Thailand's and Global Perspectives of COVID-19

Attapon Cheepsattayakorn, et al.

Medical and Research Publications

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Written by

Attapon Cheepsattayakorn

[MD FRCP FRCP (Edin)

FRCP (Glasg) FACP FCCP

FRCP (Thailand)

Consultant Pulmonologist and Senior Expert (Level 11) in Disease Control

The 10th Zonal Tuberculosis and Chest Disease Center, Chiang Mai Thailand

Faculty of Medicine Western University Thailand

Department of Disease Control Ministry of Public Health Thailand]

Ruangrong Cheepsattayakorn

[MD FRCPath (Thailand)

Associate Professor

Faculty of Medicine Chiang Mai University Thailand]

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Medical and Research Publications

First printing, 2021.

Published by

Medical and Research Publications,

124SpencerRd,Stoke-on-TrentST42BE,

United Kingdom.

www.medicalandresearch.com

Email: info@medicalandresearch.com

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CHAPTER 1

Introduction and Epidemiological Background

Abbreviations:

ACE: Angiotensin-Converting Enzyme

ARDS : Acute Respiratory Distress Syndrome

ASEAN : Association of Southeast Asian Nations

BMI : Body-Mass Index

CCDC : Chinese Center for Disease Control and Prevention

CCSA : Center for COVID-19 Situation Administration

CI : Confidential Interval

COVID-19 : Coronavirus Disease-2019

EUA : Emergency Use Authorization

FiO_2 : Fraction of Inspired Oxygen

GCSF : Granulocyte-Colony-Stimulating Factor

GISAID : Global Initiative on Sharing All Influenza Data

hACE 2 : human Anti-Converting Enzyme 2

HR : Hazard Ratio

ICU : Intensive Care Unit

IL : Interleukin

IP : Interferon Gamma-Induced Protein

IQR : Interquatile Range

MCP : Monocyte Chemoattractant Protein

MERS : Middle-East Respiratory Syndrome

MIP : Macrophage Inflammatory Protein

OR : Odds Ratio

p : Probability

PaO_2 : Partial Pressure of Oxygen

pm : post meridian

R_0 : Basic Reproductive Number

R_1 : Effective Reproductive Number

RNA : Ribonucleic Acid

RT-PCR : Reverse Transcriptase-Polymerase Chain Reaction

SARS : Severe Acute Respiratory Syndrome

SARS-CoV-2 : Severe Acute Respiratory Syndrome-Coronavirus type 2

SD : Standard Deviation

TNF : Tumor Necrosis Factor

UAE : United Arab Emirates

UK : United Kingdom

UN : United Nations

USA : United States of America

WHO : World Health Organization

Epidemiological Introduction

Coronavirus were found in the mid-1960s that can infect both humans and animals (birds and mammals), whereas seven coronaviruses are known to infect humans, such as Betacoronavirus HCoV-OC43 and HCoV-HKU1 and Alphacoronavirus HCoV-229E. These

coronaviruses primarily target on epithelial cells in the respiratory and gastrointestinal tracts through various routes of transmission, such as respiratory droplets, airborne, fecal-oral or fomites. These coronaviruses cause common colds as well as severe lower respiratory tract infections in the youngest and oldest age groups [1].

Wuhan, a city of more than 11 million residents is connected to other cities in China via high-speed railway and commercial airline flights. During January 2017 (as of January 31, 2020), there were 670,417 passenger bookings departing Wuhan, the top destinations being Shanghai of 53,214 bookings, Beijing of 51,066 bookings, and Kunming of 40,120 bookings [2]. Wuhan is connected internationally via both direct and indirect flights [3, Table 1]. COVID-19 was first identified from a patient with pneumonia, related to the cluster of acute respiratory illness cases from Wuhan, China with close relation to SARS-CoV and genetically clusters within the genus Betacoronavirus, subgenus Sarbecovirus [4, 5]. With bases on the epidemiological characteristics of respiratory infections caused by SARS-CoV and MERS-CoV, its incubation period of 2 to 7 days and up to 14 days is possible. Approximately 20 % of the laboratory-confirmed cases are seriously or critically ill and at least 4 confirmed cases have died [6].

Airport	Seats
Suvarnabhumi, Thailand	11,558
Changi, Singapore	10,680
Narita, Japan	9,080
Don Mueang, Thailand	9,000
Hong Kong International Airport	7,078
Incheon, South Korea	6,430
Kansai, Japan	6,272
Macau International Airport	6,145
Phuket, Thailand	6,116
Kota Kinabalu, Malaysia	6,048

Table 1 : Top 10 International Destinations from Wuhan city, China (December 2019 to January 2020)

(Source: South China Morning Post. Available at : multimedia.scmp.com/infographics/news/china/article/3047038/wuhan-virus/index.htlm (accessed on January 4, 2021)

On December 31, 2019, the Wuhan Municipal Health Commission in Wuhan City, Hubei province, China reported a cluster of 27 pneumonia cases of unknown etiology, including 7 severe cases, with a common reported connection with Wuhan's Huanan Seafood Wholesale Market (a wholesale fish and live animal market selling different animal species) [7, 8]. These cases presented with several infectious respiratory disease, such as fever, dyspnea, and bilateral pulmonary infiltrates on chest roentgenograms. Chinese authorities placed all cases under isolation, initiated contact tracing activities and hygiene and environmental sanitation activities at this market, which was closed to the public on January 1, 2020. At that time (as of January 1, 2020), no significant human-to-human transmission and no cases among healthcare workers were reported by the Chinese authorities. Between December 31, 2019 and January 20, 2020, 295 COVID-19-laboratory-confirmed cases, including 4 deaths, have been reported [9]. During this period (December 31, 2019-January 20, 2020), of the 295 laboratory-confirmed cases, 291 cases were reported by China (270 cases in Wuhan City, 5 cases in Beijing, 14 cases in Guangdong, and 2 cases in Shanghai) [9]. Fifteen healthcare workers in Wuhan were the reported cases during that period [10]. During that period (December 31, 2019-January 20, 2020), Wuhan City reported that 169 cases were hospitalized, of which 35 cases were seriously and 9 cases were critically ill [11]. During the period between January 9, 2020 and January 19, 2020, in Guangdong, China, 2 of the 14 reported cases had not travelled to Wuhan, China, but had a history of contact with laboratory-confirmed cases were the first confirmed human-to-human transmission cases [12], whereas the other four laboratory-confirmed cases were outside-China-travel-related [13-15]. Of the four reported deaths (January 9, 2020-January 19, 2020), all were in China with the ages ranked between 61 to 89 years [11, 13, 16, 17]. For the majority of the reported cases, the history of exposure to the Wuhan's Huanan Seafood Wholesale Market or other live markets is unknown [15]. Nevertheless, on January 19, 2020, first COVID-19-infected case of 35-year-old man presented to an urgent care clinic in Snohomish County, Washington was detected and reported in the United States [18]. Among 6,680 healthcare works employed at the Hadassah Hebrew University Medical Center (HHUMC), a two-campus medical centers in Jerusalem that established a proactive, periodic screening program for SARS-CoV-2 (COVID-19) for all

personnel revealed that 689 (10.3 %) were infected, mostly due to COVID-19-community exposure, and trends in incidence among these healthcare workers were similar to that in the Jerusalem population, from the beginning of the epidemic through January 31, 2021 [19, 20]. Men are more affected than women, whereas the ACE-2 receptor gene is identified on the X chromosome, not Y chromosome. The incidence by sex may have been to do with [8].

New Waves of COVID-19 in Europe

For considering the new 2021-COVID-19 pandemic in Europe (Figure 1), Germany extended existing control measures, including keeping cultural, leisure and sporting facilities shut through to April 18, 2021. During the Easter holidays between April 1, 2021 and April 5, 2021, all private gatherings were capped at two households of up to five people, plus children under 14 and supermarkets will remain closed, only opening their doors on Easter Saturday [20]. France has run a national strategy for the majority of the past year (2020), has decided to impose regional restrictions, putting 16 of the country's 96 mainland departments on what being termed "lockdown light". Other hard-hit departments will likely follow in the coming days or weeks [20]. Across the whole of France a 7 pm-6 am curfew remains in place and cafes, theatres, bars, cinemas, restaurants, and tourist sites were closed. The French government hopes that the regionalized "lockdown light" will be enough to relieve the pressure on hospitals in the worst hit areas whereas the much-maligned French vaccine belatedly gathers speed [20]. In Italy, the whole country has been tightened restrictions since April 5, 2021, with roughly half of the country a medium-risk "orange zone" and the rest a high-risk "red zone" [20]. On April 9, 2021, Switzerland decided to extend the majority of the country's lockdown measures for fighting against the "third wave" of COVID-19 [20]. Unlike a lot of other European countries, Denmark is in a phase of easing restrictions and has just announced a plan to lift several rules currently in place over the next two months [20]. In Spain, where COVID-19 restriction are mainly decided on a regional basis, there has been a general easing of the rules across the country in recent weeks as a result of decreasing COVID-19 infection rates overall [20]. Lighter measures include allowing travel between municipalities/provinces and better opening hours and capacity limits for shops, restaurants, and bars [20]. England is currently at the starting of a four-step plan to ease lockdown restrictions [20]. After a strict post-Christmas lockdown enforced to ease pressure on

overrun hospitals. Schools are open and if all goes to plan, pubs will open their outdoor areas next month (May 2021) [20]. The plan could see all legal limits on social contact lifted by June 21, 2021, if strict conditions are met. Northern Ireland, Wales, and Scotland also have their own plans to ease restrictions [20].

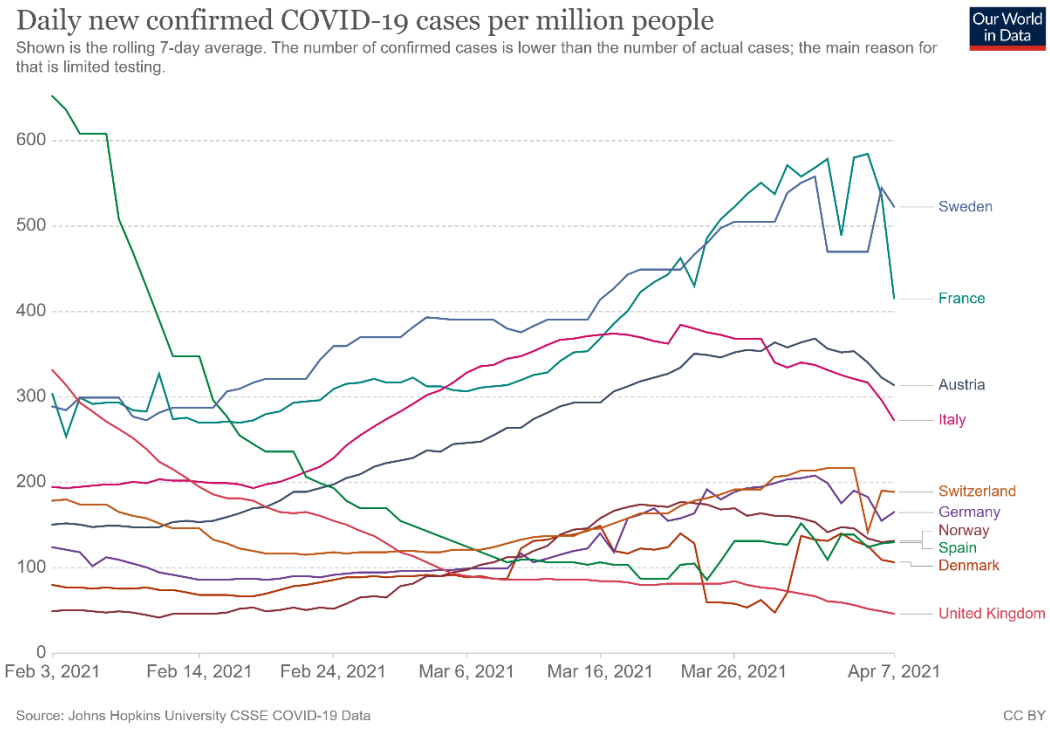

Figure 1 : Demonstrating daily new confirmed COVID-19 cases per million people in the European countries, as of April 7, 2021 [20]

(Source : The Local. Sweden's news in English. COVID-19 third wave : which countries in Europe have the tightest restrictions ? 7 pages. Available at : https://www.thelocal.se>covid-19-across-europe-have-... (accessed on April 9, 2021))

SARS-CoV-2 (COVID-19) Variants

Challen et al indicated that B.1.1.7 variant might be related to increased mortality that supplements to the central questions of the ability of an old version of the spike glycoprotein of SARS-CoV-2 (COVID-19) to produce protective antibodies against newer emerging variants

[21]. The current variants of concern, lineages B.1.351, B.1.1.7, and P1 affect the function of the spike protein and other SARS-CoV-2 proteins and can alter interaction with ACE 2 [22-23]. The first three COVID-19 vaccines with expressing spike protein and a progressing national rollout have authorization of emergency use in the United Kingdom (UK) and demonstrated protection against COVID-19 [23-25] and decreased transmission after vaccination in the preliminary report [26]. South Africa, a global leader in the use of intensive genomic sequencing since early in the COVID-19 pandemic has identified and tracked emerging SARS-CoV-2 (COVID-19) mutations [27]. The SARS-CoV-2 (COVID-19)-B.1.351 variant, first found in South Africa, has demonstrated significantly reduced neutralization by monoclonal antibodies and a considerable decrease in neutralization by postvaccination and convalescent serum [28, 29]. Second and third waves of SARS-CoV-2 (COVID-19) infection in Qatar were triggered by emergence outbreak of the COVID-19-B.1.1.7 variant that began in mid-January 2021 and COVID-19-B.1.351 variant that started in mid-February 2021. In Qatar, the B.1.1.7-variant wave peaked during the first week of March 2021, whereas the rapid expansion of the B.1.351 variant began in mid-March 2021 and continues to the present day. By viral genome sequencing started from February 23, 2021 through March 18, 2021 revealed that 44.5 % of the COVID-19 patients were infected with B.1.1.7 variant, whereas 50.0 % of the patients were infected with B.1.351 variant. After March 7, 2021, viral sequencing demonstrated either B.1.1.7 or B.1.351 variant among nearly all COVID-19-infected cases [30].

The COVID-19-B.1.617 variant (triple-mutant COVID-19 variant), first identified in India in October 2020, has been detected in more than 4,500 specimens demonstrated in an open-access database from 44 countries in all six WHO regions [31]. UK has reported the largest number of this COVID-19-B.1.617-variant-infected cases [31]. This COVID-19 variant appears to more easily transmitting than the original virus contributing to rapid increase in prevalence in several countries. The preliminary evidence indicated that this variant was more contagious, more deadly, more resistant to current vaccines and various treatments [32] and more resistant to therapy with the monoclonal antibody Bamlanivimab and limited decrease in neutralization by antibodies [31]. Nevertheless, several previous preliminary report demonstrated that antibodies from individuals infected with COVID-19 older strains or given Oxford/AstraZeneca (known as Covishield in India) or Covaxin vaccine do still prevent infections with COVID-19-B.1.617 variant [33]. India, a country of 1.3 billion people, is the world's second-most COVID-19-infected

country after the USA with nearly 23 million COVID-19 cases, and is presently reporting close to 4,000 deaths and more than 300,000 new cases daily. Due to resurgence and acceleration of COVID-19 transmission, increase in the proportion of cases of COVID-19 variants with potentially increased transmissibility, several religious and political mass gathering events, New Delhi and Mumbai, ravaged major cities have the new surge in COVID-19 cases that pushes the hospitals to bed and oxygen shortages [31]. According to the WHO's report, only 0.1 % of the positive-COVID-19 tests in India had been genetically sequenced and uploaded to the GISAID database, and by the end of April 2021, COVID-19-B.1.617.1 and -B.1.617.2 variants will be accounted for 21 % and 7 %, respectively of all sequenced specimens from India [31].

COVID-19-B.1.617.1 variant, "Indian double mutant", first detected in India in December 2020, has approximately 15 mutations compared to older COVID-19 variants and has two mutations, known as L452R and E484Q (not unique to COVID-19-B.1.617.1 variant), that might make antibodies to older COVID-19 variants or current vaccines less effective, but this must be confirmed [33]. By late March 2021, around 50 % of all reported sequences were COVID-19-B.1.617.1 variant, but the proportion decreased in April 2021 [33]. Only a very small proportion of patients with COVID-19-B.1.617.1 variant has been detected [33]. Some B-1.617.1 viruses have additional mutation in the spike protein, called "V382L" contributing to the term "triple mutant". No evidence demonstrated that these "triple mutant" viruses are deadlier or spread more rapidly [33]. COVID-19-B.1.617.2 variant, first detected in India in December 2020, has spread to many other countries and is increasing rapidly in some countries. This variant has become more common than COVID-19-B.1.1.7 variant [33]. No confirmed evidence demonstrated that COVID-19-B.1.617.2 variant has different symptoms or is any deadlier compared to COVID-19-B.1.1.7 variant, but further studies are urgently needed [33]. No evidence revealed that COVID-19 vaccines may be less effective against COVID-19, but further studies are also urgently needed [33]. COVID-19-B.1.617.2 variant has become the most common COVID-19 variant reported in India [33]. The priority for COVID-19 prevention and control should be vaccinating COVID-19 high-risk groups everywhere to maximize global protection against new COVID-19 variants and minimize the risk of COVID-19 transmission [34].

In Thailand, the incidence of SARS-CoV-2 (COVID-19) dramatically decreased when the Thai government prohibited social or physical gatherings by social or physical distancing after

the first wave of outbreak in March 2020. On March 16, 2020, the Thai government announced that the Thai New Year's national holidays (Songkran) between April 13, 2020 and April 15, 2020 would be postponed indefinitely [35]. On March 18, 2020, the Thai government started implementing a social or physical distancing policy, including the mandatory closure of all schools and universities, sporting and entertainment venues, and all stores except food markets [35]. The Thai government subsequently announced that a nationwide 10 p.m. to 4 a.m. curfew would commence on April 2, 2020. As of June 6, 2020, there had 3,104 SARS-CoV-2 (COVID-19)-related hospitalizations and SARS-CoV-2 (COVID-19)-related deaths in Thailand (fatality rate < 2 %) [36] that was lower than as it was in several countries, such as Italy (9.3 %), Iran (7.8 %), Spain (6.2 %), the United Kingdom (4.9 %), the Netherlands (4.3 %), France (4.2 %), China (4.0 %) [37].

A recent study in Thailand on clinical course and potential predictive factor for pneumonia of 193 adult laboratory-confirmed-COVID-19 patients who were hospitalized at Bamrasnaradura Infectious Institute, Department of Disease Control, Ministry of Public Health, Thailand during January 8, 2020 and April 16, 2020 revealed that 58.5 % of patients were male. The median (IQR) age was 37.0 (29.0-53.0) years with median (IQR) incubation period of 5.5 (3.0-8.0) days. The median BMI (IQR) was 23.3 (20.4-25.9) kg/m2 and 12.7 % of patients were obese. Having a history of contact with a laboratory-confirmed-COVID-19 case was 34.7 % of the patients. Approximately, 22.8 % of the patients involved a boxing stadium crowding, 20.7 % of the patients had a history of arriving from countries with widespread transmission of COVID-19 within 14 days before the onset of COVID-19 illness, whereas only one case was associated with a healthcare facility. Around 25 % of the patients had one or more coexisting medical conditions. The most common comorbidities were hypertension, diabetes, and dyslipidemia. Local transmission and imported cases were 79.3 % and 20.7 % of the patients, respectively. According to the classification of COVID-19 severity of the WHO-China Joint Mission on Coronavirus Disease 2019, approximately, 3 % of the patients were critically ill (respiratory failure, shock, and/or multiorgan failure), 14 % were severely ill (respiratory rate at least 30 breaths/minute, arterial oxygen saturation not more than 93 %, PaO2/FiO2 ratio less than 300, and/or lung infiltration more than 50 % of the lung field within 24-48 hours), 22 % were moderately ill (fever and respiratory symptoms with pneumonia on imaging, but without manifestations of severe pneumonia), and 56 % had mild illness (mild clinical symptoms without

signs of pneumonia on imaging), whereas around 5 % of the patients were asymptomatic. The incidence of COVID-19-related pneumonia, predominantly bilateral among these patients was 39 % with median (IQR) time from onset of COVID-19 illness to the detection of COVID-19-related pneumonia was 7.0 (5.0-9.0) days. At hospital presentation, fever was found in 62.7 % of the patients (39.8 % of the patients were found to have fever by a thermometer measurement), 49.2 % of the patients had cough, 28 % of the patients had coryza (including rhinorrhea and sore throat), and less than 10 % of the patients had gastrointestinal symptoms. During hospitalization, 18.7 % of the patients were administered by the nasal- cannula or face-mask oxygen supplementation, 4.7 % of the patients were administered by high-flow oxygenation, 2.6 % of the patients were administered by mechanical ventilation. The median (IQR) duration of oxygen therapy was 5.0 (2.5-11.0) days. In critical cases, more than two weeks of oxygen therapy was required. Approximately, 3.1 % of the patients had moderate to severe ARDS, while 3.6 % of the patients had COVID-19-related-acute-kidney injury. The median duration of viral RNA shedding after the onset of symptom was 16.0 (11.0-24.0) days, whereas sever COVID-19 patients had a longer viral shedding duration than the non-severe patients. Approximately, 45 days was the longest observed viral shedding, whereas the median (IQR) length of hospital stay was 12.0 (7.5-19.0) days. Recovery from COVID-19 illness were found in 189 patients (97.9 %) and 4 (2.1 %) patients were dead. The independently potential predictive factors for COVID-19-related pneumonia postulated by multivariable logistic regression among these patients were higher body temperature at the patients' hospital presentation (OR = 4.59 per 1o C increase from 37.2o C; 95 % CI : 2.30-9.17; $p < 0.001$), obesity (OR = 8.74; 95 % CI : 2.06-37.18; $p = 0.003$), and increasing age (OR = 2.55 per 10-year increase from 30 years old; 95 % CI : 1.67-3.90; $p < 0.001$) [38]. This cohort study revealed good clinical outcomes [38].

In England, a recently prospective, community-based, cohort study on the associations between BMI and the severity of COVID-19 among 6,910,695 eligible persons with mean BMI of 26.78 kg/m2 (SD = 5.59) was conducted, 5,479 (0.08 %) patients were dead, 1,601 patients were admitted to an ICU, 13,503 (0.20 %) were admitted to hospital. A linear association across the whole BMI range with ICU admission (1.10 (1.09-1.10)) was found, whereas J-shaped associations between BMI and hospital admission due to COVID-19 (adjusted hazard ratio (HR) per kg/m2 from the nadir at BMI of 23 kg/m2 of 1.05 (95 % CI : 1.05-1.05) and death (1.04 (1.04-1.05)) were identified. A significant association between BMI and age and ethnicity, with higher

HR per kg/m2 for younger persons (adjusted HR per kg/m2 above BMI of 23 kg/m2 for hospital admission = 1.09 (95 % CI : 1.08-1.10) in 20-39 years age group versus 80-100 years group = 1.01 (1.00-1.02)) and Black persons than White persons (1.07 (95 % CI : 1.06-1.08) versus 1.04 (95 % CI : 1.04-1.05)) was revealed. Slightly lower risk of admission to ICU and hospital due to COVID-19 was associated with unit increase in BMI in persons with hypertension, cardiovascular diseases, and type 2 diabetes than in those without these morbidities. Those with BMI of more than 23 kg/m2 demonstrated a linear increase in the risk of severe COVID-19 contributing to hospital admission and death [39] .

From First Wave to New Second Wave of COVID-19 in Thailand

Thailand had largely controlled the COVID-19 by mid-2020 [40] with a successful story. A new wave of COVID-19 outbreak was identified in Samut Sakhon, a province at the south of Bangkok, Thailand in December 2020 [40]. Thailand confirmed 315 new COVID-19 cases, the majority of which were local transmission, contributing its total cases to 7,694 cases and 64 deaths since its first reported case last January 2020 [40]. Samut Sakhon reported 541 additional cases of COVID-19 on January 4, 2021 [40]. The new domestic COVID-19 outbreak was hypothetically associated with illegal border migration from neighbouring Myanmar [40]. The government of Thailand had designed 28 provinces, including Bangkok, as COVID-19 high-risk zones and recommended suspension of some businesses and crowded activities, whereas some ministries and agencies had already issued several new restrictions [40]. Several field or mobile hospitals for admission of the high-risk COVID-19 exposed individuals for quarantine, laboratory testing, and clinical symptom observation had been established in these 28 provinces. The Education Ministry of Thailand had ordered all governmental and private schools and vocational training centers to close down from January 4, 2021 until the end of January 2021 [40]. The Thai Retailers Association had also announced that all shopping malls throughout the country should close at 9 pm. daily, an hour earlier than the usual closing time, whereas the authorities in Bangkok, Thailand had earlier closed entertainment venues, gyms, massage parlours, and nurseries, but keeping open shopping malls, restaurants, and public parks [40].

During the new second wave of COVID-19, a nationwide state of emergency remains in place through January 15, 2021 to facilitate the implementation of COVID-19 control measures

[41]. The authorities in several locations enforced commercial controls on top of the central government-mandated measures. Officials in Bangkok allowed food establishments to serve dine-in-customers only 06.00-19.00 daily, though they could still cater to take-away orders during the other hours. Buriram required arrivals from Bangkok and other high-risk areas to isolate at their residence or a designated facility for 14 days [41]. Additional localities may implement stricter control measures in the coming weeks if local COVID-19 activity increases [41]. Travel-Restrictions-Limited-inbound-tourist-flights are operating. Cargo, emergency, and repatriation flights and government aircraft can continue operating. Thai authorities are allowing travelers from 56 locations to enter the country without visas. Passengers must still test negative for COVID-19 within 72 hours before the trips, provide evidence of a quarantine facility booking, and isolate for two weeks at the designated facilities upon arrival. Officials have increased the length of visas from 30 to 45 days [41]. The 56 locations are Andorra, Australia, Austria, Belgium, Bahrain, Brazil, Brunei Darussalam, Canada, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, Indonesia, Ireland, Israel, Italy, Japan, Kuwait, Latvia, Liechtenstein, Lithuania, Luxembourg, Malaysia, Maldives, Mauritius, Monaco, Netherlands, New Zealand, Norway, Oman, Peru, Philippines, Poland, Portugal, Qatar, San Marino, Singapore, Slovakia, Slovenia, South Africa, South Korea, Spain, Sweden, Switzerland, Turkey, Ukraine, UAE, the UK, the USA, and Vietnam [8, 41]. People from al other locations must still obtain a special tourist visa to enter Thailand; these travelers must also quarantine at designated facilities for two weeks upon arrival [41]. The COVID-19 reports have been notified that male-infected cases are more affected than female cases, the incidence of which may have something to investigate with the fact that the ACE-2-receptor gene, which is used by SARS-CoV-2 (COVID-19) to enter the human-host cells, is identified on the X chromosome [8].

Color-Coded Risk-Classification System

The color-coded risk-classification system that categorized by the government of Thailand (The Center for COVID-19 Situation Administration) consists of four tiers based on local disease activity, ranging from "green" to "red" in order of increasing risk of infection, with high-risk zones being subject to the strictest restrictions (Figure 2). As of January 4, 2021, the

localities categorized as "red-zones" included Bangkok, Ang Thong, Chanthaburi, Chachoengsao, Chonburi, Chumphon, Kanchanaburi, Lopburi, Nakhon Nayok, Nakhon Pathom, Nonthaburi, Pathum Thani, Phetchaburi, Phra Nakhon Si Ayutthaya, Prachinburi, Prachuap Khiri Khan, Ranong, Ratchaburi, Rayong, Sa Kaeo, Samut Prakan, Samut Songkhram, Saraburi, Sing Buri, Suphan Buri, Tak, and Trat provinces [41]. The color-coded risk-classification system, zones, and the respective restrictions are as the following [41, 42]:

1)Red zone: COVID-19-infected cases = at least 51, educational institutions and entertainment venues, pubs, karaoke outlets, and bars are suspended. Crowded activities, such as meetings and seminars can take place only if the organizers have obtained approval from authorities. Convenience centers, supermarkets, exhibition halls, shopping malls, workers' dormitories, and industrial areas may open as long as they adhere to health protocol. Authorities prohibit migrant workers from leaving the areas and have set up checkpoints at entry and exit points.

2)Orange zone: COVID-19-infected cases = 11-50, public activities are prohibited. Authorities are allowing small-scale private activities, including those among friends, family, and acquaintances. Officials are limiting operating hours for commercial and industrial facilities. Transporting migrant workers out of the state is banned. Large celebrations are banned, and attendance at parties is limited.

3)Yellow zone: COVID-19-infected cases = 1-10, authorities are enacting enhanced surveillance measures. Scaled-down activities are allowed and officials are imposing rules to decrease crowding.

4)Green zone: COVID-19-infected cases = 0, authorities are permitting small-scale activities and are implementing protocols to decrease crowding.

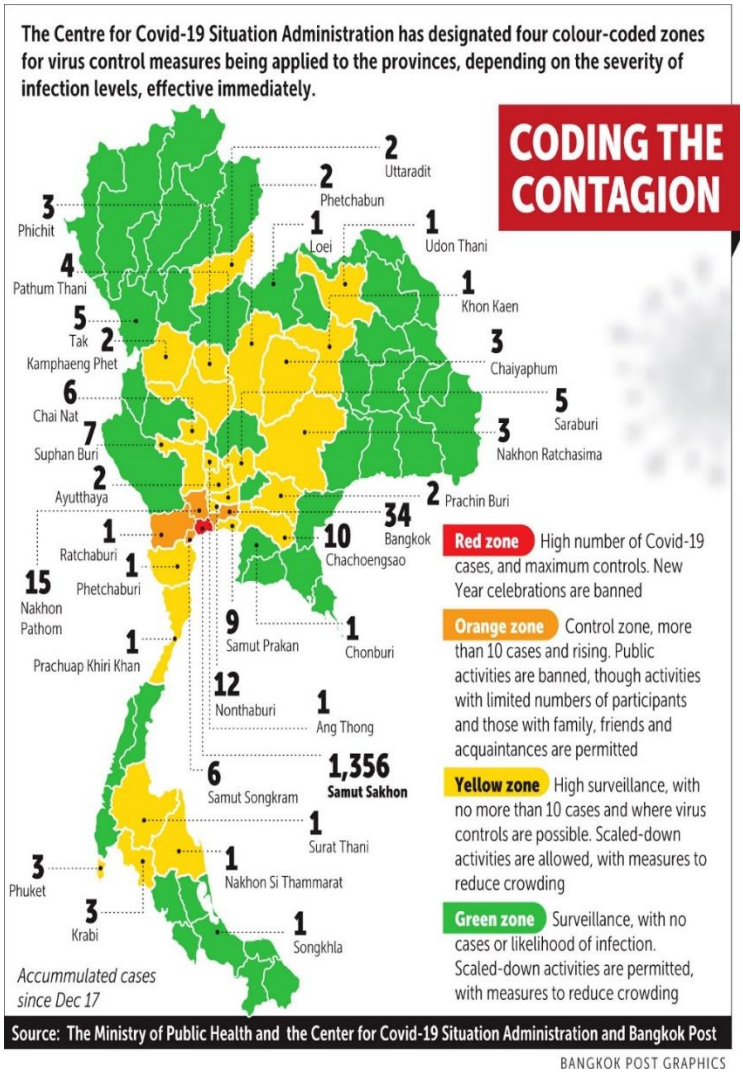

Figure 2: Demonstrating four tiers based on local disease activity, ranging from "green" to "red" in order of increasing risk of infection, with high-risk zones being subject to the strictest restrictions, as of December 31, 2020.

From Second Wave To New Third Wave of COVID-19 in Thailand

On January 4, 2021, Samut Sakhon reported 541 additional cases of COVID-19 [43]. Illegal border migration from neighbouring Myanmar was hypothetically related to the new domestic COVID-19 outbreak, defined as new second wave of COVID-19 in Thailand [43]. As of January 4, 2021, the government of Thailand had designed 28 provinces, including Bangkok, as COVID-19 high-risk (red) zones and recommended suspension of some businesses and crowded activities,

whereas some ministries and agencies had already issued several new restrictions [43, 44]. On April 5, 2021, there was an increase of 250 SARS-CoV-2 (COVID-19)-infected cases related to nightlife establishments in Bangkok, contributing to shutting of the entertainment venues in 3 districts of the capital for at least 2 weeks [45]. A private hospital in Bangkok conducted the drive-through COVID-19-infection screening since April 1, 2021 and revealed that approximately 9 % of those tested were infected [46]. The most of the infected individuals those tested at this private hospital had visited the same entertainment venues as the above group [46]. This year (2021), Thailand is likely to confront a steep increase in numbers of COVID-19 cases because people are becoming complacent about the risk of COVID-19 infection and letting their guard down. Nightlife venues give noise level that people have to stand close to each other and shout to be heard. Additionally, revelers tend to move from one party to another, potentially spreading the SARS-CoV-2 (COVID-19) [45]. During the forthcoming 2021 Songkran holidays, a lot of effort, money, and time will go into curtailing the spread of COVID-19 infection, when millions of people will travel across the country to visit their home provinces. There will be no ban on people travelling to visit their elderly relatives, except travelling to party [45]. The third and fourth waves of COVID-19 may occur, whereas it could take the country at least 2 years to achieve herd immunity [45].

On April 7, 2021, Thailand confirmed the local presence of the highly transmissible SARS-CoV-2 (COVID-19) variant B.1.1.7 first identified in the United Kingdom (UK) [47, 48] that has been identified in more than 100 countries and contributes to fuel new waves of COVID-19 infections worldwide [47]. The main source of this new third wave of the COVID-19 outbreak hypothetically was in Bangkok's Thong Lor entertainment venues [48], which could take longer (one or two months) to contain, depending on the control measure implementation [47]. The currently spreading UK- COVID-19-variant-B.1.1.7 throughout Thailand might have been carried into the country from Cambodia, either by migrant workers or Thais crossing the border [48]. The UK COVID-19-variant-B.1.1.7 was identified in individuals from China and India who entered Cambodia in February 2021 and the first case of Cambodia was reported on February 15, 2021 [48]. Since February 2021, the UK COVID-19-variant-B.1.1.7 had spread throughout Cambodia, particularly in Phnom Penh [48]. As of April 8, 2021, Cambodia has reported 2,915 confirmed-COVID-19 cases and 22 COVID-19-related deaths according to the worldometer website [48]. On April 8, 2021, Thailand reported 405 new COVID-19-infected cases, in an

outbreak that has reached 20 provinces [47]. Due to rapid spread of the latest outbreak (new third wave) to provinces far from Bangkok has contributed to the COVID-19-taskforce authorities announcing that they will shut down all entertainment venues in 41 provinces, including Bangkok for at least two weeks, beginning on April 9, 2021 [48]. The employers were asked to allow staff working from home and urged against non-essential travel [47]. When a locally-manufactured AstraZeneca vaccine becomes available, Thailand plans to begin its mass immunization campaign in June 2021 [47]. Approximately, 300,000 individuals will be immunized, mostly healthcare workers [46]. Some Thai COVID-19 experts are puzzled at how the UK COVID-19 variant evaded the country's strict quarantine system, which has assisted keeping overall cases to a relatively low 30,310 and 95 deaths [47]. The Thai army was setting up field hospitals with approximately, 3,000 beds in 10 army bases, braced for a possible surge in new COVID-19 patients [47].

On May 4, 2021, the first local case of the COVID-19-South-African-B.1.351 variant was detected in a 32-year-old Thai man after he was visited by his family who entered Thailand from Malaysia via an informal border crossing [48]. Since the beginning of May 2021, only two other COVID-19-B.1.351-variant cases, out of 81 tested cases, have been detected in similarity to those identified in Malaysia, contributing to the restriction of people movement in the nine villages in Tambon Koh Sathon of Tak Bai district of Narathiwat southern province, the COVID-19-B.1.351-variant-affected district [48-50]. Since the beginning of April 2021, four different COVID-19 variants have been detected in Thailand [49]. The COVID-19-B.1.351-variant cases in Tak Bai were hypothesized to be the first local transmission of the COVID-19-B.1.351 variant, whereas the first confirmed COVID-19-B.1.351-variant case was a Thai national who returned from Tanzania on February 15, 2021 with immediate quarantine [50, 51]. The Tak Bai COVID-19-B.1.351-variant-infected man's wife had already returned to Malaysia [50]. On May 21, 2021, 36 individuals at a construction site in Laksi district of northern Bangkok have been detected infected with the COVID-19 Indian variant (B.1.617.2), including 15 migrant workers (seven men, 8 women, average age of 46 years) at the same camp who had been detected with Indian variant earlier in the same day (21 Thais, 10 Myanmar nationals, and 5 Cambodians) [52, 53]. Most of them had mild symptoms and everyone were hospitalized [52]. Three of them were in close contact with the Indian-variant-infected workers at their home [52]. Currently, the COVID-19-Indian variant has spread to many other countries, such as Bangladesh, Nepal, Pakistan,

Cambodia, Indonesia, Malaysia, Singapore, Vietnam, and Thailand [52]. Since the beginning of April 2021, the COVID-19-Indian variant (B.1.617.2) was the majority of recently detected COVID-19 cases in Thailand that contributed to deaths increasing 7 times and COVID-19 cases increasing 4 times [53]. There were around 1,100 COVID-19-infected cases of the 1,667 workers in one camp in northern Bangkok [53]. Several camps of the 409 worker camps around Bangkok demonstrated several detected COVID-19-infected clusters [53]. About 50 % of 62,169 workers who live in Bangkok, are migrant workers [53]. Only 1.72 million individuals, most of those frontline workers or members of COVID-19 high-risk groups have received first dose of COVID-19 vaccine at a time when the detection of the COVID-19 Indian variant (B.1.617.2) comes in Thailand [53]. The government of Thailand (Center for COVID-19 Situation Administration (CCSA)) has ordered the restriction of people movement between different worker camps and checks on dormitory-living-conditions of the workers [53]. As of May 21, 2021, 3,481 new COVID-19-infected cases and 32 COVID-19-related deaths, contributed to the 735 deaths overall and the total of 123,066 COVID-19-infected cases [53]. In June 2021, Thailand is set to begin mass-COVID-19-vaccination campaign [53].

From First wave To New Second Wave of COVID-19 in Myanmar

Myanmar demonstrated a dramatic increase in the number of COVID-19 cases in the second wave on August 16, 2020 in Rakhine State, compared to the first wave of COVID-19 that reported its first case on March 23, 2020, whereas Yangon has become a major epicenter in the COVID-19 second wave [54]. Interestingly, a more infectious strain with G614 mutation of SARS-CoV-2 (COVID-19) has been identified in Myanmar [54]. ASEAN (Association of Southeast Asian Nations: South-East Asia Region and Western Pacific Region) countries, including Thailand are highly interconnected to each other and the rest of the world via trade and migration [54]. Due to weak health systems in Myanmar, Cambodia, Indonesia, Laos, the Philippines, and Timor Leste, listed as vulnerable by the United Nations (UN) [-54], the COVID-19 new outbreak in Myanmar can easily spread this contagiously infectious disease to the ASEAN countries [54]. Calls to form an ASEAN Center for Disease Prevention and Control are urgently needed [54].

Case Definition for Surveillance (As of January 17, 2020)

1.Suspected case requiring diagnostic testing

Patients with acute respiratory infection who have sudden onset of at least one of the following symptoms: cough, sore throat, shortness of breath requiring hospitalization or not. In 14 days prior to onset of symptoms, met at least one of the following epidemiological criteria : 1) Were in close contact with a confirmed or probable case of COVID-19 infection ; or 2) Had a history of travel to areas with presumed ongoing community transmission of COVID-19; or 3) Worked in or attended a health care facility where patients with COVID-19 infections were being treated [55].

2.Close Contact

Close contact is defined as : 1) Healthcare associated exposure, including providing direct care for patients with COVID-19 infection, working with healthcare workers infected with novel coronavirus, visiting patients or staying in the same close environment as a COVID-19 patient ; 2) Working together in close proximity or sharing the same classroom environment with a COVID-19 patient ; 3) Travelling together with a COVID-19 patient in any kind of conveyance ; and 4) Living in the same household as a COVID-19 patient. The epidemiological association may have occurred within a 14-day period before or after the onset of illness in the case under consideration [55].

3.Probable Case

The probable case is a suspected case for whom testing for COVID-19 is inconclusive by the result of the test reported by the laboratory section or for whom testing was positive on a pan-coronavirus assay [55].

4.Confirmed Case

The confirmed case is a individual with laboratory confirmation of COVID-19 infection, irrespective of clinical manifestations [55].

5.Criteria to Initiate Testing for COVID-19

Prompt case confirmation is essential for rapidly ensuring and effectively contact tracing, implementation of infection prevention and control measures according to national recommendations, and collection of relevant epidemiological and clinical data. Any individual fulfilling the criteria for a suspected case should be tested for COVID-19 infection. The laboratory test should be initiated immediately [55].

Classifications and Definitions of SARS-CoV-2 (COVID-19) Variants

To remain effective against the very naturally mutated viruses by powerful adaptation of the scientific responses due to the recent emergence of variants of SARS-CoV-2 (COVID-19) [56]. The United States Center for Disease Control and Prevention (US CDC) classifies the SARS-CoV-2 (COVID-19) variants as the following [57] :

1)Variant of Interest

These variants are 1.1) B.1.526 (Iota strain, designated on March 24, 2021 by the WHO (https://www.who.int>News>items), first detected in New York, USA in November 2020, 1.2) B.1.525 (Eta strain, designated on March 17, 2021 by the WHO (https://www.who.int>News>items), first detected in New York, USA and in several countries in December 2020, 1.3) B.1.617.1 (Kappa strain, designated on April 4, 2021 by the WHO (https://www.who.int>News>items), first detected in India in October 2020), 1.4) C.37 (Lambda strain, designated on June 14, 2021 by the WHO (https://www.who.int>News>items), first detected in Peru in December 2020, and 1.5) P.2 (First detected in Brazil in April 2020).

2)Variants of Concern

These variants are 2.1) B.1.1.7 (Alpha strain, designated on December 18, 2020 by the WHO (https://www.who.int>News>items), first detected in the United Kingdom in September 2020, with approximately 50 % increased transmissibility, likely increased severity based on hospital admissions and case fatality rates, minimal impact on neutralization by Emergency Use Authorization (EUA) monoclonal antibody treatments), 2.2) P.1, P.1.1, P.1.2 (Gamma strain,

designated on January 11, 2021 by the WHO (https://www.who.int>News>items) , first detected in Japan or Brazil in November 2020, with moderate impact on neutralization by EUA monoclonal antibody treatments), 2.3) B.1.351, B.1.351.2, B.1.351.3 (Beta strain, designated on December 18, 2020 by the WHO (https://www.who.int>News>items), first detected in South Africa in May 2020, with approximately 50 % increased transmissibility, moderate impact on neutralization by EUA monoclonal antibody treatments), 2.4) B.1.427 (First detected in California, USA with approximately 20 % increased transmissibility, significant impact on neutralization by some, but not all, EUA treatments), and 2.5) B.1.429 (First detected in California, USA with approximately 20 % increased transmissibility, significant impact on neutralization by some, but not all, EUA treatments). In Early May 2021, WHO have included Indian variant (B.1.617, AY.1, AY.2, AY.3, Delta strain, designated on May 11, 2021 by the WHO (https://www.who.int.>News>items), first detected in India on October 2020) in this category [31].

3)Variant of High Consequence

This variant has clear evidence that medical countermeasures (MCMS) or prevention measures have significant decreased effectiveness that are associated with previously circulating variants. The possible attributes that can impact on MCMS are demonstrated failure of diagnostics, evidence to indicate a significant reduction in vaccine effectiveness, a disproportionately high number of vaccine breakthrough cases, very low vaccine-induced protection against severe disease, significant decreased susceptibility to multiple EUA or approved treatments, and more severe clinical disease and increased hospital admissions. This variant would require public health officials announce a Public Health Emergency of International Concern (PHEIC).

