 0.6 IU/kg, depending on the degree of hyperglycemia. The entire daily dose of insulin should be evenly distributed before each of the three main meals, with 50% of basal insulin and 50% of short-or-rapid insülin (46). To lower the risk of metabolic decompensation leading to events like diabetic ketoacidosis (DKA) or hypoglycemia, more thorough BG monitoring and supportive medication is required. If hyperglycemia or fever develops, blood or urine ketones should be tested (1,39).

2.5.Therapeutic strategies in critically ill patients

Insulin is the most effective treatment for glucose management in critically sick patients, and the danger of hypoglycemia should be considered. To minimize poor clinical consequences, increased

vigilance, identification, treatment, and close monitoring of hyperglycemia should be performed. Intravenous infusion based on approved local protocols and modified according to BG until stable glycemic control is obtained should be pursued even in patients with T2D (43, 47). BG should be checked every 1–2 hours in severely ill patients who are receiving intravenous insulin. BG readings can be tested at longer intervals, such as every 4 hours, after they are stable and within the target range (48). Managing hyperglycemia in very ill patients in isolation, especially with limited staff and personal protective equipment, is one of the most difficult tasks for healthcare organizations. According to the ADA, employing electronic support to build protocols and organized order sets may help to enhance BG control (49). Secondary bacterial infection is a major issue in extremely unwell patients with diabetes and COVID-19. As a result, monitoring markers including ferritin, complete blood count, and C-reactive protein that signal worsening inflammation may be beneficial (50).

2.6. Non-insulin medications

Non-insulin hypoglycemic medicines are generally not suggested for hospitalized patients, but some medications have been investigated and may be used in certain scenarios in COVID-19 and T2D patients (47). Oral medications can be utilized in hospitalized patients with T2D, especially in those with mild COVID-19, as long as there are no contraindications and they do not cause hypoglycemia (51). It's worth noting that all experts agree that insulin should be kept on hand at home for people with T1D or T2D. Furthermore, severe conditions like COVID-19 may necessitate a higher insulin dosage (52). Some hypoglycemic medications, such as glucagon-like peptide-1 receptor agonists (GLP-1RA), dipeptidyl peptidase-4 inhibitors (DPP4i), and a thiazolidinedione (pioglitazone), can theoretically enhance results in COVID-19 patients because to their anti-inflammatory actions. Indeed, a worldwide retrospective analysis of non-hospitalized COVID-19 patients adjusted for many covariates revealed that maintaining these medications was associated with better results than quitting them (53). Because this data is observational and has not been validated, caution is advised to avoid over-interpretation.

2.7.Use of antidiabetic medications in patients with T2DM and COVID-19

The severity of Coronavirus Disease 2019 (COVID-19) is determined using the WHO clinical progression scale (54). Insulin is mostly prescribed for critically ill diabetic individuals who are infected with coronavirus 2 (severe acute respiratory syndrome) (SARS-CoV-2). Optimal glucose control with insulin infusion lowered inflammatory cytokines and improved COVID-19 severity in a statistically meaningful way (55). Uninfected patients with type 2 diabetes mellitus (T2DM) or ambulatory patients with mild COVID-19 can use metformin. It should be highlighted, however, that metformin should not be used in critically unwell patients (35).

Sulfonylurea is safe to use in people with T2DM who are not infected with COVID-19, however it is not recommended in patients who have severe COVID-19 since it can cause hypoglycemia. Thiazolidinediones have the ability to mediate cardiovascular system protection (56). Thiazolidinedione treatment, on the other hand, causes weight gain and oedema, as well as aggravating heart failure (57). These findings suggest that it should not be used in patients with severe COVID-19. Inhibitors of dipeptidyl peptidase 4 (DPP4) are one of the most commonly given drugs with few major side effects. In earlier cardiovascular outcome trials, DPP4 inhibitor medication was found to be neutral in terms of serious adverse cardiac events (58, 59). As a result, DPP4 inhibitors can be prescribed for most patients with COVID-19 symptoms ranging from mild to severe. Given the well-established benefits of glucagon-like peptide 1 (GLP1) analogues in the prevention of cardiovascular disease (CVD) and kidney disease (59, 60), these medications could be an attractive therapy option for individuals with T2DM who are at risk of CVD and kidney disease (61). Treatment with a sodium–glucose cotransporter 2 (SGLT2) inhibitor causes osmotic diuresis and possibly dehydration (62), which has been linked to acute renal injury and ketoacidosis (63). As a result, SGLT2 inhibitors should not be used in critically ill patients. ICU is for intensive care unit, and TZD stands for thiazolidinedione (35).

In conclusion, non-insulin hypoglycemic medications are not the first-line treatment for hyperglycemia in hospitalized patients, but they are being utilized well in COVID-19 patients.

Maintaining treatment with hypoglycemic medications administered at home should be explored, particularly in patients with COVID-19 that is moderate rather than severe

2.8. Considerations of specific situations

When possible, employ a temporary intravenous insulin pump with continuous insulin infusion supervised by a local diabetes inpatient team in hyperglycemic situations (43). However, there is a legitimate fear that continuous insulin infusion, which necessitates more patient attention from healthcare staff, increases the virus's exposure to these workers. For these reasons, a protocol for treating DKA with rapid-acting subcutaneous insulin every four hours and an initial dose of 0.4 IU/kg/4 h was created and is publicly available (64). A patient with COVID-19 and DKA was successfully treated with a loading dose of insulin glargine 0.15 IU/kg and insulin aspart 0.3 IU/kg, followed by insulin aspart 0.2 IU/kg every four hours until DKA resolution, according to another report (65).

2.9. Flash glucose monitoring

The FDA—Food and Drug Administration—has recognized the benefit of flash glucose monitoring in reducing healthcare personnel' exposure to SARS-CoV-2-infected patients (66). However, this is a costly procedure that necessitates training for healthcare staff as well as prudence when dealing with interferents in the readings. Patients with COVID-19 frequently have these interfering factors, such as hypoxia and paracetamol. If this strategy is followed, an acceptable objective is to keep glucose levels within range 70% of the time (52).

2.10.The transition from hospital to home

The pre-hospitalization regimen should be continued after discharge for patients with an A1c of less than 7%. If your A1c is higher than 7% and you don't have any characteristic diabetes symptoms, you might consider adding another medicine to your treatment plan. It is recommended that people with an A1c of more than 7% and symptomatic hyperglycemia start or raise their insulin dose at least one day before discharge to ensure that the new strategy is effective and safe (45).

To summarize, the COVID-19 global pandemic offers significant health risks, particularly for diabetic individuals. COVID-19 has yet to be given a specific treatment. As a result, the greatest solution is to avoid infection in the first place. Patients with diabetes should make a concerted effort to follow general preventative principles and test glucose levels more regularly, engage in physical activity, eat healthily, and reduce other risk factors .Current and future research on the best management plan for such individuals, such as the selection of glucose-lowering, antihypertensive, and lipid-lowering drugs, is critical (35).

Patients with diabetes mellitus should be informed that COVID-19 can raise blood glucose levels, and they should follow clinical guidelines for diabetes mellitus management more strictly during the COVID-19 pandemic, as outlined here. For patients and health-care providers, we offer the following general advice: Patients should be extremely cautious about taking their prescription medications (including insulin injections) and having their blood glucose levels monitored more regularly than before. Patients should see their doctor if their blood glucose levels are consistently higher than normal. In view of current worldwide quarantine rules, health-care practitioners should place a greater focus on good food intake and physical activity in diabetic patients. Patients should be urged to see their doctor right away if they have symptoms such as a dry cough, high sputum production, or fever, or if their blood glucose level suddenly rises. Furthermore, patients should closely follow their doctor's advice and be wary of statements conveyed through various forms of media (including the internet), which may or may not stand up to scientific scrutiny. Most importantly, general precautions should be closely followed by both health-care providers and their patients to limit the risk of infection in individuals with diabetes mellitus, such as social distancing, wearing a mask, washing hands, and using disinfectants. Direct physical contact between patients and medical workers poses a risk that telehealth or remote consultations may assist to mitigate. These could be further strategies for reducing the danger of SARS-CoV-2 transmission while yet providing ongoing and safe medical care to the general public (35).

Healthcare workers must be aware of the hazards of hyperglycemia in COVID-19-positive hospitalized patients, as well as how to treat it. BG monitoring on a regular basis, early detection of DKA, and glycemic management all help to save lives. Protocols tailored to the organization of each hospital are also desired. However, the benefits of stringent glucose control should be weighed against the danger of severe hypoglycemia in hospitals where comprehensive blood glucose monitoring and nurse supervision are not possible, particularly in the event of a staffing or supply shortage

Observational studies clearly reveal that insulin-assisted glycemic management reduces the risk of major negative events in COVID-19 patients, including as mechanical ventilation, ICU hospitalization, and mortality. Oral antihyperglycemic medicines are being successfully utilized in patients hospitalized because of COVID-19, especially in those who were already taking the medication regularly before becoming infected and do not have significant hyperglycemia. Insulin remains the gold standard for glycemic management in hospitals, regardless of the reason for admission.

2.11. Key Points (35)

- Diabetes mellitus and cardiovascular disease are risk factors for more severe coronavirus disease 2019 (COVID-19) disease and worse outcomes, including higher death (35).
- Effects on glucose homeostasis, inflammation, altered immunological status, and activation of the renin–angiotensin–aldosterone system are all possible pathogenetic linkages between COVID-19 and diabetes mellitus (RAAS) (35).
- During the COVID-19 pandemic, strict glucose control and the prevention of diabetes complications may be critical in diabetic people to keep susceptibility low and prevent serious COVID-19 infections (35).
- Evidence suggests that insulin and dipeptidyl peptidase 4 inhibitors can be taken safely in people with diabetes mellitus and COVID-19; metformin and sodium– glucose cotransporter 2 inhibitors may need to be discontinued in patients with a high risk of developing severe disease (35).
- Pharmacological treatments under research for the treatment of COVID-19 can alter glucose metabolism, especially in patients with diabetes mellitus; as a result, frequent blood glucose monitoring and individualized prescription adjustments are necessary (35).

2.12. Recommendations Based on Available Evidence (25)

1-In the post-COVID-19 phase, patients with diabetes mellitus should be more careful about maintaining optimal glycemic control.

2-In patients who have never had diabetes before:

- Routine glycemic testing in COVID-19 patients without confirmed in-hospital hyperglycemia or new-onset diabetes mellitus is not advised. Patients with COVID-19 admitted to the ICU and those over the age of 70 are at a high risk of developing new-onset diabetes after COVID-19, and should be screened for it three months after discharge using a fasting plasma glucose or 2-hour plasma glucose during an oral glucose tolerance test or glycated hemoglobin (HbA1C) as per the American Diabetes Association (ADA) Standards of Medical Care in Diabetes.
- Patients with COVID-19 who had documented in-hospital hyperglycemia (including steroid-induced hyperglycemia) but were not on any antidiabetic drugs at the time of discharge should be reevaluated 3 months later with a fasting plasma glucose or 2-hour plasma glucose during an oral glucose tolerance test or HbA1C, according to the American Diabetes Association's Standards of Medical Care in Diabetes.
- Patients with COVID-19 who are discharged on antidiabetic drugs and have documented in-hospital hyperglycemia (including glucocorticoid-induced hyperglycemia) should maintain glycemic control as per standard of care. Depending on the glycemic profile, the dose and

amount of antihyperglycemic drugs should be modified. Based on the blood glucose profile, anti-diabetic medications may need to be stopped. Those with verified stress-induced hyperglycemia, defined as a HbA1C level of 6.5 percent in the presence of hyperglycemia at the time of discharge, may be able to stop using antihyperglycemic drugs

- Even after recovering from COVID-19, patients with diabetes mellitus/hyperglycemia are at high risk of mucormycosis, and caregivers must be alert about this.
- Screening for other diabetes-related problems should be done on a regular basis as part of routine care.

3. COVID -19 AND OBESITY

3.1. General information

Obesity is a significant risk factor for obtaining COVID-19, as well as other viral or bacterial infections and the development of more severe forms of the disease. Obesity, especially extreme obesity, is well known to be associated with pulmonary dysfunction, which may predispose individuals to respiratory failure in the event of pneumonia (67). Increasing numbers of reports have linked obesity to more severe COVID-19 illness and death (Figure 3)(68, 73).

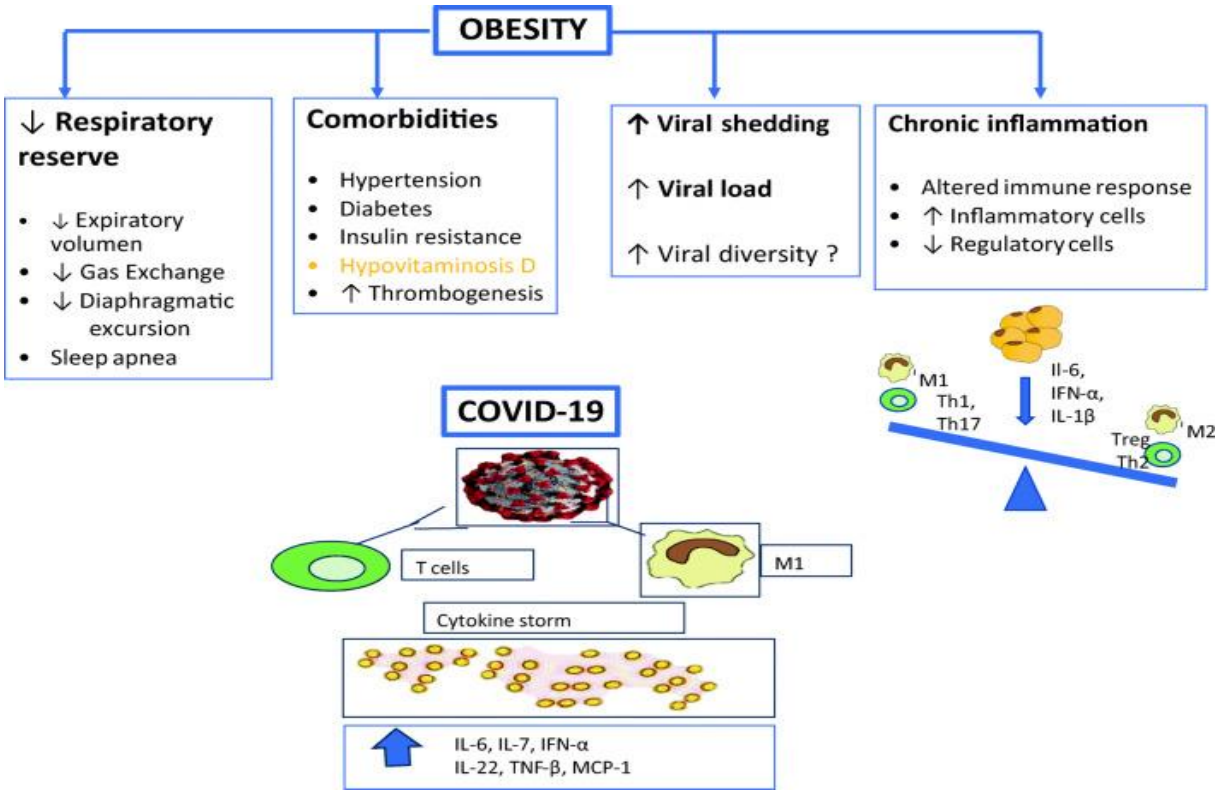

Figure 3. Potential mechanisms that link obesity to worse outcomes in COVID-19 (73). Obese individuals have 1) reduced respiratory function; 2) cardiovascular, metabolic, and thrombotic comorbidities, all of which diminish their capacity to cope with COVID-19. Obese individuals also have a 3) higher viral shedding and viral load, as well as a 4) stronger immune response due to a shift in the balance between inflammatory and regulatory cells During COVID-19 infection, the immune response is disrupted, which is increased by the obese patients' dysregulated immune system.

SARS-CoV2 enters the human body through the respiratory system and infects lung epithelial cells by binding to ACE2 with the help of other intracellular proteins like furin. Once within the cells, the viral RNA is copied, its proteins are translated through a series of biochemical processes, and new viral particles are formed and secreted by exocytosis. The virus then spreads to additional tissues that produce ACE2, with AT being a prime target because ACE2 is abundantly expressed in the lung and AT, as well

as being overexpressed in obesity. SARS-CoV causes an inflammatory response within AT, which may contribute to the cytokine storm and acute respiratory distress syndrome seen. It's been postulated that the AT of visceral organs acts as a SARS-CoV2 reservoir, resulting in enhanced viral shedding (11).

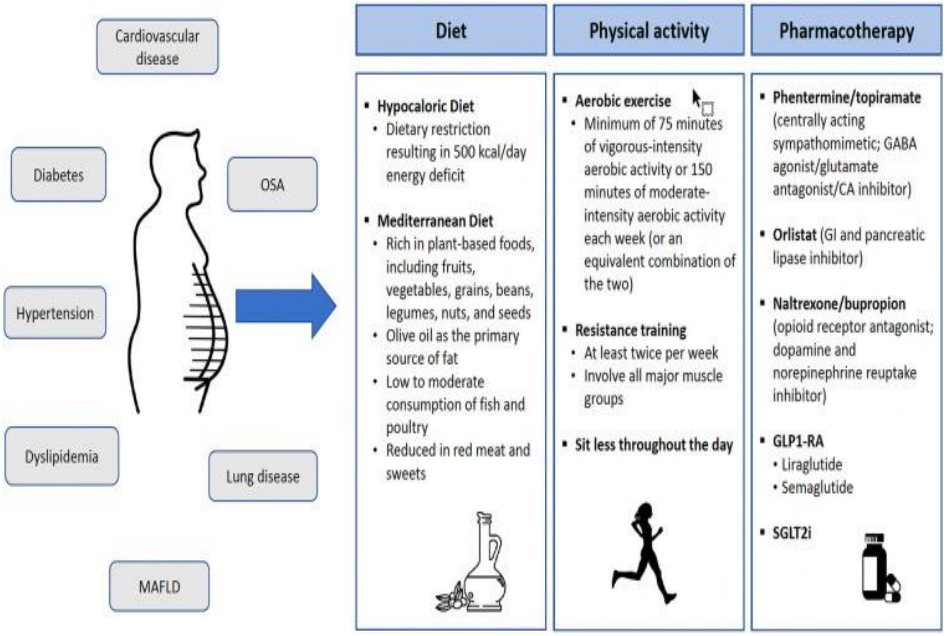

Figure 4 . Overview of lifestyle and pharmacological treatment for obesity (11). Obesity is associated with several weight-related comorbidities, including cardiovascular disease, hypertension, dyslipidemia, metabolic associated fatty liver disease, lung disease, and obstructive sleep apnea. Abbreviations: CA, carbonic anhydrase; GABA, gamma aminobutyric acid; GI, gastrointestinal; GLP1-RA, glucagon-like peptide 1 receptor agonist; MAFLD, metabolic associated fatty liver disease; OSA, obstructive sleep apnea; SGLT2i, sodium-glucose cotransporter-2 inhibitor.

Obesity patients frequently have co-morbidities such as type 2 diabetes, hypertension, cardiovascular disease, and kidney disease, all of which might negatively impact COVID-19's clinical result. Furthermore, many reasons for the link between obesity and poor COVID-19 results have been hypothesized. The first is the fact that abdominal fat has a negative ventilatory effect (69). In addition to the ventilatory deficit, poor lung perfusion owing to intravascular disseminated coagulation may contribute to respiratory dysfunction in patients with severe COVID-19 (70). Obesity is a prothrombotic disease that may contribute to a poor outcome in COVID-19 patients (71). ACE2 is also substantially expressed in the epicardial adipose tissue of obese people. Internalization of the virus into adipocytes might be aided by this. Steatosis of the liver might potentially be a factor. People with abdominal obesity and diabetes are more likely to develop non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (72). Chronic low-grade inflammation, as well as decreased adiponectin and increased leptin secretions, are hallmarks of obesity and diabetes. Obese people are also more likely to be physically sedentary, insulin resistant, and have gut dysbiosis, all of which could exacerbate the inflammatory response to SARS-CoV-2 infection. Obese people have decreased vitamin D levels, which may weaken their immunological response (36).

Given the persistent evidence that obesity increases the probability of a worse COVID-19 result, it should be noted that obese people who get ill and require hospitalization present unique care issues. These issues include venous access, positioning, transportation, and airway management, diagnostic imaging issues caused by the weight constraints of imaging machines, and the scarcity of bariatric beds in hospitals. As a result of the reasons stated above, it is vital that obese patients take all necessary precautions to avoid infection. Obesity deserves special consideration when it comes to disease prevention. Obese persons should be encouraged to increase their physical activity and adopt good eating habits, as well as develop stress-reduction and sleep-optimization measures (1).

Given the continuous nature of the pandemic and the development of a long COVID syndrome, patients who are obese or show metabolic abnormalities should be given special attention. In addition to promoting vaccination and strategies to reduce SARS-CoV-2 exposure, close and proactive clinical management with regard to proper nutrition, weight management, pharmacotherapy, and psychosocial issues is required, with the potential to significantly reduce SARS-CoV-2 morbidity and mortality. Measurement of anthropometric measures should be routinely undertaken in patients tested positive for SARS-CoV-2 as part of risk assessment in obese people who become ill. Obese patients with COVID-19 should be viewed as a higher-risk population. To avoid poor clinical outcomes, increased attention, priority on detection and testing, attentive monitoring, and sooner intense therapy should be considered in these individuals. In a pandemic Weight management, blood pressure control, and blood glucose control have always been crucial for improving cardiometabolic health and preventing serious health effects in obesity, but the risk of severe COVID-19 is now an additional reason to pay attention to these issues (Figure 4) (1,11).

In summary, Obesity, cardiometabolic dysfunction, and type 2 diabetes are now well recognized as significant risk factors for poor SARS-CoV-2 infection outcomes (extended hospital stays, mechanical ventilation, and death). While the focus has been on overt obesity (as measured by BMI), the metabolically unhealthy phenotype (characterized by dystopic fat deposition outside of the normal storage space in subcutaneous adipose tissue, as well as dysregulated adipose tissue distribution intra-abdominally and in organs such as the liver, resulting in NAFLD and abnormal metabolic markers) appears to contribute to poor COVID-19 outcomes, regardless of marked obesity (11). Although the exact mechanisms linking metabolic dysfunction to a poor clinical course are unknown, it is likely that low-grade tissue and systemic inflammation contribute to the viral-induced inflammatory response, resulting in cytokine storm, hypercoagulability, and multi-system dysfunction. In addition to promoting vaccination and strategies to reduce SARS-CoV-2 exposure, close and proactive clinical management with regard to proper nutrition, weight management, pharmacotherapy, and psychosocial issues is required, with the potential to significantly reduce SARS-CoV-2 morbidity and mortality (11).

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CHAPTER 20

CARDIOVASCULAR SYSTEM DISORDERS IN COVID-19

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INTRODUCTION

The pneumonia epidemic, which developed due to a new coronavirus detected in Wuhan, People's Republic of China in December 2019, got out of control and first spread to other parts of China. Then it spread to the whole world, especially to the European continent and then to North America(1).The World Health Organization (WHO) named this virus as "Severe Acute Respiratory Syndrome Coronavirus-2" (SARS-CoV-2) and the disease it caused as COVID-19 (CoronaVirus Disease 2019)(2,3).This disease which we are faced without having sufficient experience in its clinical course, mostly belongs to the respiratory system.Although there are symptoms of the disease, due to the cardiac damage detected in a significant portion of the patients, the cardiovascular findings of the disease began to be emphasized(4).According to the shared data ,the advanced age group with heart and vascular disease makes up the group of patients most affected by this outbreak and with the highest mortality rate. Presence of risk factors such as diabetes, hypertension and cardiovascular diseases causes the person to be more sensitive to contracting this virus and to increase the complication and death rates if infected. By affecting the cardiovascular system directly or indirectly, this virus may cause clinical conditions with high mortality, such as myocardial damage, myocarditis, acute coronary syndrome, pulmonary embolism, arrhythmia, to worsen the current situation.The virus can create a direct cytotoxic effect in humans by binding to the ACE2 receptor found in lung type 2 alveolar cells, vascular endothelial cells, myocardium, kidney proximal tubule, esophagus, bladder urothelial and ileum epithelial cells.

Cytokine storm secondary to inflammation, increased tendency to thrombosis and increased sympathetic stimulation cause the virus to affect the cardiovascular system indirectly.As a result of all these effects, COVID-19 infection causes an increased risk of myocardial damage, myocarditis and myocardial infarction (MI), heart failure, arrhythmia and venous thromboembolism (VTE)(5).Vascular inflammation, increase in thrombosis tendency, rupture of existing plaques due to cytokine storm, decrease in myocardial oxygen delivery due to hypoxia due to infection, increase in myocardial oxygen demand as a result of increase in sympathetic activity are pointed out as mechanisms that cause myocardial infarction development (5).Increase in cardiac markers (troponin and natriuretic peptide levels) and ECG changes (ST elevation, nonspecific ST-T segment changes) are seen in patients who come to the emergency department with a preliminary diagnosis of COVID-19 due to microvascular damage and accompanying myocardial involvement.

EFFECT OF COVID 19 INFECTION ON CARDIAC MARKERS

An increase in cardiac troponin indicates myocardial injury.The mechanism of the increase in troponin during COVID-19 infection is not fully understood (6).It is thought that the invasion by the virus of ACE2, which is known as the binding site of the coronavirus and which is located in the vascular endothelial cells and myocytes, may cause direct damage to the myocardium and lead to an increase in troponin.In addition, ARDS alone may be responsible for the increase in troponin.Also myocardial damage due to cytokine storm may be one of the reasons for this increase in troponin.Unfortunately, we do not have enough evidence for all of these (7).Type-I ME, which occurs as a result of activation of the prothrombotic system and plaque rupture due to the inflammatory process, or Type II MI, which occurs as a result of decreased oxygen delivery of the myocardium, are among the predicted causes.In the cases with hs-cTn increase in the studies, the relationship of troponin increase with the structural and functional abnormality of the myocardium is not clear, since the echocardiography or cardiac MRI data of the cases were not available.Although its mechanism is not fully understood, it is a fact that increase in troponin is a poor prognostic factor in COVID 19 cases.In cases infected with COVID-19, troponin elevation alone has no meaning in terms of ACS (acute coronary syndrome) (5).For the diagnosis of acute coronary syndrome, the presence of other accompanying clinical findings such as ECG changes, presence of typical symptoms, CK-MB increase and echocardiographic findings should be questioned.

Another important point is that it is not recommended to measure troponin for ACS in patients with COVID-19 unless clinical signs of acute coronary syndrome or myocardial damage are present(6).Except for Type-1 MI, the increase in troponin alone does not require the initiation of anticoagulant and antiplatelet therapy, but according to the data obtained from the studies, the opinion that cardiac troponin should be checked for risk classification and prognosis evaluation in all hospitalized cases with COVID-19 is supported.D dimer levels are also found to be increased in the vast majority of COVID 19 cases followed in the hospital.It is thought that D-dimer elevation develops as a result of the activation of the prothrombotic system due to the increase in systemic pro-inflammatory activation. Since D-Dimer elevation is an important mortality indicator in severe sepsis cases, it is recommended to be evaluated in terms of prognosis and risk classification in all hospitalized cases.