Epidemic Trend by Mathematical Modelling

Mathematical modelling for the epidemic trend of the COVID-19 outbreak in China conducted by Shen et al demonstrated that the national epidemic of COVID-19 may lead to at least a total of 8,042 (95 % Confidential Interval (CI) : 4,199-11,884) infections and at least 898 (95 % CI : 368-1,429) deaths, equivalent to a fatality rate of 11.02 % (95 % CI : 9.26-12.78 %) [58]. This fatality rate is lower than the rates of the Middle-East Respiratory Syndrome (MERS,

34.4 %) [59] and the Severe Acute Respiratory Syndrome (SARS, 14-15 %) [59-60], indicating that COVID-19 may be a less virulent strain among the coronavirus family [58].

When the epidemic of COVID-19 in China started on December 12, 2019, the basic reproductive number (R0) of COVID-19 (an indication of the initial transmissibility of the virus) was estimated to be 4.71 (95 % CI : 4.50-4.92), whereas its effective reproductive number (Re) has decreased to 2.08 (95 % CI : 1.99-2.18) as of January 22, 2020 [58]. With the assumption of no resurges of COVID-19 epidemic and the continually declining trend, Re will decrease below one within three months (77 (95 % CI : 75-80) days) of the epidemic initiation, indicating that the COVID-19 epidemic will gradually die off after this time [58]. The COVID-19 spreads during the clinical latency stage, and thus, classical models for epidemic outbreaks do not apply to the particular cases of COVID-19. An average reproductive number of 3.11 implies that the average number of secondary cases with COVID-19 is increasing [61, Figure 3]. The estimated data suggest that the average clinical latency stage lasts 7 days or more and it is longer than the median incubation period of 5.2 days (2-14 days) [61]. Therefore, the treatment of symptomatic persons can be useful. Nevertheless, the reproductive number varies over time depending on the estimation models. By stochastic methods, mathematical methods, and exponential growth, the average reproductive number are 2.44, 4.2, and 2.67, respectively [62]. With continuing efforts, the stimulation methods predict that Wuhan city, China would achieve a reproductive number of less than one in the near future [63]. In comparison with MERS and SARS, R0 of COVID-19 was similar to MERS in Jeddah (95 % CI : 3.5-6.7) and Riyadh (95 % CI : 2.0-2.8), Kingdom of Saudi Arabia, in 2014 [64] and SARS (R0 = 4.91) in Beijing, China, in 2003 [65]. Nevertheless, Zhao et al concluded that the mean estimate of R0 for the COVID-19 ranges from 2.24 (95 % CI : 1.96-2.55) to 3.58 (95 % CI : 2.89-4.39), and significantly larger than 1 if the reporting effort has been increased by a factor of between 8- and 2-fold after the diagnostic protocol released on January 17, 2020 and several medical sup[plies reached Wuhan, and indicates the potential of COVID-19 to cause outbreaks [66]. On January 30, 2020, the World Health Organization (WHO) declared the SARS-CoV-2 (COVID-19) outbreak a " Public Health Emergency of International Concern " and it declared the COVID-19 outbreak a pandemic on March 11, 2020 [67].

Conclusion

A cluster of 27 patients with unknown-etiology pneumonia was firstly reported from the Wuhan Municipal Commission in Wuhan city, China with hypothetical connection with Wuhan's Huannan Seafood Wholesale Market. This cluster of unknown-etiology pneumonia is under-invertigation by the WHO's experts. The mathematical modelling is a useful epidemiological method for predicting the epidemic trend of COVID-19. The COVID-19 pandemic waves in countries around the world could progress to increasing numbers of waves if they have inadequate and inefficient control measures of COVID-19, including their people' cooperation on COVID-19 control and prevention and balancing between the economic problem solving and the disease control.

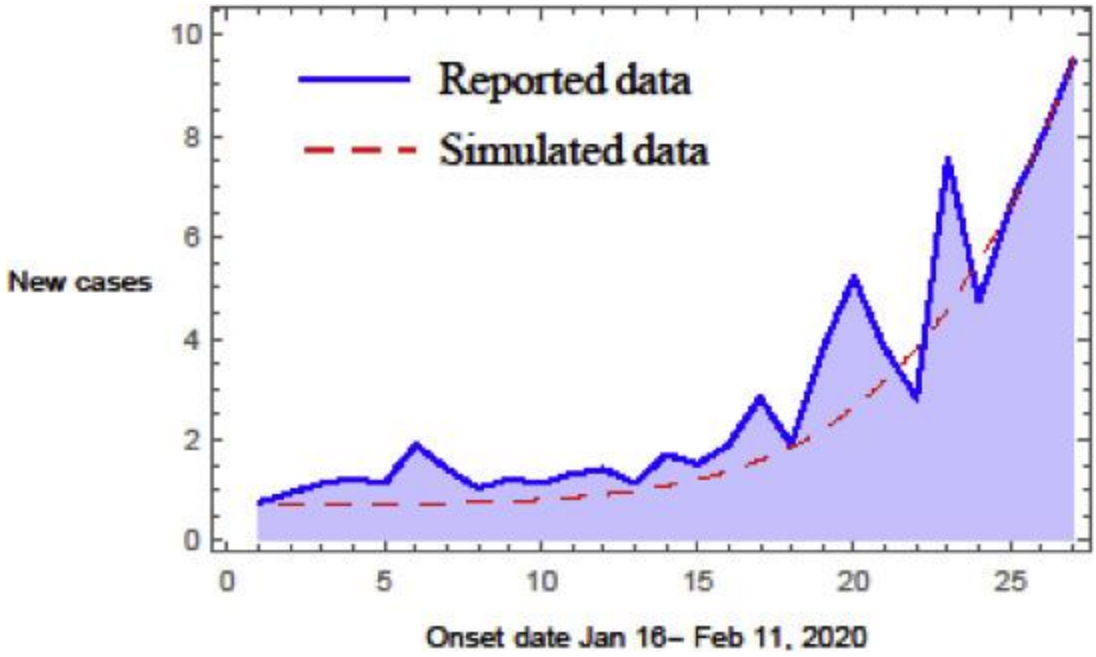

Figure 3 : Curve Fitting Results of Reproduction Model (China)

(Source : Krishna MV, Prakash J. Mathematical modelling on phase-based transmissibility of coronavirus. Infectious Disease Modelling 2020; 5 : 375-385)

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CHAPTER 2

Surface Viability of COVID-19

Abbreviations :

SARS-CoV-1 : Severe Acute Respiratory Syndrome Coronavirus-1

MERS-CoV : Middle East Respiratory Syndrome Coronavirus

Introduction

Currently, SARS-CoV-2 (COVID-19)-transmission evaluating data are limited and controversy. The majority of our knowledge is based on severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), middle east respiratory syndrome coronavirus (MERS-CoV),and influenza virus [1]. The longest survival of SARS-CoV on surfaces done by placing a very large initial virus titer sample (107 infectious virus particles) on the surfaces was 6 days [2]. SARS-CoV-1 can survive in the sputum, stool, and serum for at least 96 hours [3]. Recent study demonstrated that under controlled experimental conditions, fomite transmission of SARS-CoV-2 (COVID-19) can remain viable for days on surfaces [4]. This study demonstrated that SARS-CoV-2 (COVID-19) was more stable on plastic and stainless than copper and cardboard, and viable virus was detected up to 72 hours after application to these surfaces, although the virus titer was reduced from 103.7 to 100.6TCID50 (50 % tissue-culture infectious dose) per millimeter of medium after 72 hours on plastic and from 103.7 to 100.6 TCID50 per millimeter after 48 hours on stainless steel [4]. SARS-CoV-2 (COVID-19) remained viable in aerosols throughout 3 hours of this experiment duration [4]. Several previous studies used a sample of 107, 106, and 104 particles of infectious influenza virus on a small surface area [2, 4, 5] that are much higher concentration than those in the real-life-situation droplets, with possible several orders of magnitude of the actual virus deposition on the surfaces [6]. Dowell et al demonstrated that no viable SARS-CoV was identified on fomites [7]. Goldman E proposed that the chance of transmission through inanimate surfaces is very small, except someone touches the surface with coughs or sneeze of an infected individual after the cough or sneeze within 1-2

hours [8]. Holmes E revealed that at room temperature, SARS-CoV-2 (COVID-19) remains viable up to 4 days on glass, up to 3 days on plastic and stainless steel, up to 2 days on clothes, up to 1 day on paper or cardboard, and up to 4 hours on copper and the surface viability of SARS-CoV-2 (COVID-19) is decreased by heat and simulated sunlight [9]. The National Academies of Sciences Engineering Medicine claims that the surface viability of SARS-CoV-2 (COVID-19) are up to 3 hours on printing paper and tissue paper, up to 4 hours on copper, up to 24 hours on cardboard, up to 2 days on clothes, up to 2 days on wood, up to 4 days on paper money, up to 4 days on glass, during 3-7 days on plastic, during 2-7 days on stainless steel, and after 7 days on the outside of the surgical mask [10]. Interestingly, metals containing copper demonstrates viricidal properties [11]. Casanova et al revealed that the association between inactivation of the SARS-CoV and the relative humidity (RH) was not monotonic, at low RH (20 %) and high RH (80 %), there was greater survival or greater protective effect than at moderate RH (50 %) [12]. Kampf et al demonstrated that SARS-CoV-2 (COVID-19) can persist on surfaces up to 9 days [13]. Nevertheless, recently, Gale J demonstrated that SARS-CoV-2 (COVID-19) can survive for 28 days on smooth surfaces, such as glass found on mobile phone screen and plastic banknotes at room temperature, or 20 degrees Celsius (68 degrees Fahrenheit), compared to 17 days survival for flu virus [14]. Figure 4 demonstrates the stability of SARS-CoV-2 (COVID-19) on different surfaces.

In addition to aerosol transmission, infection via surfaces should be considered. Surface disinfection could be done with 62-72 % ethanol or 0.1 % sodium hypochlorite.

Conclusion

SARS-CoV-2 (COVID-19) can survive during 3-7 days on plastic surface, during 2-7 days on stainless steel surface, and up to 28 days on mobile phone screen and plastic banknotes at the room temperature. Its stability depends on different surface characetristics and surface temperatures.

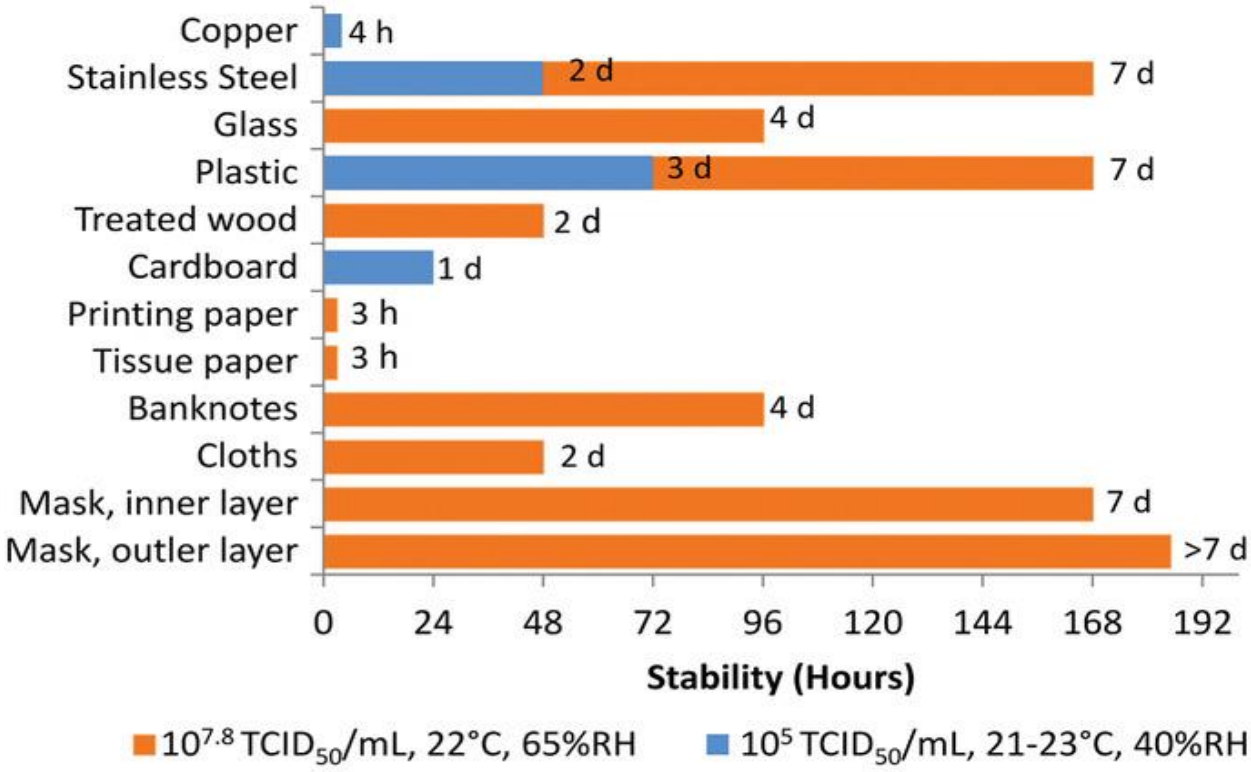

Figure 4 : Stability of SARS-CoV-2 (COVID-19) on different surfaces. Blue bars : viral titer of inoculum 105 TCID50 mL-1, 21-23 o C, 40 % RH (source data from van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. N Engl J Med 2020; 382 : 1564). Orange bars : viral titer of inoculum 107.8 TCID50 mL-1, 22 o C, 65 % RH (source data from Chin AWH, Chu JTS, Perera MRA, Hui KPY, Yen H-L, Chan MCW, et al. Lancet Microbe 2020; 1 : e10).

(Source : Ruiz-Hitzky E, Darder M, Wieklein B, Ruiz-Garcia C, Martin-Sampedro R, del Rel G, et al. Nanotechnology responses to COVID-19. Advanced Healthcare Materials 2020; 9 : 2000979. 26 pages. Published online : September 3, 2020.)

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CHAPTER 3

Personal Protective Equipment Interventions in the Era of COVID-19 Pandemics

Abbreviations :

HCP : Healthcare Personnel

HCW : Healthcare Worker

ICU : Intensive Care Unit

MHLW : Ministry of Health, Labour and Social Affairs

NIOSH : National Institute for Occupational Safety and Health

PVC : Polyvinyl Chloride

p : Probability

PPE : Personal Protective Equipment

UK : United Kingdom

US CDC : United States Centers for Disease Control and Prevention

US FDA : United States Food and Drug Administration

WHO : World Health Organization

Introduction

A recent study in China demonstrated that the rapid surge of COVID-19 in healthcare workers (HCWs) may be the lack of effective protection measures [1]. This study revealed that there were no significant differences in the use of gloves or medical masks among the three group of 1) intensive care unit (ICU) staff, 2) staff working in the fever outpatient department, general patient room, Fangcang shelter hospital, emergency department, cleaning area, imaging examination area, and transfer vehicle, and 3) staff working in the general outpatient department, community, pharmacy, and administrative area [1]. Nevertheless, all other types of personal protective equipment (PPE) (N95/FFP2 respirator, face shield/goggles, isolation gown, medical protective uniform, and positive pressure

headgear) were used most group 1 and HCWs in group 2 [1]. Skin injury was the most common type (62.3 %) of PPE-associated adverse events (87.3 %), dyspnea (61.8 %), dizziness (57.8 %), and headache (53.8 %) [1]. Greater risks of adverse events occurred in both doctors (30.2 %) and nurses (66.5 %) compared to other types of HCWs (3.3 %, both $p < 0.05$) [1]. The negative results of the reverse-transcriptase-polymerase-chain-reaction tests in all three group participants accompanying negative results of serological tests in 70 % of all participants suggested the efficacious measure of PPE for the SARS-CoV-2 (COVID-19) nosocomial transmission [1]. The need for guidance on rationalizing, prioritizing, and grading the PPE use due to HCWs' infection risk is supported by the efficacy of different PPE among HCWs in different working areas [1]. The basic emergency guidance of PPE for protecting HCWs should be issued at the earliest stage of an epidemic, not months later [1]. Although 98.6 % of HCWs revealed high adherence level to PPE protocols, PPE was commonly related to adverse events in the study participants both physically and psychologically [1].

Examples of PPE that are recommended by the World Health Organization (WHO) are as the followings. 1) Medical mask for healthcare United Kingdom, breathability, and should be preferably fluid resistance [2], 2) Face shield is made of clear plastic, adjustable band to attach firmly around the head and fit snuggly against the forehead, preferable fog resistant. Face shield completely covers the sides and length of the face and may be re-usable (made of robust material which can be cleaned and disinfected) or disposable [2], 3) Particulate respirator has minimum 94 % or 95 % of good particulate filtration [2], and 4) Non-sterile examination glove is made of nitrile (preferable), latex, and polychloroprene or polyvinyl chloride (PVC) [2]. The examination glove should be minimum 230 mm. of total length and minimum 0.05 mm. of thickness [2].

Argentina, Australia, Belgium, Brazil, Chile, Columbia, Costa Rica, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, India, Ireland, Israel, Italy, Japan, Korea, Latvia, Lithuania, Luxembourg, Mexico, Netherlands, New Zealand, Peru, Portugal, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, United Kingdom, and United States are increasing supply of protective equipment [3]. The Australian Government has strengthened the supply of PPE (including seven million P2/N95 respiratory face masks) throughout residential aged care facilities in some areas of Victoria, including

Melbourne [3]. The Government of Canada will fund US $ 50 million to purchase of PPE and other essential medical supplies and equipment to strengthen federal requirements [3]. In Estonia, there are no large PPE manufacturers. The first and second shipments of centrally procured PPEs from China arrived in Estonia on the 2nd and 7th April 2020 [3]. This PPE delivery contributed to 350,000 FFP2 protective masks, 740,000 surgical masks, and 12,100 protective coats that arrived in Estonia [3].

The Government of India funded US $ 2 billion for PPEs, testing facilities, ICU beds, ventilators, and other essential medical equipment [3]. The Japan's Ministry of Health, Labour and Social Affairs (MHLW) developed a guidelines letting the use of rain coat and snorkeling masks in case of shortage and reusing protective equipment [3]. In Mexico, FFP2 masks are used only when treatment may cause much aerosols, such as intubated case and some other medical procedures [3]. Surgical masks are considered sufficient in this country [3]. The New Zealand Government's Ministry of Health issued the guidelines on PPEs for community care providers, including aged-associated community care, hospice, disability, aged residential care, and homecare [3]. On April 13, 2020, Swiss Presidential Decree issued the regulation of the free access to PPEs, kits, tests, other medical equipment, and medicines used in the diagnosis and treatment of the disease [3]. The United Kingdom (UK) Government prepared a national stockpile of PPEs that has been released across the country [3]. The United States Food and Drug Administration (US FDA) is providing increased support to the manufacturers for increasing PPE imports [3]. The UK Government recommends the use of face masks for medical staff or face coverings in England and Scotland in addition to hand hygiene and physical (social) distancing (2 meters) for medical staff, patients or individuals and visitors in both clinical and non-clinical areas [4].

Currently, the National Institute for Occupational Safety and Health (NIOSH) recommends the application for the PPE burn out or consumption rate calculator to estimate how many days a PPE supply will last given current inventory levels and PPE consumption (burn out) rate. This application is available at the NIOSH PPE Tracker application webpage [5]. A recent study in the US demonstrated that the four main factors contributing to the US shortage of PPEs in 2020 were as the followings : 1) a major demand shock triggered by healthcare system needs as well as panicked marketplace behavior depleted inventories, 2) the federal government failed to maintain and distribute

domestic inventories, 3) a dysfunctional budgeting model in hospital operating systems incentivized hospitals to minimize costs rather than maintain adequate inventories of PPEs, and 4) major disruptions to the PPE global supply chain caused a sharp decrease in PPE exported to the US, which was already highly dependent on globally-source PPEs [6]. The United States Centers for Disease Control and Prevention (US CDC) recommends that : 1) Minimally, medical staff should wear an N95 respirator or a facemask and eye protection while they are in the patient care area, 2) Respirators should be prioritized for aerosol generating procedures, 3) Medical staff should wear gloves for contact with patients or their environment, care activities where sprays and splashes are anticipated and high-contact patient care activities that provide opportunities for transfer of COVID-19 to the hands and clothing of the healthcare personnel (HCP), 4) Medical staff should remove PPE and perform hand hygiene when they are leaving the patient care area, and 5) PPE should not be worn in the medical staff respite area [7]. Thailand developed systems to promote and support logistical coordination and cooperation, including PPEs [8]. Figure 5 and 6 demonstrate NIOSH approved N95 facial mask and its fit testing, respectively.

Conclusion

The most common adverse event of PPE wearing is facial skin injury, followed by dyspnea, dizziness, and headache, respectively. Currently, the NIOSH recommendes and implementes the NIOSH PPE Tracker application for calculation of the PPE consumption rate. This application is useful for estimation of the government's PPE budget that should be applied by the governments of all countries.

Figure 5 : NIOSH approved N95 facial mask

(Source : Holland M, Friderici CS. COVID-19 personal protective equipment (PPE) for the emergency physician. Vis J Emerg Med 2020 April; 19 : 100740. Available at : www.ncbi.nlm.nih.gov>pmc>articles>PMC7143707(accessed on January 5, 2021))

Figure 6 : Fit testing N95 facial mask with qualitative solutions (isoamyl acetate, saccharine, etc.)

(Source : Holland M, Friderici CS. COVID-19 personal protective equipment (PPE) for the emergency physician. Vis J Emerg Med 2020 April; 19 : 100740. Available at : www.ncbi.nlm.nih.gov>pmc>articles>PMC7143707(accessed on January 5, 2021))

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CHAPTER 4

Pathogenesis and Pulmonary Pathology

Abbreviations :

ACE2 : Angiotensin Converting Enzyme 2

ACP : Antigen Presenting Cell

AKI : Acute Kidney Injury

ARDS : Acute Respiratory Distress Syndrome

CI : Confidential Interval

CNS : Central Nervous System

COVID-19 : Coronavirus Disease 2019

COVAN : COVID-19-Associated Nephropathy

CT : Computed Tomography

DC : Dendritic Cell

DC-SIGN : Dendritic-cell Specific Intercellular Adhesion Molecule-3-Grabbing Nonintegrin

DC-SIGNR, L-SIGN : DC-SIGN-Related Protein

DKA : Diabetic Ketoacidosis

DLCO : Diffusing Capacity of the Lung for Carbon Dioxide

EAE : Experimental Autoimmune Encephalomyelitis

eGFR : estimated Glomerular Filtration Rate

ESR : Erythrocyte Sedimentation Rate

FGF : Fibroblast Growth Factor

G-CSF : Granulocyte-Colony-Stimulating Factor

GM-CSF : Granulocyte-Macrophage Colony-Stimulating Factor

HIV : Human Immunodeficiency Virus

HRQL : Health-Related-Quality-of-Life

ICD-10 : International Classification of Disease, 10th Revision

IFN : Interferon

IL : Interleukin

ILC : Innate Lymphoid Cell

IP : Interferon-gamma-induced Protein

KCO : Transfer Coefficient of the Lung for Carbon Monoxide

MCP : Monocyte Chemoattractant Protein

MERS-CoV : Middle-East-Respiratory-Syndrome Coronavirus

MIP : Macrophage Inflammatory Protein

MR : Magnetic Resonance

MRI : Magnetic Resonance Imaging

NK : Natural Killer

PCR : Polymerase Chain Reaction

PD-1 : Programmed Cell Death Protein-1

PDGF : Platelet-Derived Growth Factor

PRES : Posterior Reversible Encephalopathy Syndrome

PTSD : Post-Traumatic Stress Disorder

RAAS : Renin-Angiotensin-Aldosterone System

RNA : Ribonucleic Acid

RRT : Renal Replacement Therapy

SARS : Severe Acute Respiratory Syndrome

SARS-CoV : Severe Acute Respiratory Syndrome Coronavirus

SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus Type 2

TCR : T Cell Receptor

Tim-3 : T-cell Immunoglobulin and Mucin-3

TMPRSS2 : Transmembrane Protease Serine 2

TNF : Tumor Necrosis Factor

VEGF : Vascular Endothelial Growth Factor

VTE : Venous Thromboembolism

Introduction

COVID-19-infected patients demonstrated higher numbers of leukocytes, increased significantly plasma levels of pro-inflammatory cytokines, high erythrocyte sedimentation rate (ESR) and D-dimer, Interleukin (IL)-1-β, IL-1-RA, IL-7, IL-8, IL-9, IL-10, basic Fibroblast Growth Factor (FGF)-2, Granulocyte-Colony-Stimulating Factor (GCSF), Granulocyte-Macrophage Colony-Stimulating Factor (GMCSF), Interferon (IFN)-γ, Interferon-γ-induced Protein (IP)-10, Monocyte Chemoattractant Protein (MCP)-1, Macrophage Inflammatory Protein (MIP)-1-α, MIP-1-β, Platelet-Derived Growth Factor (PDGF)-B, Tumor necrosis factor (TNF)-α, and Vascular Endothelial Growth Factor (VEGF)-A. Some severe COVID-19 cases with intensive care unit admission demonstrated high levels of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1-α, and TNF-α. The main pathogenesis of COVID-19 infection was severe pneumonia [Figure 7], RNAaemia, ground-glass opacities on the chest roentgenogram (Figure 8, 9), and acute cardiac injury (Figure 10). Nevertheless, one of the COVID-19 case reports revealed leukopenia (70 % were neutrophils) and high level of C-reactive protein at 5 days of fever (39.0^{o} C) with presence of a cough and coarse breathing sounds of both lungs. Pathologic pulmonary examination of two patients with adenocarcinoma and COVID-19 infection revealed reactive hyperplasia of pneumocytes, particularly type II pneumocytes with patchy inflammatory cellular infiltration and multinucleated giant cell infiltration, fibroblastic plugs in air spaces, proteinaceous exudate, and pulmonary edema (Figure 11).

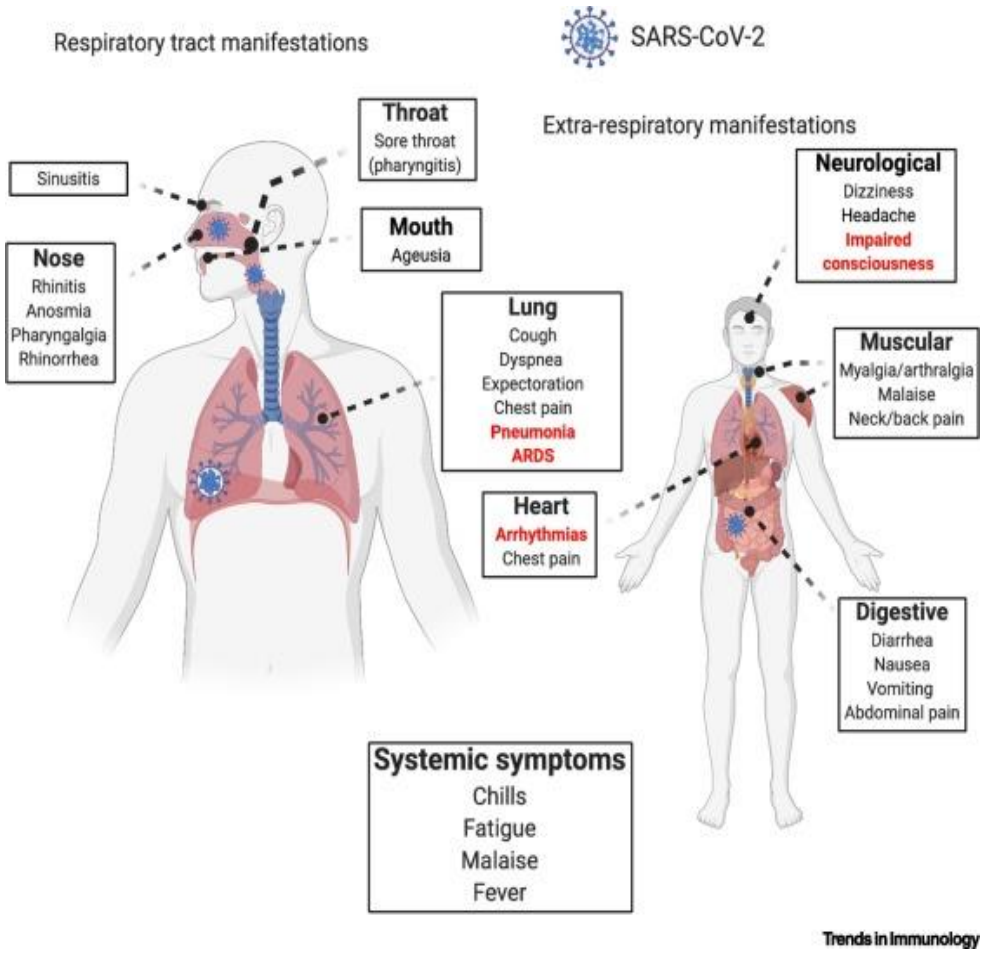

Figure 7 : Clinical symptoms of coronavirus disease 2019 (COVID-19). COVID-19 manifestations in humans have been described to incorporate multiple body systems with varying degrees of onset and severity. Both the upper respiratory tract and lower respiratory tract manifestations are often the most noticeable if a patient is not asymptomatic, in addition to systemic symptoms that are the most frequently reported regardless of disease severity. Red-highlighted signs/symptoms tend to be over-represented in severe patients, but common symptoms are also present in more advanced COVID-19. A severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus symbol denotes where a live virus and/or viral RNA has been isolated. Abbreviation: ARDS: acute respiratory distress syndrome. Figure generated with BioRender.

(Source : Harrison AC, Lin T, Wang P. Mechanism of SARS-CoV-2 transmission and pathogenesis. Trends in Immunology 2020; 41 (12) : 1100-1115. December 1, 2020. Published online : October 14, 2020. DOI : https://doi.org/10.1016/j.it.2020.10.004 Available at : www.cell>trends>immunology>fulltext (accessed on January 5, 2021)).

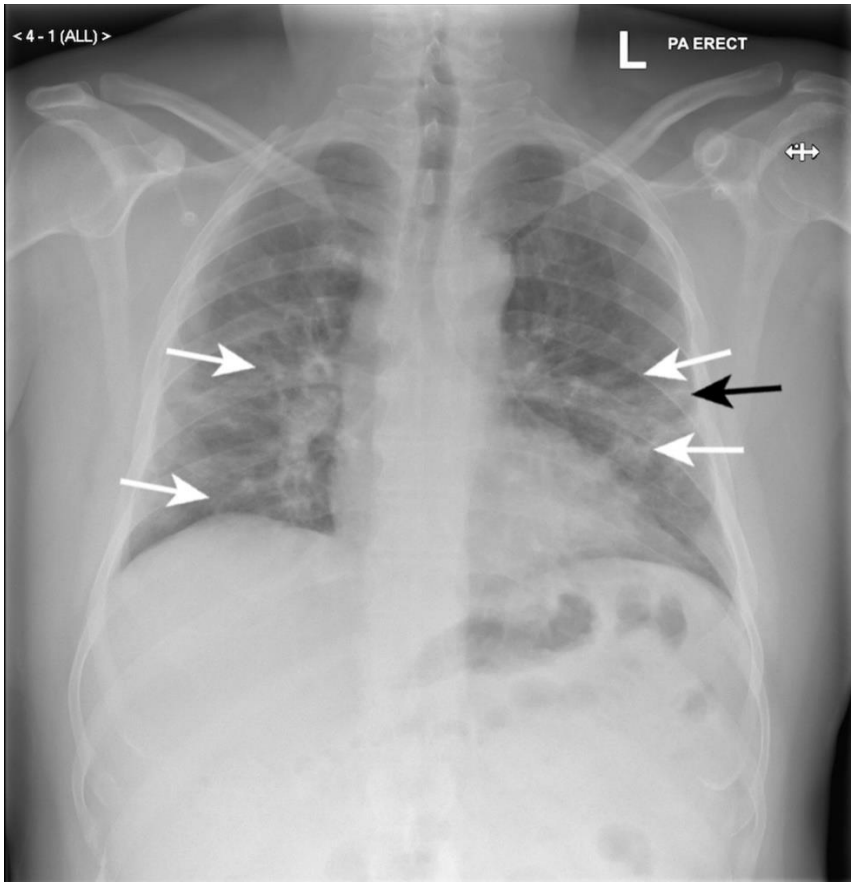

Figure 8 : Ground glass opacity. Posterior-anterior chest radiograph of patient A, a man in his 50s with covid-19 pneumonia. Features include ground glass opacity in both mid and lower zones of the lungs, which is predominantly peripheral (white arrows) with preservation of lung marking. Linear opacity can be seen in the periphery of the left mid zone (black arrow)

(Source : Cleverley J, Piper J, Jones MM. The role of chest radiography in confirming COVID-19 pneumonia. BMJ 2020; 370. DOI : https://doi.org/10.1136/bmj.m2426 Published online : July 16, 2020. Available at : www.bmj.com>content>bmj (accessed on January 5, 2021))

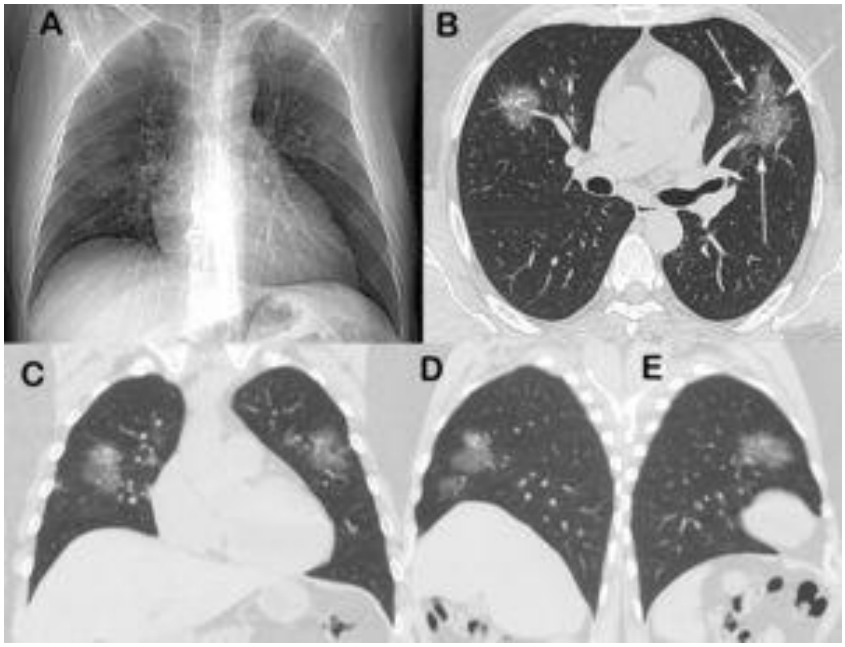

Figure 9 : (A) A posteroanterior chest radiograph was considered normal. Unenhanced chest computed tomography with axial (B), coronal (C) and sagittal (D and E) maximum-intensity projection imaging demonstrated areas of ground glass opacity, many with round and oval morphologies, in both lungs. Not also in B inter- and intralobular septal thickening with a crazy-paving pattern (arrows).

(Source : Schmitt W, Marchio E. COVID-19 : round and oval areas of ground-glass opacity. Pulmonol J 2020. 2 pages. DOI : 10.1016/j.pulmoe.2020.04.001 Available at : www.journalpulmonolgy.org>en-covid-19-round-ov... (accessed on January 5, 2021))

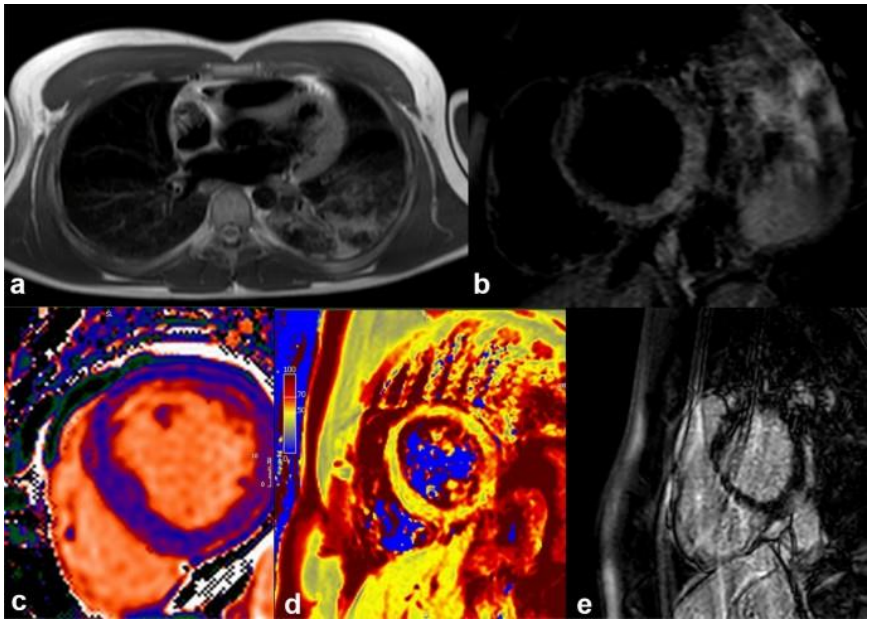

Figure 10 : A 27-year-old patient, without any significant past medical history, was admitted to a hospital with fever and chest pain. The onset of symptomatology dated back about 1 week. His initial investigation showed elevated troponin levels at laboratory tests. Electrocardiography displayed ST-segment elevation. Viral myocarditis of unknown etiology was initially suspected, but SARS-Co-V-2 as a cause was ruled out later at serology. Echocardiography was normal. A chest X-ray showed pulmonary consolidation at the left lower lobe. Cardiac magnetic resonance imaging confirmed the myocarditis (panel a). T2 STIR (panel b) showed an increased signal in mid-basal inferior and inferior-lateral segments. The analysis of T1 mapping (panel c) showed an increase in signal at the same segments (average values of 1100 ms, with reference values of 1030 ± 30 ms). T2 mapping values (panel d) showed an increased signal in mid-basal inferolateral segment (65 ms. Reference values: 52 ± 3 ms), thus indicating the presence of edema. In the sequences acquired later after contrast, an area of sub-epicardial LGE in mid-basal inferior and infero-lateral segments was observed with a concomitant involvement of the adjacent pericardium (panel e). Images processed with Circle CVI 42

(Source : Cau R, Bassareo PP, Mannelli L, Suri JS, Luca S. Imaging in COVID-19-related myocardial injury. Int Cardiovasc Imaging 2020. 12 pages. DOI : https://doi.org/10.1007/s10554-020-02089-9 Available at : link.springer.com>article (accessed on January 5, 2021))

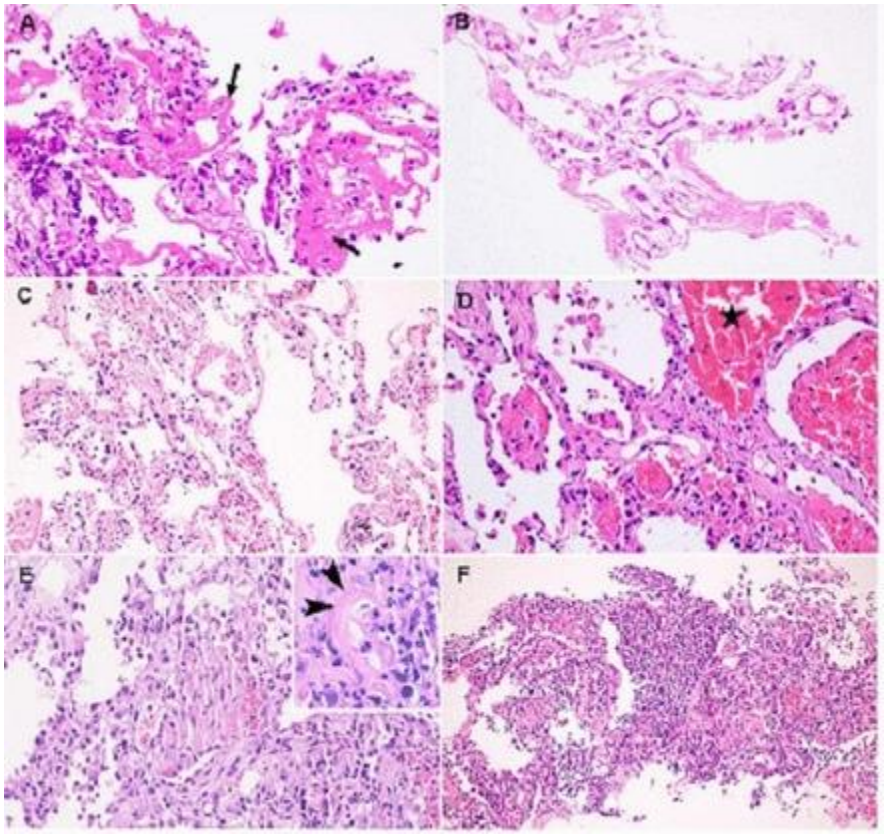

Figure 11 : Histopathological changes in lungs of COVID-19 patients. (A) Infiltration of lung tissue by mononuclear inflammatory cells, along with desquamation of alveolar epithelium and formation of hyaline membrane (arrow). (B) Hyaline membrane formation with no signs of inflammatory cell infiltrate. (C) Interstitial thickening with hyperplasia of type II alveolar epithelium. (D) Red blood cells present in alveolar lumen (asterisk) along with formation of fibrin plugs. (E) Diffuse hyperplasia of type II alveolar epithelium and presence of fibrinoid vascular necrosis (inset). (F) Infiltration of inflammatory cells, predominantly neutrophils into the alveolar lumen,indicative of broncho-pneumonia. (*Courtesy: Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, et al. Pathological study of the 2019 novel coronavirus disease (COVID) through postmortem core biopsies. Mod Pathol. 2020 Apr 14;1–8.*)

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

Mechanism of SARS-CoV-2 (COVID-19) Invasion into Host Cells

Coronaviruses are enveloped and single-stranded ribonucleic acid (RNA) viruses of approximately 30 kb with infections of various host species [1]. SARS-CoV-2 (COVID-19) are divided into four genera; α, β, γ, and δ based on their genomic structure. Alpha and beta coronaviruses infect only mammals [2]. SARS-CoV-2 (COVID-19), SARS-CoV, and Middle-East-Respiratory-Syndrome coronavirus (MERS-CoV) are classified to β coronaviruses.

In the host, the life cycle of coronavirus consists of 5 steps : 1) attachment, 2) penetration, 3) biosynthesis, 4) maturation, and 5) release. Once viruses bind to host receptors (attachment), they enter host cells, particularly type II pneumocytes via endocytosis or membrane fusion (penetration). Once viral contents are released inside the host cells, viral RNA enters the host's nucleus for replication and making viral proteins (biosynthesis) [Figure 12]. New viral particles are produced (maturation) and released.

Coronaviruses consist of four structural proteins; spike (S), membrane (M), envelop (E), and nucleocapsid (N) [3, Figure 12]. Spike protein of coronaviruses which determines the diversity of coronaviruses and host tropism is composed of a transmembrane trimetric glycoprotein protruding from the viral surface. Spike protein comprises two functional subunits; S1 subunit is responsible for binding to the host cell receptor and S2 subunit is responsible for the fusion of the viral and cellular membranes. Structural and functional studies demonstrated that the spike protein the of coronaviruses can bind to angiotensin converting enzyme 2 (ACE2) [4-6], a functional receptor for SARS-CoV [7]. ACE2 expression is high in lung (high expression on lung epithelial cells), heart, gastrointestinal tract (Figure 13) (particularly,ileum), and kidney (Figure 14) [8]. Further studies are needed for additional SARS-CoV-2 (COVID-19) binding targets.