COVID 19 AND HEMODYNAMIC INTERACTION

In severe cases, COVID-19 may cause respiratory failure, as well as adversely affect cardiac hemodynamics by directly causing the development of myocarditis. It can lead to sepsis and septic shock, similar to other infections.In the case of septic shock, hypotension due to diffuse vasodilation, systemic vascular resistance and tissue oxygenation disorders develop.This results in decreased urine output, tachycardia, and confusion.When compensatory tachycardia is combined with low systemic vascular resistance, a high-output condition emerges (8).Pulmonary artery catheterization not only provides the opportunity to evaluate cardiac hemodynamic parameters such as cardiac output, central venous pressure, but also assists in treatment management (8,9).In a case in septic shock clinic, invasive arterial monitoring can closely follow the differences in arterial traces and systemic pressure.And thanks to these data, hemodynamic evaluations of changes during dose adjustment of vasopressor therapy, fluid supplementation, mechanical ventilation and even ECMO applications can be made.Although it is said that cardiac output remains the same or increases in septic shock, in cases of COVID 19, myocardial dysfunction may occur due to arterial hypoxia and acidosis due to lung involvement.However, the clinical course of shock may be more serious due to myocarditis developing secondary to COVID-19.In this situation, left pressures will be high, cardiac output will be low.Fluid supplementation in treatment has an important place in COVID-19 infection, as in all other serious respiratory tract infections.Although the use of the central route is recommended in these cases, intravenous crystalloid fluid supplements should be carefully managed.The use of hypotonic solutions is not recommended, except in exceptional circumstances.In addition, starch-based solutions should not be used due to the risk of kidney failure (10). Aggressive fluid replacement may impair breathing, leading to further deterioration in oxygenation(11).In cases where there is no adequate response to fluid replacement, vasopressor drugs such as noradrenaline, adrenaline and dopamine are recommended.Dose titration should be done with a central venous catheter, with close monitoring of hemodynamics (systolic pressure >90 mmHg). The use of IV corticosteroids is controversial in cases of septic shock that persist despite the use of vasopressor agents.There are datas that steroid uses has a number of adverse effects in lung infections caused by influenza, SARS, MERS, RSV(12).There are many mechanisms that can cause cardiac hemodynamic disorder by affecting the cardiovascular system in COVID-19 infections (5). In the background of intense systemic inflammation and direct vascular inflammation, the risk of myocardial infarction increases due to plaque rupture and increased coagulability.Although increased sympathetic stimulation increases myocardial oxygen demand, hypoxia developing on the background of ARDS decreases oxygen delivery.A myocarditis clinic to be added to the impaired presentation-needs balance poses the risk of deterioration in cardiac functions and acute decompensation.Increased sympathetic stimulation, myocarditis, and intense systemic inflammation also increase the risk of arrhythmia.Immobility, inflammation and increased coagulability are classical risk factors that predispose to pulmonary embolism that may develop on the basis of deep vein thrombosis.

COVID-19 AND HYPERTENSION

In the publications related to COVID-19 infection, it is stated that the risk of mortality increases in the presence of a concomitant cardiovascular disease.Hypertension is also mentioned in this group

(1,4,13). Based on the knowledge that SARS-CoV-2 virus enters the cell as a result of its interaction with the angiotensin-converting enzyme-2 (ACE2) surface receptor in the cell membrane,(2) In the presence of COVID-19 infection, hypertension may be a possible risk factor, as well as ACE and angiotensin 1 receptor (AT1r) has led to the thought that antihypertensive drugs acting through the blockade may be responsible for the pathogenesis.Similar to SARS and MERS coronaviruses, the SARS-CoV-2 spike (S) protein uses the ACE2 receptor to enter the host cell (14). The virus mainly targets enterocyte and type II pneumocyte cells with high ACE2 expression(15).The S protein binds to the host cell's ACE2 receptor, then undergoes proteolytic processing by TMPRSS2, a host transmembrane serine protease, and then viral RNA enters the cell by endocytosis or by fusion to the cell membrane, infecting the cell (15-17).Based on all this information, the concern that ACE inhibitors and ARBs may increase viral attachment and infection by causing tissue level up-regulation of ACE2 receptor/protein is the main reason for recent antihypertensive treatment discussions.However, published data do not sufficiently support this hypothesis.Looking at the literature, there is not enough evidence that the use of ACE inhibitors or ARBs will worsen the existing clinic or improve the unfavorable clinic in the case of SARS CoV-2 infection.Although COVID-19 infection mainly affects the respiratory system, it can also cause cardiovascular damage. Considering all these, and considering the proven benefits of these drugs in those who already have hypertension and cardiovascular disease, it is not considered appropriate to exclude drugs from the treatment in this process (18).

APPROACH TO CASE OF ACUTE ST ELEVATION MYOCARDIAL INFARCTION WITH COVID 19

When deciding on primary percutaneous intervention in cases of STEMI in cases with a diagnosis of Covid 19, the risk of infection of the personnel to be involved in the case and the benefit to be provided on behalf of the patient should be considered (23-25).Thrombolytic therapy should be planned primarily in cases with stable clinical conditions, who present within the first 12 hours of the onset of pain, and who do not have a high-risk condition according to the above table.However, if there is a positive opinion that invasive intervention will affect the prognosis in the presence of a high-risk condition indicated in the table, and a patient with negative reperfusion despite thrombolytic therapy, a decision for percutaneous intervention should be made.

APPROACH TO CASE OF ACUTE NSTEMI INFECTED WITH COVID 19

Considering that viral infection may cause direct myocardial damage in cases infected with COVID 19, it can sometimes be difficult for a cardiologist to distinguish between COVID-19-related myocardial injury and acute coronary syndrome secondary to plaque rupture (4,1).

Table 1. High-risk conditions requiring early invasive intervention in patients admitted to hospital due to acute coronary syndrome during the COVID-19 pandemic.

High-risk conditions requiring early invasive intervention in patients admitted to hospital due to acute coronary syndrome during the COVID-19 pandemic(22)
ST Elevation Myocardial Infarction a- Cases in which thrombolytic therapy is contraindicated b- Reperfusion-negative patient unresponsive to thrombolytic therapy (for salvage percutaneous intervention) c- Presence of cardiogenic shock /Killip III patient group d- Presence of diffuse anterior MI e- Developed mechanical complications of myocardial infarction
Non-ST-Elevation Myocardial Infarction a- Developed mechanical complications b- Recurrent or persistent angina unresponsive to drug therapy c- Presence of hemodynamic instability d- Presence of life-threatening ventricular arrhythmia or cardiac arrest e- Dynamic ST-T changes (intermittent ST segment elevation)

It has been shown that there may be an increase in “high-sensitivity” troponin levels in these cases, even if there are no cardiac symptoms (6,19). Since there is no specific treatment for Covid 19-associated myocarditis, if acute coronary syndrome is not considered in line with the patient's clinical and ECG findings, troponin level is requested.It should be taken into account that it may lead to some unnecessary examination and treatment practices that "increase the risk of infection of the personnel on duty from the admission of the infected case to the coronary intensive care units to interventional angiography".For these reasons, it is not recommended to measure troponin and other cardiac markers in patients diagnosed with COVID-19 or pre-diagnosed in pandemic conditions, where effective use of healthcare personnel and personal protective equipment is important, unless there are acute coronary syndrome clinics and/or ECG findings (20). If a thorax CT request is planned in cases with suspected Covid 19, the presence or exclusion of coronary etiology can be provided by coronary CT angiography, if possible, in cases with suspected acute coronary syndrome.In addition, pulmonary CT angiography may be guiding in cases with suspected pulmonary thromboembolism, which may be accompanied by increased troponin (21). In the presence of acute coronary syndrome and ECG findings in patients infected with Covid 19, it should be considered that there is no indication for emergency invasive intervention unless there are high-risk conditions listed in the table below.

COVID-19 AND HEART FAILURE

In Covid 19 infection, as in other infections, the heart failure clinic may worsen. When patients with heart failure diagnosis and follow-up are infected with COVID 19, action should be taken according to the course of the disease.There is no proven data to require discontinuation of treatments with proven effects on mortality in heart failure, such as ACE inhibitor, ARB, mineralocorticoid receptor antagonist (MRA), and angiotensin receptor blocker-neprilysin inhibitör(ARNI), in case of COVID 19 infection.In

cases with mild signs of infection, hospitalization is not necessary, and isolation and keeping the infection under control are important, while no change is required in the existing heart failure treatment.Symptomatic treatment and, if necessary, antipyretics can be used in these patients.On the other hand, the patient should be alerted in terms of complications that may arise during the course of the disease, and referral should be considered when necessary.In cases of moderate Covid 19 pneumonia-infected heart failure who do not need oxygen support, the patient should be isolated and closely monitoredAlthough these patients do not need to change their current heart failure treatment, as in the patient group with mild signs of infection, care should be taken in terms of cardiovascular complications that may develop secondary to pneumonia in this patient group.Patients with respiratory rate greater than 30 per minute, severe respiratory distress, and room air saturation ≤93% should be considered as severely infected and caution should be exercised against the risk of ARDS development.In these patients, when mechanical ventilation is required, high end-expiratory positive pressure increases the intrathoracic pressure, resulting in a decrease in venous return and thus a decrease in cardiac output (26). If such a situation occurs in heart failure patients with low EF (Ejection fraction), more care should be taken to ensure adequate cardiac output. In case of ARDS, sepsis/septic shock, agents that may cause hypotension such as ACE inhibitors, ARBs, ARNIs, diuretics, beta blockers can be discontinued.Diuretics, ACE inhibitors and ARBs, and ARNI treatments that may cause an increase in creatinine can be discontinued if necessary, since acute renal failure clinic is also seen frequently in severe cases infected with COVID-19.Knowing whether a cardiac pathology accompanies the current picture in Covid 19 cases is important for the decision of mechanical respiratory support, circulatory support or the form of circulatory support(veno-venous ECMO, veno-arterial ECMO) to be given in these patients.The place of ECMO is limited in case of ARDS development in Covid 19 infection(27). The ECMO decision should be evaluated according to the experience of the team and the characteristics of the case.In COVID-19 cases with ARDS, especially in resistant hypoxic cases(PaO2 /FiO2<100 mmHg and/or ph<7.25), it is recommended that venous venous ECMO be applied before multi-organ failure develops.Veno-arterial ECMO method is especially prominent in COVID-19 cases developing myocarditis.Multiple organ failure, advanced age, multiple comorbidities are among the contraindications for ECMO.However, it is not recommended to be used in ventilator-assisted cases for more than 1 week.If there is no improvement after ECMO application exceeding 3 weeks, support can be terminated(28). Right heart failure may occur in conjunction with ARDS.Acute cor pulmonale, the most severe clinical form of this, can develop in approximately 25% of ARDS patients.The diagnosis of acute cor pulmonale is made as a result of echocardiographic evaluation.Since right heart failure significantly increases mortality in these patients, it is recommended to keep PEEP values within certain limits and prone position in order to prevent its development (29).

COVID-19 AND MYOCARDITIS

Myocarditis is a difficult disease to diagnose because it can occur with different clinical pictures.It is difficult to comment on the true incidence of endomyocardial biopsy, which is the gold standard in the diagnosis of myocarditis, since it is very difficult to apply in daily routine(30).In mild myocarditis cases where the ventricular functions are normal or less affected, it returns to normal spontaneously without the need for special treatment.On the other hand, dilated cardiomyopathy may develop in 30% of cases.

Diagnostic criteria that will guide the cases in which myocarditis is considered are given in Table 1.

In clinically suspected myocarditis, the diagnosis becomes clear when ≥1 clinical finding is accompanied by ≥1 diagnostic criteria and the following criteria are absent;

1. Angiographically ≥50% coronary stenosis

1.Known cardiovascular pre-excitation conditions or different cardiac or clinical pathologies (valvular disease, congenital heart disease, hyperthyroidism, etc.) can explain the syndrome.

The most common cause of myocarditis is viral infections, and it should be kept in mind that there may be many causes such as infectious, toxic, and autoimmune etiology.Although it has not been proven that SARS-CoV-2 directly infects the myocardium, the presence of T cell infiltration in the myocardium, increased interstitial edema and small areas of necrosis in biopsies suggest myocardial damage in some cases of COVID-19.There is currently no evidence-based treatment for the management of patients with suspected myocarditis secondary to COVID-19, and more controlled studies are needed.

Table 2. Diagnostic criteria in cases with suspected myocarditis(31)

Clinical presentation *Blunt chest pain, pericarditis-like or pseudo-ischemic chest pain *New onset (within days or up to 3 months) or worsening shortness of breath and/or fatigue *Subacute or chronic (more than 3 months) or progressively worsening shortness of breath and/or fatigue *Palpitations and/or symptoms of unexplained arrhythmias and/or syncope and/or obstructed sudden cardiac death *Unexplained cardiogenic shock
Diagnostic criteria **I. EKG/Holter/Stress test** *One of the following in a newly detected EKG/Holter/stress test *Atrioventricular block(I.-III.degree) or bundle branch block *ST/T wave change (ST-segment elevation or depression, T wave inversion) *Sinus arrest, asystole, ventricular tachycardia/fibrillation, atrial fibrillation, loss of R progression, abnormal Q waves, low voltage, supraventricular tachycardia
II. Myocardiocytolysis markers Troponin T/I elevation
III. Functional or structural abnormalities on cardiac imaging (echocardiography, angiography, cardiac MRI) New, unexplained left ventricular or right ventricular structural or functional abnormalities: regional wall motion or global systolic or diastolic dysfunction (with or without ventricular dilatation, with or without pericardial effusion, thrombus) not)
IV. Tissue characterization with cardiac MRI Edema and/or late gadolinium uptake (classic myocarditis pattern)

COVID-19 AND CARDIAC ARRHYTHMIAS

While arrhythmia may develop in Covid 19-related myocardial damage, cases of fulminant myocarditis with atrial and ventricular arrhythmias have also been reported(3–5).In cases followed up due to COVID-19, arrhythmias like atrial fibrillation,ventricular fibrillation,polymorphic ventricular tachycardia, Torsades de Pointes can be seen due to many reasons such as the severity of

the current infection picture, septic clinic, hypoxia, myocardial damage, metabolic disorders, use of vasopressor and inotropic agents given to support hemodynamics(32-34).In cases with continuous monomorphic or polymorphic ventricular tachycardia and ventricular fibrillation, cardioversion or defibrillation should be applied.Patients with significant QTc prolongation (more than 60 msec or greater than 500 msec from baseline) and ventricular ectopic beat should be closely monitored.In these cases, electrolyte levels should be checked and replacement should be applied if necessary.In case of drug use that may cause QTc prolongation drugs should be discontinued, heart rate should be increased if possible (with isoproterenol infusion, temporary pacing or permanent pacing if available), and anti-arrhythmic drugs such as lidocaine, which have the effect of shortening the QT duration, should be given in case of resistant arrhythmia.