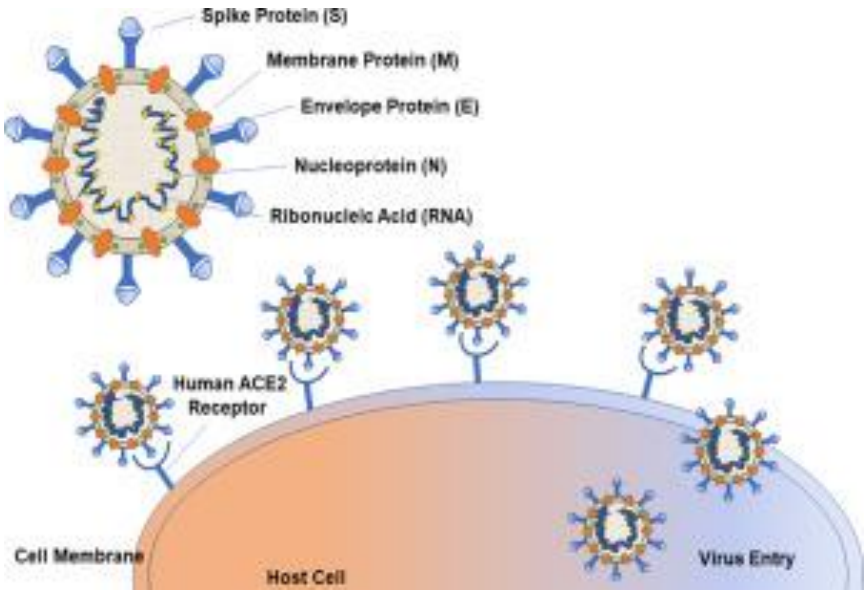

Figure 12 : Demonstrating the genomic structure of the SARS-CoV-2 (COVID-19)

(Source : Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, Atif SM, Hariprasad G, Hasan GM,Hassan MdI. Insights into SARS-CoV-2 genomic, structure, evolution, pathogenesis and therapies : structural genomics approach. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 202; 1866 (10) : 165878. Available at : www.sciencedirect.com>science>article>pii (accessed on January 5, 2021))

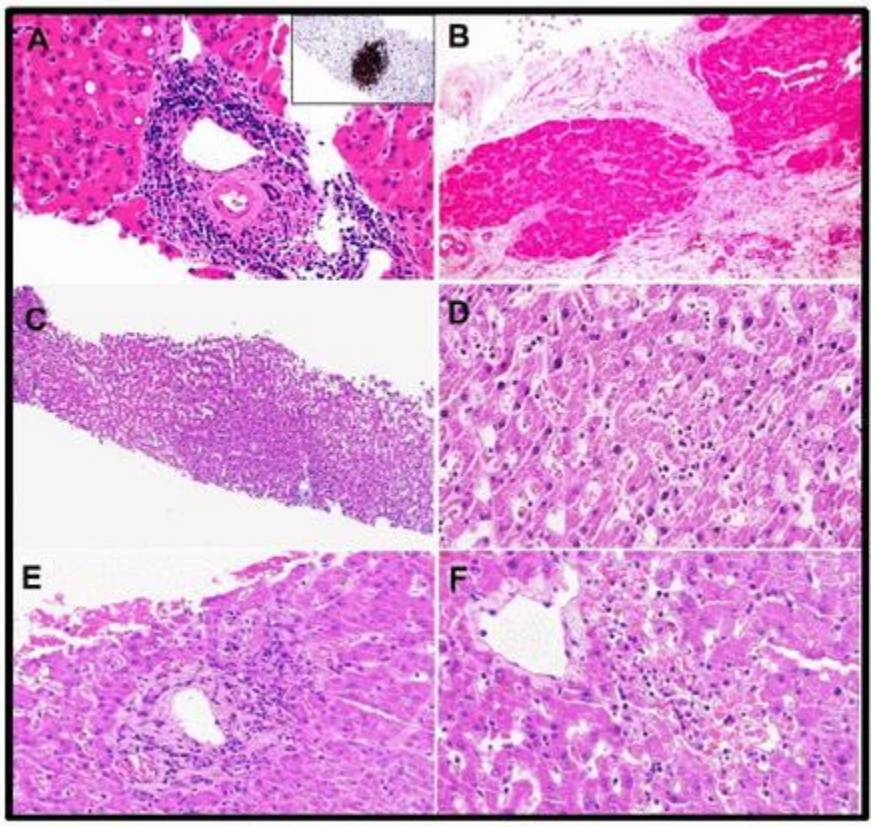

Figure 13 : (A) Hepatocytes showing glycogenated nuclei and atypical small lymphocytes densely infiltrating the area of portal triad and showing CD20 positivity (inset). Dense portal infiltration by atypical small lymphocytes (inset: CD20 immunostaining) and focal glycogenated nuclei in hepatocytes have also been observed. (B) Hepatic nodules showing fibrosis, indicative of chirrosis.Cirrhotic nodules with thick fibrosis. (C) Hepatic sinusoids are dilated and filled with lymphocytes.Mild sinusoidal dilatation with increased lymphocytic infiltration. (D) High power view showing sinusoidal lymphocytes. (E,F) Periportal and centrilobular areas show necrosis, indicative of injury. *(Courtesy: Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, et al. Pathological study of the 2019 novel coronavirus disease (COVID) through postmortem core biopsies. Mod Pathol. 2020 Apr 14;1–8.)*

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

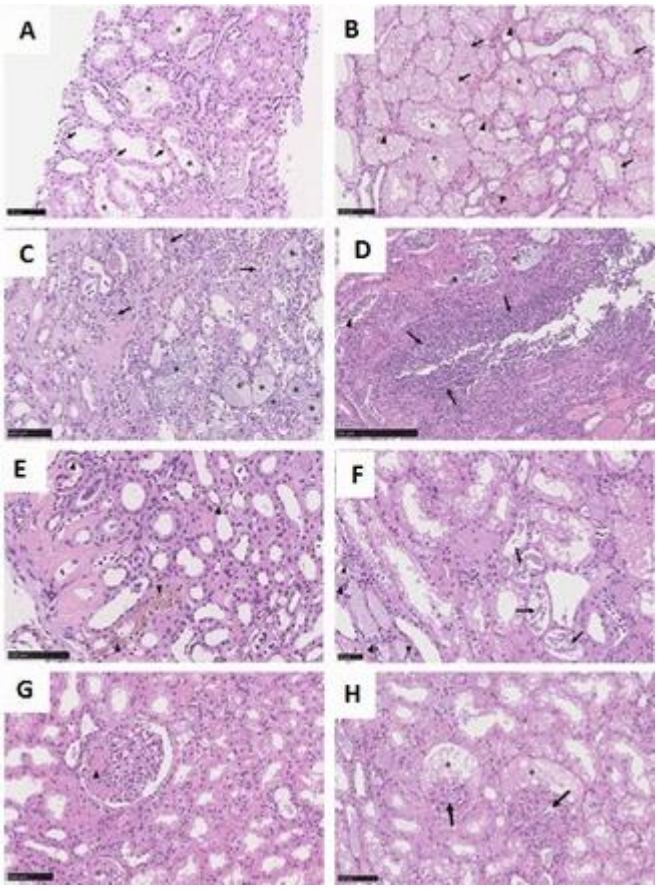

Figure 14 : Histopathological changes in kidneys of COVID-19 patients (A) Epithelium of proximal convoluted tubules shows decreased/loss of the brush border. (B)Tubular epithelial cells show vacuolar degeneration (arrows), leading to collection of necrotic debris in the lumen (asterisks). Blocked peritubular capillaries due to erythrocytic aggregates (arrowheads). (C,D) Inflammatory cells (arrowhead) infiltrate the tubules and arcuate artery (arrows), Bacterial foci (asterisks) is also observed. (E,F) Tubular deposition of hemosiderin granules, calcium deposits (arrowhead) and pigmented cast (arrow). (G,H) Glomeruli show ischaemic contraction (arrows) and fibrin thrombi (arrowhead). Bowman's space show presence of leaked accumulated plasma; hematoxylin and eosin. Bars = (F) 50 μm, (A–C, E, G, H)100 μm, and (D) 250 μm. (*Courtesy: Su H, Yang M, Wan C, Yi L-X, Tang F, Zhu H-Y, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. Kidney Int. 2020 Jul;98(1):219–2.)*

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

After binding of SARS-CoV-2 (COVID-19) to the host protein, protease cleavage is underwent by the spike protein. Activation of the spike protein of SARS-CoV-2 (COVID-19) and MERS-CoV as a two-step sequential protein cleavage has been proposed as a model that consists of cleavage at the S1/S2 cleavage site for priming and a cleavage for activation at a position adjacent to a fusion peptide within the S2 subunit " S2 " site [9-11]. Following the cleavage at the S1/S2 cleavage site, S1 and S2 subunits remain non-covalently bound and the distal S1 subunit leads to the stabilization of the membrane-anchored S2 subunit at the pre-fusion state [5]. Presumably activation of the spike protein for membrane fusion through irreversible and conformational changes is due to subsequent cleavage at the S2 site [12]. Existence of furin cleavage site (" RPPA " sequence) at the S1/S2 site is the unique characteristics of SARS-CoV-2 (COVID-19) among coronaviruses. During biosynthesis, the S1/S2 site of SARS-CoV-2 (COVID-19) is entirely subjected to cleavage in a drastic contrast to SARS-CoV spike protein that is incorporated without cleavage [5]. The expression of furin makes SARS-CoV-2 (COVID-19) very pathogenic although the S1/S2 site is also subjected to cleavage by other protease, such as cathepsin L and transmembrane protease serine 2 (TMPRSS2) [11, 13].

T-cell mediated responses against coronaviruses are antigen presentation through dendritic cells (DCs) and macrophage that can phagocytize virus-infected-apoptotic epithelial cells contributing to antigen presentation to T cells. The expression of ACE2 on (splenic) dendritic cells and pulmonary alveolar macrophages is present but limited, based on the Immunological Genome database (http://rstats.immgen.org). DCs and macrophages may be primarily infected with virus. SARS-CoV-2 (COVID-19) uses another protein to bind to antigen presenting cells (ACPs) or not should be investigated. These ACPs move to the draining lymph nodes to present viral antigens to T cells. In addition to ACE2, SARS-CoV can also bind to dendritic-cell specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN, high expression on dendritic cells and macrophages) and DC-SIGN-related protein (DC-SIGNR, L-SIGN) [14-16]. CD8+ T cells kill viral infected cells, whereas CD4+ T cells activate B cells to promote the virus-specific antibody production.

Patients with severe COVID-19 demonstrated lymphopenia, especially in peripheral blood T cells [17, 18] and increased plasma concentrations of granulocyte-colony stimulating

factor (G-CSF), interleukin (IL)-6, IL-10, macrophage inflammatory protein (MIP)-1α, monocyte chemoattractant protein 1 (MCP-1), and tumor necrosis factor (TNF)-α [17-19]. The higher levels of IL-6 are, the more severe conditions the COVID-19 patients are in. Higher expression of CD38, CD44, and CD69 is demonstrated in COVID-19 patients with activation of CD4+ and CD8+ T cells. T cells exhaustion that could have led to the progression of COVID-19 is indicated by higher percentage of checkpoint receptor Tim-3+ PD-1+ subsets in CD4+ and CD8+ T cells. Another marker for T cells exhaustion is elevation of NK group 2 member A (NKG2A) on CD8+ T cells [20]. Aberrant pathogenic CD4+ T cells with co-expressing interferon (IFN)-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) are demonstrated in severe COVID-19 patients [17]. Significant decrease in circulating T cells, the majority of infiltrating adaptive immune cells are primary cytotoxic CD8+ T cells. CD4+ T cells are also pathological cytotoxic T cells found in severe COVID-19 patients [21] with lung injury [22]. These pathological CD4+ T cells release circulating monocytes responding to GM-CSF. Significant higher percentage of CD14+CD16+ inflammatory subsets are also identified in COVID-19 patients, but they are seldom exist in health individuals. These inflammatory CD14+CD16+ inflammatory monocytes demonstrate high IL-6 expression, that accelerates the progression of systemic inflammatory response. GM-CSF, a response to virus infection can assist in differentiation of innate immune cells augment T cell function, but GM-CSF can trigger tissue damage at excess [23, 24]. Previous experimental autoimmune encephalomyelitis (EAE) models in adults revealed that GM-CSF+IFN-γ+CD4+ T cells were demonstrated upon strong T cell receptor (TCR) responses, whereas CD8+ T cells expressing GM-CSF were identified at higher percentage and secreted IL-6. Neutrophils, the majority of innate immune cells can induce lung injury [25-27].

In addition to IL-6 production, SARS-CoV-infected lung epithelial cells produce IL-8, a well-known chemoattractant for neutrophils and T cells [28]. The three main components for innate immunity in human airway are epithelial cells, pulmonary alveolar macrophages, and dendritic cells (DCs), whereas DCs are located underneath the epithelium and macrophages reside at the apical side of the epithelium [28]. The lungs of severe COVID-19 patients demonstrate infiltration of a large number of inflammatory cells [29, 30]. Due to high ACE2 expression on the apical side of lung epithelial cells in the

alveolar space [31, 32], SARS-CoV-2 (COVID-19) can enter and destroy lung epithelial cells. Significant ACE2 expression on innate lymphoid cells (ILC)2, ILC3 [33], and endothelial cells [34, 35] is also demonstrated. NK cells, a member of ILC1 constitute a majority of pulmonary ILCs, approximately 95 %, whereas ILC2 and ILC3 are responsible for mucous homeostasis. Nevertheless, there is a very limited knowledge of ILC2- and ILC3-involved coronavirus infection. Pulmonary endothelial cells represent one third of the lung cells [36]. Endothelial function includes promotion of anti-aggregation, fibrinolysis, and vasodilatation [37]. Due to a significant role playing in thrombotic regulation [37], hypercoagulable profiles that are demonstrated in severe COVID-19 patients likely suggest significant endothelial injury. Pulmonary endothelial injury can facilitate viral invasion through abnormal microvascular permeability. Pulmonary thrombosis and embolism accompanying elevation of d-dimer and fibrinogen levels have been demonstrated in severe COVID-19. The clinical features of SARS-CoV-2-infected patients vary from minimal symptoms to severe respiratory failure with multiple organ failure, in addition to pulmonary thrombosis and embolism. Computed tomography (CT) of the chest in COVID-19 patients reveals the characteristic pulmonary ground glass opacification even in the asymptomatic patients [38].

Additionally, SARS-CoV-2 (COVID-19) can invade the nervous system [39], genital system (testis) [40] and skin [41]. The hematogenous route by passing through blood brain barrier or olfactory-nerve-retrograde neuronal spread is the most common peripheral nerve involvement [42, Figure 15, 16]. Neurological imaging scans for COVID-19 patients with acute symptoms demonstrate enhanced cortical or subcortical fiber tracts and grey matter, and signs of ischemia and/or hemorrhage [43-46,]. A recent retrospective cohort study was conducted at an academic quaternary-care center and an affiliated community hospital, both in New York City, USA by performing cross-sectional neuroimaging of the brain for 278 (14 %) COVID-19 patients, with 269 (13 %) patients undergoing computed tomography (CT) of the brain, 51 (2.5 %) patients undergoing magnetic resonance (MR) imaging, and 42 (2.0 %) patients undergoing both CT and MR imaging [45, Figure 17-20]. The results of the study revealed that 58 (21 %) of 278 cases indicated acute or subacute findings, 31 cases (11 %) of cerebral infarction, 10 cases of parenchymal hematomas (3.6 %), 6 cases of cranial nerve abnormalities (2.2 %), 3 cases of posterior reversible encephalopathy syndrome

(PRES), 3 cases of probable critical illness-Associated microhemorrhage (1.1 %), 3 cases of nontraumatic subdural hemorrhages (1.1 %), and 2 cases of non-aneurysmal subarachnoid hemorrhages (0.7 %), whereas the yield of neuroimaging was higher among the 51 cases undergoing MR imaging [45]. Taste and smell impairment are the most common peripheral-nervous-system symptoms, whereas headache and dizziness are the most common central-nervous-system (CNS) manifestations [42, 43Figure 21]. Some COVID-19 patients occasionally demonstrate convulsion, stroke, symptoms of nerve demyelination, ataxia, and acute encephalopathy [44]. SARS-CoV-2 (COVID-19) RNA is detected in the brain tissue and cerebrospinal fluid accompanying generalized brain lesions of some autopsied COVID-19 patients [47-50].

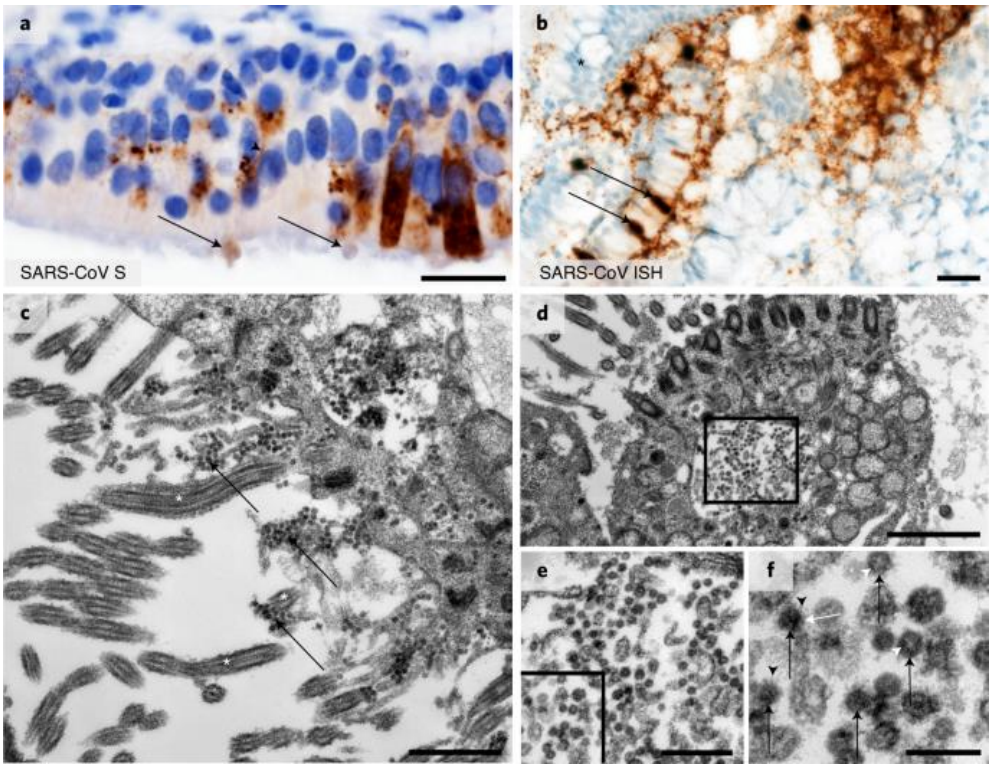

Figure 15 : Immunohistochemistry-, in situ hybridization- and electron microscopy-based detection of SARS-CoV within the olfactory mucosa.

A, CoV antigen detected by anti-SARS-CoV S protein antibodies (brown, individual P30) exhibits a cytoplasmic, often perinuclear, signal for CoV-positive cells resembling epithelial cells and cells harboring dendrite-like projections (arrowhead) with tips (arrows), which morphologically qualify as OSNs. b, SARS-CoV-2 RNA ISH showing intense signals in the

mucus layer and cells (arrows) of the epithelium (asterisk) (brown, individual P15). c–f, Ultrastructural images of re-embedded FFPE material showing numerous extracellular CoV particles (c, arrows) attached to kinocilia (c, white asterisks) and intracellular CoV particles (d–f, increasing magnification) in a ciliated cell (individual P15, punch biopsy from the area in b). In e and f, intracellular CoV particles are located within cellular compartments of different sizes and are similar in their size and substructure. In f, at high magnification, five particles in this region show a particularly well-recognizable substructure (black arrows) that includes characteristic surface projections (black arrowhead), a heterogeneous and partly granular electron-dense interior, most likely representing RNP (white arrowheads), and a membrane envelope (white arrows). Scale bars: 20 µm (a), 50 µm (b), 1 µm (c), 2 µm (d), 500 nm (e) and 200 nm (f).

(Source : Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nature Neuroscience 2020. 13 pages. DOI : https://doi.org/10.1038/s41593-020-00758-5 Available at : www.nature.com/natureneuroscience ; www.nature.com>natureneuroscience>articles (accessed on January 5, 2021))

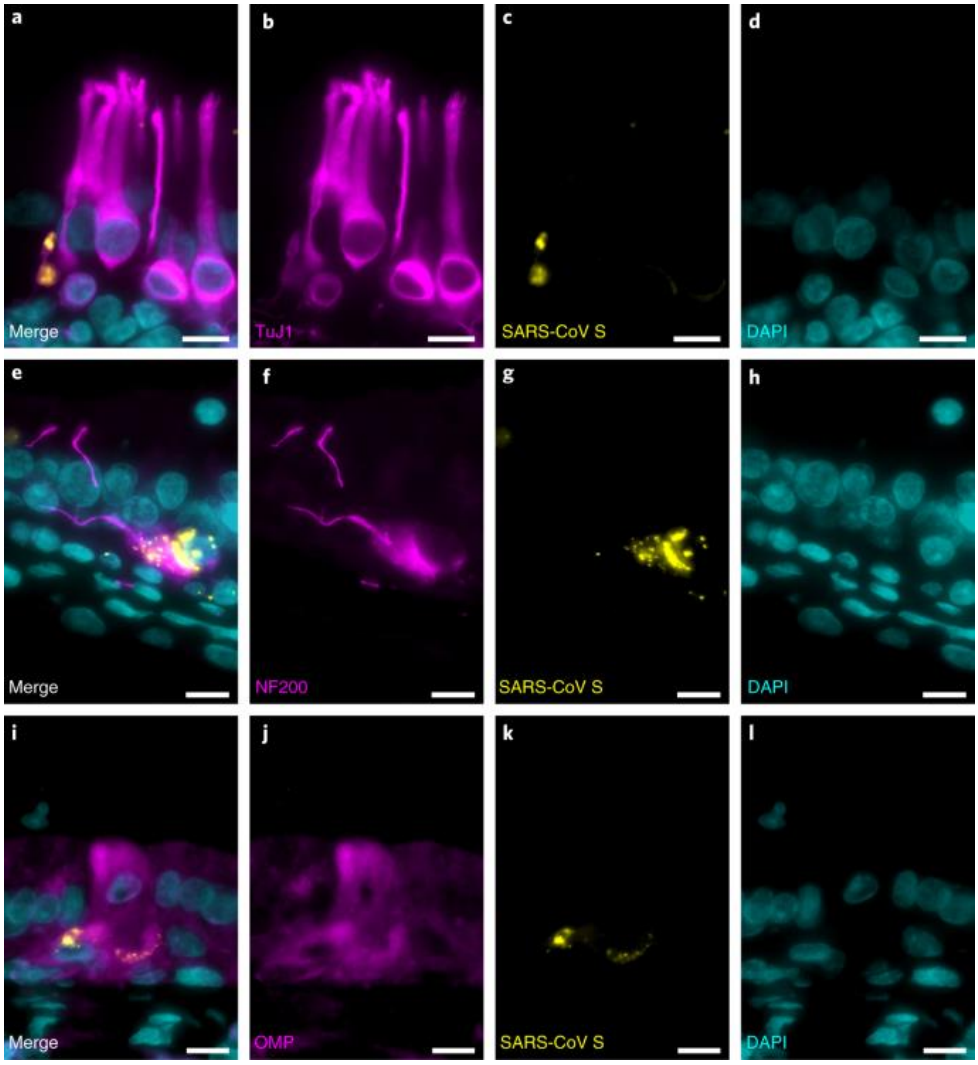

Figure 16 : a–l, Representative maximum-intensity projections of confocal (a–d and i–l) or epifluorescence (e–h) microscopy images of olfactory mucosa showing intracytoplasmic staining for SARS-CoV S protein within $TuJ1^+$ (a–d, individual P27), $NF200^+$ (e–h, individual P27) and OMP^+ (i–l, individual P27) OSNs. Staining for TuJ1, NF200 and OMP (magenta, Alexa Fluor 488) marks cells of neuronal origin, staining for SARS-CoV S protein (yellow, Alexa Fluor 555) visualizes the presence of SARS-CoV and DAPI staining (petrol) identifies all cell nuclei ($n = 3$ individuals with COVID-19 (P27, P30 and P32) were analyzed; $n = 2$ individuals without COVID-19 served as controls; shown are representative images from P27). Scale bars, (all panels) 10 µm.

(Source : Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nature Neuroscience 2020. 13 pages. DOI : https://doi.org/10.1038/s41593-

020-00758-5 Available at : www.nature.com/natureneuroscience ; www.nature.com>natureneuroscience>articles (accessed on January 5, 2021))

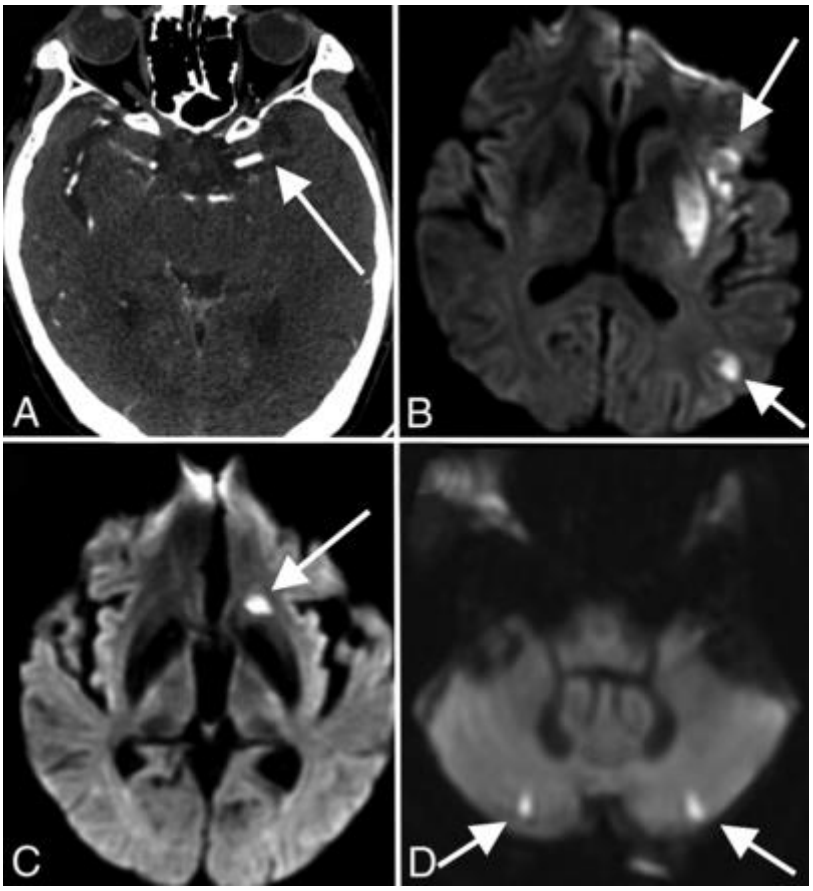

Figure 17 : Stroke. A 53-year-old man with COVID-19 with an axial CTA image (*A*) demonstrating an abrupt cutoff of the proximal M1 segment of the left MCA (*arrow*), consistent with thrombosis. The patient later underwent MR imaging, with a DWI sequence *(B*) demonstrating acute infarctions in the left MCA territory (*arrows*). An 85-year-old woman with COVID-19 and MR imaging with a DWI sequence (*C* and *D*) demonstrating acute infarctions in both the anterior (*arrow*, *C*) and posterior (*arrows*, *D*) circulations, consistent with central embolic etiology. Most patients in our cohort had stroke of either embolic or cryptogenic etiology.

(Source : Lin E, Lantos JE, Strauss SB, Phillips CD, Campion, Jr TR, Navi BB, et al. Brain imaging of patients with COVID-19 : findings at an academic institution during the height of the outbreak in New York City. Am J Neuroradiol 2020; 41 : 2002-2008. Available at : www.ajnr.org>content>early>2020/08/20>ajnr (accessed on January 5, 2021))

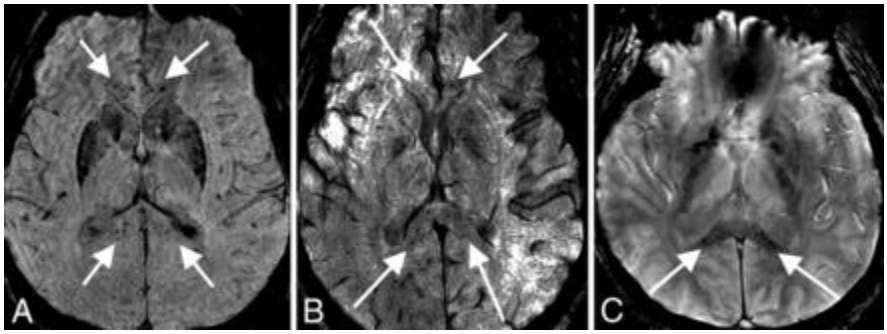

Figure 18 : Corpus callosum microhemorrhages. A 65-year-old woman (*A*), a 44-year-old woman (*B*), and a 69-year-old man (*C*) all demonstrate microhemorrhages on SWI, with a similar distribution, preferentially involving the corpus callosum *(arrows* in *A–C*), particularly the splenium. All patients had undergone mechanical ventilation before imaging. The distribution is similar to that previously described in critically ill, ventilated patients as well as in those with high-altitude cerebral edema.

(Source : Lin E, Lantos JE, Strauss SB, Phillips CD, Campion, Jr TR, Navi BB, et al. Brain imaging of patients with COVID-19 : findings at an academic institution during the height of the outbreak in New York City. Am J Neuroradiol 2020; 41 : 2002-2008. Available at : www.ajnr.org>content>early>2020/08/20>ajnr (accessed on January 5, 2021))

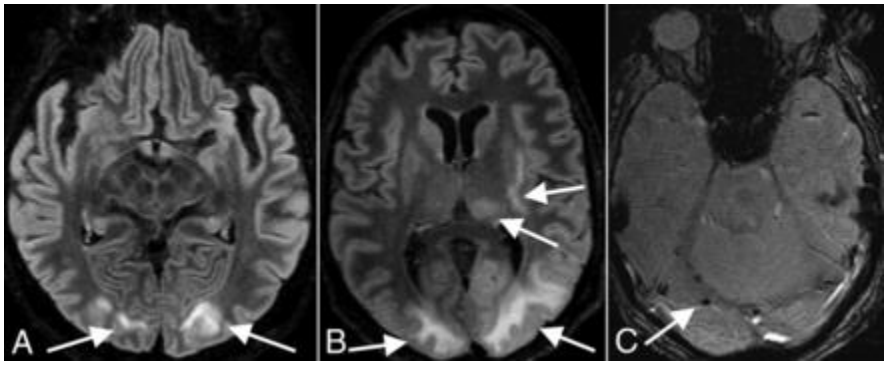

Figure 19 : Posterior Reversible Encephalopathy Syndrome (PRES). A 65-year-old woman (*A*, same patient as in Fig 18 *A*) and 63-year-old man (*B* and *C*) demonstrate a typical imaging appearance of PRES on T2-FLAIR images (*arrows* in *A* and *B*), with bilateral subcortical occipital white matter hyperintense signal, as well as more pronounced involvement of the patient in *B* with thalamic and internal and external capsule involvement. This patient also has evidence of associated right occipital microhemorrhage (*arrow* in *C*). Both patients had the typical risk factors for PRES of acute kidney injury and hypertension.

(Source : Lin E, Lantos JE, Strauss SB, Phillips CD, Campion, Jr TR, Navi BB, et al. Brain imaging of patients with COVID-19 : findings at an academic institution during the height of the outbreak in New York City. Am J Neuroradiol 2020; 41 : 2002-2008. Available at : www.ajnr.org>content>early>2020/08/20>ajnr (accessed on January 5, 2021))

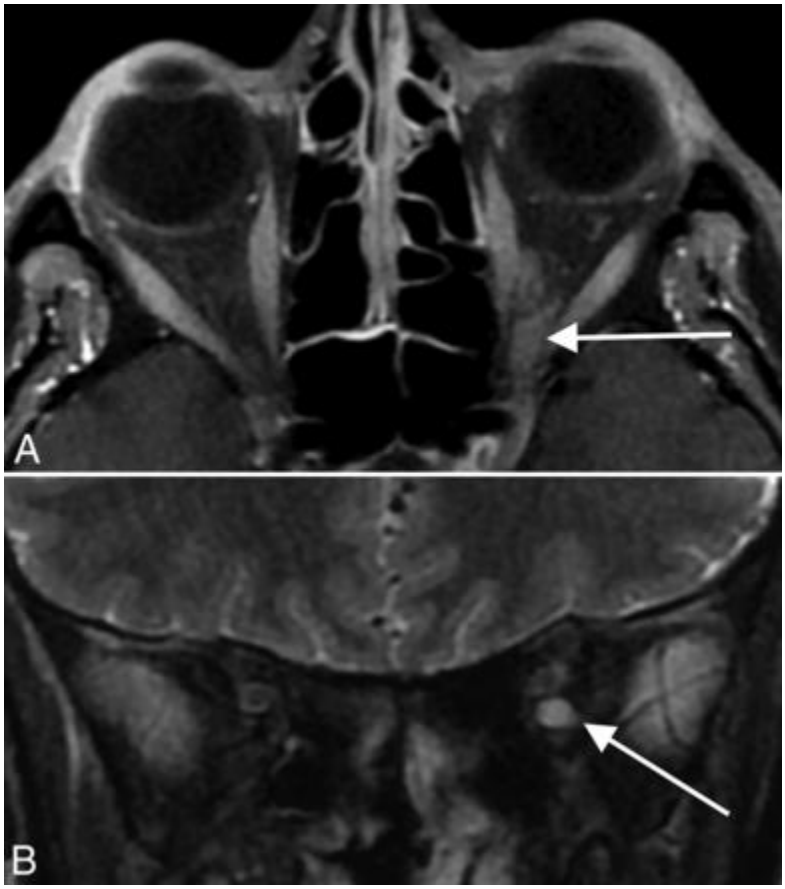

Figure 20 : Miller-Fisher syndrome. A 36-year-old male patient with a history of COVID-19 and diplopia, ataxia, and areflexia. Axial T1 postcontrast (*A*) and coronal T2 fat-suppressed (*B*) MR images through the orbits demonstrate striking enlargement, enhancement, and T2 hyperintense signal of cranial nerve III (*arrows* in *A* and *B*). The patient was clinically diagnosed with Miller Fisher syndrome and improved with intravenous immunoglobulin treatment.

(Source : Lin E, Lantos JE, Strauss SB, Phillips CD, Campion, Jr TR, Navi BB, et al. Brain imaging of patients with COVID-19 : findings at an academic institution during the height of the outbreak in New York City. Am J Neuroradiol 2020; 41 : 2002-2008. Available at : www.ajnr.org>content>early>2020/08/20>ajnr (accessed on January 5, 2021))

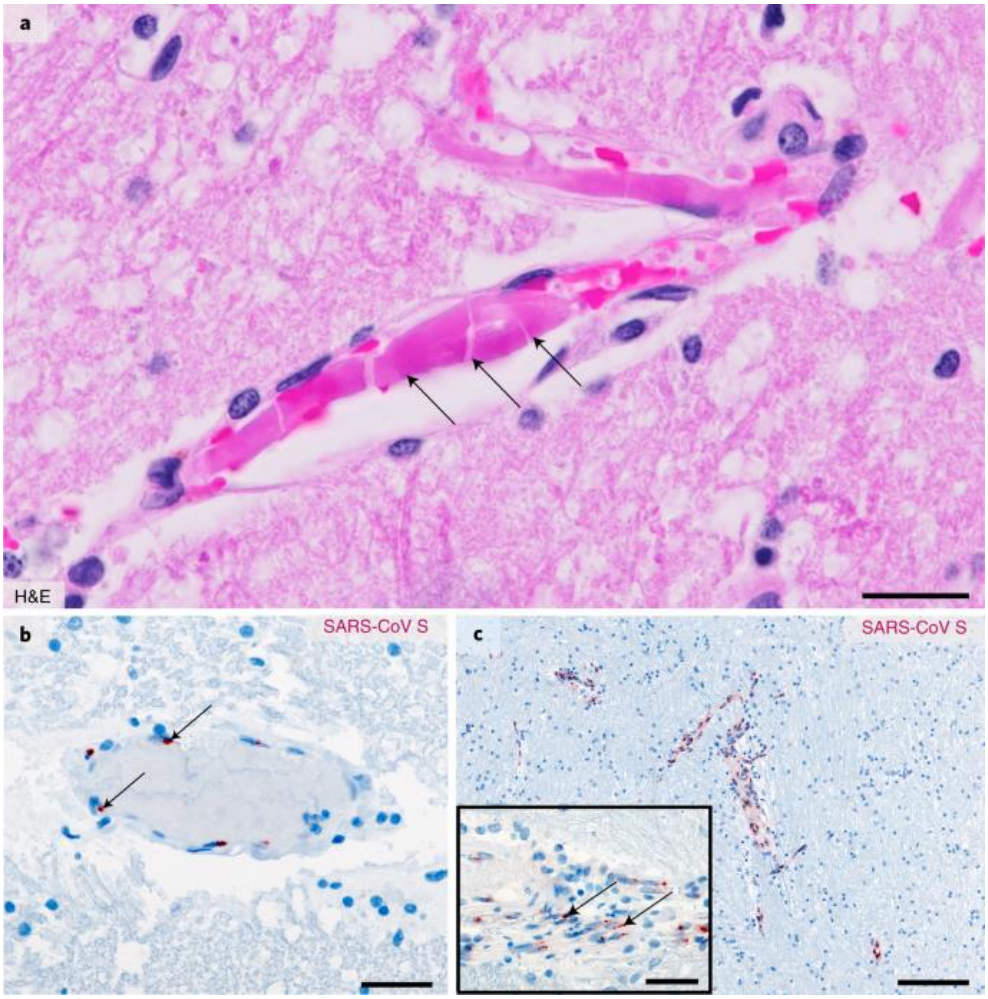

Figure 21 : Signs of (micro)thromboembolic events and SARS-CoV-2 immunostaining in the CNS of deceased individuals with COVID-19.

A, Hematoxylin and eosin (H&E)-stained FFPE section of the thalamus obtained from a deceased individual with COVID-19 (individual P26). Several small vessels exhibit fresh thrombi (pink, indicated by arrows) resulting in a large infarct of surrounding CNS tissue characterized by a substantial reduction of detectable neuronal and glial nuclei, edema and vacuolation. b,c, SARS-CoV S protein observed in the endothelial cells of small CNS vessels. Tissue with no obvious ischemic damage exhibits only sparse staining intensity in endothelial cells (b, medulla oblongata, $n = 3$ of 6; red, indicated by arrows, individual P3) when compared to endothelial cells within acute infarct areas (c, pons, $n = 3$ of 4; red, indicated by arrows, individual P4; inset depicts a magnified vessel from a different region of the same specimen exhibiting SARS-CoV S protein

deposits within endothelial cells). Scale bars: 30 µm (a), 50 µm (b), 200 µm (c) and 40 µm (inset in c).

(Source : Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. Nature Neuroscience 2020. 13 pages. DOI : https://doi.org/10.1038/s41593-020-00758-5 Available at : www.nature.com/natureneuroscience ; www.nature.com>natureneuroscience>articles (accessed on January 5, 2021))

Due to presence of ACE-2 in seminiferous tubules, Leydig cells, Sertoli cells, and spermatogonia, binding of SARS-CoV-2 (COVID-19) with the testicular-cell-expressing-ACE-2 receptors could damage the testicular tissues [51, Figure 22]. Several previous studies demonstrated that more than 90 % of the Sertoli cells expressed ACE-2 receptors, thus, Sertoli cells are more susceptible than spermatogonia [52]. Sertoli cells demonstrate higher expression of ACE-2 but lower expression of TMPRSS2, whereas spermatogonia stem cells reveal higher expression of TMPRSS2 but lower expression of ACE-2, indicating the mutual role Sertoli cells or somatic cells and spermatogonia for the SARS-CoV-2 (COVID-19) invasion [52].

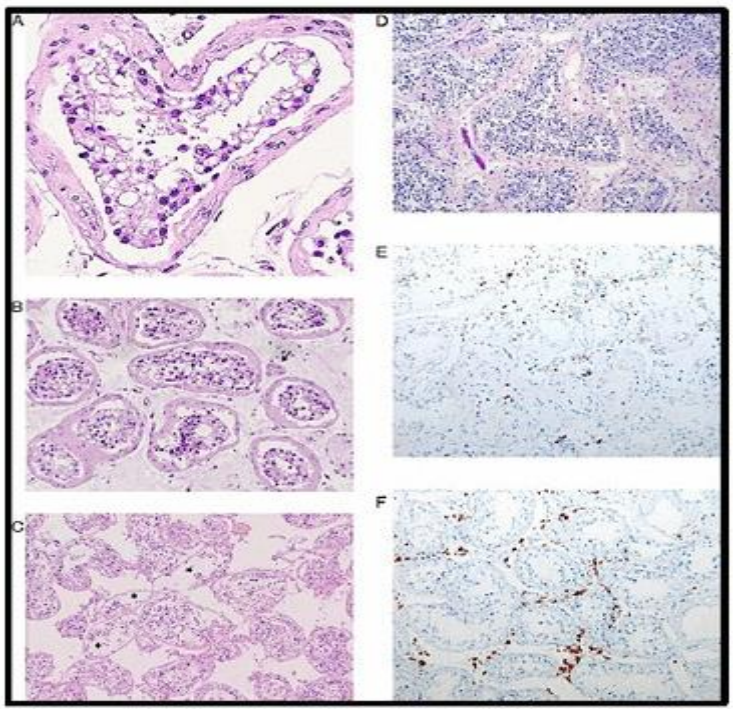

Figure 22 : Pathological changes observed in testes from patients with COVID-19. (A,B) Defoliated and oedematous Sertoli cells with vacuoles along with reduced spermatogenesis and scattered Leydig cells (arrow). (C) Tubular cells shows sloughing into the lumen (asterisks) indicative of injury. There is marked interstitial oedema. (D) Non-Covid testis with protracted disease showing interstitial edema with infiltration of inflammatory cells. (E) Immunohistochemical findings showing CD3-positive+T lymphocytes and (F) CD68-positive+ histiocytes. (*Courtesy: Yang M, Chen S, Huang B, Zhong J-M, Su H, Chen Y-J, et al. Pathological Findings in the Testes of COVID-19 Patients: Clinical Implications. Eur Urol Focus.2020 May 31; pp 1–6.)*

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

Due to vascular endothelium much expression of ACE-2, cutaneous blood vessels can easily bind the spike protein of the SARS-CoV-2 (COVID-19) and facilitate viral invasion onto the skin tissues contributing to activation of Langerhans cell with a cascade of reactivation [41, Figure 23, 24]. The skin manifestations may be urticaria, dermatitis, purpuric papulovesicular rash, pox-like vesicles, erythematous rash, livedo reticularis lesions, petechiae, pseudo-chilblains, macular/maculopapular exanthems, etc. which may be painful on the fingertips, hands, toes, feet, and trunk [53, 54]. Fortunately, most of the skin lesions heal without any residual manifestations [54].

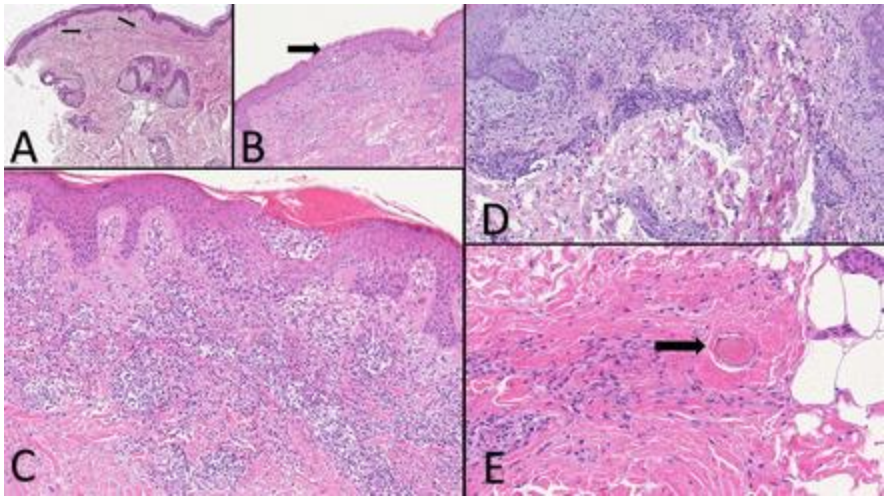

Figure 23 : Histopathological changes in skin of COVID-19 patients. (A) Arrow showing telangiectatic blood vessels in early exanthematous rash. (B) Epidermis (arrow) showing groups of Langerhans cells in the laterphase of exanthematous rash. Superficial dermis also shows perivascularinfiltration of lymphocytes. (C) An intraepidermal group of Langerhans cell seen in apapulo-vescicular rash. (D) Micrographic feature ina Maculo-papular erruption. (E) Capillary thrombosis (arrow) along with diffusehaemorrhage in an exanthemous *(Courtesy: Raffaele Gianotti, Clinical and histopathological study of skin dermatoses in patients affected by COVID-19 infection in the Northern part of Italy. Letter to the Editor. Journal of Dermatological Science. G Model DESC 3594 No. of Pages 3*.)