COVID-19 AND VENOUS THROMBOEMBOLISM

In cases infected with COVID-19, especially in severe cases, there is a predisposition to both arterial and venous thromboembolism(VTE) due to reasons such as immobilization hypoxia, mechanical ventilation, and the presence of a central venous catheter(35,36).Although the pathophysiological causes of the disease leading to thrombosis are not clear, vascular microthrombotic disease caused by sepsis, endothelial cell activation/damage after endocytosis of the virus via the ACE2 receptor, and hypercoagulability due to inflammatory process in severe cases can be counted among the causes of this thrombotic process (37,38).These thrombotic complications are an important factor that increases morbidity and mortality in COVID-19 cases.For this reason, the identification of risk groups, the follow-up of biomarkers, which diagnostic methods can be used and prophylaxis are the subjects that should be emphasized.The severity of the Covid 19 clinic is proportional to the incidence of venous thromboembolism and the severity of its course in case of development of venous thromboembolism(39).In order to prevent the development of venous thromboembolism, prophylaxis should be applied in accordance with the treatment protocols in all Covid 19 cases followed in the hospital(40).The drug of choice for prophylaxis is low molecular weight heparin(DMAH).Direct oral anticoagulants and warfarin may interact with antiviral and immunomodulatory drugs used in the treatment of Covid 19 disease.Unfractionated heparin, on the other hand, is not preferred except in exceptional cases because it requires frequent monitoring, increases the contact frequency of the healthcare personnel applying the treatment, and creates a risk of transmission.It can be preferred in cases with mechanical prosthetic valve or kidney failure.If there is no venous thromboembolism clinic in patients, it is sufficient to give prophylaxis dose.In patients using warfarin and DOAC, anticoagulant therapy should be changed to LMWH (1 mg/kg, in 2 separate doses) and unfractionated heparin in patients with prosthetic valve and severe renal failure.If there are signs of sudden onset of respiratory distress, deterioration in hemodynamics, tachycardia, and accompanying deep vein thrombosis in the follow-up of the patients, additional examination for venous thromboembolism is required.Routine VTE screening is not recommended.When deciding on the duration of anticoagulant therapy, factors such as the course of coagulation parameters, whether patients have a high risk for thromboembolism, whether thrombotic complications accompany during hospitalization should be taken into consideration.It is recommended to continue anticoagulant therapy until the coagulation parameters return to the normal range.Continuation of anticoagulation is appropriate since mobilization is limited during hospitalization due to quarantineContinuation of prophylactic anticoagulant therapy is recommended if there is no DVT/PTE diagnosed in the patient group with limited mobilization at discharge.In cases of diagnosed VTE, anticoagulant therapy should be arranged in accordance with the guideline recommendation

CONCLUSION

Covid 19 may cause new cardiovascular disease development as well as exacerbate existing cardiac diseases.Important considerations for the preventive and therapeutic use of treatment strategies should be kept in mind to mitigate the worse outcome, especially in these high-risk patients.

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CHAPTER 21

PULMONARY INVOLVEMENT OF COVID-19

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INTRODUCTION

The coronavirus disease (COVID-19), which can cause severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first discovered in Wuhan, China, in March 2020, and the WHO declared a pandemic.

Different age groups are affected by SARS-CoV-2 infection, and during both symptomatic and pre-symptomatic phases of infection, it is transferred via direct touch or respiratory droplets produced by the infected patient while coughing or sneezing. Fecal-oral transmissions are also likely, with a minimal chance of vertical transfer (if infection occurs during the third trimester of pregnancy) (1, 2).

RISK FACTORS

The prognosis of respiratory illness depends on several factors such as male gender, age (>50 yo), positive smoking history, and underlying chronic diseases in the course of COVID-19. Many studies reported that a significant number of patients had at least one underlying chronic disease. Lai et al. reported that hypertension is the most underlying chronic disease (14.9%) followed by diabetes mellitus (7.4%) and cardiovascular disease (4.2%) (3). According to a systematic review and meta-analysis older age (≥65 years old), diabetes mellitus, COPD, hypertension, male gender, Cardiovascular Diseases, and cancer were associated with a higher risk of mortality from COVID-19 infection (4).

PATHOLOGY

Under the light microscope, the histological investigation revealed congested and edematous pulmonary interstitial blood vessels with inflammatory infiltration of lymphocytes and monocytes as well as obvious thrombosis. In the alveolus cavities, serous fluid, fibrin exudate, and hyaline membrane development were seen. Monocytes and macrophages were the most prevalent exudate cells, while multinucleated giant cells were also abundant. Atypical expanded alveolar cells with big nuclei, amphiphilic cytoplasmic granules, and prominent nucleoli were also seen, indicating viral cytopathic-like alterations (5).

COVID-19 pathogenesis has been linked to thrombosis in micro and macrovascular beds, fibrin formations, blockage of alveolar passages, reduced blood vessel integrity, inflammation, multinucleation, and interferon-related reactions accompanied by the viral presence, according to certain investigations employing postmortem tissues (6-10).

RADIOLOGY

The reference standard for COVID-19 diagnosis is the real-time reverse transcription-polymerase chain reaction (RT-PCR) of viral nucleic acid. However, chest computed tomography (CT) examination is also a very crucial diagnostic tool in COVID-19 patients who presented with false-negative PCR results and reported CT sensitivity as 98% (11-13).

The most prevalent chest CT finding in COVID-19 patients was ground-glass opacity, which was followed by consolidation, according to a scientific assessment of 2,814 patients. The findings, however, can vary between people and stages of the disease. Interlobular septal thickness, reticular pattern, and crazy paving abnormalities are among the others. Air bronchogram, bronchial wall thickening, nodule, pleural effusion, and lymphadenopathy have all been seen in some studies (14).

The most common findings among the Pulmonary Function Test are impaired diffusion capacity, despite the relatively low frequency of restrictive or obstructive pulmonary dysfunction (15). Despite the absence of macro-level lung dysfunction represented as restrictive lung dysfunction or obstructive lung dysfunction, impaired diffusion capacity was more

common, which reflects the disorder of the interstitial structure and microvasculature of the lungs. This result may reflect underlying microangiopathy in the lungs as previously reported in postmortem studies in cases with COVID-19 diseases (16-18).

PULMONARY INVOLVEMENT

SARS-CoV-2 primarily targets the respiratory system, although other organs can be affected (19–21). Among the lung diseases caused by SARS-CoV-2 infections, mild pneumonia to severe pneumonia (seen in 14%), a critical disease associated with ARDS, respiratory failure and multiorgan failure (in 5%), or death (2.3%) can occur (22).

The SARS-CoV-2 infection causes alveolar injury and interstitial inflammationVirus-infected apoptotic epithelial cells are phagocytosed by dendritic cells and alveolar macrophages, and T cell responses are triggered, activating innate and adaptive immune systems (23). In patients with Covid-19 infection, serum levels of cytokines such as IL-6, interleukin 1 (IL-1), and tumor necrosis factor (TNF)- are drastically elevated (24). SARS-COV-2 infects human cells through the ACE2 receptor. When SARS-CoV-2 binds to ACE2 receptors, it causes alveolar cell destruction and injury, as well as a decrease in pulmonary surfactant, which causes an increase in lung surface tension and predisposition to ARDS (25, 26). In some cases, acute lung damage, ARDS, and septic shock can worsen quickly. The median time from the onset of early symptoms to the development of dyspnea, hospital admission, and ARDS was 5-7, 8 days (27). Despite low blood oxygen saturation (93%) and oxygen saturation as low as 50 or 60 percent, some Covid-19 patients remain stable and show no symptoms, and are referred to as silent hypoxia or happy hypoxia (28, 29).

PULMONARY EMBOLISM, PREVENTION, TREATMENT

The inflammatory condition, platelet activation, endothelial dysfunction, and blood stasis have all been shown to predispose patients to arterial and venous thromboses caused by this new coronavirus, which is responsible for this pandemic disease (30). DIC is suggested by elevated D-Dimer, thrombocytopenia, and prolonged PT. Its presentation, however, differs from that seen in sepsis, when thrombocytopenia is significantly more severe and D-dimer elevation does not reach that level reported in COVID-19 patients. COVID-19 linked coagulopathy, according to current results, is a mix of low-grade DIC and pulmonary thrombotic microangiopathy, which could have a major impact on organ failure in most individuals with severe illness (31). DIC is seen in up to 71.4 percent of COVID-19 patients who die, although only 0.6 percent of survivors have it. The key feature of this coagulopathy is a significant increase in D-dimer without a decrease in platelets or a prolonging of clotting times, suggesting thrombin production and local rather than systemic fibrinolysis. A high D-dimer level (>2.0 ug/mL) increases hospitalization (up to 3-4 times) and is linked to an increased hospital death rate (32-34).

Considering the lack of a standardized protocol for administering anticoagulant medication to patients with COVID-19, one of the critical problems confronted by physicians is the prevention and early detection of VTE (35). Infection with COVID-19 increases the risk of VTE substantially. Venous embolism screening should be performed in both severe and mild instances, including those with extremely serious clinical problems requiring admission to intensive care units and those with less severe clinical conditions (36). Both UFH and LMWH have been demonstrated to be the most effective anticoagulants for COVID-19 patients. Heparin can bind to viral proteins and participate in the down-regulation of proinflammatory cytokines in addition to its anticoagulant activity (37, 38).

CYTOKINE STORM

The severity and reactions of COVID-19 are mostly determined by immunity. T cells, B cells, macrophages, dendritic cells, neutrophils, and monocytes can all be activated by the virus, leading to the release of huge amounts of inflammatory mediators (39, 40). The immune system's immune response can result in an overproduction of proinflammatory cytokines including interleukins and cytokines (41). Excessive cytokine and interleukin release can cause a massive inflammatory response in the body, which can lead to multiorgan failure and ARDS (42). Age, gender, viral overload, genetic roles, ethnicity, and underlying chronic conditions can all influence the severity of pulmonary symptoms (43). A cytokine storm can result in acute respiratory distress syndrome, which is a leading cause of death in SARS and MERS patients (44, 45).

Del Valle et colleagues examined over 2000 serum samples from over 1400 patients hospitalized in New York City and observed signs of a proinflammatory cytokine environment in severe COVID-19. IL-6 levels were found to be linked to an increased risk of death (OR = 2.47). IL-6 was linked to inflammatory indicators such as CRP, D-dimer, and ferritin, as well as fever, but even after controlling for inflammatory markers, disease severity, and comorbidities, IL-6 was found to be connected independently with COVID-19 mortality (25). Even after controlling for other risk factors such as age, sex, hypoxia, disease severity score based on clinical assessment, and IL-6, high TNF-a, which is known to contribute to organ damage, was a robust predictor of poor outcome. (46). A meta-analysis of mean IL-6 concentrations found that patients with complicated COVID-19 had 2.9-fold higher increased levels than those with uncomplicated disease, suggesting that high IL-6 was a sign of poor prognosis in COVID-19 (47).

Tocilizumab is an IL-6 receptor blocker that was originally licensed for rheumatoid arthritis. It can successfully reverse iatrogenic cytokine storms generated by CAR T-cell treatment in patients with hematological malignancies (48). In a Chinese clinical trial, tocilizumab therapy improved fever control and respiratory functioning in 21 severe COVID-19 patients, and all participants, including two critically ill patients, recovered and were discharged from the hospital (49).

Anakinra is an IL-1 receptor antagonist that inhibits both IL-1α and IL-1β. It has been approved by the FDA and the EDA (European Drug Administration) for the treatment of rheumatic illnesses such as rheumatoid arthritis and systemic-onset juvenile idiopathic arthritis (50, 51). Anakinra can also be used to treat infection-induced cytokine storms, which improves the survival rate of patients with severe sepsis. Anakinra has a shorter half-life than other cytokine blockers, making it safer and more appropriate for severe and critically ill patients (52).

For severe COVID-19, corticosteroids may still be the most effective treatment. Corticosteroid treatment has been found in numerous studies to reduce mortality in severe COVID-19 cases. The RECOVERY Trial stands out as one of the most notable. Dexamethasone for up to 10 days reduced 28-day mortality in this trial, with the most apparent responses in those requiring mechanical ventilation (age-adjusted rate ratio 0.65) or receiving oxygen without invasive ventilation (age-adjusted rate ratio 0.65) (Rate ratio 0.82) (53). The use of dexamethasone was also associated with a lower risk of invasive mechanical ventilation or, for those who were already receiving invasive mechanical breathing, a greater probability of effective discontinuation, according to this study. The administration of dexamethasone enhanced the chances of being discharged alive from the hospital within 28 days in each of these groups (53).

Contrarily, some studies show discouraging outcomes when steroids are used in viral lung infections. Ni et al reported that the use of steroids was associated with increased mortality and length of ICU stay in patients with influenza pneumonia, with a meta-analysis enrolling a total of 6548 patients. This effect could be due to steroids' immunosuppressive effect, which leads to prolonged viremia, as well as an increased risk of bacterial superinfection. Furthermore, steroids can raise the risk of various systemic

problems such as autoimmune and cardiovascular events, as well as enhance resistance to neuromuscular blocking drugs, which are commonly used during mechanical ventilation in SARS patients (54).

CONCLUSION

The pathogenesis of COVID-19 is still not completely explained. Cytokine storms are crucial in the progression of the disease. COVID-19 is a systemic disease, and respiratory symptoms in COVID-19 patients might vary or be absent depending on a variety of circumstances, including viral overload, gender, genetic factors, immunological reactions, cytokine storm, and underlying chronic disorders.

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CHAPTER 22

IMPORTANCE OF D-DIMER IN THE COVID-19 DISEASE

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INTRODUCTION

On 29 December 2019, at a hospital in Wuhan City, China, clinicians noticed a cluster of unusual cases of pneumonia with a clear link to a market selling live fish, poultry, and animals. This event was reported to the World Health Organisation (WHO) on 31 December. Within 1 month, the causative organism was identified as a novel coronavirus, its genome sequenced and published, and reverse transcription-polymerase chain reaction tests developed (1). In humans, COVID-19 infection may be asymptomatic in carriers, or it may cause symptoms such as fever, dry cough, shortness of breath, and fatigue in virus-infected individuals. In more severe cases, COVID-19 infection can cause acute respiratory distress syndrome (SARS) and even death (2).

Conventional coagulation abnormalities in patients with COVID-19 mimic those of other systemic coagulation disorders associated with serious infections, such as disseminated intravascular coagulation, but the disease also has some distinctive features. Pulmonary embolism and deep vein thrombosis are frequently found in patients with COVID-19 (3,4). In patients with COVID-19 in the intensive care unit, systematic reviews suggest an incidence of deep vein thrombosis of 28%, although the latest reviews suggest an incidence of 19–24% when the clinical diagnosis is used and 36–46% when routine screening is used (5,6). Superficial vein thrombosis and catheter-related thrombosis are also frequently reported in COVID-19 patients. Similar to venous thrombosis, it can affect all organs, with arterial thrombosis, systemic arterial embolism, acute coronary syndrome, ischemia stroke, and limb and mesenteric ischemia reported to occur in 1-5% of COVID-19 patients (7,8).