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

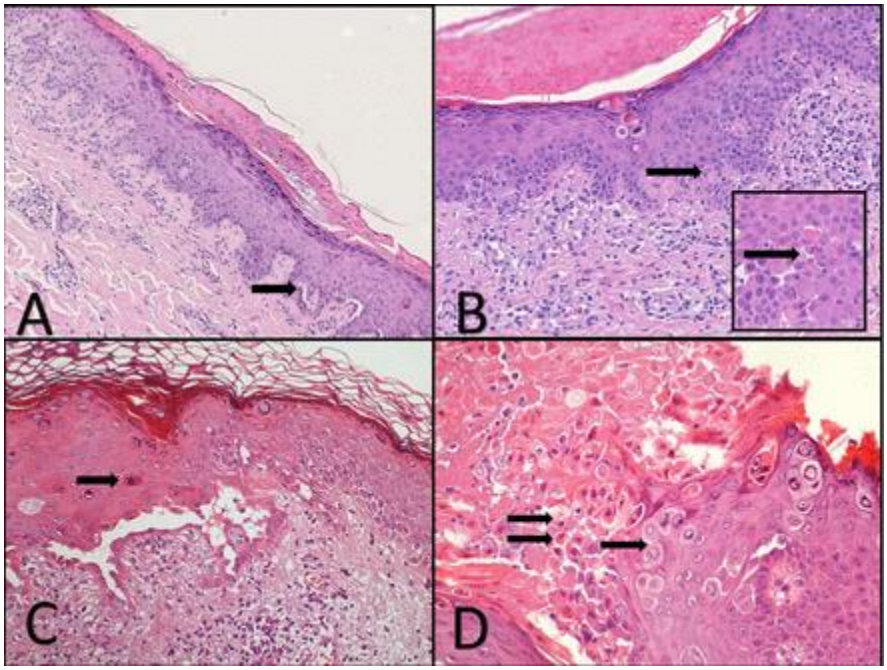

Figure 24 : Histopathological changes in skin of COVID-19 patients(A) Acanthosis with presence of cleft (arrow) observed in skin of COVID-19 patients. Parakeratosis is also observed along with abnormal keratinization. (B) Localized necrotic keratinocytes (arrow) with abnormal keratinization InsetInset, with lymphocytic infiltration (arrow). (C) Acantholytic cleft with an adjacent apoptotic keratinocyte (arrow). (D) Pseudoherpetic features (arrow) along with apoptotic keratinocytes (double arrow). (Courtesy: Raffaele Gianotti, Clinical and histopathological study of skin dermatoses in patients affected by COVID-19 infection in the Northern part of Italy. Letter to the Editor. Journal of Dermatological Science. G Model DESC 3594 No. of Pages 3.)

(Source : Deshmukh V, Motwani R, Kumar A, Kumari C, Roza K. Histopathological observations in COVID-19 : a systematic review. J Clin Pathol 2020; 0 : 1-8. DOI : https://dx.doi.org/10.1136/jclinpath-2020-206995 Available at : https://bmj.com/coronavirus/usage ; jcp.bmj.com>early>2020/08/18>jclinpath-2020-206995 (accessed on January 5, 2021))

The difference of pathophysiology between children and adults in COVID-19 is hypothesized as the following : 1) The expression level of ACE2 may differ between children and adults [32], 2) Children have a qualitatively different response to the SARS-CoV-2 (COVID-19) virus to adults [54], and 3) The simultaneous presence of other viruses in the mucosa of lungs and airways that are common in young children can contribute to SARS-CoV-2 (COVID-19) virus compete with them and limit its growth [55]. Further studies on understanding the roles of ILC1, ILC2, ILC3, and the difference in response to SARS-CoV-2 (C)OVID-19) infection between children and adults are urgently needed to develop efficient targeted therapies.

Post-Acute-COVID-19-Illness Sequelae

The World Health Organization (WHO) reported that approximately, 57.8 million COVID-19 cases, globally could have health and economic consequences [56]. There is a wide range of reported long-term symptoms, known as post COVID condition (sometimes called chronic COVID syndrome, late sequelae of COVID-19, long COVID, long haul COVID, long-term COVID-19, post COVID syndrome, post-acute COVID-19, post-acute sequelae of SARS-CoV-2 infection (as of yet, no internationally agreed definition of post COVID condition); individuals past 9-10 days post symptom onset if they have asymptomatic or mild disease, individuals characteristically do not shed SARS-CoV-2 after recovery from acute COVID-19 illness three weeks) after SARS-CoV-2 (COVID-19) infection, such as fatigue, lost of smell, persistent cough, shortness of breath, palpitations, diarrhea, abdominal pain, rash, recurrent fever, forgetfulness, depression, muscle pain (myalgia), pins and needles, chest pain, and headache [57]. Inclusion of persistence of development or symptoms of post-acute-COVID-19-illness sequelae beyond 3 or 4 weeks from the onset of symptoms of acute-COVID-19 illness has been suggested in the evolving

definition of the post-acute-COVID-19-illness timeline [58, 59], corresponding to the replication-competence of SARS-CoV-2 (COVID-19) that has not been isolated after 3 weeks of the initial COVID-19 infection [60]. Recent studies suggested the classification of the post-acute-COVID-19-illness sequelae into two categories : 1) subacute or ongoing symptomatic COVID-19 illness, that includes abnormalities and symptoms occur from 4-12 weeks beyond acute COVID-19 illness; and 2) chronic or post-acute-COVID-19 syndrome; that includes abnormalities and symptoms occur or persisting beyond 12 weeks of the onset of acute COVID-19 illness not causative to the alternative diagnoses [59, 61]. Nalbandian *et al* defined post-acute-COVID-19-illness sequelae as persistent symptoms and/or delayed or long-term complications of SARS-CoV-2 (COVID-19) infection beyond 4 weeks from the onset of initial symptoms [62, Figure 25]. Palpitation, a common persistent symptom was abnormally detected around 78 % of the post-acute-COVID-19-illness survivors who underwent cardiovascular MRI at the day 70 after the initial diagnosis of COVID-19 [63]. Thromboembolic-caused stroke can occur during the recovery phase of COVID-19 survivors, particularly in high-risk individuals [64].

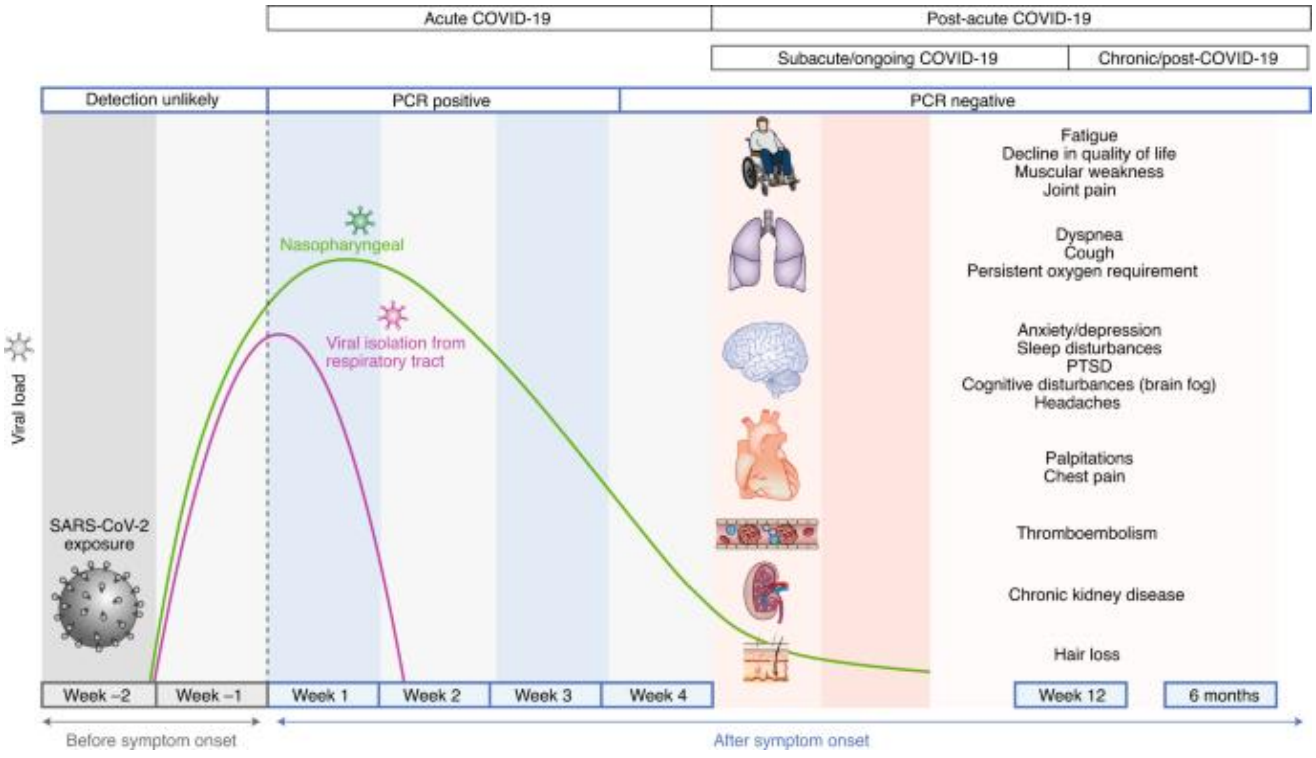

Figure 25 : Timeline of post-acute-COVID-19-illness sequelae. Acute COVID-19 usually lasts until 4 weeks from the onset of symptoms, beyond which replication-competent SARS-

CoV-2 has not been isolated. Post-acute COVID-19 is defined as persistent symptoms and/or delayed or long-term complications beyond 4 weeks from the onset of symptoms. The common symptoms observed in post-acute COVID-19 are demonstrated.

(Source : Nalbandian A, Sehgal K, Gupta K, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute- COVID-19 syndrome. Nature Medicine 2021; 27 (April) : 601-615.)

Less common and more serious long-term complications in hospitalized severe-COVID-19 patients include inflammation of the cardiac muscle (myocarditis), pulmonary function abnormalities, loss of taste, sleep disturbance or deprivation, changes in mood, and anxiety [65]. Anxiety and mood swing have been demonstrated in individuals recovering from acute-COVID-19 illness [66]. Pulmonary function abnormalities in post-acute-COVID-19-illness survivors could be due to a decrease in both diffusing capacity of the lung for carbon dioxide (DLCO) and transfer coefficient of the lung for carbon monoxide (KCO) [67]. Alveolar-capillary damage, microvascular pathological lesions, or anemia can contribute to reduction of the KCO [67]. Post-acute-COVID-19-illness pulmonary fibrosis and post-acute-MERS-associated pulmonary fibrosis were highly associated with old aging ranging from 60- to 70-years age group (13 out of 30 (43.3 %)), demonstrated by Wong *et al* [68] and Das *et al* [69]. Post-acute-COVID-19-illness pulmonary fibrosis was 1.3 times more predominant in males, compared to females, possibly explained by the effect of androgen that promotes the transcription of transmembrane protease serine 2 gene [70], and was much higher incidence in cigarette smoker (18 of 30 patients (60 %)), compared to non-smoking patients [71]. This gene impairs hose's antibody response and facilitates the fusion of the virus-hose cells [70]. Dyspnea, the most common persistent symptom of the pulmonary sequelae ranged from 42 % to 66 % prevalence at 60-100 days of following-up [72-75], supported by the result of lower median 6-minutes walking distance comparing to the normal reference values in one-fourth of the patients at 6 months in the post-acute-COVID-19-illlness Chinese study [76] that was similar to the prevalence in SARS and MERS survivors [77]. Some previous studies demonstrated that fatigue was the most common reported symptom (53 %-71 % of COVID-19 inpatients) [72, 73, 78]. A recent study in Italy among 143 post-acute-COVID-19-illness patients revealed that 87.4 % of them had at least one persisting symptom after recovery from the acute phase of COVID-19 illness, and 53 %, 43 %, 27.3 %, and 21.7 % of them were reported of fatigue, dyspnea,

joint pain, and chest pain, respectively [72]. Dyspnea without hypoxia in post-acute-COVID-19 illness patients with normal lung volumes and normal cardiac function has been due to respiratory muscle dysfunction, and could be detectable by the cardiopulmonary exercise tolerance testing [72].

A previous study conducted by Logue *et al* among 234 University of Washington-enrolled-COVID-19 patients who were contacted between August and November 2020 by a single follow-up questionnaire between 3 and 9 months after COVID-19 illness onset demonstrated that fatigue and health-related-quality-of-life (HRQL) impairment occurred in 14 % and 29 % of outpatients, respectively [79]. Daugherty *et al* demonstrated in their study among adults aged 18-65 with acute COVID-19 illness that 14 % of COVID-19-infected adults aged 65 or less (27,074 of 193,113) had at least one new type of required-medical-care clinical-post-acute-COVID-19-illness sequelae, that was 4.95 % higher than in the 2020 comparator group [80]. More than 50 clinical-post-COVID-19 illness sequelae were used-ICD-10 (international classification of disease, 10th revision) and identified following the acute-phase-SARS-CoV-2 (COVID-19) infection (defined as the date of first SARS-CoV-2 (COVID-19) diagnosis (index date) plus 21 days, a reasonable beginning to the post-acute-COVID-19-illness) in this study, including fatigue, hypercoagulability, peripheral neuropathy, amnesia (memory difficulty), encephalopathy, anxiety, myocarditis, cardiac arrhythmia, diabetes, liver-function-test abnormalities, and chronic respiratory failure were the significant greater risk for specific new clinical-post-acute-COVID-19-illness sequelae (four months after the acute phase of COVID-19 illness (index date plus 21 days)), compared to the three comparator groups (2019, 2020, and viral lower respiratory tract illness groups) (all $p < 0.001$), whereas the older COVID-19-related-and-hospitalized individuals (aged more than 50) with pre-existing medical conditions were at the greatest risk [80]. Moreover, this study indicated that non-hospitalized acute-COVID-19 adults, younger adults (aged 50 or less) with acute COVID-19 illness, and those with no pre-existing medical conditions had an increased risk of new clinical-post-acute-COVID-19-illness sequelae [80]. Fatigue was the most common post-acute-COVID-19-illnes that revealed in several previous surveys with self-reported estimates ranging from 13.6 % [79] to 77.7 % [81], depending on whether patients were hospitalized and the length of the following-up [80]. The rates over time and hazard ratios in this study were highest in the

first month of the index date and were increased to six months for some events, such as diabetes (hazard ratio : 2.47 (CI : 1.14 to 5.38)), sleep apnea (2.31 (CI : 1.23 to 4.32)), fatigue (2.20 (CI : 1.48 to 3.27)), and hypertension (1.81 (CI : 1.10 to 2.96)), indicating the sustainability of hazard for some new clinical-post-acute-COVID-19-illness sequelae several months after initial SARS-CoV-2 (COVID-19) infection [80]. Men and women were rarely different excess risk for new clinical-post-acute-COVID-19-illness sequelae, except hypercoagulability, deep vein thrombosis, kidney injury, and myocarditis, sleep apnea more commonly diagnosed in men), and anosmia and fatigue (more commonly diagnosed in women) [80].

Lungs of the post-acute-COVID-19-illness survivors demonstrated endothelial- cell-dysfunction vasculitis [82]. Detected incident-clinical sequelae that are commonly identified in other serious viral infections, such as kidney injury, stroke, and hypertension was nearly twice, compared to a normal-year-incidence in general population, suggesting more urgently needed planning for healthcare resources for management of the COVID-19 survivors' health complications [81]. Post-acute-COVID-19-illness pulmonary aspergillosis that frequently are associated with acute respiratory distress syndrome (ARDS) could be due to host factors, such as poorly controlled diabetes, anti-interleukin 6 (tocilizumab) treatment in hospitalized post-acute-COVID-19-illness patients, and long-term use of corticosteroids in severe COVID-19-associated pneumonia, in addition to damage to the respiratory epithelium directly caused by SARS-CoV-2 (COVID-19) resulting in mucociliary dysfunction and fungal invasion of the respiratory epithelium. Serum galactomannan levels, chest roentgenographic and chest computerized tomographic imaging, and fungal isolation in the bronchoalveolar lavage or bronchial washing or tracheal aspirates are the diagnostics [83]. The proposed first-line treatment for this pulmonary sequelae is isavuconazole or voriconazole [83]. Additionally, pulmonary and rhino-orbital-cerebral mucormycosis are the other two mold-related manifestations found in the post-acute-COVID-19 survivors. Biopsy with fungal isolation is the gold standard of diagnosis, whereas the initial treatment includes surgical debridement with intravenous amphotericin-B switching by oral posaconazole for avoiding nephrotoxicity [64]. Blood-stream-candida infection (invasive candidiasis), a late complication of COVID-19-related pneumonia in 15 post-acute-COVID-19-ilness patients in New Delhi, India was also reported [84].

Cardiovascular Post-Acute-COVID-19-Illness Sequelae

At 60 days of the following-up, chest pain was present around 20 % of the COVID-19 survivors [72, 85], whereas at 6 months following-up in the post-acute-COVID-19 Chinese study revealed ongoing chest pain and palpitations in 5 % and 9 % of the COVID-19 survivors, respectively [76]. Ongoing myocardial inflammation may occur at the rates as high as 60 % more than two months after the diagnosis by MRI [63]. The perpetuated mechanisms in post-acute-COVID-19-illness cardiovascular sequelae include SARS-CoV-2 (COVID-19) viral invasion, the immunologic response and inflammation affecting the structural integrity of the cardiac conduction system, pericardium, and myocardium, and downregulation of ACE 2. Autopsy studies in 39 COVID-19 cases (62.5 %) revealed SARS-CoV-2 (COVID-19) viral particles in the cardiac tissues [86] that may contribute to the cardiomyocyte death and fibro-fatty displacement of desmosomal proteins that is critical for cell-to-cell adherence [87, 88]. Persistently increased cardiometabolic demand may be occur in recovered COVID-19 patients that may be related to decreased cardiac reserve, dysregulation of the renin-angiotensin-aldosterone system (RAAS) [89]. SARS-CoV-2 (COVID-19) can induce heightened catecholaminergic state due to cytokine storming from cytokines, such as IL-1, IL-6, and TNF-α, that can prolong ventricular action potentials by modulating cardiomyocyte ion channel expression [90], in addition to the induction of resultant cardiomyopathy from SARS-CoV-2 (COVID-19) infection, and myocardial scarring or fibrosis that can contribute to re-entrant cardiac arrhythmias [91]. After SARS-CoV-2 (COVID-19) illness, autonomic dysfunction can result in inappropriate sinus tachycardia and postural orthostatic tachycardia syndrome, that has been demonstrated as a resulting adrenergic modulation [92, 93]. Abstinence from aerobic activities or competitive sports for 3-6 months until resolution of myocardial inflammation by normalization of the troponin levels or cardiac MRI and serial echocardiogram, electrocardiogram, and cardiac MRI may be considered in competitive athletes with post-acute-COVID-19-related cardiovascular complications [94, 95] and in those with persistent cardiac symptoms [96, 97]. In a previously retrospective study among 3,080 COVID-19 patients revealed that withdrawal of cardiac-guidelines-directed medical treatment was related to higher mortality in the acute

to post-acute-COVID-19 illness phases [98]. Potential harmfulness may be occur in the abrupt cessation of the use of RAAS inhibitors [99]. A low-dose beta blocker for decreasing adrenergic activity and heart rate management and anti-arrhythmic drugs (such as amiodarone) are recommended with attention in post-acute-COVID-19-illness patients with postural orthostatic tachycardia syndrome [100] and with pulmonary fibrotic changes following COVID-19 illness [101], respectively.

Hematologic Post-Acute-COVID-19-Illness Sequelae

A 2.5 % cumulative incidence of thrombosis, including ischemic stroke, intracardiac thrombus, segmental pulmonary embolism, and thrombosed arteriovenous fistula at 30 days (median duration of 23 days post-discharge) and a 3.7 % cumulative incidence of bleeding, mostly associated with mechanical falls at 30 days after hospital discharge were reported in 163 post-acute-COVID-19-illness patients from the US without post-hospital-discharge thromboprophylaxis [102]. Several previous retrospective studies in the UK revealed similar rates of venous thromboembolism (VTE) [103, 104]. A previous prospective study in Belgium in 102 post-acute-COVID-19-illness patients at 6 weeks post-hospital-discharge follow-up by assessing D-dimer levels and venous ultrasonography revealed that only one asymptomatic VTE event occurred among 8 % of subjects who received post-hospital-discharge [105]. Hypercoagulable state and hyperinflammation were consistent in COVID-19-related coagulopathy [106, 107], contributing to the disproportionately high rates of 20 %-30 % of thromboembolic events rather than bleeding events in acute COVID-19 phase [108]. The severity and duration of a hyperinflammatory state with unknown persistence are probably associated with the risk of thromboembolic events in the post-acute-COVID-19-illness phase [62]. Release of pro-inflammatory cytokines [109], disruption of normal coagulation pathway [110], complement activation [111-113], neutrophil extracellular traps [112, 114, 115], endothelial injury [82, 116-118], platelet-leukocyte interactions and platelet activation [119-121], and hypoxia [122] are the proposed mechanisms of the thrombo-inflammation. These mechanisms are similar to the pathophysiology that are present in thrombotic microangiopathy syndromes [123]. CORE-19, CISCO-19, and CORONA-VTE are the larger ongoing studies that will assist in establishing thromboembolic complications in

the post-acute-COVID-19-illness phase [124, 125]. Due to lacking the need to frequently monitor the therapeutic levels and the lower risk of drug-drug interactions, low-molecular-weight heparin and direct oral anticoagulants are preferred anticoagulation drugs over vitamin K antagonists [126, 127]. Similar to provoked VTE, for patients with imaging-confirmed VTE, at least 3 months of therapeutic anticoagulation is recommended [128, 129]. In addition to comorbidities, such as immobility and cancer, elevation of D-dimer levels (higher than two times of the upper limit of the normal value) may be benefit to risk-stratify cases at the highest risk of post-acute-COVID-19-illness thrombosis [124, 126, 127 130]. Aspirin, an alternative antiplatelet agent for COVID-19 or post-acute-COVID-19-illness thromboprophylaxis has not yet been defined and is presently studied in cases managed as outpatients [109]. In hospital-discharge-COVID-19 patients with outpatient management, extended post-hospital discharge, up to 6 weeks and prolonged primary thromboprophylaxis, up to 45 days may provide a more favorable risk-benefit ratio in COVID-19 with an increase in thrombotic events during the acute COVID-19 phase, and this is currently being studied [131, 132]. In addition to post-acute-COVID-19-illness primary thromboprophylaxis, when appropriate, ambulation and physical activity should be recommended to all patients [109].

Neuropsychiatric Post-Acute-COVID-19-Illness Sequelae

Direct viral invasion, neurodegeneration, microvascular thrombosis, neuroinflammation, and severe systemic inflammation can be the causes of post-acute-COVID-19-illness neuropathology [133-136], supported by brain parenchyma and vessel changes of possibly driven inflammation in neurons, supportive cells, and brain vasculature in COVID-19 autopsy series [137, 138]. A role in persistent brain effects of SARS-CoV-2 (COVID-19) may be played by an accumulation of memory T cells, a biomarker of immunosenescence in tissue injury and aging, accompanying with the decreased ability to respond to new antigens that are demonstrated in chronic low-level brain inflammation [139]. Cognitive-behavioral changes directly associated with the levels of immune activation [140]. Passive diffusion and axonal transport via the olfactory complex, viral invasion in the extracellular spaces of olfactory epithelium [141], and dysfunctional lymphatic drainage from

circumventricular organs [142]. Elevated peripheral blood levels of neurofilament light chain, a biomarker of brain injury [143], with a more sustained increase in severe infections [144] has been identified in post-acute-COVID-19-illness phase. PTSD or deconditioning may be mechanisms that are hypothesized in critically ill COVID-19 patients with post-acute-COVID-19-illness brain fog [145], whereas dysautonomia may be the cause of post-acute-COVID-19-illness brain fog in previously mild-COVID-19 patients [146, 147]. Approximately, 20 %-40 % of patients with previously critical-COVID-19 illness demonstrated long-term cognitive impairment [148]. Non-restorative sleep, depressive symptoms, diffuse myalgia, post-viral syndrome of chronic malaise [149, 150], late-onset headaches ascribed to high cytokine levels, and migraine-like headaches [151, 152] (frequently refractory to traditional analgesics [153]) have been reported in post-COVID-19 survivors. Around 38 % of post-acute-COVID-19-illness patients had ongoing headaches after 6 weeks [154]. At up to 6 months follow-up, approximately, 10 % of post-acute-COVID-19-illness survivors may persist loss of smell and taste [73, 74, 76, 155]. Ischemic or hemorrhagic stroke [156], hypoxic-anoxic brain damage, posterior reversible encephalopathy syndrome [157], and acute disseminated myelitis [158, 159], may contribute to required-extensive-rehabilitation permanent or lingering neurological deficits.

Endocrinological Post-Acute-COVID-19-Illness Sequelae

Endocrinological sequelae in post-acute-COVID-19 phase may be caused by iatrogenic complications, immunological and inflammatory damage, and direct SARS-CoV-2 (COVID-19) invasion, including apparently pre-existing diabetes mellitus during acute COVID-19 phase and can be long-term treated with antidiabetic agents other than insulin, although initially related to diabetic ketoacidosis (DKA) [160]. Primary deficit in insulin production may be mediated by several factors, such as infection stress response accompanying peripheral insulin resistance or inflammation [160]. Thus, reversion of COVID-19-related diabetes, nor that its outcomes difference in COVID-19 long haulers is not confirmed [161]. Lasting damage of the pancreatic β cells is still not confirmed although demonstrated ACE 2 and TMPRSS2, involving in SARS-CoV-2 (COVID-19)-cell-entry expression in pancreatic β cells [162]. Interruption of anabolic or antiresorptive

agents, vitamin D insufficiency, exposure to corticosteroids, immobilization, and bone demineralization associated with systemic inflammation are also the COVID-19 risk factors [161]. Patients with newly diagnosed diabetes mellitus in the absence of traditional risk factors for type 2 diabetes should be performed serologic testing for type 1 diabetes-related autoantibodies repeated post-prandial C-peptide measurements at the follow-up, while patients with such risk factors can be similarly treatable to ketosis-prone type 2 diabetes [163]. New-onset Graves' disease should be excluded before treating post-acute-COVID-19-illness patients with incident hyperthyroidism with corticosteroids due to SARS-CoV-2 (COVID-19)-associated destructive thyroiditis [164].

Gastrointestinal and Hepatobiliary Post-Acute-COVID-19-Illness Sequelae

Faecalibaterium prausnitzii, a butyrate-producing has been inversely related to the disease severity of COVID-19 [165, 166]. Gut microbiome alteration, depletion of beneficial commensals, and enrichment of opportunistic infectious organisms has been influenced by SARS-CoV-2 (C)OVID-19) [165, 167] same as the alteration of the gut-lung axis (course of respiratory infections) that previously recognized in influenza and other respiratory infections [168]. Viral RNA in acute COVID-19-illness phase can be detectable for a mean duration of 28 days following the onset of SARS-CoV-2 (COVID-19) infection and symptoms and can persist for a mean duration of 11 days following the negative PCR results in the respiratory specimens [169-172]. Currently, long-term sequelae of the gastrointestinal system, such as post-infectious irritable bowel syndrome and dyspepsia are ongoing investigated (US patent, NCT04691895) [62].

Renal Post-Acute-COVID-19-Illness Sequelae

Renal biopsies and autopsies in COVID-19-associated nephropathy (COVAN) patients revealed characteristically collapsing variant of focal segmental glomerulosclerosis, accompanying involution of the glomerular tuft and acute tubular injury in response to the activation of the chemokine and interferon [173-176], in addition to renal microcirculation thrombi [177]. In acute-kidney-injury-susceptible COVID-19 patients, SARS-CoV-2 acts as a second hit that is similar to HIV and other virus [175]. In

hospitalized-COVID-19 patients and mechanical-ventilation-required-critically-ill-COVID-19 patients, severe acute kidney injury (AKI) requiring renal replacement therapy (RRT) occurs in approximately 5 % and 20 %-31 %, respectively [178-181]. Short-term follow-up in several previous early studies in RRT-required patients demonstrated that around 27 %-64 % of them were dialysis independent by 28 days or ICU discharge [180, 182]. At 6 months after acute-COVID-19 phase in the post-acute-COVID-19 Chinese study, around 35 % of patients revealed reduction of the estimated glomerular filtration rate (eGFR; < 90 ml/min per 1.73 m^2) and 13 % of them developed new-onset decrease of eGFR after revealed normal eGFR during acute COVID-19 phase [76]. Approximately, 84 % of the COVID-19 survivors demonstrated renal recovery and a survival probability of 0.46 at 60 day following the acute COVID-19 phase [181]. AKI survivor clinics will provide the benefits to COVID-19 survivors with persistent impaired renal function [183, 184].

Dermatological Post-Acute-COVID-19-Illness Sequelae

A previous study of 716 COVID-19 patients revealed that 64 % and 15 % of them demonstrated dermatological features after or concurrent to other acute COVID-19 symptoms, respectively [185]. In adult COVID-19 patients, the average latency from the time of upper respiratory symptoms to dermatological manifestations was 7.9 days [186]. At 6-month-follow-up in the post-COVID-19 Chinese study, only 3 % of patients were identified a skin rash [76], whereas hair loss was the predominant dermatological feature, approximately 20 % of the patients [73, 76, 187]. *Telogen effluvium* resulting from SARS-CoV-2 (COVID-19) or a stress response can be the causes of hair loss [76]. Dermatological sequelae may be from the significant role of the potential immune or inflammatory mechanisms of COVID-19 [188]. The skin rash manifestations include urticarial rash (treated with low-dose systemic corticosteroids combined with non-sedating antihistamines), purpuric " vasculitic " patter (treated with topical corticosteroids for mild cases; systemic corticosteroids for severe cases), livedo reticularis/racemose-like pattern (wait and see), chilblain-like acral pattern (wait and see), papulovesicular exanthem 9wait and see), confluent erythematous/maculopapular/morbilliform rash (treated with topical corticosteroids for mils cases; systemic corticosteroids for severe cases) [185, Figure 26, 27], in addition to erythema

multiforme-like eruption [189], pityriasis rosea-like rash [190], multi-system inflammatory syndrome in children [191], anagen effluvium [192], and a pseudoherpetic variant of Grover disease [193].

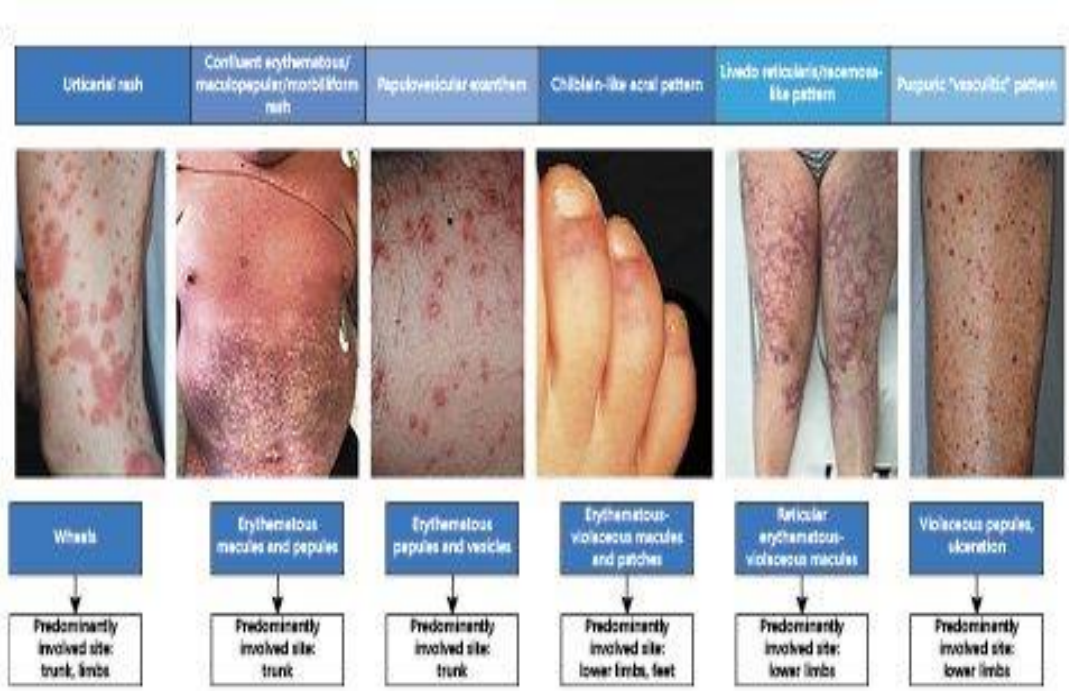

Figure 26 : Demonstrating various dermatological manifestations in acute COVID-19 and post-acute-COVID-19 patients

(Source : Genovese G, Moltrasio C, Berti E, Valerio-Marzano A. Skin manifestations associated with COVID-19 : current knowledge and future perspectives. Dermatology 2021; 237 : 1-12. Published Online : November 24, 2020. DOI : 10.1159/000512932)

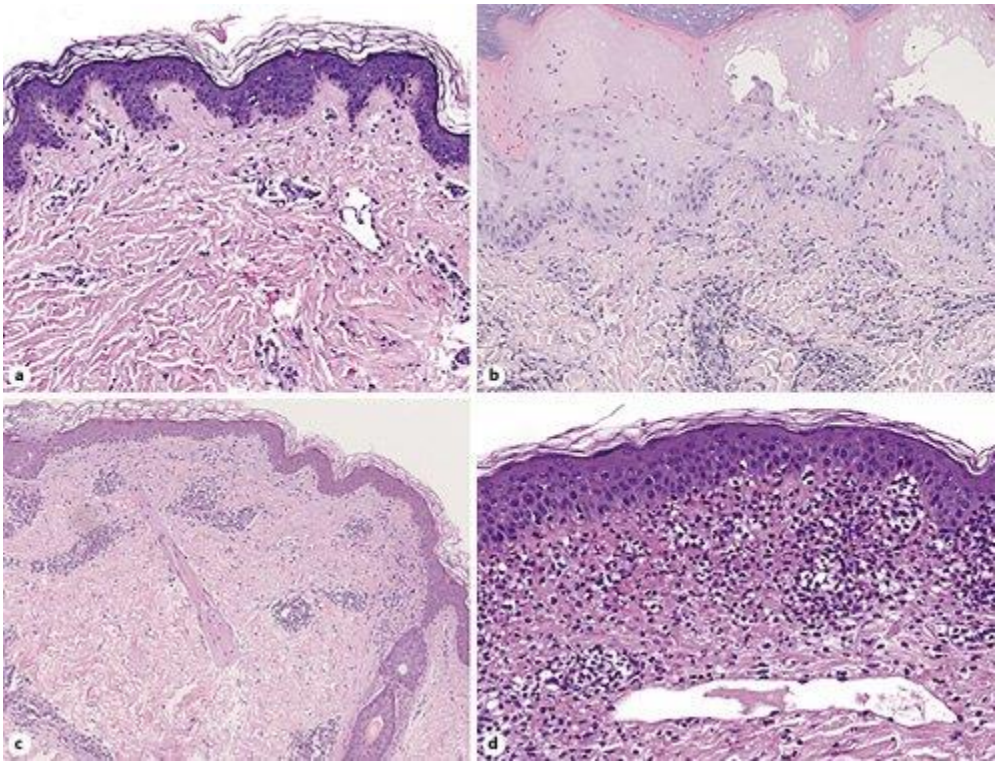

Figure 27 : Demonstrating histopathological features of the main cutaneous patterns associated with COVID-19. a Urticarial rash. b Confluent erythematous maculopapular/morbilliform rash. c Chilblain-like acral lesions. d Purpuric "vasculitic" pattern.

(Source : Genovese G, Moltrasio C, Berti E, Valerio-Marzano A. Skin manifestations associated with COVID-19 : current knowledge and future perspectives. Dermatology 2021; 237 : 1-12. Published Online : November 24, 2020. DOI : 10.1159/000512932)

Ophthalmological Post-Acute-COVID-19-Illness Sequelae

Ophthalmic-related COVID-19 illness can be presented in acute COVID-19 illness phase or post-acute-COVID-19-illness phase [194]. A previous study on ocular findings in 64 COVID-19 survivors (128 eyes, 7-mild-to-moderate, 33-severe, 24-critical disease) were evaluated 82 +/- 36.4 days after the onset of COVID-19 symptoms [195]. Approximately, 15.6 % of them demonstrated diabetic retinopathy, and two patients revealed discrete white-yellowish dots in the posterior pole with hyperreflective changes at ellipsoid layers, outer segment, and retinal pigment epithelium level [195, Figure 28]. Approximately, 10.9 % of

them had dry eye disease [195]. In critical group, the mean+/-standard deviation of intraocular pressure was 14.16 +/- 1.88 mmHg, whereas the severe group revealed 12.51 +/- 2.40 mmHg, both in left eyes ($p = 0.038$) and right eyes ($p = 0.02$) [195]. There was no sign of uveitis. The median interquatile range of the visual acuity and distant best-corrected visual acuity were 0.1 (0-0.2) and 0 (0.0.1), respectively [195]. The SARS-CoV-2 (COVID-19) RNA has been isolated from ocular tissues [194]. The COVID-19 manifestations of the eyelids, ocular surface and anterior segment of the eyes include follicular conjunctivitis (7.7 %-8.6 % of incidence) [194, 196-199, Figure 29], viral keratoconjunctivitis, hemorrhagic and pseudomembranous conjunctivitis, childhood conjunctivitis, episcleritis, dryness (6.9 %-37 % of incidence) [196-199], eye pain (10.3 %-31.2 % of incidence) [196-199], eye discharge (6.9 %-29.6 % of incidence) [196-199], eye redness (10.8 %-24.1 % of incidence) [196-199], eye tearing (9.7 %-22.2 % of incidence) [196-199], foreign body sensation in the eyes (6.0 %-18.5 % of incidence) [: 196-199], photophobia (2.6 %-16.1 % of incidence) [: 196-199], eye itchiness (9.6 %-15.7 % of incidence) [196-199], blurred vision (4.8 %-12.8 % of incidence) [196-199], burning sensation of the eyes (8.4 % of incidence) [196-199], eyelid margin hyperemia (34.5 % of incidence) [196-199], crusted eyelashes (24.1 % of incidence) [196-199], Meibomian orifices abnormality (20.7 % of incidence) [196-199], eye chemosis (3.4 % of incidence) [196-199], and episcleritis (2.2 % of incidence) [196-199]. COVID-19 manifestations of the posterior segment of the eyes include central retinal vein occlusion [194, Figure 30], central retinal artery occlusion [194, Figure 31], acute macular neuroretinopathy and paracentral acute middle maculopathy [194, Figure 32]. COVID-19 manifestations of the retina include vitritis and outer retinal abnormalities and acute retinal necrosis [194]. COVID-19 manifestation of the uvea includes serpiginous choroiditis [194]. COVID-19 manifestations of neuro-ophthalmic lesions include papillophlebitis, optic neuritis [194, Figure 33], Adie's tonic pupil, Miller-Fisher syndrome and cranial nerve palsy, cerebrovascular accident with vision loss, and neurogenic ptosis [194]. COVID-19 manifestations of the orbits include dacryoadenitis, retino-orbital pain, orbital cellulitis and sinusitis, orbital mucormycosis, and orbital histiocytic lesion [194]. Further investigating of COVID-19-neurological-mechanism of invasion should be performed [200].

Conclusion

Spike protein of SARS-CoV-2 (COVID-19) is the major protein that involves the pathogenesis of COVID-19, by capturing with the ACE-2 receptor of the host cells. Lungs and respiratory tract are the major organs involved by the SARS-CoV-2 (COVID-19), in addition to cardiovascular system, blood coagulation system, hemopoietic tissues, kidneys, liver and gastrointestinal tract, reproductive organs, central, peripheral and autonomic nervous system, neuropsychiatric system, endocrinological system, skin, opthalmological organs, etc. Respiratory tract is the most common route of SARS-CoV-2 (C)OVID-19) transmission.

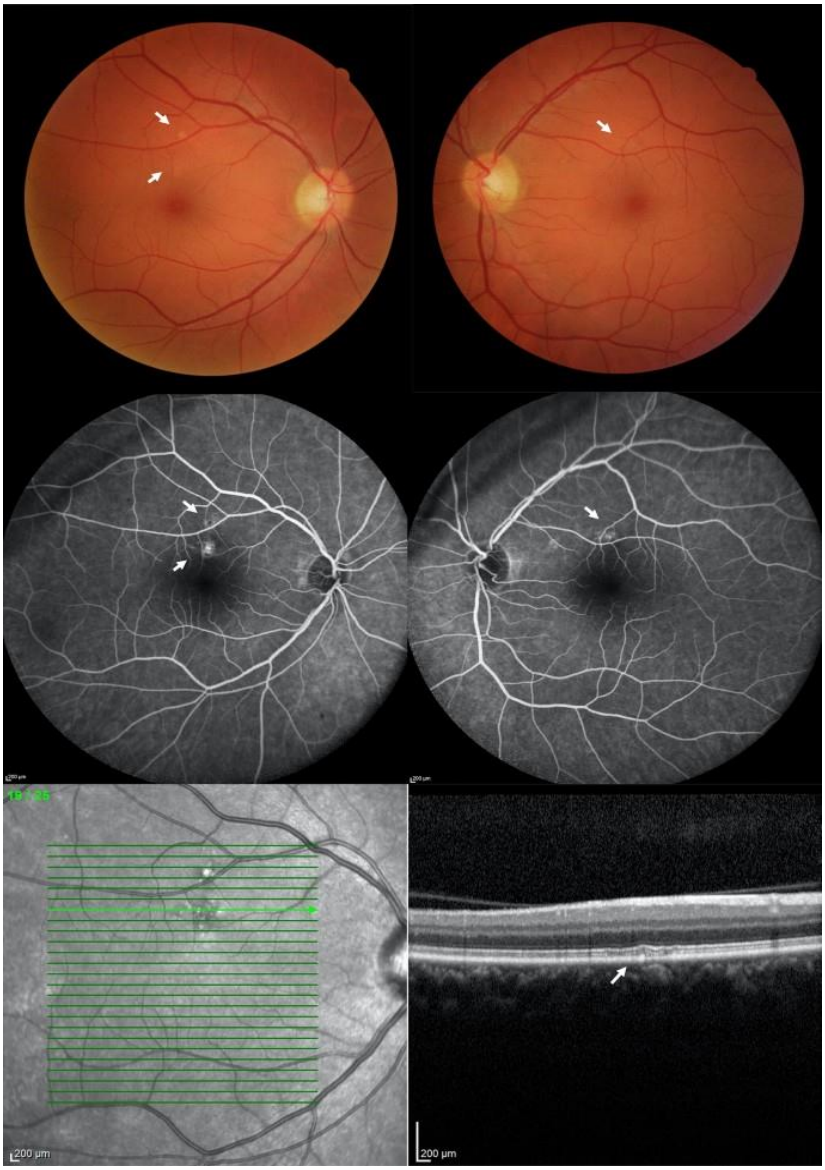

Figure 28 : Demonstrating ocular fundus multimodal imaging of a 48-year-old man (critical case) 128 days after first symptoms of COVID-19. Color fundus pictures of both eyes showing white-yellowish dots (arrows). Midphase fluorescein angiography pictures of the RE (middle left) and LE (middle right) showing transmission hyperfluorescence in the retina lesions 195 days after first symptoms of COVID-19. Optical coherence tomography (OCT) of the right eye shows hyporreflectivity in the retinal pigment epithelium and ellipsoid layers, and discontinuation of photoreceptors' outer segments (arrow).

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

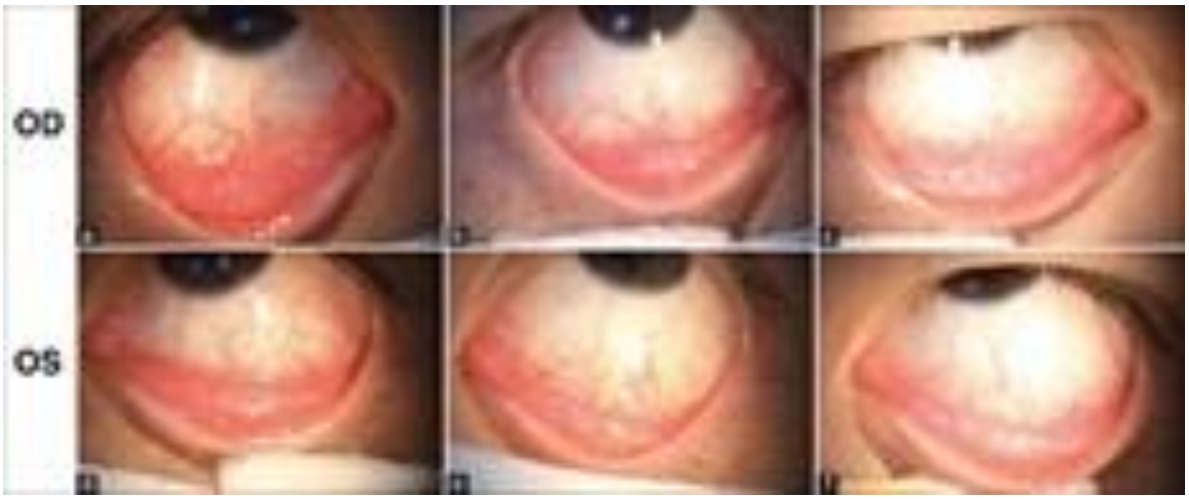

Figure 29 : Demonstrating follicular conjunctivitis following COVID-19: A 30-year-old man developed bilateral follicular conjunctivitis 13 days after mild COVID-19 infection. Slit lamp examinations showed evidence of acute viral conjunctivitis. (a and d)The examination on illness day 13 showed moderate conjunctival injection and inferior palpebral conjunctival follicles. (b and e) Examinations on illness day 17 and (c and f) illness on day 19 demonstrated that treatment with ribavirin eye-drops gradually improved the patient's symptoms. (Reproduced with permission from Chen L, Liu M, Zhang Z, Qiao K, Huang T, Chen M, Xin N, Huang Z, Liu L, Zhang G, Wang J. Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. Br J Ophthalmol. 2020;104:748-51)

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

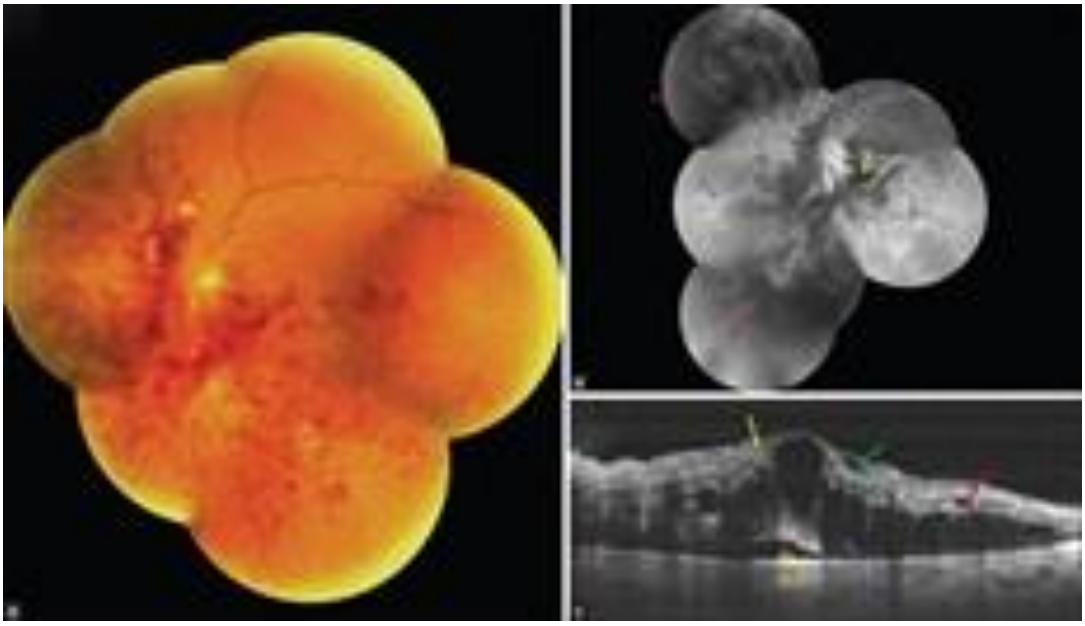

Figure 30 : Vasculitic retinal vein occlusion as a manifestation of COVID-19: A 52-year-old patient presented with the diminution of vision in the left eye 10 days after he tested positive for SARS-CoV-2. (a) Fundus photograph demonstrating inferior hemiretinal vein occlusion with superonasal branch retinal vein occlusion. (b) Fundus fluorescein angiogram showing the presence of dilated tortuous vein in inferior and superonasal quadrants with late phases showing staining and leakage from the vessel walls (Blue arrow), multiple areas of hypofluorescence corresponding to retinal hemorrhages clinically, suggestive of blocked fluorescence (Yellow arrow) and areas of hypofluorescence suggestive of capillary nonperfusion (Blue arrow) in involved quadrants. The macular region and optic disc also showed hyperfluorescence in late phases suggestive of leakage. (c) Spectral domain optical coherence tomography illustrating the presence of serous macular detachment (Orange arrow), cystoid macular edema, cysts located in outer nuclear layer (Blue arrow), inner nuclear layer (Red arrow) and ganglion cell layer (Green arrow) and disorganization of retinal inner layers (Yellow arrow) (Reproduced with permission from Sheth JU, Narayanan R, Goyal J, Goyal V. Retinal vein occlusion in COVID-19: A novel entity. Ind J Ophthalmol 2020;68:2291-3).

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

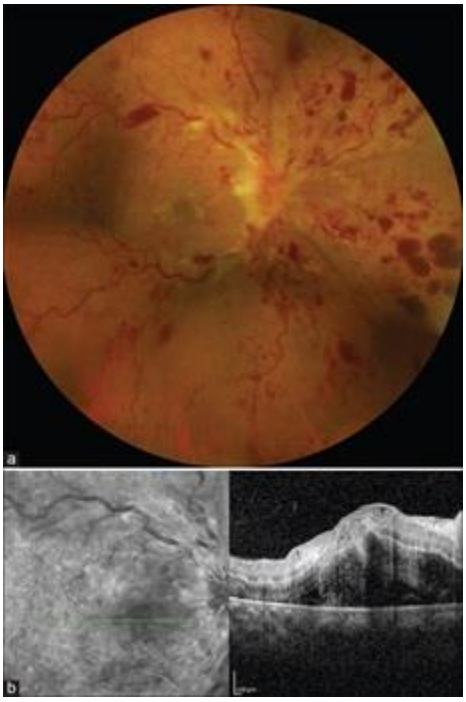

Figure 31 : Combined central retinal artery and vein occlusion following COVID-19: A 32-year-old lady, known hypertensive with past history of COVID-19, presented with sudden onset, painless diminution of vision in the right eye. Examination showed right eye visual acuity of finger counting at 50cm and RAPD. (a) Fundus photograph showing retinal hemorrhages in all quadrants, dilated tortuous vessels and optic disc edema. (b) SD-OCT showing neurosensory detachment with intraretinal fluid and hyper-reflectivity of inner retinal layers. (Contributed by Rajashree Salvi and Shrinivas Joshi, M M Joshi Eye Institute, Hubli, India)

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

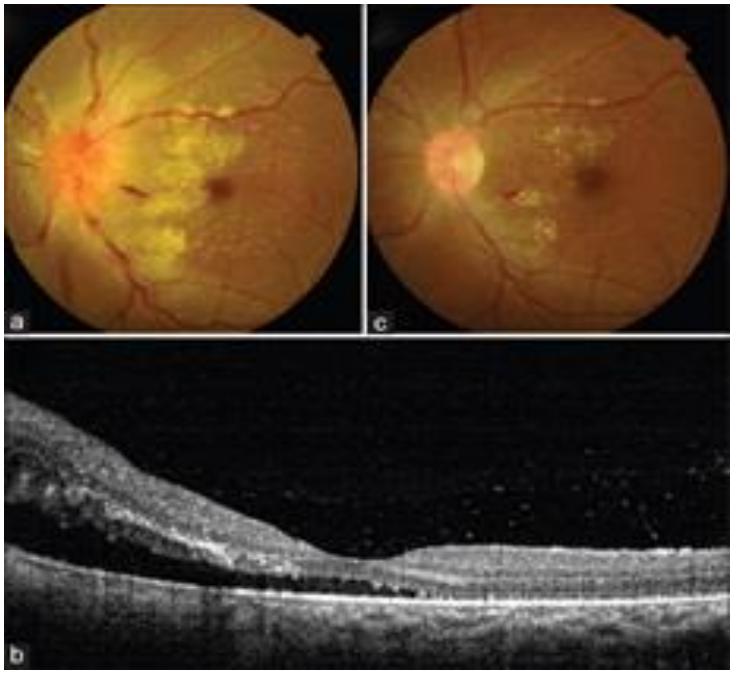

Figure 32 : Demonstrating acute macular neuroretinopathy following COVID-19: A 28-year-old woman presented with diminution of vision in left eye seven days after recovering from a mild COVID-19 infection. Vision was 6/36 in left eye with RAPD. (a) Fundus examination showed vitritis 1+, blurred disc margins, hard exudates over macular area and internal limiting membrane folds. (b) SD-OCT showed neurosensory detachment and outer retinal hyperreflective foci. She was managed with tapering doses of oral steroids and topical steroid and homatropine. (c) After 1 month, vision had recovered, disc edema had subsided with resolving exudates. (Contributed by Debdulal Chakraborty, Vitreoretina Services, Disha Eye Hospitals, Kolkata, India

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

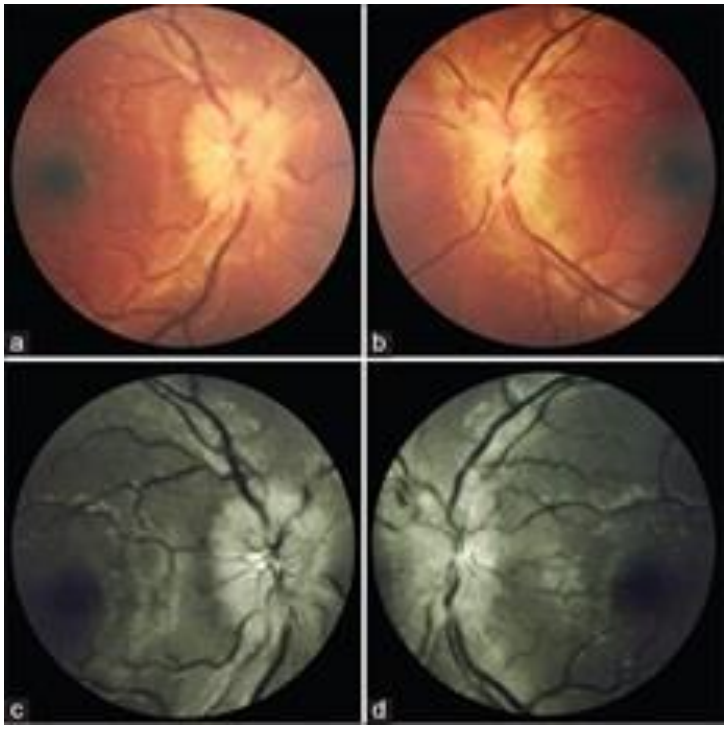

Figure 33 : Bilateral atypical optic neuritis after a mild COVID-19 infection: A 34-year-old female presented with complaints of gradual blurring of vision in right eye with pain on eye movements since 1 week and history of a similar episode 3 weeks back in left eye, which improved spontaneously. She had recovered from a mild COVID-19 infection 2 weeks before the onset of ocular symptoms. On examination, her uncorrected visual acuity was 20/200, N24 in right eye, and 20/25, N6 in left eye. Pupil examination revealed a Grade III RAPD in right eye. (a and b) Fundus photograph and (c and d) red-free imaging showing bilateral disc oedema, more in the right eye. (Contributed by Rachna Vinaya Kumar, Paediatric ophthalmology, Neuro ophthalmology and Adult Strabismus Services, Apollo Eye Institute, Apollo Hospitals, Hyderabad, India)

(Source : Sen M, Honavar SG, Sharma N, Sachdev MS. COVID-19 and eye : a review of ophthalmic manifestations of COVID-19. Indian Journal of Ophthalmology 2021; 69 : 488-509)

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CHAPTER 5

Immuno-Epidemiological Parameters and Immunogenetics of COVID-19

Abbreviations :

ACE: Angiotensin-Converting Enzyme

ARDS : Acute Respiratory Distress Syndrome

ASC : Apoptosis-Associated Speck-Like Protein with a Caspase-Recruitment Domain

ATP : Adenosine Triphosphate

BALF : Bronchoalveolar Lavage Fluid

CoV: Coronavirus

COVID-19: Coronavirus Disease 2019

2019-nCoV: 2019-Novel Coronavirus

DAMPS : Damage-Associated Molecular Patterns

FCN : Ficolin

GISAID : Global Initiative on Sharing All Influenza Data

HLA : Human Leukocyte Antigen

ICU : Intensive Care Unit

IFITM1 : Interferon-Induced Transmembrane Protein-1

IFN : Interferon

IL : Interleukin

ISGs : Interferon-Stimulated Genes

MERS: Middle-East Respiratory Syndrome

MRC: British Medical Research Centre

NK : Natural Killer

NKR : Natural Killer Receptor

OR : Odds Ratio

PD-1 : Programmed Death Ligand-1

PAMPS : Pathogen-Associated Molecular Patterns

PBMCs : Peripheral Blood Monocytes

pDC : Plasmacytoid Dendritic Cells

RBD : Receptor Binding Domain

RNA: Ribonucleic Acid

SARS: Severe Acute Respiratory Syndrome

Tim-3 : T-cell Immunoglobulin and Mucin-3

TNF : Tumor Necrosis Factor

WHO: World Health Organization

Introduction

As of February 2020, 526 laboratory-confirmed cases have been reported across 25 other countries [1]. Around 15% of reported cases are severe, 3% are critical, and 82% are mild symptoms[2]. There is no expected cross protection by a common human coronavirus infection [3]. COVID-19 complications target especially the elderly [3]. Despite a low risk of COVID-19 complications, a mild clinical presentations of the disease allow a larger chain of transmission through various populations [3].

Detection of viral ribonucleic acid (RNA) in the plasma is approximately 15% [4] and viral detection in stool of the most severe cases reveals possibility of fecal transmission [5]. Isolation of COVID-19 in human saliva [6], nasopharynx and lower respiratory tract has been reported [7]. Incomplete understanding of the pathogenesis of COVID-19 infection is due to lacking lung biopsies or post-mortem sample investigations [3]. Being capable of producing T regulatory cell cytokine (interleukin (IL)-10 and T helper 17 (TH17) cell cytokines (IL-6 and IL-23) of the innate immune cells, but they are unable induce T helper 1 (TH1) cell cytokines (type I interferons (IFNs), IFN-γ, and IL-12) [8]. The diversity of the T-cell receptor (TCR) repertoire decreased with age, whereas a new lineage of oligoclonal T cells that express natural killer (NK)-related receptors (NKR) is formed [9]. The immunological features in neonates and children are more prominent than in adults, in addition to the experimental

evidence. Overwhelming inflammatory reaction or pro-inflammatory response to the SARS-CoV-2 infection in aging population is a logical possibility [10]. The mean age of COVID-19 patients is 52.4 years [11], whereas children and adolescents are the least likely group to be infected with the COVID-19, occurring in only 2 % of cases 19 years of age or younger. When the younger-age group get sick, they will get a mild form of COVID-19 without serious complications, with an average death rate of 0.2 % [12]. Male cases are more than two-thirds of the reported COVID-19 cases and are more than 1.5 times more likely to die from COVID-19 (death rate : 2.8 % vs. 1.7 %) [13]. This sexual distinction of the anti-viral immunity is because of the genomic factors, hormonal factors, and environmental factors. The unanswered questions include the pathophysiology of pulmonary clinical infection, influenza and other viral co-infection, and the rate of bacterial complications [3].

Human Leukocyte Antigen Map of COVID-19

Genotye of human leukocyte antigen (HLA) is a significant factor in activation and differential regulation of T cells, including disease duration and transmission [14]. A recent study on HLA binding affinity of 48,395 unique peptides from the SARs-CoV-2 (COVID-19) proteome for assessing the cross-protective-immunity potential conferred by previous exposures to common human coronaviruses (i.e. 229E, NL63, OC43, and HKU1) demonstrated that alleles HLA-A*02 : 02, HLA-B*15 : 03, and HLA-C*12 : 03 were the top presenters of conserved peptides. Fifty-six different HLA alleles, especially HLA-B*46 : 01 revealed no impressive binding affinity (<500 nm) to any peptides of conserved SARS-CoV-2 (C)OVID-19), indicating a concomitant lack of potential for cross-protective immunity from other human coronaviruses. Considering the entire proteome of SARS-CoV-2 (COVID-19), HLA-A and HLA-C alleles expressed the relative largest and smallest capacity to present SARS-CoV-2 (COVID-19) antigens, respectively. No appreciable global correlation between conservation of the SARS-CoV-2 (COVID-19) proteome and its predicted MHC binding affinity, indicating a lack of selective pressure for the capacity to present coronavirus epitopes ($p = 0.27$, Fisher's exact test). peptide presentation appears to be independent of estimated time of peptide production during SARS-CoV-2 (COVID-19)

life cycle, with indistinguishable early and late SARS-CoV-2 (COVID-19) peptide presentation [14].

Positive T cell assays in all retrieved SARS-CoV-2 (COVID-19) proteins in a recent study on 19 epitopes by HLA binding assays demonstrated that five characteristic alleles, HLA-A*02 : 01, HLA-B*40 : 01, HLA-DRA*01 : 01, HLA-DRB1*07 : 01, and HLA-DRB1*04 : 01 were positive. The Chinese population covering alleles was 32.36 % and 59.76 % globally [15]. Increased severity towards the closely related SARS-CoV diseases in persons with HLA-B*46 : 01 was demonstrated in several previous studies [16]. HLA-haplotype dirrences may influence the immune response to SARS-CoV-2 (COVID-19) infection and some associations between HLA haplotypes and increased disease severity could be possible. Thus, HLA genotyping may help in identifying persons at risk. To predict susceptibility to disease severity and assisting in future vaccination strategy plan, COVID-19 testing along with HLA genotyping is highly recommended.

Proximal Origin of COVID-19

The two distinct characteristics of the COVID-19 genome are: 1) the highly variable spike (S) protein of COVID-19 has a polybasic (furin) cleavage site at the S1 and S2 boundary via the insertion of twelve nucleotides, and 2) based on structural modelling and early biochemical experiments, COVID-19 is optimized for binding the human ACE2 receptor. This event contributes to the acquisition of the three predicted O-linked glycans around the polybasic cleavage site [17].

The most variable part of the virus genome, the six residuals in the receptor binding domain (RBD) of the spike protein of SARS-CoV and SARS-related coronaviruses appear to be critical for binding to the human ACE2 receptor and the determining host range [18]. Five of these six residuals are mutated in COVID-19 compared to its most closely related virus, RaTG13 sampled from a *Rhinolophus affinis* bat to which it is approximately 96% identical [19]. COVID-19 binds with high affinity to human ACE2 was demonstrated in recent binding studies [20]. COVID-19 is not the genetic engineering product appears to be the result of selection on human or human-like ACE2 [17]. COVID-19 are derived from a common ancestor due to all COVID-19 sequenced genomes have the well adapted RBD and the polybasic cleavage site. Malayan

pangolins (*Manis javanica*) illegally imported into Guangdong province, China contain a coronavirus (CoV) that is similar to COVID-19 [21, 22]. Nevertheless, no sufficient evidences demonstrated that pangolin CoV has been identified to be sufficiently similar to COVID-19 across its entire genome for supporting direct human infection [17].

Phylogenetic Analysis of COVID-19

A previous study reveal that the COVID-19 was introduced into the human population in Wuhan, China in early December 2019 and has an epidemic doubling time of about 7 days. The study demonstrated substantial heterogeneity in the number of secondary infections caused by each COVID-19-infected case that indicated by a high level of over-dispersion in the reproduction number [23]. By phylogenetic analysis, there was a common ancestor to SARS-CoV-2 (COVID-19), human SARS-CoV, and the bat SARS-CoV converge. The envelope (E), membrane (M), nucleocapsid (N), and spike (S) structural viral proteins implied a high degree of shared identity in range of 97.7-100 % between the SARS-CoV-2 (COVID-19) and bat coronaviruses that supports the animal descend of SARS-CoV-2 (VID-19) [24].

Thailand's Experiences : First Wave Outbreak and Pattern of SARS-CoV-2 (COVID-19)

The SARS-CoV-2 (COVID-19) first wave of outbreak initiated in early March 2020 and peaked between March 22, 2020 and March 29, 2020. There was a overall huge decline in the cases since March 20, 2020 [25]. A recent study on SARS-CoV-2 (COVID-19) isolates from Thailand that investigated the genotypes of the SARS-CoV-2 (COVID-19) from February 2020 to April 2020 with basing on the genome sequences available in GISAID, nucleotide variation in the four regions of the SARS-CoV-2 (COVID-19) genome for conducting viral tracking and identifying sites of origin of outbreaks in Thailand revealed five main clusters, defined as L, S, G, V, and O types, based on genetic variations and amino acid changes by selection of the sequences of partial ORF1ab (nucleotides 8,596-8,927 and 13,259-16,269), S (nucleotides 21,320-25,541), ORF3a to E (nucleotides 25,902-26,549), and ORF9b to ORF10 (nucleotides 28,101-29,682) (Figure 34, 35) [25].

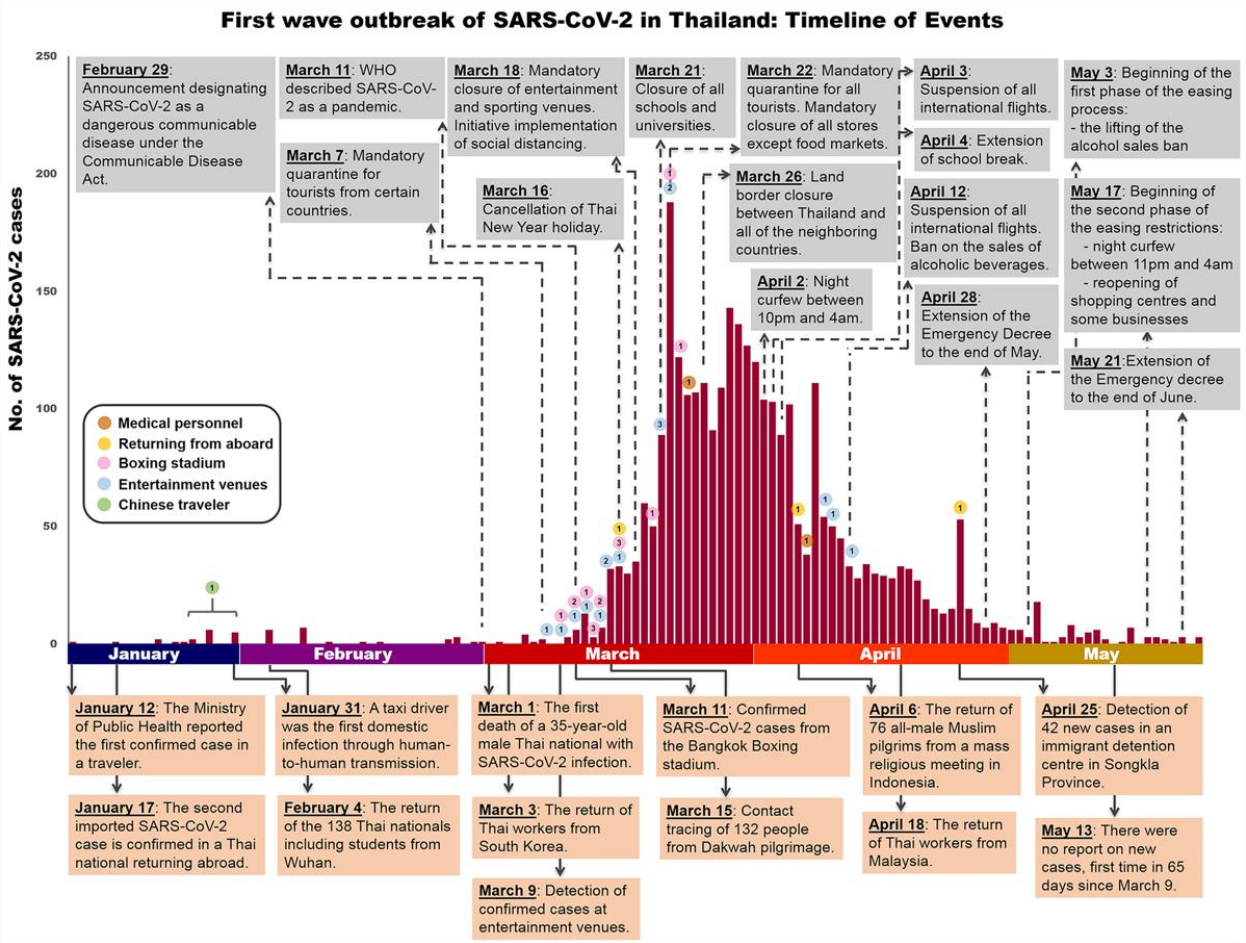
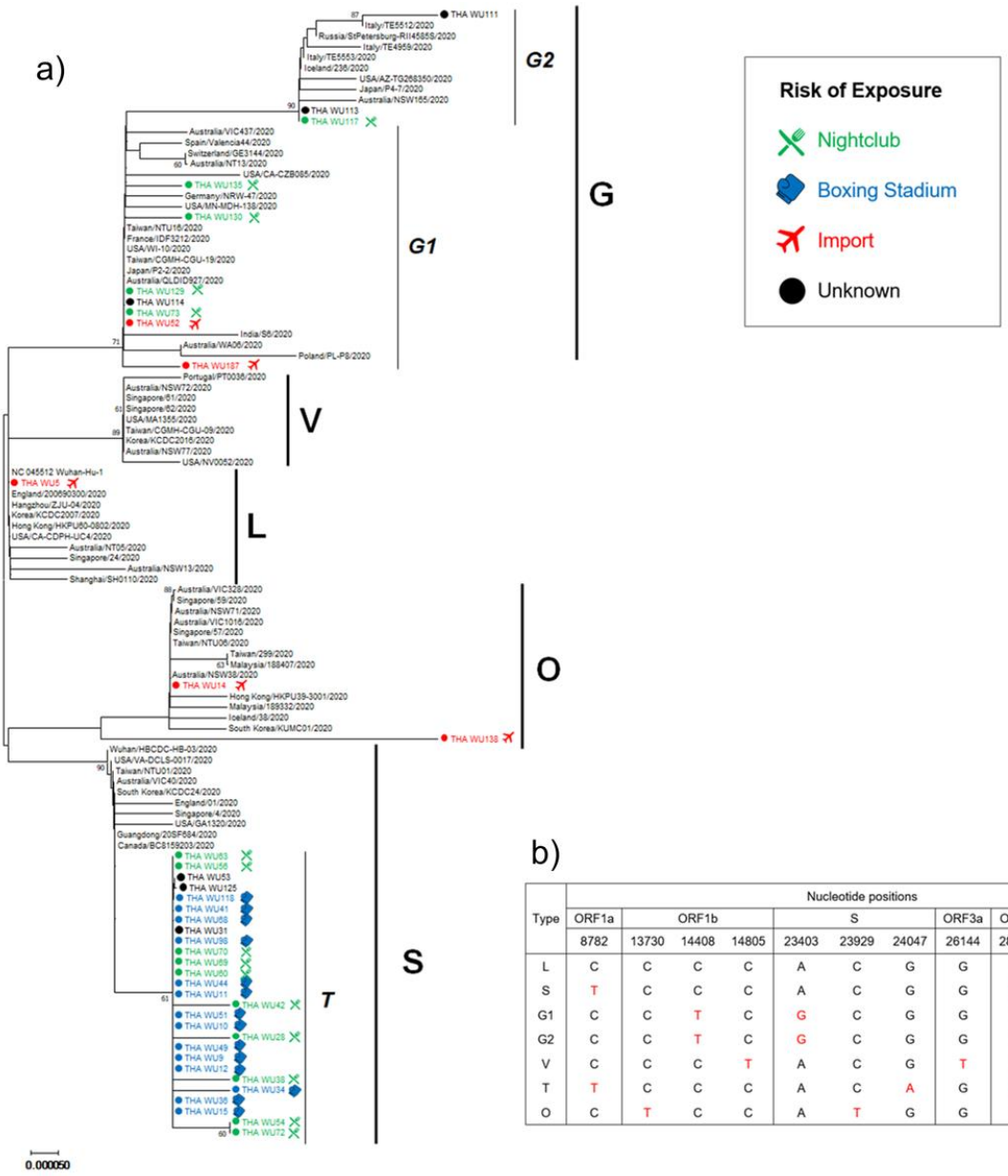


Figure 34 : The first wave of SARS-CoV-2 (COVID-19) outbreak in Thailand : Timeline of events and the number of samples.

(Source : Puenpa J, Suwannakarn K, Chansaenroj J, Nilyanimit P, Yorsaeng R, Auphimai C, et al. Molecular epidemiology of the first wave of severe acute respiratory syndrome coronavirus 2 infection in Thailand in 2020. Research Square 2020. Preprint. 16 pages. DOI : https://doi.org/10.21203/rs.3rs-34516/v1 Available at : www.researchsquare.com>article (accessed on August 10, 2020))



b)

Type	Nucleotide positions										
	ORF1a	ORF1b			S			ORF3a	ORF8	ORF9b	ORF14
	8782	13730	14408	14805	23403	23929	24047	26144	28144	28311	2881-3
L	C	C	C	C	A	C	G	G	T	C	GGG
S	T	C	C	C	A	C	G	G	C	C	GGG
G1	C	C	T	C	G	C	G	G	T	C	GGG
G2	C	C	T	C	G	C	G	G	T	C	AAC
V	C	C	C	T	A	C	G	T	T	C	GGG
T	T	C	C	C	A	C	A	G	C	C	GGG
O	C	T	C	C	A	T	G	G	T	T	GGG

Figure 35 : Type of viral variations with exposure history; a) Phylogenetic tree of concatenated sequences, including partial ORF1ab (nucleotide position 8,596-8,927 and 13,259-16,269), S (nucleotide position 21,320-25,541), ORF3a to E (nucleotide position 25,902-26,549), and ORF9b to ORF10 (nucleotide position 28,101-29,682) The blanket demonstrated the five main types. b) The pattern of nucleotide substitution change and type of SARS-CoV-2 (COVID-19).

(Source : Puenpa J, Suwannakarn K, Chansaenroj J, Nilyanimit P, Yorsaeng R, Auphimai C, et al. Molecular epidemiology of the first wave of severe acute respiratory syndrome coronavirus 2 infection in Thailand in 2020. Research Square 2020. Preprint. 16 pages.

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During the early period of the SARS-CoV-2 (C)OVID-19) outbreak in China, genetic variations of L and S types were identified and confirmed SARS-CoV-2 (COVID-19)-imported cases from China during January 2020 and February 2020 [25]. One specimen collected in January 2020 in this study from Thailand revealed L type that closely related to the SARS-CoV-2 (COVID-19) strain circulating in China at that time [25]. All of the specimens that related to the first SARS-CoV-2 (COVID-19) outbreak in a boxing stadium and entertainment venues in Bangkok, Thailand during March 2020 demonstrated type L (branching off from type S-originating from China) that has not been identified in other countries [25]. This finding suggested local transmission in Bangkok, Thailand [25]. After the first outbreak in Thailand in March 2020, type T was detected less frequently and limiting the imported cases. This may be due to Thai government's intervention policies, such as mandatory closure of entertainment and sporting venues, the land border closure, and suspension of all international flights (partial city-lockdown, state quarantine, local quarantine, and self-quarantine) [25]. Nevertheless, several cases who included multiple genetic variants of SARS-CoV-2 (COVID-19), such as types G1, G2, and O recently returned from outside of Thailand had positive SARS-CoV-2 (COVID-19) testing [25]. In March 2020, these cases were identified as having type O and classified as imported cases as well as returned travelers and the religious-pilgrimage group from the southern region of Thailand [26]. In May 2020, a new cohort of imported cases of a group of migrant workers in the southern region of Thailand were identified as type G2 [27]. This may be multiple introductions of SARS-CoV-2 (COVID-19) and an outbreak in the southern region of Thailand [25]. Most study cases from Thailand demonstrated mild febrile illness without sequelae, but with multiple origins of SARS-CoV-2 (COVID-19) that are similar to the identified pattern in Shanghai, China [28]. Understanding SARS-CoV-2 (COVID-19) genetic variations will assist more accurate future trend prediction and the more informed Thai government's development of intervention policies.

ABO Blood Type Associated COVID-19

A recent study conducted by Zhao *et al* on the correlation compared between the ABO blood group among 1,775 COVID-19-infected patients and 3,694 normal individuals from Wuhan city, China and 23,386 normal persons from Shenzhen city, China revealed that blood group A had a specific significant higher risk for COVID-19 compared to non-blood group A groups (albeit modest effect size, odd ratio (OR) = 1.20, $p = 0.02$), while blood group O had a significant lower risk for COVID-19 compared to non-O blood groups (OR = 0.67, $p < 0.001$) [29]. Tanigawa *et al* compared blood group O frequencies between the Shenzhen controls and the UK biobank Chinese group and demonstrated that the study results were consistent with the study results conducted by Zhao *et al*. Nevertheless, Tanigawa *et al* found that the frequency of blood group O was different between the Wuhan controls and the UK Biobank Chinese group ($p = 0.00121$) indicating careful consideration of inferences regarding ABO blood group differences [30].

COVID-19 Induced Inflammation

In COVID-19, acute respiratory distress syndrome (ARDS) and multiple organ failure related to cytokine storm is the primary cause [31, Figure 7], a phenomenon of the rapid production of large amounts of cytokines in response to infection [32]. A cytokine storm and tissue damage is observed via initial delay in innate immune cell secretion of cytokine and chemokine with subsequent surge in pro-inflammatory cytokines and chemokines (CCL2, CCL5, IFNs, IL-1β, IL-6, IL-8, MCP-1) by the activated macrophages and other recruited lymphocytes is observed in COVID-19, contributing to the recruitment and activation of adaptive immune cells (neutrophils, NK cells, and T cells) along with further production of pro-inflammatory cytokines [32, Figure 36, 37]. In COVID-19 patients, elevated serum cytokines are G-CSF, GM-CSF, IFN-γ, IL-1β, IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, IP10, MCP-1, MIP-1A, MIP-1B, and TNF-α [33]. Particularly, macrophage-secreting IL-1, IL-6, and Tumor Necrosis Factor (TNF)-α are significantly higher in severe patients [33]. IL-6, a multifunctional cytokine and primary role in cytokine storm involves the formation of follicular helper T cells, generation of plasma cells, differentiation of Th 17 cell subsets, and inhibition of IFN-α that suppress CD8+ cytotoxic T cells [34]. As demonstrated by *PD-1* and *Tim-3* expressions, T cell-mediated immune response might be suppressed during cytokine storm by IL-6-inducing T cell exhaustion [35]. Role of IL-6

in COVID-19 disease severity has been demonstrated by IL-6-positive association with disease severity and increasing IL-6 levels [35-42]. A recent report from Germany revealed that COVID-19 cases had 22-fold increased risk of respiratory failure with median time to mechanical ventilation of 1.5 days with increasing IL-6 level of at least 80 pg/ml [43] and higher serum IL-6 levels were also reported even 24 hours before death [44, 45]. Thus, IL-6 could be used for early detection the risk of respiratory failure in COVID-19 patients.

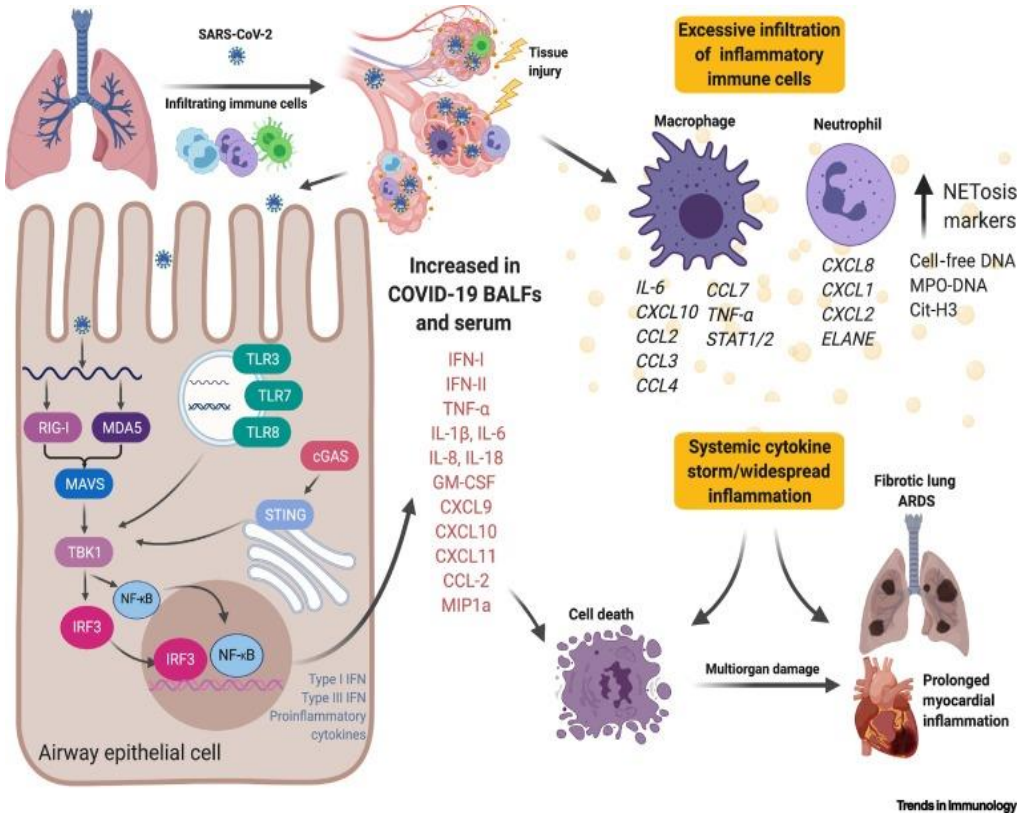

Figure 36 : A brief overview of lung pathology in COVID-19 patients. Following inhalation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into the respiratory tract, the virus traverses deep into the lower lung, where it infects a range of cells, including alveolar airway epithelial cells, vascular endothelial cells, and alveolar macrophages. Upon entry, SARS-CoV-2 is likely detected by cytosolic innate immune sensors, as well as endosomal toll-like receptors (TLRs) that signal downstream to produce type-I/III interferons (IFNs) and proinflammatory mediators. The high concentration of inflammatory cytokines/chemokines amplifies the destructive tissue damage via endothelial dysfunction and vasodilation, allowing the recruitment of immune cells, in this case, macrophages and neutrophils. Vascular leakage and compromised barrier function promote endotheliitis and lung edema, limiting gas exchange that then facilitates a hypoxic environment, leading to

respiratory/organ failure. The inflammatory milieu induces endothelial cells to upregulate leukocyte adhesion molecules, thereby promoting the accumulation of immune cells that may also contribute to the rapid progression of respiratory failure. Hyperinflammation in the lung further induces transcriptional changes in macrophages and neutrophils that perpetuate tissue damage that ultimately leads to irreversible lung damage. Recent evidence suggests that systemic inflammation induces long-term sequela in heart tissues. Abbreviations: BALF, bronchoalveolar lavage fluid; IRF3, interferon regulatory factor 3; NF-κB, nuclear factor-κB; RIG-I, retinoic acid-inducible gene I; STAT1/2, signal transducer and activator of transcription 1/2; STING, Stimulator of interferon genes. Figure generated with BioRender.

(Source : Harrison AC, Lin T, Wang P. Mechanism of SARS-CoV-2 transmission and pathogenesis. Trends in Immunology 2020; 41 (12) : 1100-1115. December 1, 2020. Published online : October 14, 2020. DOI : https://doi.org/10.1016/j.it.2020.10.004 Available at : www.cell>trends>immunology>fulltext (accessed on January 5, 2021)).

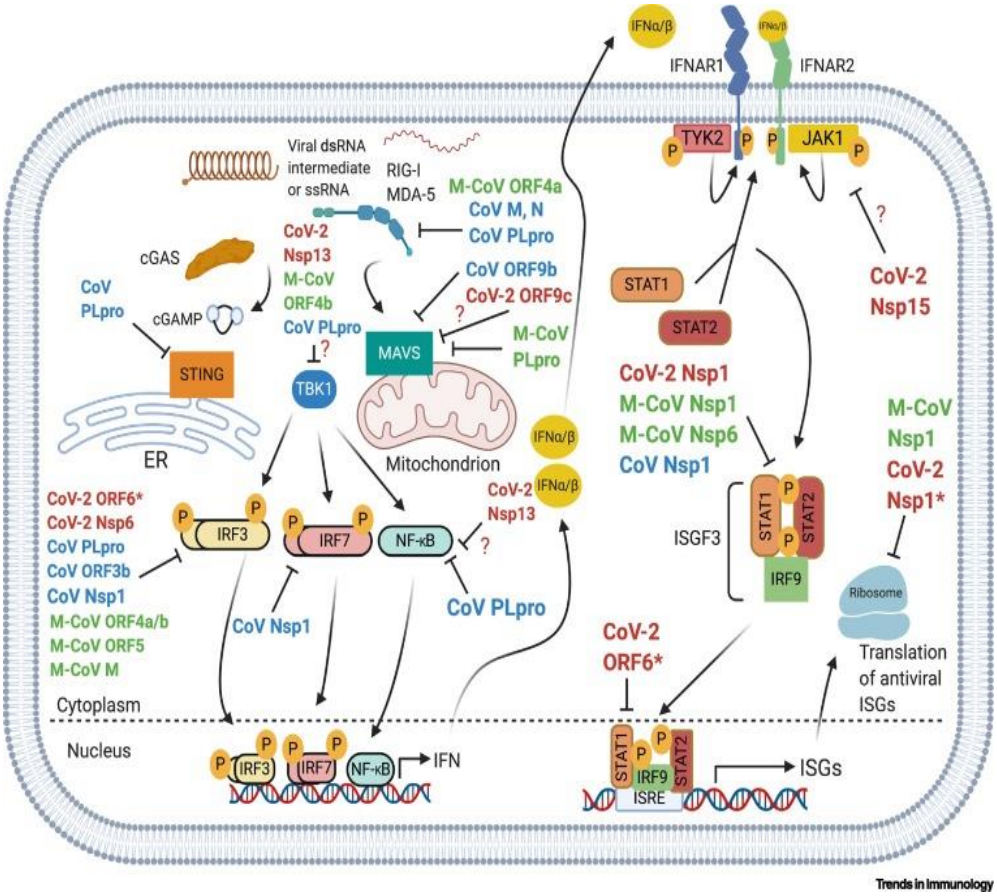

Figure 37 : Evasion of the pattern recognition receptor-type 1 interferon (PRR-IFN-1) pathway by coronavirus. A simplified schematic of the canonical IFN response after sensing

RNA viruses. Viral nucleic acid is first recognized by PRRs (e.g., retinoic acid-inducible gene I; RIG-I) that perpetuate signal transduction through an adaptor complex on the mitochondrial (mitochondrial antiviral-signaling protein; MAVS) or endoplasmic reticulum (Stimulator of interferon genes; STING) membrane surface. Here, the PRR–adaptor interactions recruit kinases that converge into a large complex, leading to phosphorylation of interferon regulatory factor 3/7 (IRF3/7) and nuclear factor-κB (NF-κB), transcription factors that enter the nucleus and transcribe IFN genes. Type-I and type-III IFNs then signal in an autocrine or paracrine manner through the Janus kinase 1 (JAK1)/signal transducer and activator of transcription 1 and 2 (STAT1/2) pathway, culminating in antiviral IFN-stimulated gene (ISG) transcription. Listed here are SARS-CoV (CoV), SARS-CoV-2 (CoV-2), and MERS-CoV (M-CoV) IFN-I antagonists, which render these viruses resistant to IFN responses. IFN-III is also implicated in exhibiting potent antiviral effects in lung/intestinal tissues, but the underlying evasion strategies of this pathway for these viruses are currently unknown. SARS-CoV proteins are highlighted in blue, while functions of SARS-CoV-2 and MERS-CoV proteins are highlighted in red and green, respectively. ? denotes that a SARS-CoV-2 protein bound a member of that signaling pathway, but further work is necessary to confirm its immunological mechanism. SARS-CoV-2 proteins with * denotes functional conservation with SARS-CoV. Figure generated with BioRender.