In COVID-19-related coagulopathy, concentrations of interleukin (IL)-6, von Willebrand factor (VWF), and D-dimer are elevated with increasing severity of the disease. The incidence of thrombosis is higher in severe cases. Two theories have been suggested for the mechanisms underlying COVID-19-related coagulopathy (7). The first is that SARS-CoV-2 infects endothelial cells and causes thrombosis with vascular inflammation. İn situ hybridization, immunohistochemistry, and electron microscopy have shown SARS-CoV-2 itself and viral particles in endothelial cells. These findings support the theory that SARS-CoV-2 directly infects endothelial cells and impairs their anti-thrombogenic features. Damage to endothelial cells by a viral infection can easily be understood as a cause of thrombosis (8,9).

A second theory is that SARS-CoV-2 does not directly infect endothelial cells, but rather that cytokine storms are responsible for thrombosis as excessive immune responses. One study found that cultured endothelial cells were resistant to SARS-CoV-2 infection (9). On the other hand, infection of alveolar macrophages and alveolar epithelial cells with SARS-CoV-2 causes these cells to produce markedly high levels of IL-6, IL-8, tumor necrosis factor (TNF)-α, and chemokine ligand 8 (CXCL8) such as results cytokines or chemokines. Ultimately causing a cytokine storm (10). Cytokine storms are thought to cause platelet activation, endothelial damage, coagulation activation, and as a result, a decrease in endothelial anti-thrombogenic activity and an increase in prothrombogenic activity. In addition, it has been reported that the SARS-CoV-2 spike protein alone stimulates endothelial activation, resulting in cytokine release and complement activation (11).

High fibrinogen and D-dimer levels are frequently observed in laboratory abnormalities consistent with hypercoagulation and are considered the two most important markers. Also, elevated D-dimer levels, PT prolongation, and thrombocytopenia suggest secondary fibrinolysis following coagulation activation in COVID-19 infection (3). Patients with a three to fourfold increase in D-dimer levels should be hospitalized even in the absence of other symptoms. Because this indicates increased thrombin formation with potential thrombotic risk (12). Coagulation laboratory tests in COVID-19 patients differ from those found in disseminated DIC (intravascular coagulation). Although increased D-dimer levels are suggestive of DIC, the D-dimer concentrations generally observed in COVID-19 are much higher than those observed in DIC (13).

In the standard DIC table, a decrease in platelet count and an increase in prothrombin times (PT) are observed. In COVID-19 patients, however, most standard coagulation tests are generally relatively normal at baseline, despite hyperfibrinogenemia (14).

Routine coagulation tests are performed to determine the deterioration of the coagulation system in patients admitted to the intensive care unit. In addition to these tests, fibrinogen may be useful in COVID-19 patients. Fibrinogen is an acute-phase protein, so fibrinogen concentrations may be higher than normal (15).

1. D-DIMER STRUCTURE AND FORMATION

D-dimer is a complex protein molecule produced during plasmin-mediated cross-linked fibrin degradation. When D-dimer formation begins, fibrin molecules are formed after thrombin-mediated cleavage of fibrinogen, a soluble glycoprotein found in plasma. Thrombin cleaves the polymerization site of a fibrinogen molecule, thus making the site open to binding of other fibrinogen or fibrin molecules. In this way, several split fibrin molecules are linked together in an overlapping manner. Thrombin molecules remain attached to fibrin molecules during their polymerization, while simultaneously activating fibrinogen-bound plasma factor XIII. The complex of thrombin molecules, plasma factor XIII, and fibrin polymers together trigger the formation of factor XIIIa (16,17).

Plasma factor XIIIa is involved in the cross-linking of fibrin molecules. Next, plasminogen interacts with fibrin molecules, which leads to the formation of plasmin. The plasmin molecules produced, in turn, bind to the fibrin molecules and mediate the degradation of bound fibrin into products commonly known as fibrinogen degradation products (FDPs). Plasmin also mediates terminal degradation of cross-linked fibrin molecules into soluble fragments containing DDE fragments. The DDE fragment is a D-dimer molecule non-covalently attached to the E fragment. The plasmin molecule further cleaves the DDE fragment into DD and E fragments. The D-dimer (DD fragment + E fragment) is a soluble complex that circulates in plasma until it is eliminated by the renal pathways and the reticuloendothelial system. The half-life of D-dimer circulating in plasma is 8 hours, and it can be detected in blood only 2 hours after thrombus formation. The formation of D-dimer occurs only during the formation and degradation of cross-linked fibrin molecules, which occurs during coagulation and fibrinolytic events. Therefore, D-dimer molecules serve as a marker of thrombotic and fibrinolytic activity (17,18).

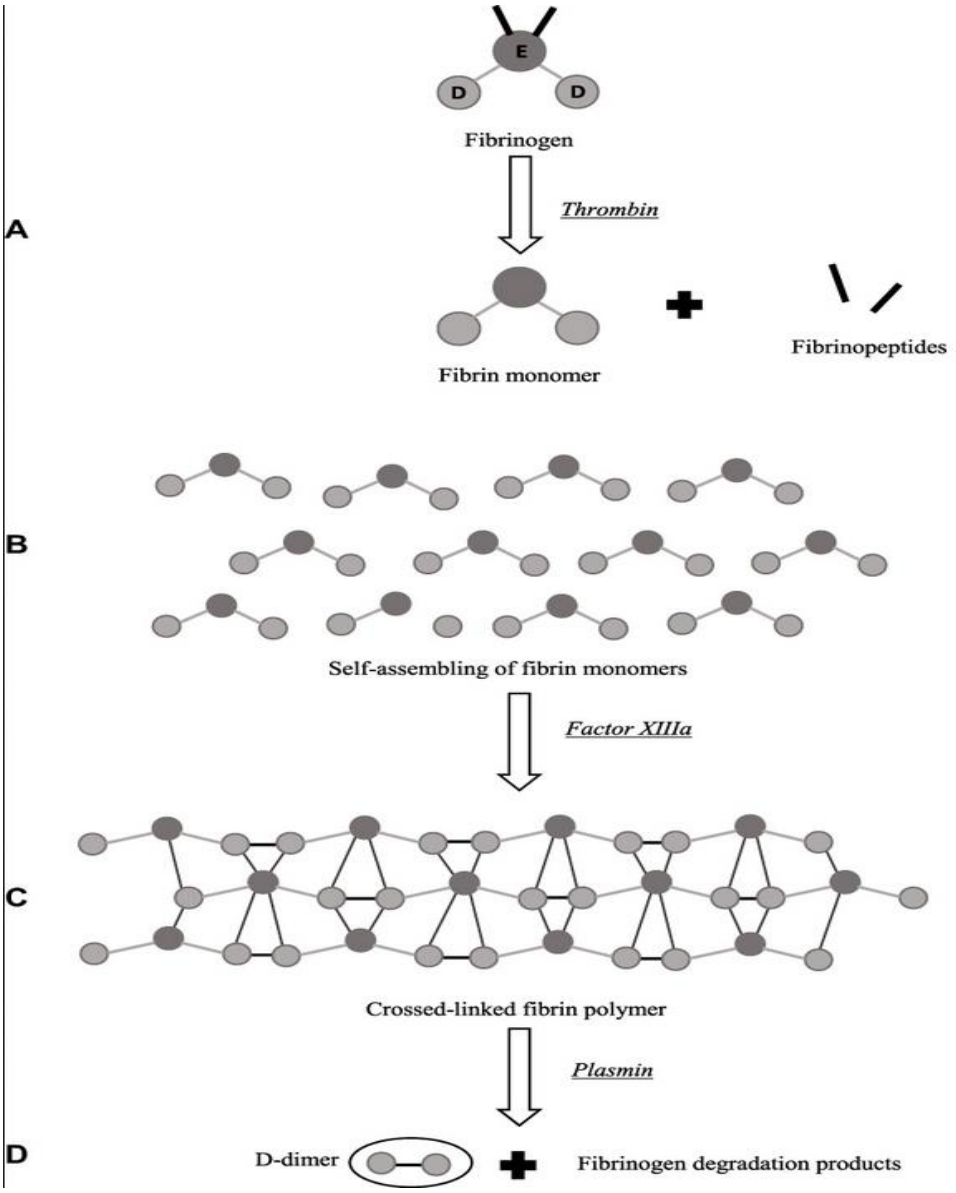

Figure 1 The process of D-dimer generation: 1) Thrombin cleaves fibrinopeptides from fibrinogen monomer (A); 2) Fibrin monomers aggregate (B); 3) Fibrin monomers are cross-linked by factor XIIIa, that stabilizes the fibrin polymer (C); 4) fibrin is degraded by plasmin, releasing D-dimer and fibrinogen degradation products (FDP) (D) (19).

D-Dimer is a low-specific laboratory test. D-dimer level tends to increase in a few clinical situations, so its use in the Emergency Department (ED) may be affected by a low diagnostic specificity. Lippi et al collected D-dimer values from 1647 patients who reached the emergency department regardless of their pretest clinical probability for VTE. In patients with high D-dimer levels, the most common diagnosis was infection (15.6%), followed by VTE (12.1%), syncope (9.4%), heart failure (8.9%), trauma (8.2%), and cancer (5.8%) have shown that they are watching (20).

2. ROLE OF D-DIMER IN THE EVALUATION OF COVID 19 PATIENTS

D-dimer is the major degradation component of fibrin and is used as a biomarker of coagulation and fibrinolysis. In theave been reported in the majority of patients hospitalized with COVID-19. D-dimer levels peak about 5 days after hospitalization and are higher in critically ill patients or those who die later. An in-depth investigation of D-dimer levels in 2,377 hospitalized COVID-19 patients found a relationship between baseline and peak d-dimer levels and critical illness, thrombosis, acute kidney injury, and all-cause death. (21,22). D-dimer levels could be used to screen for thrombosis, with higher levels of d-dimer found in patients with COVID-19 and venous thromboembolism (VTE) than in those without venous thromboembolism, both in multiple individual studies and in a systematic review of 47 studies. Another review of 71 studies suggested that d-dimer levels could be used to identify COVID-19 patients needing CT pulmonary angiography to diagnose pulmonary embolism using a d-dimer cut-off level of ≥1,000 μg/L (23). In a prospective study in 803 patients hospitalized with COVID-19, all receiving anticoagulant thromboprophylaxis, a protocolized escalation of anticoagulation dose based on a combination of illness severity, body weight, and D-dimer level was associated with reductions in mortality (6.3% versus 11.8%; P = 0.02) and thrombotic events (4.4% versus 10.7%; P = 0.002) compared with patients treated off-protocol (24).

Since D-dimer levels are directly related to the rate of plasmin formation and degradation, any pathological condition that increases the rate of plasmin production and degradation will also increase D-dimer levels. In addition, pathologies that support chronic inflammation such as asthma, rheumatoid arthritis, and cancer also lead to increased D-dimer levels. Some studies have suggested that increased D-dimer levels in individuals with severe COVID-19 may be associated with severe disease, higher rates of thrombotic activity, and higher mortality rates in such patients (25).

Zheng et al. reported in their study that D-dimer levels greater than 500 μg/L indicate abnormally high blood coagulation in COVID-19 patients and are significantly associated with poor outcomes in such patients (26).

Chen et al. showed how D-dimer levels can be used as a marker to predict the in-hospital mortality rate of COVID-19 patients. Based on the study results, they determined a D-dimer threshold higher than 2140 μg/L to predict the prognosis of COVID-19 patients at admission. They reported that this cut-off value can be used effectively to predict in-hospital mortality with a sensitivity of 88.2% and a specificity of 71.3% (27).

In 2020, Guan et al. reported the results of a large retrospective study showing for the first time the correlation between abnormal D-dimer levels and disease severity in COVID-19 patients presented. By setting a D-dimer level cutoff greater than 500 μg/L, they reported that a significant proportion of COVID-19 patients with severe disease exhibit abnormally high D-dimer levels compared to those with only mild or moderate disease (28). Similarly, Tang et al. reported that COVID-19 patients with severe diseases showed approximately 3.5 times higher D-dimer levels than patients with only mil COVID-19 setting, d-dimer has been widely investigated and elevated levels h

d or moderate diseases (14). In addition, Wang et al. found that D-dimer levels in deceased COVID-19 patients were more than double the D-dimer levels in severely ill COVID-19 patients (29). Yao et al. showed a significant correlation between disease severity and D-dimer levels in patients categorized as severe COVID-19 based on oxygenation indices, lung CT scans, and relevant clinical guidelines (30).

3. CONCLUSION

Pathophysiologically, the coagulopathy of COVID-19 has not yet been fully elucidated, although the underlying mechanisms may partially overlap with those of bacterial sepsis-induced coagulopathy and DIC. Despite some similarities with other coagulopathy conditions, coagulopathy in COVID-19 shows distinctive features: prolongation of PT and aPTT and decreased antithrombin activity are less common

in COVID-19 compared to coagulopathy from bacterial sepsis. Thrombocytopenia is relatively rare, while thrombocytosis occurs in the most severe cases. However, one of the typical features of the coagulation disorder in COVID-19 is the marked increase in D-dimer in line with the severity of the disease (31).

In conclusion, D-dimer levels are directly related to disease severity among COVID-19 patients. New coronavirus infections promote inflammatory and coagulation reactions, leading to increased rates of thrombotic events. Evaluation of D-dimer levels seems useful in predicting the prognosis of COVID-19 patients. Laboratory tests for D-dimer and proinflammatory cytokines can help classify COVID-19 patients according to the severity of their illness. This, in turn, can help to manage such individuals adequately and more efficiently.

More comprehensive and multicenter studies are needed to better elucidate the relationship of D-dimer levels with proinflammatory cytokine levels and thrombotic pathways in COVID-19 patients. Finally, it seems important for physicians to use anticoagulant treatments to prevent increased thrombotic activity in COVID-19 patients.