(Source : Harrison AC, Lin T, Wang P. Mechanism of SARS-CoV-2 transmission and pathogenesis. Trends in Immunology 2020; 41 (12) : 1100-1115. December 1, 2020. Published online : October 14, 2020. DOI : https://doi.org/10.1016/j.it.2020.10.004 Available at : www.cell>trends>immunology>fulltext (accessed on January 5, 2021)).

Apoptosis of aged T cells that express high TNFR1 (receptor of TNF-α) may be contributed by TNF-α, a pro-inflammatory or a pro-apoptotic cytokine [46]. Significant higher levels of TNF-α among aging patients (> 60 years) with decreased T cell counts, increased levels of *PD1* and *Tim-3* (T cell exhaustion markers), and role as a negative regulator of T cell proliferation or survival of TNF-α may be demonstrated. This study results indicate the role of TNF-α as a negative regulator of T cell proliferation or survival [31]. Nevertheless, a negative correlation of TNF-α with T cell count were reported by few previous studies [33, 47, 48], and no difference in TNF-α levels in patients with COVID-19 was reported [49, 50]. IL-1β and its family (IL-18, IL-33) are significant players in ARDS to increase the recruitment of immune cells subsequent production of cytokines [51]. To develop Th17 cells and assist in Th17 mediate immune response and increased

vascular permeability, IL-1β and TNF-α are required [51]. Increasing IL-17 and GM-CSF, cytokines of the Th17 pathway in patients with severe COVID-19 [52] have investigators urgently study the role of Th17 [53]. Th17 cell-increased expression in the peripheral blood of patients with COVID-19 indicates a player in the COVID-19 cytokine storm as reported in the patients with MERS and SARS [54]. Some Th17 pathway-specific cytokines, such as GM-CSF, IL-1β, IL-17, and TNF-α are elevated in severe COVID-19 patients [52]. Elevated count of Th17 cells, activated CD4+, and CD8+ T cells [55] was demonstrated in severe COVID-19 patients, whereas a decrease in Th17 subset suggested by low IL-17 secretion urges the need to investigate the Th17 specific response [56]. An increased IFN-γ, IL-1β, IP-10, and MCP-1 serum concentrations contribute to the facilitation of Th1 cell response and further cytokine storm aggravation like the occurrence in MERS-CoV and SARS-CoV [57, 58]. A previous study on three patients with COVID-19 demonstrated that only before deterioration of the respiratory function that IL-1 expressed significant expression changes, whereas only after occurrence of respiratory symptoms, the other pro-inflammatory cytokines were induced. These IL-1 expression changes indicate the association between the IL-1 pathway and the initial progression of COVID-19-associated pulmonary immunopathology and the IL-1 receptor signaling in respiratory epithelium-inflammatory damage [59]. Identification of increased anti-inflammatory cytokines of Th2 cells (IL-4 and IL-10) in SARS-CoV-2 (COVID-19) patients was reported without clarification [60].

In severely ill-COVID-19 patients (approximately 15 % of patients with COVID-19), there were significant elevation of the levels of serum G-CSF, IL-2, IL-7, IL-10, IL-17, MCP-1, MIP-1A, and TNF-α, indicated diversity of the cytokine profile in the two groups and cytokine storm in disease severity and progression involvement [33]. The transition from mild to severe COVID-19 accompanied by cytokine storm. Peripheral blood monocytes (PBMCs) of 4 covid-19 patients were collected at the periods of pre-Intensive Care Unit (ICU) stay, ICU stay, and post-ICU stay, by using single-cell transcriptome sequencing demonstrated that there was a significant increase in plasmacytoid dendritic cell populations and monocytes in the ICU-stay specimens [61]. Reported signature of gene in the ICU-admitted patients' specimens demonstrated elevation of DDX58, IRF8, interferon-stimulated genes (*ISGs*) expression, such as *IFITM1*, and *TLR7* in comparison to the pre-

and post-ICU specimens, indicating a significant viral-load-regulated-type I interferon response (Type-1 IFN response) in onset of disease progression and ARDS [56, 61]. Thus, evidence of dampened or delayed type response of interferon in the initial phase of COVID-19 is demonstrated with subsequent increasing active viral replication that is a portion of pathogenesis of SARS-CoV [62, 63].

Dysregulation of Immune Cell Subset

COVID-19-viral-replication-induced pyroptosis contributes to release of Damage-Associated Molecular Pattern (DAMPS), IL-1β, and Pathogen-Associated Molecular Pattern (PAMPS), for examples, adenosine triphosphate (ATP), apoptosis-associated speck-like protein with a caspase-recruitment domain (ASC) oligomers, and viral nucleic acid that induce pro-inflammatory cytokine and chemokine production, for examples, IL-6, IL-10, MCP-1, MIP-1α, MIP-1β from alveolar macrophages and adjacent alveolar epithelial cells [33, 58]. Therefore, the resultant microenvironment attracts both pro-inflammatory cascade sets and innate and adaptive immunity cells [33, 58]. Pulmonary macrophages play the primary role in both effective host immunity against COVID-19 that contribute to cytokine storm by secretion of IP-10, MCP-1, MCP-1α, etc. and uncontrolled immunology of COVID-19 [64]. Dysregulations of major host immune include recruitment of pro-inflammatory cells, for examples, monocytes and neutrophils, viral load-induced hyperinflammation, and dampened type-1 IFN response was demonstrated in a recent study [63]. For controlling viral replication and induction of effectively adaptive response, type-1 IFN response is critical.

Single cell RNA-sequencing-based characterization of bronchoalveolar lavage fluid (BALF) from three patients with severely ill, three patients with mild COVID-19 and eight healthy subjects demonstrated that a monocyte-derived *FCN-1*$^{+}$ macrophages were predominant [65]. Elevation of the level of $CD14^{+}$ and $CD16^{+}$ monocyte subset was revealed in COVID-19 patients, particularly in ICU-admitted patients [66]. In the critical patients, reduced levels of IFN-α and IFN-β accompanying with high levels of IL-6 and TNF-α with significant impaired type-1 IFN response were demonstrated in 50 varying-severity COVID-19 patients involving profiling of cytokine levels, whole blood transcriptome, and immune cells in a previous study. Additionally, this study demonstrated

a significant 6 *ISGs* downregulation that specify type-1 IFN response in patients with severe COVID-19 and reduction of pDC population [67].

Rapid Increase of a SAR-CoV-2 (COVID-19) Variant with Multiple Spike Protein Mutations

The new variant (B.1.1.7.) of SARS-CoV-2 (COVID-19), picked up by the COVID-19 Genomics UK (COG-UK) consortium [68] that defined by multiple spike protein mutations (D1118H, S982A, T716I, P681H, D614G, A570D, N501Y, deletion 144, deletion 69-70) as well as mutations in other genomic regions was present in the South-East England during the late December 2020 [69]. With an estimated potential to increase the reproductive number (R) by at least 0.4 with an estimated increased transmissibility of up to 70 %, this COVID-19 variant is significantly more transmissible than previously circulating COVID-19 variants [69]. Currently, there is no indication of increased infection severity observed associated with this COVID-19 variant, but the challenging assessment is that the majority of reported patients were under 60 years old, who are less likely to develop severe symptoms [70]. None of the previously identified SARS-CoV-2 variant have been demonstrated to cause increased infection severity. In Singapore, a clade 19B variant with lower infection severity was identified in the Spring and then disappeared [71]. This new COVID-19 variant have been reported in Denmark, the Netherland [69], including a family of 4 Britons who arrived from Kent, South-East England and were reported in Thailand on January 3, 2021 [72], and were in quarantine at a private hospital in Bangkok, Thailand with no risk of spread [72, 73].

Conclusion

SARS-CoV-2 (COVID-19) infection has evolved to become a pandemic, in contrast to infections with SARS and MERS, whereas SARS-CoV-2 (COVID-19) has demonstrated having the similarities of genome sequence, receptor affinity, pathogenesis, and disease manifestation. It is necessary to assess the COVID-19 disease severity before rapid

disease progression. Nevertheless, our knowledge of SARS or MERS has not enough to restrain current COVID-19 pandemic.

SARS-CoV-2 (COVID-19) involves both humoral (interleukins, cytokines, etc.) and cellular immune (T-cell lymphocytes, etc.) responses, whereas IL-6 plays the major role in developing cytokine storm. The majority of its variants occur via the S protein mutations.

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CHAPTER 6

Laboratory Testing Methodology

Abbreviations:

Ag : Antigen

CI : Confidential Interval

COVID-19 : Coronavirus Disease 2019

Ct : Cycle threshold

EUA : Emergency Use Authorization

EUL : Emergency Use Listing

FDA : Food and Drug Administration

IgA : Immunoglobulin A

IgG : Immunoglobulin

IgM : Immunoglobulin

MERS-CoV : Middle-East-Respiratory-Syndrome Coronavirus

N : Nucleocapsid

NAATs : Nucleic Acid Amplification Tests

qRT-PCR : quantitative Reverse-Transcriptase-Polymerase-Chain Reaction

RATs : Rapid Antigen Tests

RBD : Receptor-Binding Domain

RNA : Ribonucleic Acid

RT-PCR : Reverse-Transcriptase-Polymerase-Chain Reaction

S : Spike protein

SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus type 2

RT-PCR : Reverse Transcriptase-Polymerase Chain Reaction

USA : United States of America

WHO : World Health Organization

Introduction

Rapid collection of the following specimens should be considered: when possible, specimens from both lower and upper respiratory tracts should be collected. For lower respiratory tract: expectorated sputum, endobronchial aspirate, and bronchoalveolar lavage. For upper respiratory tract: nasopharyngeal aspirate or nasal wash, oropharyngeal swab, and nasopharyngeal swab. The additional specimens for later testing are: when serological testing is available (serum: acute and convalescent specimens-possibly 2-4 weeks after acute phase). The other additional specimens for later testing are blood, urine, and feces [1].

Currently, the specific test recommended by the World Health Organization (WHO) for the diagnosis and confirmation of COVID-19 is real-time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) supported by the study on detection of COVID-19 infection that conducted by Corman *et al* [2]. A single positive test should be confirmed by a second RT-PCR assay targeting a different COVID-19 gene. A single negative COVID-19 test, particularly if specimens obtained from upper respiratory tract or a positive test result for another respiratory pathogen, indicates that the result does not exclude COVID-19 infection. If there is a strong suspicion for COVID-19 infection, another specimen should be tested with primary and secondary RT-PCR assays. When possible, sequence information should be generated from positive specimens [1].

COVID-19 Reinfection and Laboratory Testing

Generally, the immunity development that responds to a pathogenic microorganism occurs around 1-2 weeks with a non-specific innate response followed by body producing antibodies (humoral responses), immunoglobulins in combination with production of T-cells or cellular immunity. The virus in the body will be eliminated by this combined adaptive response. The definitive viral elimination by their protective role from viral reinfection is yet unidentified [3]. Around eighty known distinct genotypical variants of SARS-CoV-2 (COVID-19) have been identified [4]. Several previous studies demonstrated that SARs-CoV-2 (COVID-19) can persistently present in the feces of the patients, whereas no oral-fecal transmission markers were identified [4]. A previous study in Beijing, China revealed that the virus can persist in the sputum

for 39 days after becoming pharyngeal-swab negative [4]. IgM-negative, IgG-positive antibody response, and non-detectable viral ribonucleic acid (RNA) after discharging and consequent positive-SARS-CoV-2 (COVID-19)-RNA test, remained negative IgM, and positive IgG antibody tests was demonstrated in three readmitted asymptomatic-COVID-19 patients in Tongji Hospital, China [4]. Due to the highest titers of SARS-CoV-2 RNA reaching within 7-10 days of clinical symptom onset and declining thereafter, the upper respiratory tract (posterior nasopharyngeal tonsil region) swabs should be performed [4]. At least one episode of swab during a 14-day post-hospital discharge period of quarantine for patients with COVID-19 was recommended [4].

The highest positive rate of COVID-19 ranked from bronchoalveolar lavage fluid (BALF) (93 %), sputum (72 %), nasal swab (63 %), fibrobronchoscopic brush biopsy (46 %), pharyngeal swab (32 %), feces (29 %), and blood (1 %) was demonstrated in a previous study conducted on 205 patients with 1,070 samples [4]. Another previous study revealed that the highest levels detected SARS_CoV-2 (COVID-19) RNA by the reverse transcriptase-polymerase chain reaction (RT-PCR) reveals as the following : 1) Nasal swab (Mid-turbinate swab, anterior nares swab) and oropharyngeal swab, and 2) Rectal swab in first and second week of the clinical symptom onset, respectively [4]. In comparison of the accuracies of the RT-PCR and computed tomography (CT) of the chest in COVID-19 patients by several previous studies demonstrated 59 % versus 88 %, 71 % versus 98 %, 92 % versus 44 %, and 10 % versus 96.1 %, respectively [4]. Clinicians should differentiate the COVID-19 recurrences from several secondary complications, such as persistence of traces of viral RNA (detectable in respiratory specimens up to 6 weeks after onset of clinically-cured patients' symptoms), super-infection, or pulmonary embolism [5]. Nevertheless, some patients did not develop SARS-CoV-2 (COVID-19) antibodies more than 21 days after presenting severe symptoms, reported by a previous study [5]. An inappropriate immune response can facilitate an inflammatory rebound and could establish an explanation to the clinical symptom recurrence [5]. Currently, there is no large-scale study conducted on the effectiveness of the antibodies protected against the subsequent human COVID-19 infection that will be critically supported by the World Health Organization (WHO) [3].

Antibody Responses and Viral Loads

Patients with various severities of COVID-19 (coronavirus disease-2019) demonstrates different viral shedding patterns and antibody responses [6, Figure 38]. Due to finding of IgM in tissues outside the respiratory tract in severe COVID-19 patients, detection of urinary and other body fluid antibody responses could be used as a biomarker to determine disease severity [6]. Strong cross-reactivities were detected between SARS-CoV-2 (COVID-19) and SARS-CoV, but not MERS-CoV (middle-east-respiratory-syndrome coronavirus) that is significant information for the differential diagnosis [6]. In comparison to mildly ill patients, severely ill patients have more prolonged viral shedding in various tissues and have more IgM response [6]. A recent study among 94 patients with COVID-19 from the Guangzhou Eight People's Hospital, China demonstrated that SARS-CoV-2 (severe acute respiratory sundrome-coronavirus-2) (COVID-19) viral load (VL) peaked at 0.7 days before the symptom onset, whereas SARS-CoV VL peaks on average 10 days after the onset of symptoms [7-10]. There is no different of viral load kinetics between mild and severe COVID-19 patients [10]. Similarly, a pervious study among 5,000 COVID-19 patients from Lombardy, Italy revealed no VL difference between asymptomatic carriers and symptomatic patients [11], whereas a previous study among 76 COVID-19 patients in Nangchang, China demonstrated that hospitalized severe-COVID-19 patients tend to have a high VL and a longer virus-shedding periods, in comparison to mild patients [12]. Liu *et al* demonstrated that mild COVID-19 patients had significantly lower VLs compared with severe patients [12]. Wölfel *et al* revealed that COVID-19 patients had upper respiratory VL peaks within the first week of symptoms [13]. Patients in this study continued to have active viral replication in upper respiratory tract tissue detected by PCR despite 100 % seroconversion of the patient cohort by day 14 and symptom cessation [13]. Severe COVID-19 patients had significant prolonged symptomatic duration [13]. ICU patients remained PCR positive with a prolonged symptom duration, compared with non-ICU patients [14]. Severe symptoms in COVID-19 patients are likely not associated with high viral titers [15]. Acute respiratory distress syndrome (ARDS), multiple organ failure, related-immunologic hyperactivation (high level of various cytokines, like interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, lymphocyte activation, T-helper 17 differentiation, severe lymphopenia) seems to be associated with patient deterioration [16, 17].

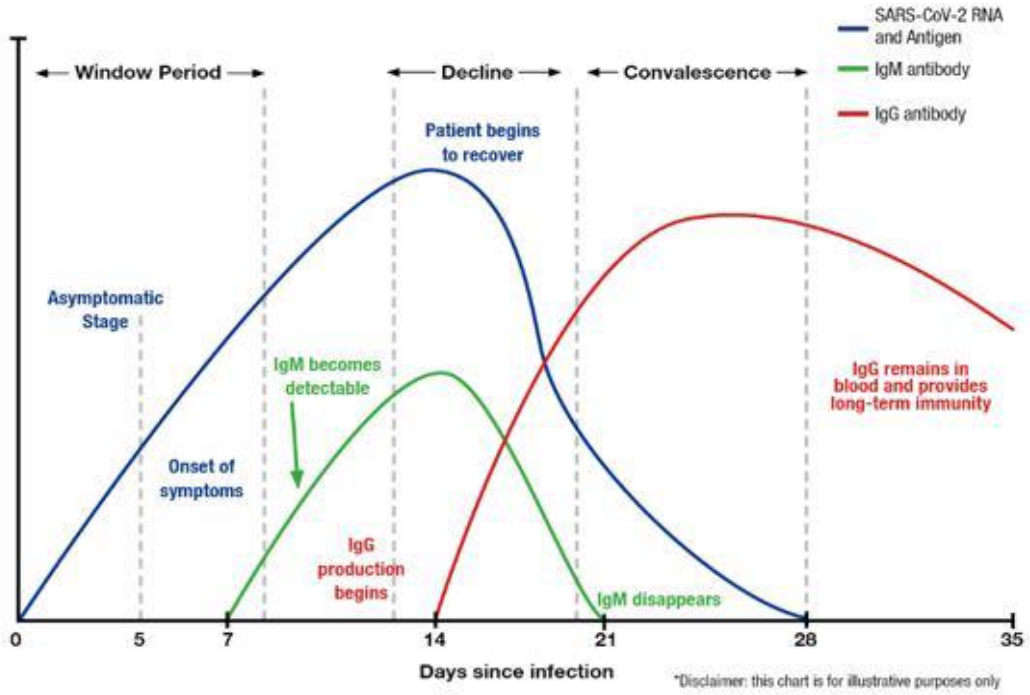

Figure 38 : Demonstrating the levels of SARS-CoV-2 (COVID-19) RNA and antigen, IgM and IgG antibodies after infection

Technology-based-polymerase-chain reaction (PCR) allows calculation of VL that is associated with transmission risk and viral disease severity [18]. Pujadas *et al* recently demonstrated that there was an independent association between high VL and the mortality of 1,145 COVID-19 patients (hazard ratio 1.07 (95 % Confidential Interval (CI) 1.03-1.11, *p* (probability) = 0.0014, Cox proportional hazards model adjusting for age, sex, asthma, atrial fibrillation, coronary artery disease, chronic kidney disease, chronic obstructive pulmonary disease, diabetes, heart failure, hypertension, stroke, and race), with a 7 % increase in hazard for each log transformed copy per millimeter (ml) [19]. By univariate survival analysis, the study demonstrated that there was a significantly statistical difference in survival probability between those with low VL (p = 0.0003) and those with high VL (greater than the overall mean $\log_{10}$ VL of 5.6 copies per ml), with a maximum follow-up of 67 days and a mean follow-up of 13 days (standard deviation (SD) 11) [19]. VL might affect isolation measures on the basis of infectivity [19]. Nevertheless, no current actual studies have evaluated the association between VL and mortality in large patient cohort [20-22]. Antibody responses against N or S protein of SARS-

CoV-2 (COVID-19) are associated with neutralizing antibody titers that may be useful for passive transfusion therapy in COVID-19 [6].

Serological Tests for COVID-19

Characteristically, after infection, antibodies are detected in the blood of individuals, particularly individuals with few or mild symptoms. In patients with varying symptoms of COVID-19 and negative results of reverse-transcriptase-polymerase-chain reaction (RT-PCR) tests, the testing has a significantly clinical role when nasopharyngeal swabs are taken more than 5 days after symptom onset [23, 24].

Immunoglobulin M (IgM) rises soonest, whereas IgA and IgG persist. IgG alone. The maximum sensitivity for IgM alone, IgA alone, and IgG alone appear during the days 15-21 after the symptom onset that are 75.4 % (64.3-83.8), 98.7 % (39.0-100), and 88.2 % (83.5-91.8), respectively [25], whereas the specificity at all times for IgM alone and IgG alone are 98.7 % (97.4-99.3) and 99.1 % (98.3-99.6), respectively [25] . The sensitivity and specificity of the antibody tests are critical due to false negative rates of RT-PCR that are between 2 % and 29 % [25]. A previous study on immunological assessment of SARS-CoV-2 (COVID-19) infections in China revealed that 81.1 % (30/37) and 62.2 % (23/37) of asymptomatic individuals tested positive for IgG and IgM, respectively and 83.8 % (31/37) and 78.4 % (29/37) of the symptomatic patients tested positive for IgG (around 3-4 weeks after COVID-19 exposure) and IgM, respectively [26]. In acute phase that the viral ribonucleic acid (RNA) can be identified in a respiratory sample, IgG levels in symptomatic patients were significantly statistical higher than those in the asymptomatic individuals [26].

The pre-test probability of infection has much influence on the interpretation of the serological test results not only influenced by the accuracy of the test itself. When screening suggestive symptomatic individuals, the pre-test probability will be much higher, compared to asymptomatic persons [27].

COVID-19 screening is essentially amounted by non-specific indication and population-based policies on testing. In consequences of testing with uncareful consideration, this risks the potential harm. In more affluent populations, the rates of testing will be higher [28] that limits the

estimates of seroprevalence. The Royal College of Pathologists (RCPath) developed seven principles for production of a COVID-19 testing strategy. Testing being carried out for a purpose is one of these RCPath's principles [29]. Nevertheless, denial of requesting SARS-CoV-2 (COVID-19) antibody tests for reassurance should be cautioned [30, 31].

In eliminating COVID-19, a combination of B and T cell immunity is likely to involve for production of protective-immunity memory [27]. Nevertheless, currently, several longitudinal studies demonstrated waning of antibody levels [32]. With a lower antibody levels, whether the protective immunity will be sustained is questionable [27]. A recent study revealed that produced antibodies can provide long-term immunity, whereas non-neutralizing antibodies can be generated. Antibody enhancement, a phenomenon that can facilitate a more severe-secondary infection [33]. This phenomenon is not to date with SARS-CoV-2 (COVID-19),but it has been demonstrated in other coronaviruses [33].

Several immune-based assays were developed against different SARS-CoV-2 (COVID-19) viral proteins as the followings : 1) Entire Spike (S) protein, IgG antibody from patient serum can cross-react with SARS-CoV and MERS-CoV [34], 2) S1 subunit of Spike (S) protein, IgA, IgG antibodies from patient serum can cross-react with SARS-CoV only [34], 3) Receptor-binding domain (RBD), IgG antibody from patient serum can cross-react with SARS-CoV only [34], and 4) Nucleocapsid (N), IgG antibody from patient serum can cross-react with SARS-CoV only [34].

IgG antibody responses sustained for at least 34 months after outbreak in persons with laboratory-confirmed MERS-CoV infection [35], whereas IgG levels in SARS-CoV-infected individuals were sustained for more than two years [36, 37]. Neutralizing antibodies that associate with the numbers of virus-specific T cells have been detected in most COVID-19 convalescent patients [38-41]. Long *et al* demonstrated in their study that IgG antibody and neutralizing antibody levels initiate decreasing within 2-3 months after infection in the majority of persons with recovery from SARS-CoV-2 (COVID-19) infection [26]. Nevertheless, an analytical study of the dynamics of neutralizing antibody titers demonstrated reduced neutralizing antibodies around 6-7 weeks after illness onset [42].

Serological Test Interpretation

The pre-test probability of infection has much influence on the interpretation of the serological test results not only influenced by the accuracy of the test itself. When screening suggestive symptomatic individuals, the pre-test probability will be much higher, compared to asymptomatic persons [27, Figure 38 , Table 2].

Test Results			Clinical Interpretation
RT-PCR	IgM	IgG	
positive	negative	negative	May be in the window period of infection
positive	positive	negative	Early-stage of infection
positive	positive	positive	Active phase of infection
positive	negative	positive	Late phase or recurrent stage of infection
negative	positive	negative	Early stage of infection or false positive for RT-PCR
negative	negative	positive	Recovered
negative	positive	positive	Recovery phase or false positive for RT-PCR

Table 2 : Demonstrating clinical interpretation of molecular and serological tests

Serological Testing Pitfalls

COVID-19 screening is essentially amounted by non-specific indication and population-based policies on testing. In consequences of testing with uncareful consideration, this risks the potential harm. In more affluent populations, the rates of testing will be higher [28] that limits the estimates of seroprevalence. The Royal College of Pathologists (RCPath) developed seven principles for production of a COVID-19 testing strategy. Testing being carried out for a purpose is one of these RCPath's principles [29]. Denial of requesting SARS-CoV-2 (COVID-19) antibody tests for reassurance should be cautioned [30, 31].

Immunity and Antibody Tests

In eliminating COVID-19, a combination of B and T cell immunity is likely to involve for production of protective-immunity memory [27]. Nevertheless, currently, several longitudinal studies demonstrated waning of antibody levels [32]. With a lower antibody levels, whether the protective immunity will be sustained is questionable [27]. A recent study revealed that produced antibodies can provide long-term immunity, whereas non-neutralizing antibodies can be generated. Antibody enhancement, a phenomenon that can facilitate a more severe-secondary infection [33]. This phenomenon is not to date with SARS-CoV-2 (COVID-19),but it has been demonstrated in other coronaviruses [34].

Immune-based Assays developed against different SARS-CoV-2 (COVID-19) Viral Proteins

Several immune-based assays were developed against different SARS-CoV-2 (COVID-19) viral proteins as the followings : 1) Entire Spike (S) protein, IgG antibody from patient serum can cross-react with SARS-CoV and MERS-CoV [43], 2) S1 subunit of Spike (S) protein, IgA, IgG antibodies from patient serum can cross-react with SARS-CoV only [43], 3) Receptor-binding domain (RBD), IgG antibody from patient serum can cross-react with SARS-CoV only [43], and 4) Nucleocapsid (N), IgG antibody from patient serum can cross-react with SARS-CoV only [43].

Novel Diagnostic Methods For Detecting SARS-CoV-2 (COVID-19) Variants

Recently, there were variations of SARS-CoV-2 (COVID-19) developed and transmitted in the United Kingdom (UK) (201/501Y.V1/B1.1.7), South Africa (20H/501Y.V2/B.1.351), and Brazil (P.1/201/501Y.V3/B.1.1.248). The SARS-CoV-2 (COVID-19) variants in the UK can be detected by current molecular methods, such as qRT-PCR [44], but there are no data on impact on molecular assay performance for detection SARS-CoV-2 (COVID-19) variants in South Africa and Brazil. SARS-CoV-2 (COVID-19) variants may impact molecular assays that target S gene sequence of SARS-CoV-2 (COVID-19) [44]. There are no data on impact on serological antibody tests for detecting SARS-CoV-2 (C)OVID-19) variants in the UK, South Africa, and

Brazil, but there is potential for the performance of a assay detecting antibodies to viral spike protein or nucleocapsid to be affected [44]. Considering the performance of antigen-based tests (including rapid lateral flow devices), five SARS-CoV-2(COVID-19) rapid antigen tests are all able to detect the SARS-CoV-2ID-19) variants in the UK [43], but no evaluation studies are available for detecting SARS-CoV-2COVID-19) variants in South Africa and Brazil [44]. Recently, additional novel assays based on the isothermal amplification of viral nucleic acids, in combination with clustered regularly interspaced short palindromic repeat (CRISPR)-based detection methods have been developed. These methods do not require thermal cycling and are more rapid than RT-PCR and are considered as point-of-care tests for SARS-CoV-2 (COVID-19) detection [45-47].

IgG antibody responses sustained for at least 34 months after outbreak in persons with laboratory-confirmed MERS-CoV infection [48], whereas IgG levels in SARS-CoV-infected individuals were sustained for more than two years [49, 50]. Neutralizing antibodies that associate with the numbers of virus-specific T cells have been detected in most COVID-19 convalescent patients [38, 51, 52]. Long *et al* demonstrated in their study that IgG antibody and neutralizing antibody levels initiate decreasing within 2-3 months after infection in the majority of persons with recovery from SARS-CoV-2 (COVID-19) infection [15]. An analytical study of the dynamics of neutralizing antibody titers demonstrated reduced neutralizing antibodies around 6-7 weeks after illness onset [53]. Isothermal amplification is a useful alternative to thermal-cycling-based nucleic acid amplification [54], whereas RdRp/Hel assays are highly sensitive methods for detection of SARS-CoV-2 (COVID-19) [55]. Other novel diagnostic technologies for detecting SARS-CoV-2 (COVID-19) are nanotechnology-based-reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) [56] and multiplex SARS-CoV-2 antibody immunoassays [57]. Variation of the levels of SARS-CoV-2 (COVID-19) RNA and antigen, IgM and IgG antibodies after infection and clinical interpretation of the molecular and serological tests are demonstrated in Figure 1 and Table 1, respectively [58]. The conventional chest radiography provides the sensitivity approximately 60 % for initial detection of pulmonary diseases in 1,014 COVID-19 patients [59], whereas the sensitivity of the chest computed tomography (CT) for COVID-19-related pulmonary diseases was 97 % among positive RT-PCR-COVID-19 patients and 75 % of COVID-19 patients with negative RT-PCR results revealed positive chest CT scans

(308 of 413 patients) [60]. Thus, chest CT scans [61] and chest radiography [62] could be adjunctive tests, in combination with repeated RT-PCR assays and serological tests.

Serological Detection of SARS-CoV-2 (COVID-19) in Saliva

Saliva samples [63-65] and dried blood spots [66-68] have been used successfully for detecting antibodies against several infectious diseases although serum is the typical sample type. Saliva sampling allows potential self-collection and substantial scale of testing. IgG antibody titer for Hepatitis B correlates well between saliva and plasma [65]. A recent study conducted by Randal and colleagues using multiplex SARS-CoV-2 antibody immunoassay-based on Luminex technology for testing 167 saliva and 324 serum samples, including 134 and 118 negative saliva and serum samples, respectively, collected before the COVID-19 pandemic, and 33 saliva and 206 serum samples from patients with RT-PCR-confirmed SARS-CoV-2 (COVID-19) infection demonstrated that matched saliva and serum SARS-CoV-2 (COVID-19) antigen-specific IgG responses were statistically correlated [69]. The saliva anti-nucleocapsid (N) protein IgG response resulted in the highest sensitivity (100 % sensitivity at at least 10 days post-SARS-CoV-2 (COVID-19) illness onset), whereas the saliva anti-RBD IgG response resulted in 100 % of specificity [69]. The temporal kinetics of IgG, IgA, and IgM in saliva of RT-PCR-confirmed-SARS-CoV-2 (COVID-19)-infected patients were consistent with those demonstrated in serum [69]. A recent meta-analysis of the sensitivity of the COVID-19 (SARS-CoV-2 viral RNA) diagnostic testing in saliva specimens in comparison to the sensitivity of the nasopharyngeal swab (NPS) tests demonstrated that the sensitivity for saliva tests was 91 % (CI = 80-99 %), whereas the sensitivity of the NPS tests was 98 % (CI = 89-100 %) [70]. Saliva could be an alternative valid strategy to serum for detecting antibodies against SARS-CoV-2 (COVID-19).

The effective antibody-mediated immunity is not enough evidence to guarantee the protective mechanism against re-infected-COVID-19. The type of specimen collection and technical errors, the methods used before patient discharging, and the presence of fecal viral RNA without evidence of viral replication in fecal swab should be considered. Viral culture, inflammatory target monitoring, genomic comparison of SARS-CoV-2 (COVID-19) strains involving both episodes of infection (at least one episode of the laboratory test during a 14-day post-hospital discharge period of quarantine period for patients with COVID-19), and evaluation

of innate and adaptive immunity are recommended for understanding of the recurrences of COVID-19. Further urgent studies should be identification of the parameters associated between the viral load and clinical parameters, such as certain comorbidities, symptom severity, hospital admission and direct hospital discharge, hospital length of stay, intensive-care-unit (ICU) admission, length of need for oxygen support, and overall survival. Further exploration quantitative VLs from lower respiratory tract tissue and blood in severe COVID-19 patients may prove to be a better predictor for clinical outcomes. Future studies will address SARS-CoV-2 (COVID-19) VL dynamics and the quantitative association with neutralizing antibodies, cytokines, pre-existing conditions, and therapies. Serological data greatly supplement the laboratory results from the quantitative reverse-transcriptase-polymerase-chain reaction (qRT-PCR), the design of virus elimination programs (seroepidemiology), discovery of the monoclonal antibodies, and development of SARS-CoV-2 (COVID-19) vaccines, particularly, the saliva tests could offer a promising alternative tests to the NPS tests for the COVID-19 diagnosis. Further diagnostic accuracy studies are urgently needed for improvement of their sensitivity and specificity.

Rapid Antigen Testing for Detection of SARS-CoV-2 (COVID-19) Infection

N and S proteins of SARS-CoV-2 (COVID-19) are the main immunogens among the four structural proteins (E, M, N, and S) [71]. The N protein-IgG ELISA provides a sentivity of 94.7 % that is significant higher than that of the S protein-IgG ELISA [72]. Antibodies against N proteins are longer-lived and have greater volume, in comparison to E, M, and S proteins [72]. Approximately, 100 companies are manufacturing or developing rapid antigent tests (RATs), one of the four types (virus isolation with cell cultures, serological testing, RATs, and molecular techniques) [72] for SARS-CoV-2 (COVID-19) detection [73]. Most RATs for SARS-CoV-2 (COVID-19) detection use a simple-to-use lateral flow test format that commonly use for influenza, malaria, and HIV testing as a sandwich immunodetection [74]. The testing results are interpreted by the operator within 10 to 30 minutes after collecting the respiratory specimen and applying it to the test strip [75]. In comparison to the nucleic acid amplification tests (NAATs), a decreasing sensitivity is found in the trade-off for simplicity of RATs for SARS-CoV-2 (COVID-19) operation [74]. As of September 11, 2020, only three companies submitted documents towards WHO's Emergency Use Listing (EUL) procedure, two tests have been approved by

Japan's Pharmaceutical and Medical Devices Agency, and only four tests have received United States Food and Drug Admoinistration (FDA) Emergency Use Authorization (EUA) [76, 77]. In comparison to NAATs in respiratory specimens (nasal or nasopharyngeal swabs), the specificity is consistently high (> 97 %), whereas the sensitivity ranges from 0 to 94 % [78-87]. When the cycle threshold (Ct) values are equal to or less than 25 or the SARS-CoV-2 (COVID-19) viral loads are more than 10^6 genomic virus copies/mL that frequently present in the pre-symptomatic period (1-3 days before the symptom onset) and the first 5-7 days of the acute COVID-19 illness phase [88-90]. Kweon *et al* conducted a study on evaluation of the diagnostic accuracy of the two newly developed, point-of-care, RATs, the ichroma COVID-19 Ag™ and AFIAS COVID-19 Ag for detecting SARS-CoV-2 (COVID-19) infection by serially collecting 200 nasopharyngeal samples from 38 COVID-19-infected patients and 122 samples from negative control group [-91]. The study revealed that both RATs demonstrated the sensitivity of 91.3 % to 100 % for samples with Ct < 25, whereas the specificity was 98.7 % to 98.9 % for AFIAS COVID-19 Ag and 100.0 % for ichroma COVID-19 Ag™ [91]. The sensitivity of AFIAS COVID-19 Ag and ichroma COVID-19 Ag™ for all targeted genes (*E*, *N*, and *RdRP*) was higher for samples collected before 7-days post-symptom onset than for those collected 8-14 post-symptom onset [91]. Stohr *et al* studied the sensitivity and specificity of the two self-tsting kits (BD Veritor System-BD RAT, n = 1,604 and Roche SARS-CoV-2 RAT, n = 1,611) with lateral flow assay compared to the qRT-PCR with Ct-value below the Ct-value cut-off demonstrated 78.0 % (95 % CI : 72.5-82.8) and 99.4 % (95 % CI : 99.0-99.6), respectively [92]. A test with the sensitivity of 80 % performed and implemented by at least 70 % of the population once a week was estimated to decrease the reproductive number of SARS-CoV-2 (COVID-19) from the basic reproductive number (R_0) of 1.5 to the effective reproductive number (R_e) below 1.0 [92].

The WHO has announced the general recommendations for the use of RATs for SARS-CoV-2 (COVID-19) detection as the following : 1) SARS-CoV-2 (COVID-19) RATs that meet the sensitivity of 80 % or higher and the specificity of 97 % or higher, compared to a NAATreference assay where NAAT prolonged turnaround times preclude clinical utility or is unavailable; 2) Appropriate scenarios for the use of SARS-CoV-2 (COVID-19) RATs (responding to suspected COVID-19 outbreaks in remote settings, semi-closed communities, and institutions where NAAT is not immediately available, supporting outbreak investigations; testing of asymptomatic cases of cantacts may be considered even if the RATs is not specifically

authorized for this use, due to asymptomatic cases having been shown to have viral loads similar to symptomatic cases; where there is widespread community transmission; and monitoring trends in COVID-19 incidence; 3) Initial introduction of RATs into clinical use; 4) in institutions where confirmed testing with NAAT in not feasible, and indications that RAT results may be incorrect should raise about validity suspicions; and 5) Use of RATS is not recommended in populations or settings with low expected prevalence of SARS-CoV-2 (COVID-19) (for examples; elective surgery, blood donation, screening at point of entry) [74]. The WHO also recommends the selection of RATs for procurement and implementation that includes 1) Quality of available data to validate the test; 2) Reported performance; 3) Manufacturing capacity and further evidence of quality; 4) Manufacturing quality and regulatory status; 5) Distribution and technical support; 6) Storage conditions, shelf-life, and shipping; 7) Availability, completeness, and clarity of instructions for use; 8) Cost of RATs; 9) Contents of RAT kit; and Specimen collection requirements [74]. Additionally, the WHO recommends the implementation considerations that include 1) Strictly following the supplier-recommended procedures; 2) Biosafety requirements for operators must be in place; 3) Each of these RATs has a specifically indicated method for specimen processing after collection; 4) Specimen collection is one of the most critical factors affecting performance of RATs; 5) Use of instrumented detection systems demands additional training requirements and sufficient infrastructure; and 6) Post-market surveillance with regulatory oversight [74]. Several variables may impact on RAT clinical performance that include 1) Pre-analytical influencers (sample type and way of sampling; collection device, transport media, and volume versus direct testing without dilution by transport media; time to test and storage/transport conditions, the time delay before processing); 2) Analytical influencers (Viral load of the specimen and viral load distribution in respective cohort (represented by SARS-CoV-2 (COVID-19) viral RNA copies/mL or Ct), analytical sensitivity and specificity of the PCR reference standard,PCR assay specifics, for examples, different targeted genes (*E*-/*N*-/*RdRp*-gene, etc., and across-laboratory differences (for examples, the definition of a positive specimen starting at Ct < 38 or < 40); and 3) Clinical parameters of the tested individual (days post-symptom onset of sampling, asymptomatic versus symptomatic status, the definition of symptoms " suspicion of SARS-CoV-2 (COVID-19) infection, and severity of symptom [93]. The sensitivity and specificity of the rapid SARS-CoV-2 (COVID-19) antigen (N protein) detection results are demonstrated in Table 3 [72].

Country; Reference	Test; Manufacturer	Specimen Type	Method	Sensitivity; Specificity (%) (95 % CI)
Belgium; 86	COVID-19 Ag Respi-Strip assay (Coris BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	30.2 (21.7-39.9); 100
Belgium; 94	COVID-19 Ag Respi-Strip assay (Coris Bioconcept Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	30 (16.7-47.9); 100
Chile; 78	SARS-CoV-2 antigen test (Bioeasy Biotechnology Co., Shenzhen, China)	Nasopharyngeal, oropharyngeal swabs	Fluorescence immunochromatographic test	93.9 (86.5-97.4); 100 (92.1-100)
France; 95	COVID-19 Ag Respi-Strip assay (Coris BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	50.0 (39.5-60.5); 100 (91.8-100)
Germany; 96	SARS-CoV-2 rapid antigen test (Roche, Switzerland)	Nasopharyngeal swabs	Lateral flow assay	70.7 (59.0-81.0); 96 (89.0-99.0)
USA; 97	Sofia 2 SARS antigen FIA (Quidel Corporation)	Nasopharyngeal swabs	Lateral flow immunofluorescent sandwich assay	80 (68-88); Not available

Table 3: Demonstrating the sensitivity and specificity of the rapid antigen (N protein) detection tests for SARS-CoV-2 (COVID-19)

Recently, the United States Centers for Disease Control and Prevention established an interim guidance for RATs for detecting SARS-Cov-2 (COVID-19) infection in a community setting [98, Figure 39]. If the individual has a low risk of SARS-CoV-2 (COVID-19) infection, a positive RAT result from an asymptomatic individual in a community setting may need confirmatory testing (for examples; a low risk of SARS-CoV-2 (COVID-19) infection would be an individual who is fully COVID-19 vaccinated, or has had a SARS-CoV-2 (COVID-19)

infection in the last 3 months, or has had no known exposure to a COVID-19-infected individual within the last 14 days) [98]. If a asymptomatic individual has a high risk of SARS-CoV-2 (COVI-19) infection, a negative RAT result may need confirmatory testing (for examples; a high risk of SARS-CoV-2 (COVID-19) infection would be an individual who is not fully COVID-19 vaccinated, or has not had a SARS-CoV-2 (COVID-19) infection in the last 3 months, or has had close contact or suspected exposure to SARS-CoV-2 (COVID-19) within the last 14 days) and should consider performing serial RATs every 3-7 days for 14 days [98]. A suspected COVID-19-symptomatic individual with a positive RAT result can be interpreted that the individual is infected with SARS-CoV-2 (COVID-19) [98]. For fully COVID-19 vaccinated individual with a positive RAT, the healthcare providers should inform the public health authorities and ideally, a separate specimen would be collected and sent to a laboratory for public-health-purpose viral sequencing [98]. A positive RAT result in a symptomatic individual with a low risk of SARS-CoV-2 (COVID-19) infection (for examples; for examples; a low risk of SARS-CoV-2 (COVID-19) infection would be an individual who is fully COVID-19 vaccinated, or has had a SARS-CoV-2 (COVID-19) infection in the last 3 months, or has had no known exposure to a COVID-19-infected individual within the last 14 days) may need confirmatory testing [98]. A symptomatic individual with a negative RAT result should be performed serial RATs every 3-7 days for 14 days, in addition to confirmation with a laboratory-based NAAT [98]. In a symptomatic individual with a negative RAT result, the negative RAT result may not need confirmatory SARS-CoV-2 (COVID-19) testing [98]. If a symptomatic individual with a negative RAT result had not any known exposure to SARS-CoV-2 (COVID-19), the individual do not need to quarantine as well as for asymptomatic individual with a negative RAT result who had been fully COVID-19 vaccinated, or had a SARS-CoV-2 (COVID-19) in the last 3 months, or have has close contact or suspected exposure to a COVID-19-infected individual within the last 14 days [98].

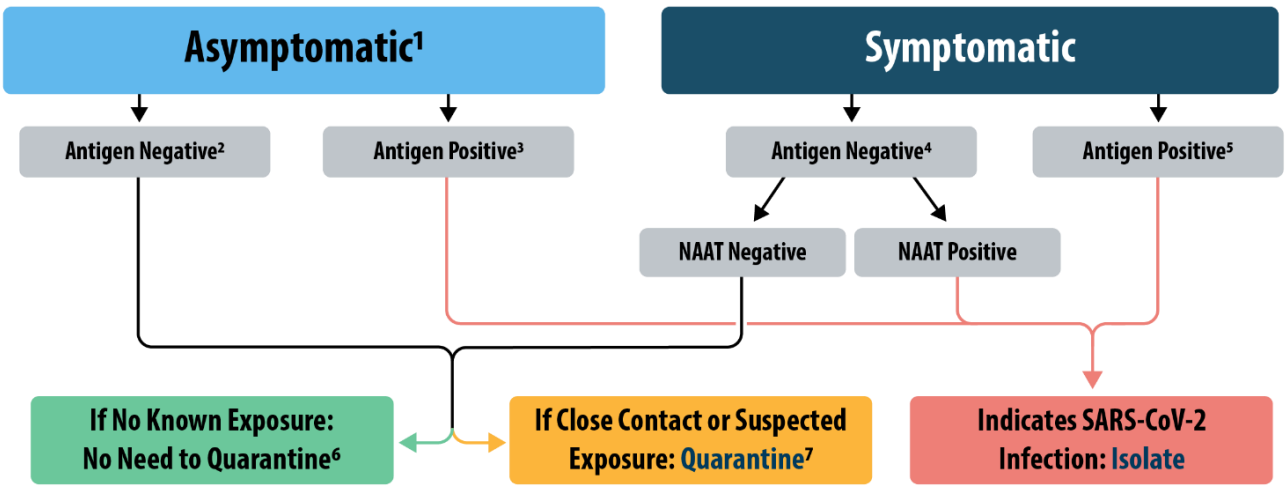

Figure 39 : Demonstrating RATs algorithm for community setting

(Source : United States Centers for Disease Control and Prevention. Interim guidance for antigen testing for SARS-CoV-2. Updated on May 13, 2021. Available at : https://www.cdc.gov>lab>antigen-tests-guidelines (accessed on July 31, 2021))

Conclusion

Currently, which SARS-CoV-2 (COVID-19) antigens are most appropriate for serological testing and whether the direct detection of viral antigens in the clinical specimens is a more sensitive-rapid-accurate-immunological diagnostic method for SARS-CoV-2 (COVID-19) remain unknown. S1 subunit protein of SARS-CoV-2 (COVID-19) is the S protein as an antigen for the serological diagnosis of SARS-CoV-2 (COVID-19) infection, whereas the S2 subunit protein plays a significant role in the cross-reactivity when the whole S protein is utilized as an antigen. Combinations of both S and N proteins have revealed improvement of the laboratory results through multiantigen protein arrays in comparison to each individual protein antigen. RATs become useful diagnostic tool for the SARS-CoV-2 (COVID-19)-early detection. They should be used in conjunction with the molecular methods and further urgent studies should be focused on strategies to improve the accuracy and sensitivity and post-implementation evaluation of the diagnostic accuracy.

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CHAPTER 7

Novel Therapeutic Interventions for COVID-19 Pneumonia, Vaccine Candidates, Medicinal Plants and Healthy Immune Nutrients

Abbreviations:

AACR : The American for Cancer Research's COVID-19 and cancer task force

ACE 2 : Angiotensin-Converting Enzyme 2

ADCC : Antibody-Dependent Cellular Cytotoxicity

ADE : Antibody-Dependent Enhancement

ADEM : Acute Disseminated Encephalomyelitis

AEFI : Adverse Events Following Immunization

AIDS : Acquired Immunodeficiency Syndrome

ARDS : Acute Respiratory Distress Syndrome

ASCO : American Society of Clinical Oncology

BAF : B-cell Activation Factor

BAL : Brochoalveolar Lavage

BAU : Binding Antibody Unit

CAR : Chimeric Antigen Receptor

CC_{50} : Half Maximal Inhibitory Concentration

CDC : Centers for Disease Contol and Prevention

CI : Confidential Interval

CMIA : Chemiluminescent Microparticle Immunoassay

Com-COV : Comparing COVID-19 Vaccine Schedule Combinations

COVID-19 : Coronavirus Disease-2019

DNA : Deoxyribonucleic Acid

DPT : Diphtheria vaccine, Tetanus toxoids and whole-cell Pertussis vaccine

EBV : Epstein-Barr virus

ESMO : European Society for Medical Oncology

ET : Endothelin

EUA : Emergency Use Authorization

FDA : Food and Drug Administration

FiO_2 : Fraction of Inspired Oxygen

FND : Functional Neurological Disorder

GBS : Guillain-Barre´ Syndrome

GMT : Genomic Mean Titer

GRADE : Grading for Recommendations, Assessment, Development and Evaluations

HIV : Human Immunodeficiency Virus

IC_{50} : 50 % Cytotoxic Concentration

ICI : Immune Checkpoint Inhibitor

ICU : Intensive Care Unit

IDSA : Infectious Disease Society of America

IL : Interleukin

INF-α : Interferon-alpha

IRAEs : Immune-Related Adverse Events

IRR : Incidence Rate Ratio

ISSR : Immunization-Stress-Related Response

IU : International Unit

MERS : Middle-East-Respiratory Syndrome

MERS-CoV : Middle-East-Respiratory-Syndrome Coronavirus

MHC : Major Histocompatibility

MMR : Measles, Mumps, and Rubella vaccine

mRNA : messenger-Ribonucleic Acid

MS : Multiple Sclerosis

MT : Transverse Myelitis

NAC : N-acetylcysteine

NADPH : Nicotinamide Adenine Dinucleotide Phosphate

NCCN : National Comprehensive Cancer Network

NHS : National Health Service, England

NIH : National Institute of Health

OR : Odds Ratio

p : Probability

PaO_2 : Partial Pressure of Oxygen

PNES : Psychologic Non-Epileptic Seizures

PRNT50 : 50 % Plaque Reduction Neutralization Test

RBD : Receptor-Binding Domain

RdRp : RNA-dependent RNA polymerase

RNA : Ribonucleic Acid

RR : Relative Risk

RT-qPCR : Reverse Transcriptase-quantitative Polymerase Chain Reaction

SAEs : Serious Adverse Events

SARS-CoV : Severe Acute Respiratory Syndrome-Coronavirus

SiCRES : Siriraj Institute of Clinical Research

SOT : Solid Organ Transplant

TBI : Total Body Irradiation

tIgG EU : Immunoglobulin G titer Equivalent Unit

TKIs : Tyrosine Kinase Inhibitors

TMSPSS2 : type II transmembrane serine protease

TNF : Tumor Necrosis Factor

Tregs : T regulatory lymphocytes

UCLA : University of California, Los Angeles

UCSD : University of California, San Diego

UK : United Kingdom

US : United States

USA : United States of America

UV : Ultraviolet

VAERS : Vaccine Adverse Event Reporting System

VS : Versus

ZO : Zonula Occludens

25-OHD : 1,25 $(OH)_2$D-vitamin D

WHO : World Health Organization

Introduction

Patients with COVID-19 pneumonia were detected in Wuhan city, China since late December 2019. More and more cases have been identified in other areas outside Wuhan city of China and abroad, particularly in Italy, Iran and other European countries, including the United Kingdom and the United States. Since December 2019, and as of March 29, 2020, 657,140 cases of COVID-19 and 30,451 deaths have been reported [1]. Currently, there is no effective treatment for COVID-19-infected patients. Some recent clinical studies demonstrated that alveolar epithelial cells and capillary endothelial cells of the patients' lungs were damaged contributing to acute lung injury. Post-mortem transthoracic needle biopsies from a COVID-19 patient with roentgenographic bilateral ground-glass opacities that was consistent with alveolar damage and respiratory failure revealed that there were organizing-phase diffuse alveolar damage, reactive type II pneumocyte hyperplasia with denuded alveolar lining cells, intra-alveolar fibrous exudates, chronic inflammatory infiltrates, loose interstitial fibrosis, and intra-alveolar loose fibrous plugs indicating organizing pneumonia [2]. Inhibition of pulmonary inflammatory response is hypothesized to be the key to cure the patients with COVID-19 pneumonia.

Chloroquine and Hydroxychloroquine

Chloroquine, a potential broad-spectrum antiviral agent found in 2006, can interfere with the virus's ability to replicate in two ways. First, the rug enters compartments called "endosomes" within the cell membrane. Endosomes tend to be slightly acidic, the chemical structure of the drug boosts their pH, making the compartments more basic. Many viruses acidify endosomes in order to breach the cell membrane, release their genetic material and initiate replication. Chloroquine blocks this critical step. Second, chloroquine also prevents SARS-CoV from plugging into a receptor called "angiotensin-converting enzyme 2 (ACE 2)" on primate cells, demonstrated in the 2005 report [3,4]. The virus sets off a chemical process that alters the structure of the ACE 2 receptor and allows the virus to infect when the virus inserts its spike protein into the ACE 2 receptor. Chloroquine blocks COVID-19 infection at low-micromolar concentration, with a half-maximal effective concentration (EC_{50}) of 1.13 μM and a half-cytotoxic concentration (CC_{50}) greater than 100 μM. An appropriate dose of chloroquine is to be undermined in this process. The investigators expect that whatever pertained to SARS-CoV-1 might apply to SARS-CoV-2 (COVID-19) [3, 4].

In February 2020, Manli Wang, a virologist in a research group of the Chinese Academy of Sciences demonstrated that chloroquine successfully inhibited the spread of SARS-CoV-2 (COVID-19) in cultured human cells. Preliminary reports from China, South Korea, and France indicated that the treatment was effective in COVID-19-infected patients. Some hospitals in the United States have initiated administering chloroquine in treating severely COVID-19-infected patients. Additionally, the United States Food and Drug Administration (US FDA) is organizing a large clinical trial to formally evaluate the chloroquine's effects [3, 4]. The US FDA will take all steps to ensure chloroquine remains available for patients who take it to treat severe and life-threatening illnesses, such as lupus. Nevertheless, due to the overdose of chloroquine can contribute to acute poisoning or death in humans and a short supply of chloroquine in China, Wang's research team also studied the closely related drug "hydroxychloroquine" that shares a similar chemical structure and reveals lower toxicity in animals than chloroquine and also remains widely available in treating lupus and rheumatoid arthritis. Wang and colleagues reported in the journal "Cell Discovery" published on March 18, 2020 that hydroxychloroquine can prevent SARS-CoV-2 (COVID-19) replication. Seven clinical trials had been registered in the Chinese Clinical Trial Registry to test hydroxychloroquine's effectiveness against COVID-19 infection

[3, 4]. The University of Minnesota, United States is studying whether taking hydroxychloroquine can protect persons living with COVID-19-infected patients from catching the virus themselves [5]. According to the American Society of Health-System Pharmacists, both chloroquine and hydroxychloroquine have been in short supply since earlier March 2020. Nevertheless, on March 19, 2020, a drug company from Germany donated 3 million tablets of chloroquine phosphate to the US federal government.

In a single protocol was studied from early March 2020 to March 16, 2020 , thirty-six French COVID-19-confirmed patients were included to receive 600 mg of hydroxychloroquine daily. Azithromycin was added to the treatment protocol depending on their clinical presentation [6]. Nasopharyngeal swabs for testing their viral load were performed daily in a hospital setting. Absence and presence of COVID-19 at Day6-post inclusion was considered the end point. Eight patients presented with lower respiratory tract symptoms, 22 cases presented with upper respiratory tract infection, whereas 6 cases were asymptomatic. Twenty cases were treated and demonstrated a significant decrease of the viral load at Day6-post inclusion, whereas adding azithromycin to hydroxychloroquine was significantly more efficient in viral load reduction [6].

Remdesivir

Remdesivir, a nucleoside analogue with a broad-spectrum antiviral activity and as being in the US clinical trials with near approval for use by the US FDA has been also studied in France by Gautret *et al* from Marseille University. The investigators feel optimistic about the French research data [7]. Previous studies conducted by the researchers from the University of Alberta, Canada and Gilead Sciences, Inc. involving cell cultures and animal models has demonstrated that remdesivir can block the replication of a variety of coronaviruses, hypothesized by blocking the RNA-dependent RNA polymerase, a particular enzyme that is required for viral replication [8]. Remdesivir potently blocks COVID-19 infection at low-micromolar concentration and has a high selectivity index (half-maximal effective concentration (EC_{50}), 0.77 μM; half-cytotoxic concentration (CC_{50}) > 100 μM; SI > 129.87) [9]. A previous study in the US reported that remdesivir treatment demonstrated promising results [10]. For evaluating the efficacy and safety of remdesivir in patients with COVID-19 disease, a randomized placebo-controlled, double-blind, multicentric phase III clinical trial was initiated on February 6, 2020 in China. The subjects in the

study group received a initial dose of 200 mg of remdesivir and a subsequent dose of 100 mg for 9 consecutive days through intravenous infusion in addition to routine treatment. The control group received routine treatment and the same dose of a placebo. By the end of April 2020, the clinical trial is expected to be concluded [11-14]. Remdesivir was developed by the US pharma giant Gilead Sciences, Inc. and previously was tried to treated Middle-East-Respiratory Syndrome (MERS) and Ebola. The Credit Suisse pharma team declares that remdesivir is the most advanced novel drug in treating patients with COVID-19 disease, but concerning about the supply [15].

Other Compound Candidates

On February 4, 2020, investigators in China announced that darunavir inhibited COVID-19 viral replication at a concentration of 300 μM *in vitro* and its inhibition efficiency was 280-fold that in the control group. Type II transmembrane serine protease (TMSPSS2) inhibitors and BCR-ABL kinase inhibitor-imatinib are other potential drugs. TMSPSS2 inhibitors would block the entry of the cellular protease, TMPRSS2 into the target cells via ACE 2 receptor in organ with having high ACE 2 expression, such as kidney and intestine. Imatinib inhibits the fusion of virions with the endosomal membrane (anti-coronal activity) [16]. On January 25, 2020, 30 drugs with potential antiviral activity against COVID-19 performed through the drug screening in silicon and an enzyme activity test, cinanserin, cyclosporin A, TDZD-8, PX-12, tideglusib, ebselen, shikonin, carmofur, disulfiram, chalcone, polydatin, deoxyrhapontin, montelukast, raltegravir, maribavir, elvitegravir, bortezomib, abacavir, presatovir, enzaplatovir, fosamprenavir, tipranavir, darunavir, atazanavir, remdesivir, ritonavir, carfilzomib, lopinavir, saquinavir, and indinavir were reported by a joint research team of the Shanghai Institute of Materia Medica and Shanghai Tech University [16].

The 6th edition of China national Guidelines for COVID-19 treatment, issued on February 18, 2020, recommends interferon-α (IFN-α), lopinavir/ritonavir, ribavirin, chloroquine phosphate and arbidol for treatment agents. IFN-α is administered via vapor inhalation at a dose of 5 million Units (and 2 ml of sterile water for infection) for adults, 2 times daily. Lopinavir/ritonavir is administered orally at a dose of 400 mg/100 mg for adults, 2 times daily. Ribavirin is administered via intravenous infusion at a dose of 500 mg each time for adults, 2 to 3 times daily in combination with IFN-α or lopinavir/ritonavir. Chloroquine phosphate is administered orally at a dose of 500

mg (300 mg for chloroquine) each time for adults, 2 times daily. Arbidol is administered orally at a dose of 200 mg, each time, 3 times daily. Arbidol effectively inhibits COVID-19 infection at a concentration of 10-30 μM *in vitro*. The duration of all treatment options is no more than 10 days [16].

Favipiravir

On February 15, 2020, favipiravir, a new type of RNA-dependent RNA polymerase (RdRp) inhibitor that was first approved in Japan in March 2014 for establishing preparedness against the possible outbreak of novel or re-emerging influenza virus infections was approved for treatment of COVID-19 disease in China. Currently, favipiravir is undergoing clinical trials in treating COVID-19 disease. In addition to favipiravir's anti-influenza virus activity, it can block the replication of alpha-, arena-, bunya-, filo-, flavi-, and other RNA viruses. Favipiravir inhibits RNA polymerase activity by the conversion of favipiravir into an active phosphoribosylated form (favipiravir-RTP) in cells and is recognized as a substrate by viral RNA polymerase. A clinical trial on favipiravir for the treatment of COVID-19 disease was initiated by the Third People's Hospital of Shenzhen and the Clinical Medical Research Center of the National Infectious Diseases, China on February 14, 2020 achieved the promising results. The preliminary results from a total of 80 patients with COVID-19 disease, including the control group and the experimental group demonstrated that favipiravir had more potent antiviral activity than that of lopinavir/ritonavir. Favipiravir treatment group demonstrated no significant adverse reactions and had significantly fewer adverse effects than the lopinavir/ritonavir group [16].

Molnupiravir

Updated Molnupiravir (MK-4482/EIDD-2801, an orally investigational antiviral drug) clinical development program was provided by Merck (MSD, NYSE : MRK) and Ridgeback Biotherapeutics, as of April 15, 2021 (available at : https://merckcovidresearch.com/). Data from a previously completed Phase IIa dose-ranging study in outpatients and the two ongoing placebo-controlled Phase II/III trials were analyzed to evaluate molnupiravir administered twice daily for five days. They decided to continue MOVe-OUT, Phase III (Part II) in COVID-19 outpatients

and not to proceed to Phase III of MOVe-IN due to general having a longer duration of symptoms prior to study entry that contributed to molnupiravir being unlikely to demonstrate a clinical benefit in COVID-19 inpatients in MOVe-IN by evaluation of the 800 mg dose of molnupiravir twice a day. During the period from participant randomization through the day 29, 302 mild-to-moderate-COVID-19-symptom participants with 18 years of age or older and all sexes, including individuals with high risk for poor COVID-19 outcomes (individuals with symptom duration of 5 days or less, aging, obese, or diabetic individuals) had been enrolled in Phase II portion of the MOVe-OUT study. Merck plans to initiate enrolling participants in Phase III portion (Part II) of MOVe-OUT by late April 2021 or early May 2021 and the final data is estimated to be available in September 2021 or October 2021. In the second half of 2021, starting an evaluation of molnupiravir clinical program for COVID-19-post-exposure prophylaxis and submission of pending favorable results from molnupiravir-MOVe-OUT for an Emergency Use Authorization will be occurred. Molnupiravir is not genotoxic or mutagenic, demonstrated in data from several *in vivo* mammalian system studies [17]. This trial has been registered in the US patent system, entitled " The safety of molnupiravir (EIDD-2801) and its effect on viral shedding of SARS-CoV-2 (END-COVID) " with the ClinicalTrials.gov Identifier of NCT04405739. The actual study start date of trial, estimated primary completion date of trial, and estimated study completion date of trial are June 16, 2020, May 28, 2021, and May 28, 2021, respectively. Department of Drug Innovations at Emory (DRIVE), LLC, a non-profit biotechnology company wholly owned by Emory University, USA invented molnupiravir [18]. Based on the preliminary results of this study, Merck and Ridgeback Biotherapeutics are advancing a Phase III program in outpatients with COVID-19 to achieve their large global network of clinical sites.

Interleukin-6 Inhibitors

As of March 27, 2020, the 7th edition of the Chinese Clinical Guidance for COVID-19 pneumonia diagnosis and treatment published by the China National Health Commission on March 4, 2020 included an interleukin (IL)-6 receptor inhibitor (a humanized anti-IL-6 receptor antibody) "tocilizumab" as an therapeutic option for severe COVID-19 patients, patients with extensive pulmonary lesions and IL-6 level elevation [19]. High IL-6 level is observed in COVID-19 patients for at least 2 weeks after disease onset. This IL-6 inhibitor demonstrated the positive outcomes in 21 severe COVID-19 patients with severe pulmonary inflammation. There are

several ongoing or planned studies for the US Food and Drug Administration (FDA)-approved IL-6 inhibitors in patients with COVID-19 as the following : 1) NCT04310228 (multicenter, open label, randomized control trial (3 arms)), favipiravir+tocilizumab, compared with favipiravir alone or tocilizumab alone, date of primary completion-May 2020); 2) NCT04306705 (retrospective cohort study (3 arms), tocilizumab with standard of care, compared with continuous renal replacement therapy with standard of care or standard of care alone, date of primary completion-May 2020); 3) NCT04322188 (observational, case-control study, siltuximab, compared with standard of care, date of primary completion-May 2020); 4) NCT04317092 (single-arm, multicenter, phase II, observational cohort study, tocilizumab, no comparator, date of primary completion-December 2020); 5) NCT04321993 (open label, phase II, non-randomized study, lopinavir/ritonavir, hydroxychloroquine sulphate, baricitinib, sarilumab, no comparator, date of primary completion-February 2021); 6) NCT04315298 (double blind, phase II/III, randomized control trial (3 arms), high dose sarilumab, low dose sarilumab, compared with placebo, date of primary completion-March 2021); 7) NCT04320615 (multicenter, open label, randomized control trial (4 arms), intravenous tocilizumab, subcutaneous tocilizumab, subcutaneous sarilumab, compared with standard medical care, date of primary completion-June 2021); 8) NCT04320615 (multicenter, double blind, phase III, randomized control trial, tocilizumab, compared with placebo, date of primary completion-August 2021) [19]. A trial "COVACTA" on tocilizumab will recruit about 330 COVID-19 patients around the world, with expected start date sometime in early April 2020 [20]. A drug company in the US has announced the initiation of a randomized controlled clinical trial of sarilumab, an antibody to the interleukin (IL)-6 receptor, to evaluate whether the modification of the lung inflammatory response by therapeutic intervention provides the benefit to COVID-19-infected patients [16].

Although none of the IL-6 inhibitors are mentioned by the World Health Organization (WHO), the Society of Critical Care Medicine, and the European Society of Intensive Care Medicine, a number of professional bodies have included tocilizumab as a therapeutic option for selected sever COVID-19 patients as the following : 1) Chinese Clinical Guidance for COVID-19; 2) The Italian Society of Infectious Diseases and Tropical Diseases COVID-19 Guidelines; 3) Michigan Medicine (University of Michigan); and 4) The Society for Immunotherapy of Cancer. Several other US FDA-approved IL-6 inhibitors currently are in trials : ALX-0061, ARGX-109, BMS945429(ALD518), clazakizumab, CPSI-2364, elsilimomab, FE301, FM101,

olokizumab (CDP6038), olokizumab, sirukumab (CNTO136), and sirukumab. Other promising drugs includes Janus kinase (JAK) inhibitors (baricitinib, inositol-requiring transmembrane kinase/endoribonuclease (IRE1α)), tylophorinebased compounds, and tracolimus [21].

Ivermectin

Bryant *et al* conducted searching the bibliographic databases up to April 25, 2021 for assessing the efficacy of ivermectin (an antiparasitic agent) of treatment in decreasing mortality, in secondary outcomes, and in chemoprophylaxis, among persons with , or at high risk of COVID-19 infection and demonstrated that 24 randomized controlled trials involving 3,406 participants were included in the study. Assessment of the certainty of the evidence is performed by using the GRADE approach. Among meta-analysis of 15 trials revealed that ivermectin decreased risk of death compared with control group (no ivermectin) (average risk ratio = 0.38, 95 % CI : 0.19-0.73); m = 2,438; I^2 = 49 %; moderate-certainty evidence). This study result was confirmed in a trial sequential analysis by using the same DerSimonial-Laired method that underpinned the unadjusted analysis. The low-certainty evidence demonstrated that ivermectin prophylaxis decreased COVID-19 infection, whereas the secondary outcomes provided less certain evidence. Nevertheless, low-certainty evidence indicated that there may be no benefit with ivermectin for need for mechanical ventilation [22]. As of March 31, 2021, the WHO recommended that ivermectin only be used within the clinical trials and the current evidence is inconclusive until more data is available [23].

Convalescent Plasma

"Convalescent plasma " therapy has been working on some time by the US FDA. This is not a proven treatment, but is a possible treatment. The immunoglobulins in the previously COVID-19-exposed individuals' circulating virus-free convalescent plasma could potentially provide a benefit to severely COVID-19-infected patients [24].

Mesenchymal Stem Cells

Currently, several trials on mesenchymal stem cells in treating patients with COVID-19 pneumonia are ongoing. For examples, trial sponsored by Innovative Precision Medicine Group (IPM), China, Wuhan Houshenshan Hospital, Wuhan, China, Tianjin Haihe Hospital, VCANBIO CELL and GENE ENGINEERING CORP., LTD., China, Shenzhen Third People' s Hospital, and Fifth Affiliated Hospital, Sun Yat-Sen University, China (NCT04252118); trial on human umbilical cord mesenchymal stem cell treatment for COVID-19-pneumonia patients sponsored by Wuhan Union Hospital, China and Wuhan Hamilton Bio-technology Co., Ltd., China (NCT04273646); trial on inhalation of mesenchymal stem cell exosomes in treating COVID-19-pneumonia patients sponsored by Ruijin Hospital, China, Shanghai Public Health Clinical Center, Wuhan Jinyintan Hospital, Wuhan, China, and Cellular Biomedicine Group Ltd. (NCT04276987); trial on human umbilical cord mesenchymal stem cells for treatment of COVID-19-pneumonia patients sponsored by Puren Hospital Affiliated to Wuhan University of Science and Technology and Wuhan Hamilton Bio-technology Co., Ltd. (NCT04293692); trial on dental pulp mesenchymal stem cell treatment for COVID-19-pneumonia patients sponsored by CAR-T (Shanghai) Biotechnology Co., Ltd. (NCT04302519); and trial on mesenchymal stem cells in treating patients with COVID-19 pneumonia sponsored by Beijing 302 Hoppital, VCANBIO CELL and GENE ENGINEERING CORP.,LTD., China, Wuhan Huoshenshan Hospital, Wuhan, China, Tianjin Haihe Hospital, Shenzhen Third People's Hospital, Fifth Affiliated Hospital, Sun Yat-Sen University, China, Wuhan Union Hospital, China, and West China Hospital (NCT04288102) [13].

Promising Medicinal Plants

Andrographis paniculata

Andrographis paniculata (Green chiretta) (Figure 40), a medicinal plant, known as "Indian echinacea" is an herb used in traditional Chinese medicine and ayurveda. *Andrographis paniculata* is native to India and Sri Lanka but is naturalized in many tropical countries, such as Thailand, Malaysia, Java, and Borneo where it grows isolated patches on roadside, near drain, in between wall cracks, lowlands, hillside, coastlines, and other cultivated or disturbed areas, such as wastelands. It is a bitter-tasting herb rich in compounds known as andrographolides, a major bioactive phytoconstituent found in various parts of *Andrographis paniculata*, particularly in the

leaves [25-29] that is hypothesized to have antiviral, anti-inflammatory, and antioxidant properties. The chemical name of andrographolide is 3α, 14, 15, 18-tetrahydroxy-5β, 9βH, 10α-labda-8, 12-dien-16-oic acid γ-lactone with its molecular formula and weight are $C_{20}H_{30}O_5$ and 350.4 (C 68.54 %, H 8.63 %, and O 22.83 %), respectively [25-29]. This herb is said to act as a natural immune-booster [30]. Other major bioactive compounds are flavonoids, polyphenols, and diterpenoids [31]. *Andrographis paniculata* is commonly known as the "King of bitters". In Thailand, it is known as "Fah-Thalai-Jone". In China, it is known as "Chuan-Xin-Lian". In India, it is known as "Kalmegh". In Japan, it is known as "Senshinren". In Malaysia, it is known as "Hempedu bumi". In Scandinavian countries, it is known as "green chiretta" [32]. In mice, the extract and purified andrographolide were reported to stimulate an innate immune response [33]. In murine immature dendritic cells with experimental autoimmune encephalomyelitis, andrographolide inhibited NF-kappa B activation [34]. In murine-T cells stimulated with concanavalin A *in vitro*, andrographolide reduces IFN-γ and IL-2 production [35]. Furthermore, in macrophages stimulated by lipopolysaccharide, andrographolide inhibits the production of IL-12 and TNF-α [36]. *Andrographis paniculata*, 25 μg/mL of ethanolic extract and 5 μg/mL of andrographolide effectively exhibits the expression of Epstein-Barr virus (EBV) lytic protein, Rta, Zta, and EA-D, during the viral lytic cycle in P3HR1 cells [37]. A previous study demonstrated that *Andrographis paniculata* has the most inhibitory effects against dengue type 1-infectd Vero E6 cells [38]. *Andrographis paniculata* also has antibacterial, antimalarial, insects-larvicidal and -ovicidal, renoprotective, hepatoprotective, antihyperglycemic, hypolipidemic, antifertility, antioxidant, anti-inflammatory, anticancer, anti-platelet aggregation, anti-NF-kappa B (NF-kB) transcription, and antipyretic and anti-analgesic effects [39]. Pre- and post-treatments of the extract of *Andrographis paniculata* after surgical procedures and operations, particularly angioplasty procedures, significantly prevent the blood vessel constriction, demonstrated by an increase of blood-clotting time, hence reducing the risk of subsequent closing blood vessels [40]. Several previous studies demonstrated antidiarrheal [41] and anti-HIV effects of *Andrographis paniculata* [42]. A previous study on 152 Thai adults with pharyngotonsillitis demonstrated that *Andrographis paniculata* at a dose of 6 g/day for 7 days relieved the symptoms of sore throat and fever similar to the efficiency of acetaminophen [43]. A previous study conducted by Ca´ceres and colleagues demonstrated that 4-days treatment of *Andrographis paniculata* extract SHA-10 decreased the intensity of the symptoms of sore throat (OR = 2.3; 95 % CI : 1.69-3.14), sleeplessness (OR = 1.71; 95 % CI : 1.38-2.11), and tiredness (OR = 1.28; 95 % CI : 1.07-1.53),

compared with control group [44]. A previous study in mice challenged with ADP (700 mg/kg) can markedly lowering the mortality rate from 90 % to 60 % that were treated with andrographolide concentrations of 22 µg/kg and 55 µg/kg, respectively, confirming the effects of andrographolide on prevention of thromboembolism [45]. *Andrographis paniculata* is most widely used to treat cold and flu symptoms and is also used to treat other diseases and symptoms, such as human immunodeficiency-virus infection (HIV)/acquired immunodeficiency syndrome (AIDS), infections, parasitic infestations, sinus infections, cancer, rheumatoid arthritis, hepatic problems, cardiac diseases, anorexia, allergies, ulcers, and skin diseases. Nevertheless, there is not enough scientific evidence to support the use of *Andrographis paniculata* for most of these health benefits. Some preliminary studies demonstrated that *Andrographis paniculata* may offer the health benefits, such as upper respiratory tract infections and ulcerative colitis [46].

Andrographis paniculata may trigger adverse side effects like fatigue, headache, nausea, diarrhea, and allergic reaction. *Andrographis paniculata* should not be administered intravenously due to possible acute renal injury. Individuals using some medications, such as anti-hypertensive medicines, chemotherapy drugs, blood-thinning drugs, etc. should consult a clinician before using *Andrographis paniculata*. Little is known about the safety of using *Andrographis paniculata*. There is no single recommended dose of *Andrographis paniculata* due to various dose studies. Some previous studies revealed that for relief of sore throat, a dose of 3-6 grams *Andrographis paniculata* was used once a day. For ulcerative colitis, *Andrographis paniculata* extract, 1,200-1,800 milligrams was used once a day for eight weeks. For common cold, a combination product (4-5.6 milligrams Andrographolide and 400 milligrams Siberian ginseng) was used three times daily, whereas another previous study demonstrated using Andrographis extract (KalmCold) 200 milligrams once a day for 5 days [46]. Recently, some Asian countries claimed that *Andrographis paniculata* may kill the COVID-19 and they will initiate studies on this issue in 2020 as soon as possible. Meanwhile a joint research team of the Shanghai Institute of Materia Medica and Shanghai Tech University conducted a research and demonstrated that Chinese herbal medicines, such as *Radix Sophorae Tonkinensis* and *Rhizoma Polygoni Cuspidati* may contain ingredients against COVID-19. Until now, it has been difficult to get the polymerase complex that contains multiple proteins to function in a test tube [31]. A recent study in Thailand conducted by Sa-ngiamsuntorn *et al* in human lung epithelial cells demonstrated that *Andrographis paniculata* extract and its major component andrographolide

expressed anti-SARS-CoV-2 (COVID-19) activity at 25$TCID_{50}$ in SARS-CoV-2 infected Calu-3 cells by significant inhibition of the production of infectious virions with the $IC_{50}$of 0.036 µg/mL and 0.034 µM, respectively [47].

Various experimental and clinical pharmacological actions of andrographolide include anti-inflammatory, anti-hyperglycemic, anti-hypoglycemic, anti-cancer, antioxidant, anti-viral, particularly SARS-CoV-2 (COVID-19) and HIV. Favorable cytotoxicity profiles and potent anti-SARS-CoV-2 activities promote further development of *Andrographis paniculata* extract and andrographolide for monotherapy or in effectively combined drug regimens against SARS-CoV-2 (COVID-19). *In vitro* study of effects of *Andrographis paniculata* extract and andrographolide on SARS-CoV-2 (COVID-19) conducted by Phoomamorn *et al* from the Department of Medical Services, Ministry of Public Health, Thailand with plaque reduction assay demonstrated that both *Andrographis paniculata* extract and andrographolide inhibited viral replication (IC_{50} < 1 µg/mL and 15.6 µg/mL by the viral inactivation test, respectively; IC_{50} = 3.02 µg/mL and 0.54 µg/mL by the antiviral test, respectively), but both had no inhibitory effects on SARS-Co-V-2 (COVID-19) by the cell production test [48, unpublished work, 2020].

Clinical Research Project of Effects of *Andrographis paniculata* on SARS-CoV-2 (COVID-19) in Thailand

Recently, a clinical double-blinded-randomized-control trial of effects of *Andrographis paniculata* on SARS-CoV-2 (COVID-19) has been conducted by Wanarat *et al* from the Department of Thai Traditional and Alternative Medicine, Ministry of Public Health, Thailand (phase 1 (preliminary study) : Andrographolide, 6 patients with mild COVID-19 (3 male participants and 3 female participants), determination of appropriate dosage of Andrographolide (stage 1 : standard treatment plus Andrographolide 180 mg/day for 5 days, stage 2 : standard treatment plus Andrographolide 300 mg/day for 5 days (depending on the severity of COVID-19, levels of the pro-inflammatory cytokines, and adverse side effects, andrographolide was administered at 6.00, 14.00, and 22.00 am.), safety measurement and monitoring, the pharmacokinetics of *Andrographis paniculata*, levels of pro-inflammatory cytokines and metabolomic profiles; phase 2 (clinical trial study) : double blinded- randomized-clinical trial, duration of the COVID-19 recovery, COVID-19 viral loads, assessment of the study group in

comparison to control (placebo) group. Assessment of treatment results of day 1, day 2, day 3, day 4, and day 5 were evaluation of clinical symptoms by the visual analog scale, measurement of viral loads and RT-qPCR for SARS-CoV-2 (COVID-19) by blood sampling at 2 hours after first dose of Andrographolide, and monitoring the electrocardiographic changes and the adverse drug-side effects on day 1; evaluation of clinical symptoms by the visual analog scale on day 2; evaluation of clinical symptoms by the visual analog scale, measurement of viral loads and RT-qPCR for SARS-CoV-2 (COVID-19) by blood sampling at 2 hours after first dose of Andrographolide, and monitoring the electrocardiographic changes and the adverse drug-side effects on day 3; evaluation of clinical symptoms by the visual analog scale on day 4; and evaluation of clinical symptoms by the visual analog scale, measurement of viral loads and RT-qPCR for SARS-CoV-2 (COVID-19) by blood sampling at 2 hours after first dose of Andrographolide, and monitoring the electrocardiographic changes and the adverse drug-side effects on day 5 of treatment. The study results revealed that the average visual analog scale (cough severity, cough frequency, sore throat severity, sputum quantity, nasal discharge quantity, myalgia severity, headache severity, dyspnea, and diarrhea) was 17.0+/-5.7. The profiles of the pro-inflammatory cytokines were increased in hs-CPR and increased in serum ferritin, LDH, and ESR on day 5 of Andrographolide treatment, compared to levels before Andrographolide treatment in 5 study participants (p-value < 0.05, Paired t-test, not available data of one study participant). All 5 study participants had decreased SARS-CoV-2 (COVID-19) viral loads (not available data of one study participant). For consideration of the drug safety, one study participant had increased alanine aminotransferase (ALT) 1.7 times of the normal value, one study participant had increased aspartate aminotransferase (AST) and ALT, but were not higher than the normal value, and the adverse drug-side effects were not identified in all study participants. In conclusion, Andrographolide 180 mg/day might relieve the COVID-19 clinical symptoms, might reduce the COVID-19 viral loads, might have anti-inflammatory effects in COVID-19 patients. Overdosage use of Andrographolide can contribute to hepatic impairment [49, unpublished work, 2021].

Figure 40 : *Andrographis paniculata* (Green chiretta)

(Source : indiamart.com)

Boesenbergia rotunda (Finger Root)

Generally, *Boesenbergia rotunda* (Finger Root) is a herb grown in China and Southeast Asia, including Thailand [50] and its rhizome [Figure 41, 42] is used for medicines [51]. It consists of several bioactive compounds, such as Panduratin A, 5-Hydroxy-7-methoxyflavanone (Pinostrobin), 2′,6′-Dihydroxy-4′-methoxychalcone (Pinostrobin chalcone), 5, 7-Dihydroxyflavanone (Pinocembrin), Boesenbergin, Camphor, and Linalool [51], whereas Panduratin A in *Boesenbergia rotunda* extract demonstrated the potent anti-SARS-CoV-2 (COVID-19) activity in Vero E6 cells coupled with plaque reduction assay [50]. Among 122 Thai natural herbs of this *in vitro* study, the study results of *Boesenbergia rotunda* extract revealed drastical inhibition in Vero E6 cells with IC_{50} of 3.62 µg/mL (CC_{50} = 28.06 µg/mL) and 0.81 µM (CC_{50} = 14.71 µM), respectively after COVID-19 infection and at the the pre-entry phase suppressed SARS-CoV-2 (COVID-19) infection with IC_{50} of 5.30 µM (CC_{50} = 43.47 µM) [50]. The purified Panduratin A and its extract may be the promising anti-SARS-CoV-2 (COVID-19) infection and therapeutic aspect with economic advantage during the COVID-19 pandemic [50].

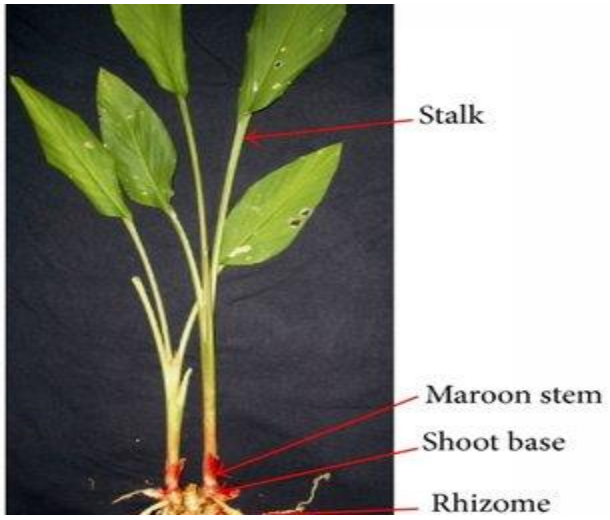

Figure 41 : Demonstrating the rhizome, shoot base, maroon stem, and stalk of *Boesenbergia rotunda* (Finger Root)

(Source : researchgate.net (accessed on August 31, 2021))

Figure 42: Demonstrating the rhizome of *Boesenbergia rotunda* or Finger Root

(Source : sibrape.com.br (accessed on August 31, 2021))

Vaccine Candidates

Presently, there are three main types of COVID-19 vaccines (1) mRNA vaccines, 2) Protein subunit vaccines, and 3) Vector vaccines) that are or soon will be undergoing Phase 3 (large-scale) clinical trials in the United States [52].

Individuals' past infections with common coronavirus probably did not induce a B cell memory for producing antibodies that can neutralize SAR-CoV-2 (COVID-19). Nevertheless, by analysis of linear 9 aa epitopes in the current studies reveals that these common human coronaviruses are expected to induce CD8+ T cells that may potentially kill COVID-19-infected cells and can assist eradicate the virus [53]. Trials on several vaccine candidates, currently are also ongoing, for examples; phase I trial sponsored by Moderna Therapeutics, CanSino Biologics, Arcturus Therapeutics (Preclinical stage), BioNTech (Preclinical stage), CureVac (Preclinical stage), GlaxoSmithKline (Preclinical stage), Inovio Pharmaceuticals (Preclinical stage), Johnson & Johnson (Preclinical stage), and Pfizer (Preclinical stage), Sanofi (Preclinical stage). Usually, vaccine development takes more than 5 years and requires much capital investment. There is no guarantee of success though the traditional pharma giants' experience involving seasonal flu, particularly their specializing in mRNA molecules that are used to instruct the human body to produce its own response to combat a range of diseases [54]. Significant questions are whether the history of recent infections with common coronaviruses, or individuals' major histocompatibility (MHC) alleles affect individuals' resistance to COVID-19 [53]. Consideration of pre-existing MHC-I-based immunity derived from previous infections with common coronaviruses should be needed.

As of December 28, 2020, three COVID-19 vaccines in the United States are in progress or being planned in Phase 3 (large-scale) clinical trials : 1) AstraZeneca's COVID-19 vaccine, 2) Janssen's COVID-19 vaccine, and 3) Novavax's COVID-19 vaccine [52]. Presently, the United States Centers for Disease Control and Prevention recommends two COVID-19 vaccines that are authorized in the United States: 1) Pfizer-BioNTech's COVID-19 vaccine and 2) Moderna's COVID-19 vaccine [52].

Scientists have been studying with COVID-19 mRNA vaccines for decades and began designing the mRNA instructions for human cells to build the unique spike protein into an mRNA vaccine [52]. COVID-19 mRNA vaccines contain material from SARS-CoV-2 that causes COVID-19 that provides human cells instructions for how to make a harmless protein that is

unique to the SARS-CoV-2 (COVID-19) [52]. COVID-19 mRNA vaccines will be rigorously evaluated for safety [52]. Protein subunit vaccines compose of harmless proteins of SARS-CoV-2 (COVID-19) that cause COVID-19 instead of the entire virus [52]. COVID-19 vector vaccines contain a weakened version of live SARS-CoV-2 (COVID-19), a different virus than the one that causes COVID-19 [52]. This weakened live virus has genetic material from SARS-CoV-2 (COVID-19) that causes COVID-19 inserted in viral particle, called " a viral vector " [52]. All but one Phase 3-clinical-trial-COVID-19-vaccines in the United States use two dose-shots, whereas one vaccine of Johnson and Johnson only needs one dose-shot could be available in March 2021 [52]. Figure 43 and Table 3 demonstrates the development of COVID-19 vaccines (as of September 16, 2021) and status of COVID-19 around the world (as of January 14, 2021), respectively.

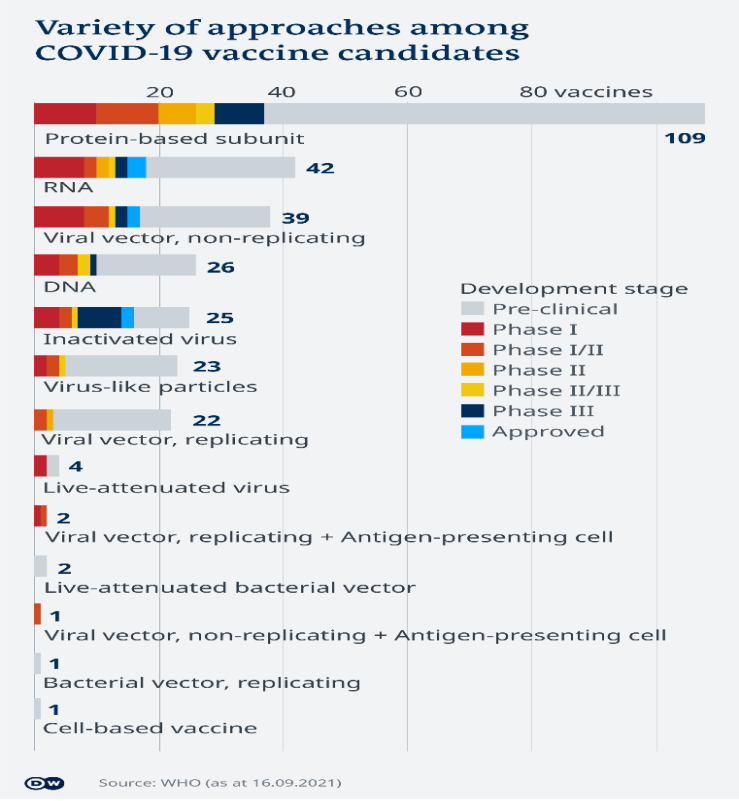

Figure 43: Demonstrating the development of COVID-19 vaccine.