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CHAPTER 23

COVID-19 EFFECTS ON OTHER ENDOCRINE GLANDS (ADRENAL–PITUITARY-PARATHYROID-GONAD)

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INTRODUCTION

The pandemic of coronavirus disease 2019 (COVID-19) continues to have a substantial impact on worldwide health care systems, inflicting terrible mortality and morbidity. Many organs and biological systems are affected by SARS-Cov-2 infection, either directly from virus destruction or indirectly through effects that can have systemic consequences (1). As time goes on and our understanding of this novel respiratory virus grows, it becomes obvious that its effects go beyond the respiratory system. The COVID-19 coronavirus, severe acute respiratory syndrome coronavirus 2, gains cellular entry via the angiotensin-converting enzyme 2 (ACE2) receptor. Various extensive symptoms are related to the presence of the ACE2 (Angiotensin converting enzyme 2) receptor in these organs, which is thought to be at the heart of COVID-19 pathophysiology. ACE2 receptor expression has also been found in the hypothalamus, pituitary, thyroid, gonads, and pancreatic islets, among other endocrine organs (2,3).

ACE2 mRNA is found in a variety of endocrine glands in humans, including the pancreas, thyroid gland, ovaries, and testes (3). TMPRSS2 mRNA is also found in the pancreas, thyroid gland, ovaries, and testes, which is important (3). As a result, the endocrine system not only has the essential ACE2 receptor, but also the TMPRSS2 protein to allow the SARS-CoV-2 virus to enter the cell. In conclusion, there is mounting evidence that COVID-19 makes the endocrine system particularly sensitive to both destruction and functional changes (4).

COVID-19 has the potential to disrupt the operation of many endocrine glands and metabolic processes, putting individuals at risk of acute or late-onset endocrine or metabolic dysfunction. During the previous two years, a large amount of fresh data has arisen with the goal of improving our understanding of COVID-19, its symptoms, and management. the current state of knowledge on COVID-19 endocrine symptoms Several endocrinopathies have been documented in patients with COVID-19 infection. Hypopituitarism, SIADH, central diabetes insipidus, thyroiditis, thyrotoxicosis, hypothyroidism, low T3 syndrome, hyperglycemia, adrenal insufficiency, orchitis, and sperm morphology changes are among them. The majority of the data comes from single case reports and case series. Furthermore, preexisting endocrine problems or metabolic processes can increase the risk of developing COVID-19 or a more severe clinical presentation and outcome (5).

Because the endocrine system is a concern during the COVID-19 pandemic, various clinical problems must be addressed. It should be determined whether one patients with COVID-19 are more likely to develop acute or late-onset endocrine diseases or dysfunction; 2. underlying endocrine diseases or dysfunctions are risk factors for poor prognosis once the infection has occurred; and 3. pandemic-related community and healthcare service restrictions and reorganization may contribute to changes in the epidemiology of endocrine diseases or dysfunctions or affect their management.

As a result, in this special chapter, the available data on the main components of COVID 19's endocrine and metabolic profile, as well as their clinical implications on the endocrine glands including adrenal, pituitary, parathyroid, and gonad in order to assist clinicians in implementing appropriate evaluation and care of afflicted individuals will be discussed (Figure 1) (4,6).

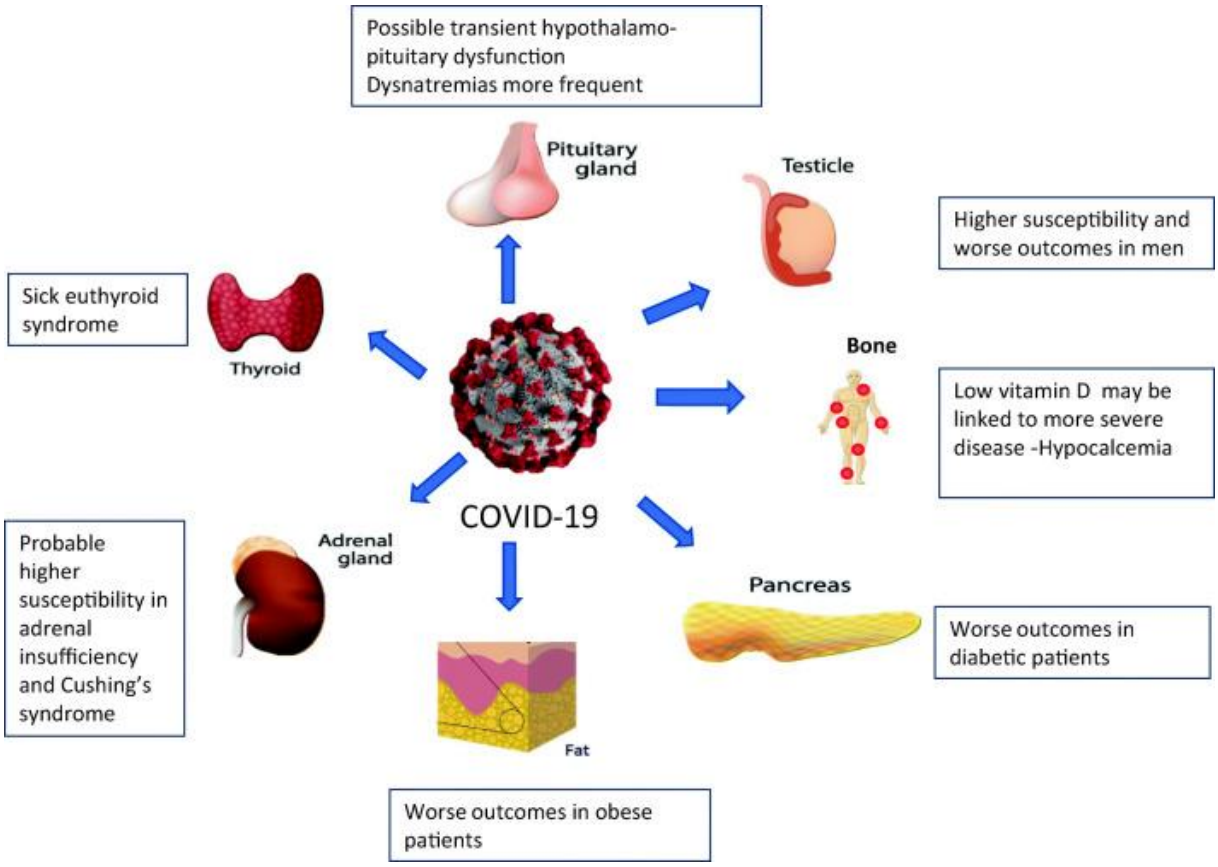

Figure 1: Different endocrine glands/organs that can be affected by COVID-19: 1) Pituitary: possible hypothalamic-pituitary disfunction and alterations in antidiuretic hormone metabolism. 2) Thyroid: sick euthyroid syndrome; 3) Adrenal: probable higher susceptibility to COVID-19 in adrenal insufficiency and Cushing's syndrome; 4) Bone. Low vitamin D may be linked to more severe disease Increased risk of hypocalcemia. 5) Testicle: Higher susceptibility and worse outcomes have been reported in men; 6) Diabetes. Worse outcomes in diabetic patients; 7) Obesity. Worse prognosis in obese patients (6).

1. COVID-19 AND ADRENAL GLAND

ACE2 and TMPRSS2 are colocalized in adrenocortical cells. The high vascularisation and blood supply of the adrenal glands makes them particularly sensitive to endothelial dysfunction and bleeding as a result of sepsis-induced organ damage. As a result, patients with COVID-19 have already had adrenal endothelial impairment, bilateral haemorrhages, and infarctions (7).

Multiple variables can contribute to the development of adrenal insufficiency in COVID-19 patients, changing corticotroph function post-COVID-19. Although it is generally acknowledged that short-term steroid therapy (less than 3 weeks) does not produce corticotroph insufficiency, it can arise in some predisposed individuals after only a few days of low-dose treatment. Pharmacokinetic variations and glucocorticoid receptor sensitivity account for the majority of individual heterogeneity. Patients in critical care units also have abnormal glucocorticoid metabolism, as previously described. Antiretroviral medications, such as ritonavir, may elevate plasma steroids by blocking cytochrome P450, resulting in hypercortisolemia and, through a negative feedback loop, endogenous corticotroph insufficiency in COVID-19 patients. Among the organs directly affected by SARS-CoV-2, adrenal damage has also been reported (8,9). As a result, it's critical to look at adrenocortical function in COVID-19 individuals.

The activation of the HPA axis by cytokines results in greater adrenal perfusion and a higher risk of bleeding, as well as immunomodulation toward a Th-2 helper T cell response, which

causes adrenal insufficiency induced by viral infections (10). It's been suggested that adrenal injury is caused by vasculopathy caused by a “cytokine storm” (7) The development of ACTH-inactivating antibodies is another mechanism of adrenal failure. SARS-CoV peptides are structurally similar to ACTH and cause "molecular mimicry" against the adrenocorticotrophic hormone (ACTH) As a result, secondary adrenal insufficiency may emerge as a result of this infection (11). The development of functional adrenal insufficiency throughout the severe course of COVID-19 is possible (12).

Infections are more common in patients with primary adrenal insufficiency, such as Addison's disease and congenital adrenal hyperplasia (13). Patients with PAI have an ineffective innate immune response, which is characterized by a reduction in cytotoxic natural killer cells, as well as a subsequent failure of IgG-mediated activation due to shedding of CD16, the surface receptor for the disease (14). As a result, PAI is thought to increase the chance of COVID-19 infection (15). Furthermore, a stress-induced rise in serum cortisol is necessary for priming the immune system; hence, a lack of cortisol rise in patients with PAI may put them at a higher risk of progressing to the critical stage (16). Furthermore, COVID-19 can hasten the onset of acute adrenal crisis in PAI patients, increasing the risk of complications and fatality. As a result, individuals with PAI, like those with diabetes, must exercise extreme caution in the face of the continuing epidemic. To be able to manage their diseases effectively and safely, all patients should be given enough self-management assistance. Support for self-management can be provided and conveyed by mailshot, video, text, email, phone call, or videoconferencing, as needed. All patients (and their caretakers) must be taught how to apply sick day regulations, which require them to raise their customary glucocorticoid replacement dosage while they are sick. A stress dose of glucocorticoid with 20 mg hydrocortisone delivered orally every six hours can be considered in patients with PAI who develop an acute COVID-19 infection. An adrenal crisis will not be precipitated as a result of this workout. Patients with worsening COVID-19 require parenteral glucocorticoids; a proposed protocol includes a 100 mg hydrocortisone intramuscular injection, followed by a continuous intravenous infusion of 200 mg hydrocortisone every 24 hours, or 50 mg hydrocortisone boluses every 6 hours until this can be established. Hypokalemia has been recorded in individuals with COVID-19, hence serum potassium should be closely monitored in this situation (17). Glucocorticoids and mineralocorticoids should be maintained on hand in sufficient quantities. Patients who regularly use modified-release hydrocortisone should maintain a supply of immediate-release, conventional oral hydrocortisone on hand in case of an emergency (15). Because hydrocortisone pills are sometimes difficult to come by, an equivalent dose of oral prednisolone can be used instead. Fludrocortisone acetate tablets (the most commonly used mineralocorticoid) are heat labile and should be stored between 2 and 8 degrees Celsius. In addition, all patients should have access to an up-to-date hydrocortisone emergency self-injection kit, and the patient and a caregiver should be comfortable administering the injection themselves.

Other important issue to remember how to manage patients on steroide treatment , it is crucial to execute an adequate dosage tapering in individuals infected with COVID-19 who are being treated with large doses of steroids. However, if a stressful event occurs (such as illness, accident, or surgery), the dose must be raised. Prednisolone 5.0–7.5 mg/day, hydrocortisone 20 mg/day (fractionated in 2–3 doses), or cortisone acetate 25 mg/day are used to maintain physiological glucocorticoids concentrations (fractionated in 2 administrations). Hydrocortisone is often associated with a faster recovery of HPA function due to its short half-life. Unfortunately, there are no standards or consensus statements on how to taper steroids in this circumstance. As a result, regular monitoring of blood pressure, weight, serum sodium levels, and clinical symptoms that indicate adrenal insufficiency should be part of the patient's management. Finally, non-endocrinologists should be aware that blood cortisol levels must be monitored in the morning prior to steroid therapy (18).

Accorging to available data, most patients' adrenal function is intact, and elevated cortisol levels within the first 48 hours of admission are linked to increased mortality. While there have been cases of adrenal insufficiency in COVID-19 individuals due to acute vascular problems (e.g. hemorrhage/thrombosis), corticosteroid production has not been affected. Furthermore, while the symptoms of protracted COVID-19 are comparable to those of adrenal insufficiency, even in individuals treated with dexamethasone, there is little evidence of glucocorticoid shortage (4).

Table 1: Recommendations based on the evidence available (20)

❖ The regular measurement of blood cortisol/ ACTH in post-COVID-19 patients is not recommended.
❖ Patients with COVID-19 who were treated with dexamethasone as part of the RECOVERY study (6 mg once a day for a maximum of 10 days) did not have compromised adrenal function, hence they did not need an adrenal function examination in the post-COVID-19 environment.
❖ Patients with COVID-19 who took steroids for 3 weeks during the acute phase of the disease are unlikely to have clinically substantial hypothalamic-pituitary-adrenal (HPA) axis suppression and hence do not need to have their HPA function evaluated.
❖ In individuals taking the equivalent of 15 mg or more of prednisolone for more than 3 weeks, suppression of the HPA axis is unavoidable. As a result, after tapering and ceasing glucocorticoids, the HPA axis may be assessed.
❖ In post-COVID-19 patients with surrogate signs of AI, such as recent onset anorexia, involuntary weight loss, diarrhea, hyponatremia and/or hyperkalemia, and/or eosinophilia, morning serum cortisol/ACTH levels can be estimated.
❖ At 12 weeks following discharge, patients with COVID-19 who have biocheically documented AI during the acute phase of the disease should have a morning serum cortisol/ACTH test calculated with/without Synacthen stimulation test, with hydrocortisone withheld for 24 hours previous to the test.
❖ In the absence of a prior history of glucocorticoid intake, any documentation of central AI should initiate a search for additional anterior pituitary hormone deficits.

To summarize, glucocorticoids serve a critical function in priming the immune system as a result of infection or injury. Hypothalamic-hypophysis activity also enhances immune suppression and lowers cytotoxic damage and potential autoimmune reactions. According to this intriguing point of view, patients with primary or secondary hypocortisolism are more likely to have a poor prognosis if glucocorticoid supplementation is not appropriately sustained throughout the infection's various stages, including COVID-19 (Table 1)(19,20).

2. COVID-19 AND PITUITARY

In COVID-19, pituitary disorders were also reported to be clinically relevant [96]. The occurrence of neurologic symptoms in individuals infected with SARS-CoV2 and the expression of ACE2 on hypothalamus and pituitary cells imply that coronaviruses infect the central nervous system. The SARS-CoV-2 may also target the hypothalamic-pituitary (HP) axis (21). Coronavirus infections can damage the hypothalamus and pituitary, as well as the central nervous system. Because vascular endothelium has a high expression of ACE2 receptors (22), the pituitary gland's vascular supply is sensitive to injury during COVID-19 infection. In fact, compared to the general population, patients with Cushing's disease had a greater incidence of SARS-Cov-2 infection, according to many studies. COVID-19 severity was shown to be higher in individuals with active illness (23), implying that chronic uncontrolled

hypercortisolism may play a role in this clinical setting (24). Furthermore, hypopituitarism, pituitary apoplexy, hyponatremia, and hypophysitis were recently reported as major hallmarks of a specific involvement of the pituitary in the endocrine phenotype of COVID-19 (24,25). Furthermore, comorbidities such as diabetes mellitus, obesity, and spinal fractures might infect hypopituitarism patients, predisposing them to severe COVID-19 (26,27).

Pituitary apoplexy is a clinical and surgical emergency syndrome produced by a pituitary haemorrhage and blood infarction, which frequently occurs in the presence of a pituitary macroadenoma. Pituitary apoplexy is a rare occurrence (incidence 0.17/100,000/year, prevalence 6.2/100,000) that affects up to 12% of people with pituitary adenoma, especially those on anticoagulant therapy or those with prolactinomas that are just starting to grow. dopamine–agonists. Patients main symptoms include severe headache, reduced visual field, and ocular palsy, which are caused by a tumor haemorrhagic and necrotic mass squeezing the surrounding optic structures and extending into the cavernous area; hypopituitarism is a common complication in this clinical scenario due to the severe damage of the pituitary gland (28, 29).

In individuals with COVID-19 infections, many incidences of pituitary apoplexy have been reported. These individuals' clinical indications and symptoms were consistent with or indicative of apoplexy: headache, visual abnormalities, occasionally with neurological signs or vomiting. Males and females under the age of 50 were both impacted. Different pituitary functions were involved in varying degrees, ranging from normal anterior pituitary function to insufficiency in one or more pituitary functions. Apoplexy, as previously mentioned, can be a direct result of SARS-CoV-2 infection in the central nervous system, most likely due to the virus's endothelial effects, which cause thrombocytopenia, coagulopathy, and platelet dysfunction (30).

Furthermore, multiple case reports have been published that show COVID-19-induced hypothalamic and pituitary damage in a variety of clinical settings, including panhypopituitarism, central diabetes insipidus, and a syndrome of inappropriate antidiuretic hormone leading in hyponatremia. Hormonal disturbances of the pituitary gland, including reduced prolactin, follicle-stimulating hormone, luteinizing hormone, and increased thyroid-stimulating hormone production, were reported in SARS-CoV patients, possibly due to injury to the adenohypophysis endocrine cells (5, 31-34).

Hyponatremia affects 20–50 percent of COVID-19 infected patients, however it is usually mild (130–134 mmol/L). Dysnatremia has been linked to poorer outcomes. Several cases of SIADH have been documented in patients with COVID-19, but the true prevalence of SIADH is difficult to estimate because antibiotics, steroids, positive pressure ventilation, gastric ionic losses, and sodium depletion can all contribute to hyponatremia in these individuals. Hypersecretion of IL-6, which promotes vasopressin release, could potentially cause SIADH

(Table 2) (20,34,35).

Table 2: The evidence available in COVID-19 (20)

❖ Patients with COVID 19 who experience new onset headache and visual disturbance after recovery should be suspicious of pituitary apoplexy and should have a noncontrast computed tomography of the head conducted as soon as possible. Pituitary apoplexy should be considered more frequently, especially in those with an underlying pituitary adenoma, pregnant women, and those using antiplatelet medicines (20).
❖ Patients with COVID 19 who have pituitary apoplexy in the acute phase of the disease but no anterior pituitary hormone shortage should be reevaluated for incident hormone deficiencies at 6 weeks (20).
❖ Patients with COVID 19 who were diagnosed with pituitary apoplexy in the acute phase of the disease and were found to have 1 or more anterior pituitary hormone deficiencies and were supplemented with the appropriate hormones should be reevaluated at 6 weeks to see if any other incident hormone deficiencies had occurred (20).

3. PARATHYROID GLANDS AND CALCIUM-PHOSPHORUS METABOLISM AND OSTEO-METABOLIC PHENOTYPE

Although there is no information on the direct effect of COVID-19 on the parathyroid glands or bones, tissue samples collected from deceased COVID-19 infected individuals have revealed SARS-CoV-2 RNA in parathyroid gland acidophilic cells. Furthermore, increased expression of angiotensin converting enzyme 2 (ACE2) receptors was found in acidophilic cells of parathyroid glands, suggesting that the parathyroid gland could be a target for SARS-CoV-2. Hyperphosphatemia and low parathyroid hormone levels have been reported, which could imply COVID-19-related parathyroid dysfunction (36-38).

During the spread of the pandemic, several studies have shown that the endocrine and metabolic features of COVID-19 were strongly relevant clinical manifestations (1). Some papers have now highlighted an emerging osteo-metabolic phenotype of COVID-19, which might influence COVID-19 severity and clinical outcomes. This phenotype is typically characterized by widespread acute hypocalcaemia and chronic hypovitaminosis D, as well as a high prevalence of morphometric vertebral fractures (39,40.41). Calcium appears to be involved in endosome trafficking, viral internalization into host cells, and the process of viral fusion to the cell membrane of many enveloped viruses, including coronaviruses (42). Vitamin D (D vit) and its active metabolites are necessary for maintaining homeostasis balance in a variety of tissues and systems, including bone, skin, heart and arteries, pancreas, muscles, and brain, and it also has immune-modulative actions (43,44).

Hypocalcemia and morphometric vertebral fractures among those hospitalized for COVID-19 are other characteristics of metabolic derangements, in addition to low vitamin D levels. Low vitamin D levels and hypocalcemia have been documented in up to two-thirds of hospitalized individuals. These biochemical abnormalities could be linked to a lack of compensatory PTH response caused, at least in part, by the host's inflammatory response, as well as a number of clinical indicators of disease severity, such as the need for mechanical breathing, ICU hospitalization, and mortality (45-49).

Hypocalcemia has been noted often in COVID-19 patients. Hypocalcemia was discovered to be an independent risk factor for hospitalization. Patients with hypocalcemia required considerably higher hospitalization time and admissions to high-dependency units/ICUs than those without hypocalcemia. Nonetheless, persons with nonsevere COVID-19 have been observed to have a significant prevalence of asymptomatic hypocalcemia (and hypophosphatemia), the clinical significance of which is unknown. Despite this, hypocalcemia is a common consequence and a predictor of more severe COVID-19 forms,

with an unexpectedly high rate of silent fractures reported in these patients. Two-thirds of COVID-19 patients had hypocalcemia, according to a study described several mechanisms for this finding, including vitamin D deficiency, hypoalbuminemia, impaired intestinal absorption of calcium, hypoxic tissue damage with subsequent increase in calcium influx, and impaired secretion of, and response to parathyroid hormone (PTH) secondary to increased levels of inflammatory cytokines, rather than singling out COVID-19-related direct parathyroid hormone (50-54).

A significant prevalence of radiographic thoracic vertebral fracture (affecting one-third of hospitalized patients) is also part of COVID-19's osteo-metabolic profile. Vertebral fractures were proposed as a marker of disease fragility, and their severity was found to be a significant predictor of mortality. The necessity of keeping any form of anti-osteoporosis medication for people with osteoporosis and COVID-19 was highlighted by these findings (45, 55, 56)

Interestingly, a widespread shortage of vitamin D in Southern European countries was mentioned as one of the possible risk factors for severe COVID-19 from the start of the epidemic. Low vitamin D levels enhance the likelihood of severe COVID-19 symptoms as a result of these metabolic diseases, but also as an independent cause]. The link between hypovitaminosis D and these outcomes is most likely due to a weakened innate and adaptive immune system. Studies demonstrating the effectiveness of vitamin D administration in preventing respiratory infections back up this theory. The molecular linkages between vitamin D deficiency and an increased risk and severity of SARS-CoV-2 infection are currently being researched. Until now, vitamin D status has been shown to predict the degree of pulmonary involvement in various cross-sectional investigations. Finally, vitamin D supplementation appears to play an important role in disease prevention, however some pilot studies have found that giving vitamin D to COVID-19 hospitalized patients reduces the severity of the condition enhance clinical outcomes in COVID-19 (41,57,64-67). Recommendations based on the evidence available in post-COVID-19 patients, we do not advocate routine serum calcium measurements (20). Patients with COVID-19 who have symptomatic hypocalcemia during the acute stage of the disease should have their blood calcium levels checked again 2 weeks after discharge. Recommendations based on the evidence available in post-COVID-19 patients, 25-hydroxyvitamin D levels can be approximated, and vitamin D should be supplied as needed (20).

4. COVID-19 AND GONAD

Several studies from various nations have found that women have a lower prevalence of poor outcomes than men (68,69). Baseline health status (i.e., chronic comorbidities), lifestyle (i.e., cigarette smoking), and hormonal and immunologic factors could all have a role in this gender dimorphism (70). SARS-CoV-2 relies on ACE2 and TMPRSS2 gene expression to function, however androgens boost ACE2 and TMPRSS2 gene expression independent of levels. Estrogen, on the other hand, may suppress ACE2 mRNA expression in differentiated airway epithelial cells. As a result, viral entrance appears to be easier in males, which could explain the gender difference in COVID-19 susceptibility. Females have higher cell-dependent and humoral responses to infection, as well as faster pathogen clearance than males. The sexual variation in COVID-19 disease severity and mortality could be explained by the immunomodulatory effects of sex hormones (71). The X chromosome contains multiple genes that regulate immune system response, and this biological situation could explain why immune system control differs by gender (72-74) Estrogens and progesterone (P4) may help to prevent COVID-19 from progressing further (75).

4.1. SARS-COV-2 INFECTION AND TESTIS FUNCTION

The hypothalamic-pituitary-testicular (HPT) axis is in charge of regulating reproductive activity and directing the release of sex hormones produced both centrally and peripherally. This is a very active axis, and its activity lasts a lifetime; nevertheless, external stimuli can readily change its behavior. Viruses like COVID-19 can disrupt the hypothalamic-pituitary-adrenal, thyroid, and gonad axes quickly, and their impairment can lead to sexual dysfunction in men (11,76). This impact can be induced directly by viral invasion or indirectly through systemic reactions (77). Although the brain and testicles are largely shielded from external influences by mechanisms known as the blood-brain and blood-testicular barriers, some viruses can slip through them and induce inflammation (77,78).

In earlier SARS virus assaults, serum prolactin, FSH, and LH levels were higher than healthy controls, whereas estradiol and progesterone levels were lower, indicating primary hypogonadism. During the current SARS-CoV-2 infection, however, there have been mixed results. Prolactin and luteinizing hormone levels were high in males with COVID-19, while testosterone and follicle-stimulating hormone levels were low, indicating primary testicular injury during active disease, according to a study (71). Because acute and severe illness can suppress the HPT axis and lower circulating testosterone, testosterone levels in COVID-19 patients are not a reliable indicator of testicular function (79). Overall, despite the fact that the testes are vulnerable to SARS-CoV-2 and there is evidence that patients with SARS-CoV-2 have lower testosterone levels than patients with other critical illnesses, the evidence to date suggests that any drop in testosterone levels after recovery from acute illness resolves spontaneously (4). Hypogonadism is defined as "failure of the testes to produce physiological amounts of testosterone and/or a normal quantity of spermatozoa due to disease at one or more levels of the HPT axis," according to the Endocrine Society Guidelines.

Hypogonadism can be primary, caused by testicular dysfunction, or secondary, caused by hypothalamic/pituitary disorders, and the two can occur together in some cases. During SARS-Cov-2 infection, all kinds of hypogonadism described above can be present. In the case of the hypogonadism patient with COVID-19, the condition can affect him or her in a variety of ways (79-81). Testis Function with SARS-CoV-2 Infection The impact of viral infection on testicular function includes the hypothalamus-pituitary-testis axis, local inflammation in the testis, and the effect of fever on testicular function (82). Patients with SARS-CoV-2 infection had a higher frequency of hypogonadism. Hypogonadism was secondary in the majority of these cases (85%) (83). SARS-CoV-2's direct effect on hormone and sperm testicular production, on the other hand, appears to be important in the development of hypogonadism. On both Sertoli and Leydig cells, ACE2 receptors were found. NRP1 expression was also shown to be greater in testicular cells. The presence of ACE2 receptors in sperm has also been documented. COVID-19 infection may have a greater impact on male fertility than previously assumed because SARS-CoV-2 infects not only testis cells but also sperm (84,85).

In COVID-19, T was identified as a key hormone with a putative bivalent impact (86,87). Normal serum T levels, on the one hand, may promote a large viral entrance into host cells and facilitate SARS-CoV-2 systemic spread. Lower T levels, on the other hand, as seen in elderly and comorbid men, may predispose them to a bad prognosis due to a putative involvement of male hypogonadism in generating cardiovascular events and amplifying immunological and coagulative responses (70, 88). Observational pilot investigations have indicated decreased serum levels of T in COVID-19 men with poor prognosis after hospital admission, possibly in the context of initial testicular injury, supporting these assumptions (89-91). As a result, blood T concentrations could be a biomarker for poor prognosis in hospitalized patients, and could be used to identify individuals who need more extensive therapy earlier. Additionally, T replacement therapy should be continued in patients with male hypogonadism, according to general recommendations (79), and therapies aimed at lowering T or increasing estradiol serum levels in men with confirmed COVID-19 should be introduced with caution until specific clinical trials are completed.

4.2. SARS-CoV-2 INFECTION AND OVARY FUNCTION

Despite the prevalence of ACE-2 receptors in the ovaries and oocytes, COVID-19's acute and chronic effects on the female hypothalamic-pituitary-gonadal (HPG) axis are not fully known. COVID-19 appears to be equally prevalent in both sexes (92). The polycystic ovarian syndrome is a systemic disorder that predisposes to a higher cardiometabolic risk than the general population, according to available data (93). Furthermore, because women with PCOS are subjected to hyperandrogenism, and because androgens promote the expression of TMPRSS2, they may be more susceptible to COVID-19 development once infected (94). According to a recently published observational retrospective study, women with PCOS have a 28 percent higher chance of catching SARS-CoV-2 infection than women without PCOS, and the former should be advised to follow hygienic precautions to avoid being burdened during the COVID-19 pandemic (95). Menstrual abnormalities, including irregular menstruation and extremely heavy periods/clots, were also identified among women with post-COVID-19 syndrome in an international cohort study (96).

Furthermore, despite reports of changes in menstruation and reproductive health, gonadal function appears to be prone to disruption and remains understudied, particularly in women. Finally, as the long-term effects of COVID-19 becoming a greater concern for health-care systems, the amount to which endocrine dysfunction contributes to long-COVID is unknown, and so represents a key subject for future research (4).

4.3. SUMMARY AND RECOMMENDATIONS

Summarizes the factors linked to increased male prevalence and mortality, as well as the impact of SARS-CoV-2 on the male reproductive system (Table 3) (71). The evidence of a relationship between COVID-19 and female (Table 4) (71). Recommendations Based on Available Evidence is shown (Table 5) (20).

Table 3: The factors associating the higher prevalence and mortality rate in males and effects of SARS-CoV-2 on the male reproductive system (71).

Aging and comorbid diseases
• As people get older, their testosterone levels start to drop.
• A higher prevalence of comorbid conditions, such as diabetes, heart disease, and cardiovascular disease.
• Men are more likely to develop COPD, which has a more severe course.
Sexual selectivity and genetic factors
• ACE2, SARS-major CoV's route of entrance into target cells, is more highly expressed in males than in females.
• The X chromosome contains both the androgen receptor and ACE2 genes, which are involved in inflammation.
• Androgen receptor sensitivity for testosterone differs between males and females, resulting in metabolic repercussions in the opposite direction.
• Immune modulation is influenced by testosterone.
• Male lungs are more susceptible to disease development.
Behavioral and social differences, and other factors
• Males are more likely to smoke and drink alcohol than females.
• Women are more conscientious about hand cleanliness and are more likely to seek preventive medical attention.
• In business and travel situations, men have more intimate contact.

SARS-CoV-2-related factors

- Viral clearance of SARS-CoV-2 is delayed in male subjects.
- Male lungs have higher ACE2 expression levels than female lungs.
- High ACE2 and TMPRSS2 expression in testicular Leydig cells causes testosterone levels to drop even more, causing increased morbidity during acute illness.
- SARS-CoV-2 may cause a reduction in testosterone levels by affecting the hypothalamic-pituitary-testicular axis

Table 4: The evidence of a relationship between COVID-19 and female (71)

ACE2/TMPRSS2 expression and estrogen

- ACE2 is found in human ovaries, oocytes, and endometrial tissue.
- TMPRSS2 is more widely expressed, with co-expression of ACE2 and TMPRSS2 in testicular, endometrial, and placental cells, as well as nonhuman primate ovarian cells.
- ACE2 may influence the synthesis of estradiol and progesterone, as well as ovulation and oocyte maturation in humans, as well as endometrial regeneration and myometrium activity.
- In differentiated airway epithelial cells, E2 can influence the expression of ACE2.

COVID-19 estrogen and sexual dimorphism

- After COVID-19 infection, female patients have a lower illness morbidity and fatality rate than male patients.
- Sex hormones' immunomodulatory effects appear to be the most essential element in women's reduced mortality rates.
- Females have more cell-dependent and humoral immunity
- Females respond to infection and immunization in a more cell-dependent and humoral manner than males.
- In females, pathogen clearance is faster than in males because leukocyte activity and macrophage phagocytosis are more efficient.

COVID-19 and female reproduction

- Early pregnancy loss is uncommon, according to limited evidence.
- Pregnant women with COVID-19 have a lower rate of maternal morbidity, particularly in the respiratory system and preterm birth, than non-infected mothers, and the pregnancy outcomes of women with severe COVID-19 infection are similar to those of non-infected moms.
- Early clinical evidence suggests that SARS-CoV-2 is not transmitted vertically from mother to infant.

Estrogen as a treatment of COVID-19

- Emerging research suggests that estrogens can reduce lung inflammation, and that they may also help to prevent and treat COVID-19.
- New COVID-19 estrogen therapy trials are now underway.

Table 5: Recommendations Based on Available Evidence (20).

❖ During the acute phase of COVID-19, males with biochemically proven hypogonadism (either primary or secondary) should have blood total testosterone, LH, and FSH tests performed 3 months following discharge.
❖ Men with COVID-19 who have normal gonadal function throughout the acute phase of the disease do not need to have their gonadal hormones reevaluated on a regular basis.
❖ Men with COVID-19 whose gonadal function was not assessed during the acute phase of the disease do not need to have their gonadal hormones checked on a regular basis at follow-up.
❖ Regardless of the gonadal state during the acute phase of the disease, men who have recovered from COVID-19 and are experiencing new-onset erectile dysfunction and/or low/loss of libido should have serum total testosterone, LH, and FSH testing conducted. In such men with normal gonadal function, a psychiatric opinion should be obtained to rule out psychogenic erectile dysfunction.
❖ Following recovery, males with hypogonadism (low serum total testosterone) and low/normal LH/FSH levels should be checked for other anterior pituitary hormone deficits. In addition, the amount of serum prolactin should be determined.
❖ After recovery, males with hypogonadism (low blood total testosterone level) and increased LH/FSH levels should be monitored for the possibility of primary testicular failure.
❖ Men of reproductive age may undergo a semen analysis in any scenario if they intend to father children.

As a result, patients should be followed up on following COVID-19 to check for gonad function and even fertility. Finally, sex hormones, particularly estrogen, have the ability to control inflammation and so may be useful in the prevention and treatment of COVID-19. Even androgen-modulating medications could be investigated as a potential COVID-19 therapy (71).

5. COVID-19 VACCINATION AND ENDOCRINE SYSTEMS

COVID-19 vaccination should not be handled differently in patients with stable endocrine diseases such as autoimmune thyroiditis, Graves' disease, Addison's disease, pituitary adenomas, diabetes type 1 and 2, and obesity as compared to the general population," according to a statement from the European Society of Endocrinology. (1) ” In fact, COVID-19 vaccination should be given first priority to people who have diabetes (97).

The endocrine system's reciprocal influence on COVID-19 immunization has not been thoroughly investigated. However, in people with diabetes, blood glucose monitoring is required more frequently than normal for several days following immunization, and glucocorticoid dosage may need to be temporarily increased in people with AI (98). COVID-19 vaccinations may be linked to thyroid problems, according to new research. Following vector-based or mRNA-based SARS-CoV-2 vaccines, multiple cases of thyrotoxicosis have been documented, with symptoms ranging from SAT and silent thyroiditis to Graves' disease. Thyroid dysfunction could be caused by a variety of causes, including adjuvant-induced autoimmune/inflammatory syndrome (ASIA syndrome), molecular mimicry between human and viral proteins, the "self-adjuvant" action of mRNA, and immunological disruption from external stimuli (99-103). Subjects who experience involuntary weight loss, palpitations, tremors, and/or neck pain following immunization with vector-based or mRNA-based SARS-CoV-2 vaccines should be suspected of having thyrotoxicosis, and a TFT should be conducted (20).

6. COVID-19 SUMMARY AND PERSPECTIVES

COVID-19 can cause a variety of issues in clinical endocrinology. First, endocrine derangements and hormonal imbalances could appear as new symptoms or as a relapse of prior endocrine illnesses. It is critical to identify the date and mode of symptom onset in these situations, especially in hospital settings. In order to provide further scientific information regarding the problem, it may also be useful to rule out any possible links between these clinical disorders and a recent SARS-CoV-2 infection. Long-term follow-up could be relevant in this case to check for endocrine abnormalities in people who recovered from COVID-19 (e.g. autoimmune thyroiditis, male hypogonadism, erectile dysfunction) (104). Second, pre-existing endocrine and metabolic diseases, as well as their treatment, may increase the chance of getting SARS-CoV-2 infection or worsening COVID-19 clinical progression. There is no evidence that endocrine illnesses increase the risk of getting or transmitting SARS-CoV-2, even though women with PCOS appeared to be more vulnerable to infection in some reports. However, certain of them are more likely to acquire COVID-19 at a faster rate. Patients taking glucocorticoid replacement medication for adrenal insufficiency, calcium-metabolism imbalance, or uncontrolled endocrine illnesses like hypercortisolism, for example, may be at a higher risk of poor prognosis or death than the general population (104).

Because this risk is linked to the severity of endocrine dysfunction, patients must get specialized education in order to effectively manage chronic therapy and avoid SARS-CoV-2 infection. Because these patients are considered at-risk, any endocrine abnormalities should be treated as soon as feasible in the event of a SARS-CoV-2 infection, both in the hospital and at home. Potential interactions between continuing endocrine disorder medication and contingent COVID-19 treatment should also be examined. This is the situation with hypercortisolism individuals, for whom the danger of drug interactions might result in negative consequences such as hypoglycemia, QT prolongation, hypoglycemia, and hepatotoxicity (104). Last but not least, the ongoing epidemic has impacted the care of endocrine illnesses. The outbreak is still being categorized as a pandemic, and it could be years before the disease becomes endemic and more manageable (104). Long-term healthcare constraints are expected to impede the management of endocrine illnesses over time, resulting in diagnostic delays and disease control loss. The most significant source of this load is a lack of follow-up appointment scheduling. Case triaging could be a useful approach in dealing with the tough circumstance by prioritizing patients who require non-deferrable examination for diagnostic or therapeutic objectives (104,105). Non-urgent elective appointments, laboratory and imaging tests, and non-urgent surgery should all be safely postponed. Patients and caregivers should employ remote consultations and digital telehealth solutions for less serious cases, minor consultations, and educational/informative purposes.

Finally, despite the ongoing appearance of novel and more transmissible SARS-CoV-2 variants that may cause concerns, vaccines may be a resource in this historical setting (106). (107). Different types of vaccinations are now being researched, although some have been approved for clinical use (108), demonstrating that they are safe and effective in clinical trials, particularly in reducing the risk of severe disease and death (109-111). There are currently no data on vaccine safety and efficacy in certain groups of at-risk patients, particularly those with endocrine illnesses. In patients with endocrine problems, however, there are no legitimate concerns or real concerns that vaccinations may have lesser efficacy or safety concerns.

As a result, they can follow the same guidelines as the general public, as recommended by the European Society of Endocrinology (112). Due to the possibility of side effects (e.g., fever, myalgia, or arthralgia), patients with adrenal insufficiency may require additional glucocorticoid dosage with vaccination delivery (113). Patients with endocrine illnesses, particularly those with PAI, CD, CS, acromegaly, hypopituitarism, hypogonadal men, and polycystic ovary syndrome, should be included in the vaccination program, maybe with priority. Adequate vaccination coverage among these people could lower clinical risks and allow for safer access to healthcare facilities for diagnosis and treatment (104).

Endocrine dysfunction should be evaluated among other symptoms and late consequences of SARS-COV-2 infection. Some problems, including SIADH, pituitary infarction, and acute adrenal insufficiency, can prove fatal. In patients with SARS-COV-2 infection and post-COVID-19 syndrome, hormonal measures should be followed. Up to three months after discharge, 50-70 percent of patients hospitalized owing to SARS-COV-2 infection develop Post-COVID-19 syndrome. The following criteria are suggested for distinguishing the three types of post-COVID syndrome: acute postCOVID-19, with symptoms lasting 5 to 12 weeks; long post-COVID, with symptoms lasting 12 to 24 weeks; and chronic post-COVID-19, with symptoms lasting more than 24 weeks after the infection (114).Neurocognitive, autonomic, gastrointestinal, respiratory, and musculoskeletal abnormalities are all indications of post-COVID syndrome. Heart palpitations, chronic rhinitis, sleeplessness, chest pain, cough, anxiety, nausea, abdomen pain (115), exhaustion, headache, concentration problems, hair loss, and dyspnea have all been reported by patients (116). Endocrine problems, such as hypothalamic, pituitary, and thyroid gland dysfunction, may be linked to several post-COVID symptoms. Long-term effects of SARS-CoV-2 infection include decreased fertility and sexual function, particularly in men (117). In patients with SARS-CoV-2 infection and post-COVID-19 syndrome, there are no recommendations for hormonal testing. Because the number of persons with COVID-19 and post-COVID-19 syndrome is increasing, it is necessary to design minimal diagnostic schemes for the types of hormonal measurements and the time intervals at which they should be conducted.

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CURRENT COMPREHENSIVE APPROACH TO COVID-19

Today, it has been accepted by all health institutions that the COVID-19 pandemic is still an epidemic that continues to affect the whole world and that a new variant also emerged, that has a high mortality rate and causes serious economic and sociological losses. The COVID-19 pandemic has shown us that there are many aspects and new information that we need to learn over time. Some countries have taken precautions regarding vaccination, quarantine, mask, medicine, closure, and travel during this pandemic process and kept them on their agenda. In this process, our country also has our production of the "TURCOVAC" vaccine and gained new scientific experiences. How long this process will continue and whether other viral outbreaks will occur has become the subject of curiosity and concern for everyone. We would also like to point out that; the role and effort of health workers in the fight against the pandemic in our country and all over the world has been great. Day and night, they devoted a lot of effort and still do. WHO, our Ministry of Health, health-care professionals, hospitals, and all scientists are working hard to understand, end, and bring prosperity to the process. Our book is prepared for this purpose; We hope that it will make scientific contributions to all humanity in the fight against COVID-19 and new variants that may arise in the future. We believe that this book, which has been diligently prepared by faculty members from Karamanoğlu Mehmetbey University, Faculty of Medicine, and other Medical Faculties, will alter light on future studies.

We can also say that this book, prepared by our faculty members by adding their current experiences during the pandemic process, is one of the leading scientific publications about the COVID-19 pandemic. We would like to thank all our esteemed Faculty Members who contributed to the preparation of the book for their contribution and congratulate them sincerely. We wish all of us healthy, happy, and peaceful days.

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