(Source : World Health Organization, as of September 16, 2021)

	Manufacturer	Name of Vaccine	NRA of Record	Platform	EOI accepted	Pre-submission meeting held	Dossier accepted for review*	Status of assessment**	Anticipated decision date***
1.	Pfizer BIONTECH	BNT162b2/COMIRNATY	EMA	Nucleoside modified mNRA	✓	✓	✓	Finalized	31/12/20
2.	Zhifei Longcom, China	Recombinant Novel Coronavirus Vaccine（CHO Cell）	NMPA	Recombinant protein subunit	Not accepted Product in Phase I/II				
3.	IMBCAMS, China	SARS-CoV-2 Vaccine, Inactivated (Vero Cell)	NMPA	Inactivated	Not accepted, still under development				
4.	AstraZeneca OXFORD	AZD1222	Core – EMA Non-COVAX	recombinant replication defective chimpanzee adenovirus expressing the SARS-CoV-2 S surface glycoprotein	✓	✓	✓	In progress Core data Non-Covax. Covax data to be reviewed as EMA post approval change	Earliest by EMA End of Jan- Feb 2021 (non-Covax) Additional nodes in March/ April for Covax
5.	SK BIO AstraZeneca OXFORD	AZD1222	MFDS KOREA	=	✓	✓	Tentative 18 and 29 Jan 2021 (CMC for SK Bio)	Core data (non-covax) in progress	Earliest 2nd half Feb 2021

Table 4 : Demonstrating Status of COVID-19 Vaccines within WHO EUL/PQ Evaluation Process, as of January 14, 2021 (Sinovac vaccine has been approved since June 2021)

(Source : World Health Organization. COVID-19 Vaccines : Status of COVID-19 Vaccines. Available at : www.who.int>...>Coronavirus disease (COVID-19) (accessed on January 15, 2021))

Vaccine Efficacy on SARS-CoV-2 (COVID-19) Variants

A study demonstrated in early March 2021 the efficacy of various COVID-19 vaccines produced by many manufacturers in symptomatic SARS-CoV-2 (COVID-19) patients and patients infected with SARS-CoV-2 (COVID-19) variants as the following products (vaccine name), used technology, doses, efficacy against symptomatic disease, and efficacy against variants (B.1.1.7 (first detected in the United Kingdom) and B.1.351 (first detected in South Africa)) : 1) Bharat Biotech (Covaxin), inactivated virus, 2 doses, unknown, unknown, unknown; 2) Sinovac (CoronaVac), inactivated virus, 2 doses, 50.4 %, unknown, unknown; 3) Sinopharm (BBIBP-CorV), inactivated virus, 2 doses, 79.34 %, unknown, unknown (but reports of weekend effect); 4) John & Johnson (Ad26.COV2.S), viral vector, 1 dose, 72 %, unknown, 57 %; 5)

Novavax (NVX-CoV2373), protein, 2 doses, 95.6 %, 85.6 %, 60 %; 6) CanSinoBio (Convidecia), viral vector, 1 dose, 65.7 %, unknown, unknown; 7) Gamaleya (Sputnik V), viral vector, 2 doses, 91.6 %, unknown, unknown; 8) Moderna and the National Institute of Health (NIH) (mRNA-1273), mRNA, 2 doses, 94.5 %, unknown (but reports of reduction in neutralizing antibodies), unknown; 9) Oxford and AstraZeneca (AZD1222), viral vector, 2 doses, 82.4 % (12 weeks between doses), 74.6 %, to be confirmed (unconfirmed reports as low as 10 %); 10) Pfizer and BioNTech (Comirnaty), mRNA, 2 doses, 95 %, unknown, and unknown; respectively [55].

SARS-CoV-2 (COVID-19) variants of concern might be related to changes in both morbidity and mortality. Changes in both morbidity and mortality in COVID-19-infected individuals may due to suppression of the host immune response, altered viral transmission dynamics, or higher viral loads that might worsen the clinical outcomes. Current COVID-19 vaccines are based on the SARS-CoV-2 spike protein, whereas the SARS-CoV-2 (COVID-19) variants contain mutations in the spike protein that contributes to spurring vaccine efficacy concerns [56]. Among 168 cases with SARS-CoV-2 (COVID-19) infection, the serious adverse events from AZD1222 was found in 79 cases in the vaccine group and 89 cases in the control group [55]. Transverse myelitis was identified in two cases that later determined to be unlikely to be associated [55], whereas, there is unknown serious adverse events from CoronaVac in phase III trials [55]. More serious adverse events were found in the control group than in the vaccine group [55]. Recently, The World Health Organization (WHO) recently stated that the Oxford/AstraZeneca vaccine offers protection against severe COVID-19, COVID-19-related hospitalization, and COVID-19-related death in the context without SARS-CoV-2 (COVID-19) variants, particularly B.1.351 [57]. The South Africa trials demonstrated lower vaccine efficacy compared with trials in other countries where B.1.351 variant was not dominant [58], whereas a recent study demonstrated that a two-dose regimen of the ChAdOx1 nCoV vaccine did not protect against mild-to-moderate B.1.351 COVID-19 variant [59]. Unfortunately, the difference of vaccine efficacy of Ad26COV2.S COVID-19 vaccine were demonstrated in the United States and South Africa (72 % vs 57 %) [60]. Two trials of COVID-19 vaccines were carried out, the first trial with the recombinant, replication-incompetent adenovirus serotype 26 vector SARS-CoV-2 vaccine (Ad26.COV2.S) in South African moderate-to-severe-COVID-19 and severe-to-critical patients, demonstrated the significant efficacy of 64.0 % (95 % CI : 41.2-78.7) and 81.7 % (95 % CI : 46.2-95.4) when the predominant circulating strain was the B.1.351 variant, respectively [61].

The second trial in South Africa included 800 participants by using the BNT162b2 messenger RNA vaccine revealed satisfied efficacy, with nine cases (with six confirmed strains of the B.1.351 lineage) in the placebo group and no COVID-19 cases in the vaccine group [62]. A trial on 2 doses of BNT162b2 mRNA COVID-19 vaccine (Pfizer/BioNtech vaccine) efficacy was conducted among 6,680 healthcare workers who were employed at the Hadassah Hebrew University Medical Center (HHUMC) in Jerusalem, Israel demonstrated that since first dose of vaccination, the incidence of COVID-19 infection among vaccinated healthcare workers (no. of COVID-19-infected healthcare workers (tested at HHUMC or community clinics) per 1,000 healthcare workers) at week 1 (5,297 participants received a first dose of vaccine), week 2 (5,247 participants received a first dose of vaccine), at week 3 (5,200 participants received a first dose of vaccine), at week 4 (5,164 participants received a first dose of vaccine, 4,864 participants received second dose of vaccine, and 300 participants did not receive second dose of vaccine), at week 5 (5,050 participants received a first dose of vaccine, 4,934 participants received second dose of vaccine, and 116 participants did not receive second dose of vaccine), at week 6 (4,947 participants received a first dose of vaccine, 4,793 participants received second dose of vaccine, and 154 participants did not receive second dose of vaccine), and at week 7 (4,079 participants received a first dose of vaccine, 4,069 participants received second dose of vaccine, and 10 participants did not receive second dose of vaccine) were 9.4, 9.0, 5.6, 2.1 (at week 4-received second dose = 1.4, at week 4-did not receive send dose = 13.3), 0.6 (at week 5-received second dose = 0.6, at week 5-did not receive second dose = 0), 0.4 (at week 6-received second dose = 0.4, at week 6-did not receive second dose = 0), and 1.2 (at week 7-received second dose = 1.0, at week 7-did not receive second dose = 100.0), respectively [63]. In this trial, two doses of the Pfizer/BioNTech vaccine was administered 21 days apart that started on December 20, 2020 [63]. By December 20, 2020, 5,297 of 6,252 (84.7 %) healthcare workers who had not been previously infected with COVID-19 were vaccinated within 8 weeks [63]. Another trial in Qatar among COVID-19-B.1.1.7-variant-infected and COVID-19-B.1.351-variant-infected individuals who received BNT162b2 mRNA (Pfizer/BioNTech) vaccine demonstrated the percentage of the vaccine efficacy (95 % CI) after one dose of vaccination (892 B.1.1.7-variant-infected participants), at least 14 days after second dose of vaccination (50 B.1.1.7-variant-infected participants), after one dose of vaccination (1,329 B.1.351-variant-infected participants), at least 14 days after second dose of vaccination (179 B.1.351-variant-infected participants), after one dose of vaccination (30 B.1.1.7-variant-severe, -critical, or -fatal disease participants), at least 14

days after second dose of vaccination (0 B.1.1.7-variant-severe, -critical, or-fatal disease participants), after one dose of vaccination (45 B.1.351-variant-severe, -critical, or -fatal disease participants), at least 14 days after second dose of vaccination (0 B.1.351-variant-severe, -critical, or -fatal disease participants), after one dose of vaccination (139 participants with any severe, critical, or fatal disease SARS-CoV-2), and at least 14 days after second dose of vaccination (3 participants with any severe, critical, or fatal disease SARS-CoV-2) were 29.5 (22.9-35.5), 89.5 (85.9-92.3), 16.9 (10.4-23.0), 75.0 (70.5-78.9), 54.1 (26.1-71.9), 100.0 (81.7-100.0), 0.0 (0.0-19.0), 100.0 (73.7-100.0), 39.4 (24.0-51.8), and 97.4 (92.2-99.5), respectively [64]. A cohort study from December 16, 2020, through February 9, 2021 on the mRNA-1273 vaccine (Moderna) and BNT162b2 vaccine (Pfizer) efficacy among 36,659 healthcare workers in California (UCSD and UCLA), USA who received the first dose of vaccine, and 28,184 of these healthcare workers (77 %) received the second dose of vaccine. The results of this cohort study on 36,659 participants who were eligible for SARS-CoV-2 (COVID-19) testing demonstrated that the new SARS-CoV-2 (COVID-19) infection (total of 379 new SARS-CoV-2 (COVID-19)-infected cases, total of 14,604 tested-and-vaccinated participants) at days 1-7, days 8-14, days 15-21, days 22 or later, after the first dose and before second dose of vaccination were 145 (5,794 COVID-19-tested-and-vaccinated participants, 35,673 (97.3 %) eligible-for-COVID-19-testing participants) , 125 (7,844 COVID-19-tested-and-vaccinated participants, 34,404 (93.8 %) eligible-for-COVID-19-testing participants), 57 (7,958 COVID-19 tested and vaccinated participants, 32,667 (89.1 %) eligible-for-testing participants), and 15 (4,286 COVID-19-tested-and-vaccinated participants, 32,327 (88.2 %) eligible-for-COVID-19-testing participants) cases, respectively, and at days 1-7, days 8-14, and days 15 or later after the second dose of vaccination were 22 (5,546 COVID-19-tested-and-vaccinated participants, 23,100 (63.0 %) eligible-for-testing participants), 8 (4,909 COVID-19-tested-and-vaccinated participants, 16,082 (43.9 %) eligible-for-testing participants), and 7 (4,167 COVID-19-tested-and-vaccinated participants, 14,990 (40.9 %) eligible-for-testing participants), respectively [65]. This cohort study results suggests that the efficacy of these vaccines must be maintained outside the trial setting. Even with a surge of the COVID-19-B.1.1.7 variant, the major decrease of new COVID-19-infected cases among those who received two doses of the vaccine was observed in up to 80 % of COVID-19-infected cases [66]. Even in the presence of a high rate of SARS-CoV-2 (COVID-19) infection in the communities, widespread and effective vaccination among healthcare workers provides safe environment [66]. Nevertheless, data from these trials emphasize the critical importance of continued public health

control measures (daily symptom screening, regular testing, social (physical) distancing, and facial masking), even in high incidence of vaccination environments, until herd immunity is achieved [65].

The WHO recommends on the effectiveness of COVID-19 vaccines in the context of SARS-CoV-2 (COVID-19) variants that : 1) The existing mechanism for tracking and evaluating COVID-19 variants that may affect vaccine composition must be enhanced, 2) Priority should be given to vaccinating high-risk groups everywhere, 3) Enhanced genomic surveillance must be backed by rapid haring of genetic and meta-data to allow for global coordination and response, 4) The manufacturers must be prepared to adjust to the SARS-CoV-2 (COVID-19) viral evolution, 5) Trials must be designed and maintained to allow any changes in efficacy to be assessed, and 6) Governments and donors, as well as development banks, should further support COVAX [57].

The WHO recommendations on COVID-19 vaccines in the context of SARS-CoV-2 (COVID-19) variants contribute to the plans of the next steps on COVID-19 vaccine production, such as Novavax, whose first-generation vaccine has not been authorized yet in the United States, announced on January 28, 2021 that it was working on developing a booster, a combination bivalent vaccine, or both to protect against SARS-CoV-2 (COVID-19) variants; Moderna announced on February 24, 2021 that a booster vaccine candidate based on B.1.351 had been shipped to NIAID for a phase 1 trial; and Pfizer and BioNTech announced on February 25, 2021 that they had started evaluating the safety and immunogenicity of a third dose of their vaccine to observe whether it would boost immunity to SARS-CoV-2 (C)OVID-19) variants, particularly B.1.351 [56].

It is hard to predict long-term risk of immune escape. From experience with avian coronavirus, vaccines against one variant will protect against similar variant, but not always against highly divergent variants. As SARS-CoV-2 (COVID-19) variants are too divergent, similar to flu vaccines, COVID-19 vaccines will be changed. It might be more robust that multivalent vaccines include the viral nucleoprotein in long term.

Modifying COVID-19 vaccines would probably be the most straightforward step in involving SARS-CoV-2 (COVID-19) variants. More challenging will be deciding when and how to deploy

COVID-19 vaccines 2.0. Much more proactively rapid identification and characterization of variants of concern will be provided by the national and global surveillance.

The National Health Service, England recommends COVID-19 vaccines in people aged 80 and over, some people aged 70 and over, some people who are clinically extremely vulnerable, people who live or work in care homes, and health and social care workers [67]. Mostly, the side effects of COVID-19 vaccines are mild and should not last longer than one week that include a sore needle-went arm, tired feeling, headache, achy feeling, and felling or being sick [67]

COVID-19 Vaccines, Thrombosis and Thromboembolism

Due to concerns about thrombosis or thromboembolism, the European Union's top pharmaceutical regulators concluded on April 18, 2021 after more than 12 European countries [68], including Bulgaria, Denmark and Norway [69] stopped distribution of the AstraZeneca/Oxford COVID-19 vaccine that the AstraZeneca/Oxford COVID-19 vaccine, the particularly important vaccine accounting for more than 90 % of distributed COVAX's vaccines [70] was safe [71]. The European Medicines Agency (EMA) stated that the vaccine was not associated with an increase in the overall risk of thromboembolic events or blood clots [71, 72]. The vaccine's benefits in protecting persons from COVID-19 with the associated risks of deaths and hospital admissions outweigh the possible risks, supported by the World Health Organization (WHO)' s announcement on April 17, 2021 and recommended the continuation of the vaccine [68-70], according to its own global database of safety report and data from 27 million doses of AstraZeneca/Oxford COVID-19 vaccine administered in India [70]. The rate of clotting conditions after COVID-19 vaccination are fewer than expected [70]. Bulgaria, Denmark, Iceland, Norway, Italy, Austria, and Thailand (temporary stopped using the vaccine) have stopped using certain batches of the vaccine as a precautionary measure [69]. Following the announcements on April 18, 2021 from the EMA and the United kingdom (UK) regulators that the AstraZeneca/Oxford COVID-19 vaccine is safe and immunization should continue, more than 12 countries restarted their AstraZeneca/Oxford COVID-19 vaccination programs, including Indonesia, Italy, Germany, UK, Australia, and Mexico [69-71]. In the UK, more than 11 million individuals have already received at least one dose of the AstraZeneca/Oxford COVID-19 vaccine and there has been no evidences of excess blood clots or deaths occurring, whereas Germany had

signed a deal for 30 million doses with Pfizer/BioNTech in September 2020 [69]. Europe's drug regulator also has backed the AstraZeneca/Oxford COVID-19 vaccine and promotes the campaign " COVID-19 can be deadly and vaccination saves lives " [69]. France also restarted AstraZeneca/Oxford COVID-19 vaccination program, but only for those older than 55 years following an EMA's finding that it could not exclude and increased thromboembolism risk in individuals younger than 55 years, based on a background rates of blood clotting and a review of cases in those who are and are not vaccinated [70]. Nevertheless, Norway, Sweden, and Denmark are continuing their pauses, whereas they collect more information [70]. Due to being about to receive shipments of the COVAX's vaccine, Cameroon suspended use of the AstraZeneca/Oxford COVID-19 vaccine [70].

In Europe, there have been around 30 cases with thromboembolic events, including a report that a 50-year-old man had died in Italy with the development of deep vein thrombosis (DVT), whereas approximately 5 million Europeans have already received the AstraZeneca/Oxford COVID-19 vaccine [69]. In Norway, in early March 2021, four persons developed blood clots a few days after having the AstraZeneca/Oxford COVID-19 vaccine [71]. Later, another individual in Austria was hospitalized with a clot on the lung and finally died, 10 days after vaccination [71]. In Denmark, a death of vaccinated person involving a blood clot has been reported [71]. The medical researchers in Germany had demonstrated an association between the AstraZeneca/Oxford COVID-19 vaccine and one especially rare type of blood clot hypothesized to have occurred in a "very small number" of persons who have received the vaccine [71-73]. The incidence of this type of blood clot is 3 to 4 persons per million persons in the general unvaccinated population [71-73]. There is no associated increase in cases in the vaccinated group [71-73]. Schultz *et al* demonstrated five healthcare workers, 32-54 years of age developed venous thrombosis and thrombocytopenia 7 to 10 days after receiving the first dose of adenoviral vector vaccine (AstraZeneca/Oxford COVID-19 vaccine) [72]. All these patients had high levels of antibodies to platelet factor 4-polyanion complexes and they had no previous exposure to heparin [72]. Hypothetically, they represent a rare vaccinated-associated variant of spontaneous heparin-induced thrombocytopenia that refers to as vaccine-induced immune thrombotic thrombocytopenia due to these five cases occurred in a population of more than 130,000 vaccinated individuals [72].

Nevertheless, AstraZeneca stated that there was no evidence of an increase risk of thrombocytopenia, deep vein thrombosis (DVT), or pulmonary thromboembolism in any defined age group (particularly in patients ages 20 to 50 years [68]), gender, batch or in any particular country [71]. The type of found blood clots included cerebral venous sinus thrombosis and disseminated intravascular coagulation (DIC) [68]. Blood clots occur in approximately one in 1,000 persons [71]. Pulmonary thromboembolism and deep vein thrombosis are estimated one to two adults per 1,000 adults per year in the United States [68]. This order of magnitude is higher than the Europe's 37 such events out of 17 million AstraZeneca/Oxford COVID-19 vaccine recipients [68]. Several vaccinated persons also presented with thrombocytopenia and having blood schistocytes [68]. These complications occurred 7 to 14 days after vaccination [68]. In the European Union countries, there were 7 cases of DIC and 18 cases of cerebral venous sinus thrombosis (presenting with headache, blurred vision, and weakness of part of the face or limbs [71] suspected of association with the AstraZeneca/Oxford COVID-19 vaccine, as of March 18, 2021 [68].

The scare causes understandable anxiety for those who have already had their AstraZeneca/Oxford COVID-19 vaccine, or who may be waiting for their second dose of vaccination. Further studies are urgently needed to identify exact association between thrombosis or thromboembolism events and the COVID-19 vaccination.

Transforming COVID-19 Proteins into Nanoparticles COVID-19 Vaccines

It was hypothesized that by converting the RBD into a nanoparticle (similar in size to the virus itself) that would generate higher levels of neutralizing antibodies and its ability to generate an immune response would increase instead of letting it remain in its natural form as a small protein. A group of scientists developed a technology that easily converted small, purified proteins into particles by using small nanoparticles formed from naturally-occurring fatty components, or liposomes. A the new study included cobalt-porphyrin-phospholipid (CoPoP), a special lipid within the liposomes that enables the RBD protein to rapidly bind to the liposomes that generate an immune response by forming more nanoparticles. A previous study revealed that when the RBD was converted into nanoparticles, it maintained its correct, three-dimensional shape and the particles were stable in incubation conditions similar to those in the human body. High antibody

level were induced when laboratory mice and rabbits were immunized with the RBD particles. For enhancing the immune response, only the approach with particles containing CoPoP gave strong responses, whereas other vaccine adjuvant technology does not have the capacity to convert the RBD into particle-form. This method could assist information of future vaccine design that targets this specific antigen [73, 74].

Shinde and colleagues conducted a 2a-b trial in South Africa on the efficacy of NVX-CoV2373 COVID-19 vaccine against the COVID-19-B.1.351 variant by randomization of assigned HIV-negative adults between the ages of 18 and 84 years or medically stable HIV-positive participants between the ages of 18 and 64 years in a 1:1 ratio to receive two doses of either the NVX-CoV2373 vaccine (5 µg of recombinant spike protein with 50 µg of Matix-M1 adjuvant) or placebo [75] . Among 6,324 participants who underwent screening, 4387 subjects received at least one dose of vaccine or one injection of placebo. About 30 % of the participants were seropositive for ARS-CoV-2 (COVID-19) at baseline. Among 2,684 baseline-seronegative participants (6 % this participants were HIV-positive and 94 % of this participants were HIV-negative), the trial results in 15 participants in the vaccine group and 29 participants in the placebo group demonstrated that two doses of the NVX-CoV2373 vaccine, an adjuvanted, recombinant nanoparticle vaccine provided an efficacy of 49.4 % (95 % CI : 6.1-72.8) against the 5 symptomatic B.1.351-variant-COVID-19 patients [75]. Among HIV-negative participants, vaccine efficacy was 60.195 % CI : 1-80.1) and post hoc vaccine efficacy against COVID-19-B.1.351 variant was 51.0 % (95 % CI : -0.6-76.2) [75]. In the vaccine group, preliminary local and systemic reactogenicity events were more common, whereas serious adverse events were rare in both vaccine and placebo groups [75]. At the time of this NVX-CoV2373 vaccination trial in South Africa, the trial sponsor " Novavax " reported that all five cases with severe B.1.351-variant-COVID-19 were the placebo group, contributing to not receiving the regulatory approval [76]. Nevertheless, the efficacy and safety profiles, favorable storage conditions, and global manufacturing partnerships make NVX-CoV2373 vaccine an attractive candidate [76].

Nasal Spray Delivery of COVID-19 Vaccines

As of March 29, 2021, Oxford University, UK launched a phase I trial investigating the nasal spray delivery of its AstraZeneca (AZ)-partnered COVID-19 vaccine (ChAdOx1 nCoV-19). The study will be conducted at the Oxford University's Jenner Institute and is aimed to determine the ability of improvement of protection against mild COVID-19 and transmission, including monitoring the safety of the delivery technique and any adverse reactions after vaccination by intranasal administration of AZ-vaccine via an intranasal spray device, compared to the currently intramuscular injection delivery as part of the national roll-out of the same vaccine. Thirty healthy participants with 18-40 years of age will be enrolled in the early stage of trial. The study participants' symptoms will be followed-up by filling in an electronic diary card of any symptoms following vaccination with having blood and nasal swabs taken at most of the follow-up visits for a total of four months of the study participants' following-up [77]. As of March 10, 2021, Rokote Laboratories Finland Ltd., a newly founded, Finnish academic spin-out company is developing and bringing to the world markets a nasal spray COVID-19 vaccine, based on gene transfer technology. An adenovirus vector is used in this vaccine to deliver a cloned DNA strand that causes nasopharyngeal cells to produce SARS-CoV-2 (COVID-19) viral protein inducing an immune response with good results in animal models. This technology of gene therapies has been successfully used in clinical trials treating cancer and cardiovascular diseases. The clinical trial will be began in Finland within a few months [78].

The red algae-derived NasitrolTM nasal spray, based on iota-carrageenan (a sulfate polysaccharide synthesized by red algae) and produced by Amcyte Pharma demonstrated antiviral activity and clinical efficacy in the common cold treatment. Active substance, iota-carrageenan of Nasitrol is thought to exert antiviral activity via its interaction with the COVID-19 viral surface that prevent viral entry and capturing viral particles released by host infected cells. Nasitrol is formulated to decrease the COVID-19 viral load in the upper respiratory tract, preventing from viral proliferation and lung spreading. The University of Tennessee Health Science Center, USA revealed in its previous in vitro study that the NasitrolTM formulation can inhibit SARS-CoV-2 (COVID-19). In the new study of Amcyte Pharma (NCT04590365), carried out in 394 clinically-healthy-not-yet-been-COVID-19-vaccinated clinicians, nurses, and other medical professionals who provided care

to COVID-19 patients at 8 hospital ICUs. Four daily doses of Nasitrol spray or placebo were administered to randomly assigned participants for 21 days with the primary end point of clinical COVID-19 infection, confirmed by RT-qPCR testing, after 21 days of treatment. The study has been also carried out in Argentina at the Cesar Milstein Research Institute and the CEMIC University Hospital in Buenos Aires, and sponsored by the Ministry of Science, Technology and Innovation of Argentina. The preliminary report of the study revealed that the incidence of COVID-19 infection was significantly lower in the Nasitrol group, compared to the placebo group, 1 % versus 5 %, respectively [79].

Neurological and Other Adverse Side Effects after COVID-19 Vaccination

The development of COVID-19 vaccines with wide range of the used technology platforms [80] began as soon as the SARS-CoV-2 (COVID-19) was described and published in early January 2020 [81]. Some of these used-COVID-19-technology platforms have not previously used [82]. Human vaccines against coronaviruses have not been previously licensed, whereas the Pfizer/BioNTech vaccine has become the first licensed [83]. More than past decades, vaccine hesitancy has steady increased due to fear of post-vaccination side effects, whereas some reports demonstrated the post-immunization side effects, the majority of demyelinating diseases, such as multiple sclerosis (MS) from Hepatitis B vaccine [84], acute disseminated encephalomyelitis (ADEM) from influenza vaccine [85] and human papillomavirus vaccine [86], transverse myelitis from rabies vaccine [87], and optic neuritis from yellow fever vaccine [88]. Other frequently reported neurological adverse effects after vaccination included Guillain-Barre´ syndrome (GBS) from influenza vaccine [89], oral polio vaccine [85], and tetanus vaccine [90], encephalopathy from whole-cell pertussis vaccine [91] and influenza vaccine [85], seizure from diphtheria vaccine, tetanus toxoids and whole-cell pertussis vaccine (DPT) [85] and measles, mumps, and rubella vaccine (MMR) [92], and autism from MMR vaccine [93]. Functional neurological disorder (FND), a brain-based disorder, may be physically or emotionally triggered by vaccination, medical or surgical procedures, and injury and exacerbated by psychological, environmental, and/or biological factors [94]. Some previous studies indicated that post-vaccination demyelination was most likely acting as triggers of clinical disease expression in persons who already had an underlying disease process [94, 95]. Due to given the speed of COVID-19 vaccine

development, concerns among people exist [85] contributing to the willingness of people getting COVID-19 vaccines varies from 55 % to 90 % [96-98]. The World Health Organization (WHO) announced that by February 2021, none of the 11 COVID-19 vaccine candidates had finished the phase III endpoints per protocol [99].

The landscape of the 11 COVID-19 vaccine candidates is demonstrated in the aspects of : 1). Vaccine platform, 2). Vaccine candidate name,3) developer, 4) number of doses, 5) outcomes stem cell transplantation in treating COVID-19 in humans, 6) published sample size, 7) Phase III trial sample size, 8) neurological adverse effect, and 9) severe adverse effect, as the following [85] :

1)1) Inactivated, 2) CoronaVac, 3) Sinovac, 4) 2, 5) Phase I/II in the Lancet Infectious Diseases [100], 6) 743 (143+600), 7) 13,060 in Brazil; 13,000 in Turkey; 1,620 in Indonesia, 8) none, and 9) Phase I : one case of urticaria 48 hours after the first dose of in the 6 µg group in the days 0 and 14 vaccination cohort.

2)1) Inactivated, 2) no name announced, 3) Wuhan Institute of Biological Products/Sinopharm, 4) 2, 5) Phase I and II in JAMA [101], 6) 320 (96 + 224), 7) 15,000 in UAE; 600 in Morocco, 8) none, and 9) Phase I : one case with swelling and pain of left knee joint and subcutaneous hematoma and another case of acute appendicitis, in high dose group Phase II : one case of fever (39.0o C), another case of skin laceration from left eyebrow arch to hair source, multiple skin contusion at nasal back.

3)1) Inactivated, 2) BBIBP-CorV, 3) Beijing Institute of Biological Products/Sinopharm, 4) 2, 5) Phase I/II in The Lancet Infectious Diseases [102], 6) 640 (192 + 448), 7) 15,000 in UAE; 3,000 in Argentina, 8) none, and 9) Phase II : one placebo recipient in the 4 µg days 0 and 21 group reported grade 3 fever.

4)1) Inactivated, 2) Covaxin, 3) Bharat Biotech 4) 2, 5) Bharat Biotech statement, 6) 1,100, 26,000 in India, 8) not available, and 9) not available

5)1) Non-replicating viral vector, 2) AZD1222, 3) University of Oxford/AstraZeneca, 4) 2, 5) Phase I/II in the Lancet [103] and Phase III in the Lancet [104], 6) 1,077 + 11,636, 7) 40,051, 8) some cases of transverse myelitis were reported in Phase III trial, 2 of them was

considered irrelevant, and 9) one case of hemolytic anemia in the controlled vaccine (a meningococcal conjugate vaccine); one death case in Brazil in Phase III trial

6)1) Non-replicating viral vector, 2) Ad5-nCoV, 3) Cansino Biological Inc./Beijing Institute of Biotechnology, 4) 1, 5) Phase I and Phase II in the Lancet [105], 6) 616 (108 + 508), 7) 500 in Russia; 40,000 in Pakistan, Saudi Arabia, and Mexico, 8) none, and 9) Phase I : nine participants (two (6 %) in the low dose group, two (6 %) in the middle dose group, and five (14 %) in the high dose group) had an episode of severe fever. One (3 %) from the high dose group reported severe fever along with severe symptoms of fatigue, muscle pain, and dyspnea. One case in the high dose group reported severe fatigue and joint pain.

7)1) Non-replicating viral vector, 2) Gam-COVID-Vac (Sputnik V), 3) Gamaleya Research Institute, 4) 2, 5) Phase I/II in the Lancet [106], phase III in the Lancet [107], 6) 76 + 21,977, 7) 40,000 in Russia, 8) Data not be published yet, and 9) few serious adverse events, data not be published yet.

8)1) Non-replicating viral vector, 2) JNJ-78436735 (formerly Ad26.COV2.S), 3) Janssen Pharmaceutical companies, 4) 1/2, 5) Phase I/IIa data in the pre-print server MedRxiv [108], 6) 796 (402 + 394), 7) 90,000, 8) none, and 9) 3 cases (0.8 %) in cohort 1a (aged 18 to) and one case in cohort III (older than x) had grade 3 local adverse effect; 41 cases (10.9 %) in cohort Ia, 5 cases (20 %) in cohort 1b (aged 18 to 65) and 3 cases in cohort III (older than 65) had grade III systemic adverse effect

9)1) Protein subunit, 2) NVX-CoV2373, 3) Novava, 4) 2, 5) Phase I data in The New England Journal of Medicine [109], 6) 131, 7) 10,000 in the UK, 8) none, and 9) first injection : two cases (2 %), one each in group D (received 25-µg doses of rSARS-CoV-2 plus Matrix-M1, including three sentinels), had severe adverse events (malaise, fatigue, and headache); second injection : one case, in group D, had a severe local event (tenderness), and 8 cases, one or two cases in each group, had severe systemic events.

10)1) RNA, 2) mRNA-1273, 3) Moderna/NIAID, 4) 2, 5) Phase I data in the New England Journal of Medicine adult [110]/older adult [111], Phase III data in the New England Journal of Medicine [112], 6) 45 + 30,420, 7) 30,000, 8) none, and 9) one participant of transient urticaria related to the first vaccination. Three cases with severe event(s) not specified.

11)1) RNA, 2) BNT162, 3) BioNTech/Fosun Pharma/Pfizer, 4) 2, 5) Phase I data in the New England Journal of Medicine [113], Phase II/III data in the New England Journal of Medicine [114], 6) 332 + 43,448, 7) 43,998, 8) none, and 9) none.

Basic Platforms of COVID-19 Vaccines

1.Inactivated Vaccine

This vaccine platform, one of the most traditional platforms uses the attenuated virus that requires stimulation of the cellular immunity using adjuvants (mostly using alum adjuvant) [115]. Currently, no adjuvant-related severe adverse effect has been reported [85]. A febrile reaction, a common adverse reaction that might be due to high level of inflammatory cytokines related with more immune responses and a febrile reaction [116]. Nevertheless, no febrile seizure was reported in COVID-19 clinical trials [85]. Symptoms of immunization-stress-related response (ISRR) and psychologic non-epileptic seizures (PNES-clinical manifestations of ISRR) that can be easily spread by sound, sight, or oral communication to others were also reported in this vaccine platform [117]. A potential trigger of PNES could be from the circumstantial stress and profound psychological distress [118]. This serious adverse event contributed the Brazilian drug administration authority to abruptly stop the Sinovac vaccine clinical trials. Nevertheless, the Brazilian drug administration authority re-started the Sinovac vaccine clinical trials, once it was established that the trial participant committed suicide [85].

2.Viral Vector Vaccine

This vaccine platform uses recombinant DNA techniques that consist of a recombinant virus, in which genes encoding viral antigen (s) have been cloned. The viral vector vaccine will enter host cells to produce antigen without new virus particles formed. Due to a broad range of viral tropism, high level of transgene expression, and high transduction efficiency, this adeno-based virus vectors or recombinant adenovirus are widely used [119]. This viral-vector vaccine platform was used against Ebola virus without seriously identified side effects by using the vesicular stomatitis virus-based Ervebo vaccine [120]. This viral

vector platform was successful in developing MERS vaccine without reporting severe adverse events within 12 months [121, 122]. Following a report of a case of transverse myelitis (TM), a viral-vector-platform ChAdOx1 nCOV-19 clinical trial stopped [123]. After the confirmation of MS in the volunteer, the global clinical trials resumed [123]. There were 3 cases of TM identified among 11,636 participants (one case of possible vaccine-related-idiopathic-short segment spinal cord demyelination, other two cases being likely to be irrelevant or pre-existing, all four deaths being vaccine-unrelated, caused by fungal pneumonia, homicide, blunt force trauma, and road traffic accident) [63], according to the interim analysis of their phase III trial [122-124]. Janssen Ad26.COV2.S vaccine, another promising viral-vector-platform vaccine candidate, released its safety data via the United States Food and drug Administration (FDA) briefing document. There were one case of facial paralysis and one case of GBS in vaccine group, but these two cases were hypothesized to have insufficient data to for vaccine causal relationship determination [63]. A precise gene delivery into the host cell with a energetic immune response with avoidance of handling of infectious viral particles had been demonstrated by the viral-vector vaccine platform, whereas the viral-vector prior exposure could decrease the vaccine efficacy. The integration of the viral genome into human host genome has contributed to the concerns about cancer induction. Due to a common feature of similarity to neural cells transformed by adenovirus [124], findings of the molecular mimicry between human proteins and SARS-CoV-2 (COVID-19) spike protein and the hypothesis that autoimmune reactions could be triggered by a cross-reaction with an adenovirus vector itself or with proteins coded by the RNA vaccines, individuals' sera vaccinated with adenoviral COVID-19 vaccines are suggested to be tested for the possible presence of antibodies Against neuronal proteins [125]

3.Protein Subunit Vaccine

A protein subunit vaccine, based on recombinant antigenic proteins or the synthetic peptides, is composed of at least one type of heterologous-expression-systems-produced viral antigen [126], contributing to being safer without the infectious virus involvement [127]. The prime targets for the subunit vaccine platform are S protein and its antigenic fragments [127]. This vaccine platform needs an adjuvant to support the immune responses due to low

immunogenicity of this vaccine platform [85]. NVX-CoV2373 (phase III) was the only candidate of this vaccine platform that was composed of trimeric full-length SARS-CoV-2 spike glycoproteins and Matrix-M1 adjuvant (a saponin-based adjuvant composed of Quillaja saponins, phospholipid, and cholesterol) [109]. Several previous studies demonstrated a local transient immune response with activation and recruitment of central immune cells to distant lymph nodes, resulted by the therapeutic doses of Matrix-M1 adjuvant [128]. This adjuvant has been used in the vaccine development of Ebola, respiratory syncytial virus, and influenza virus, but none has yet been accepted and licensed [128]. No neurological adverse-side effect was identified, according to phase I- and I/II-NVX-CoV2373-trial published data. A COVID-19 case with transient fever was reported, whereas fatigue and joint pain were the most identified severe systemic events [85]. Further evidence to confirm the long-term effect in human from this vaccine platform is urgently needed. Recently, a clinical phase IIa-b trial on safety and efficacy of NVX-CoV2373 COVID-19 vaccine among 4,387 at-least-one-vaccine-injection participants of 6,324 underwent-COVID-19-screening participants (30 % of SARS-CoV-2-baseline-screening participants having SARS-CoV-2-baseline-seropositivity, 2,684 SARS-CoV-2-baseline-seronegative participants having 6 % HIV-positive and 94 % HIV-negative) in 18 sites in South Africa (ClinicalTrials.gov number, NCT04533399, funded by Novavax and the Bill and Melinda Gates Foundation) demonstrated that 15 vaccinated participants and 29 placebo participants predominantly developed mild-to-moderate COVID-19 (vaccine efficacy, 49.4 %; 95 % CI : 6.1 to 72.8). Among HIV-negative participants, the vaccine efficacy was 60.1 % (95 % CI : 19.9 to 80.1). SARS-Cov-2 (COVID-19)-B.1.351 variant was identified in 38 (92.7 %) of 41 sequenced isolates. Among the HIV-negative participants, post hoc vaccine efficacy against SARS-CoV-2 (COVID-19)-B.1.351 variant was 51.0 % (95 % CI : -0.6 to 76.2). Serious adverse events were rare in both vaccine and placebo groups, whereas preliminary local and systemic reactogenicity events were more common in the vaccine group [129]. In preventing COVID-19, NVX-CoV2373 COVID-19 vaccine was efficacious, particularly in HIV-negative individuals.

4.Nucleic Acid Vaccine

Nucleic-acid vaccine platform, consisting of mRNA or DNA, is able to be adapted rapidly when confronts the emergence of new viruses [85]. This vaccine platform introduces an mRNA sequence coding for the S protein or RBD by interfacing with human host's cells, producing specific antigen outside the host's cell surface, and activation of the immune system [130]. This vaccine platform does not contain inactivated pathogen or viral particles, thus, it is non-infectious. It is safer, compared to the traditional vaccine platform. Once the protein is produced, the RNA strand in this platform vaccine is degraded. RNA does not integrate itself into the host genome. Modified nucleosides are included in this vaccine platform to prevent mRNA degradation [130]. Lipid nanoparticle, a carrier molecule, is needed to enter the mRNA into cells. Approximately, 2 % of this platform vaccine recipients developed a severe fever [130]. Due to the limited available data, further studies on the adverse side effect in this vaccine platform are urgently needed. The first license COVID-19 vaccine in this platform, Comirnaty (BNT162b2) was developed by Pfizer and BioNTech [130]. According to the data released by the US FDA [114] demonstrated that no neurological adverse event was identified in the vaccine group (18,555 completed the two doses schedule among a total of 37,796 enrolled participants, 43,448 phase II/III-received-vaccine-injection participants) [131]. In placebo group, one of the four deaths was caused by hemorrhagic stroke [131]. Other mRNA platform vaccine in the fact sheet published by the US FDA [132], mRNA-1273, developed by Moderna demonstrated the vaccine efficacy of 94.1 % among the vaccine group of 14,134 participants and severe adverse events among 1.0 % of the participants in both vaccine group and placebo group. A case of Bell's palsy was reported at day 32 after vaccination, but there was no report of this case in the published phase III data [112].

A recent prospective observational study on safety and efficacy in phase III trials of the Oxford-AstraZeneca (ChAdOx1 nCoV-19, a viral-vector platform vaccine) COVID-19 and the Pfizer/BioNTech (BNT162b2, a nucleic-acid platform vaccine) vaccines was conducted between December 8, 2020 and March 10, 2021 by examination of the proportion and probability of self-reported systemic and local side-effects within 8 days of vaccination among 627,383 UK individuals using the COVID Symptom Study program application who received one dose of the ChAdOx1 nCoV-19 vaccine or one or two doses of the BNT162b2 vaccine. All data analyses were adjusted by sex, age (55 years or below vs >

55 years), comorbidities (binary variable, with or without comorbidities), obesity (BMI < 30 kg/m2 vs at least 30 kg/m2), and healthcare worker status (binary variable). Of total participants, 345,280 participants reported being vaccinated one dose of ChAdOx1 nCoV-19 and 282,103 participants received one dose of BNT162b2, of whom 28,207 vaccinated a second dose of BNT162b2. After the first dose of ChAdOx1 nCoV-19, after the first dose of BNT162b2, and after the second dose of BNT162b2 demonstrated the systemic side-effects of 33.7 % (116,473 of 345,280), 13.5 % (38,155 of 282,103), and 22.0 % (6,216 of 28,207) of the participants, respectively, and demonstrated the local side-effects of 58.7 % (104,282 of 177,655), 71.9 % (150,023 of 208,767), and 68.5 % (9,025 of 13,179), respectively. These data indicated that the systemic side-effects were more common (1.6 times after the first dose of ChAdOx1 nCoV-19 vs 2.9 times after the first dose of BNT162b2) among previously SARS-CoV-2 (COVID-19)-infected individuals (1.4 times after the first dose of ChAdOx1 nCoV-19) than those without known previous SARS-CoV-2 (COVID-19) infection (1.2 times after the first dose of BNT162b2). In consideration of SARS-CoV-2 (COVID-19) infection after vaccination with these two vaccine platforms, 2.99 % (3,106 of 103,622) of vaccinated individuals and 10.84 % (50,340 of 464,356) of unvaccinated or controlled participants tested positive for SARS-CoV-2 (COVID-19) infection. At 12, 21-44, and 45-59 days after the first dose, the significant decreases in COVID-19 infection risk was targeted at 60 % (95 % CI : 49-68) for ChAdOx1 nCoV-19, 69 % (95 % CI : 66-72) for BNT162b2, and 72 % (95 % CI : 63-79) for BNT162b2, respectively [133].

COVID-19 Vaccination in Solid Organ Transplant Recipients

Vaccine such as influenza vaccine, is administered in stable transplant recipients, although live attenuated virus vaccines are contraindicated, generally due to risk of disseminated infection [134, 135]. Neither efficacy, safety, nor durability are well known in transplant recipients due to exclusion of them from recent COVID-19 vaccine trials [134, 135]. Currently, there are no SARS-CoV-2 vaccine platforms using attenuated live virus approved in phase III trials. Nevertheless, if they are approved for use, concerns, including potential decrease in efficacy in immunocompromised patients, potential for vaccine-related allograft rejection, unknown durability of the immune response, and long-term safety data still exist. Due to experience with neither the influenza vaccine nor the adjuvant

recombinant zoster vaccine having been related to allograft rejection, successful administration of influenza and adjuvanted recombinant zoster vaccines to stable transplant recipients, and unanticipated vaccine-related adverse events to the allograft having not borne out, the influenza and adjuvanted recombinant zoster vaccines are able to be extrapolated to COVID-19 vaccines [135, 136]. In immunocompromised host, concerns for adenoviral vector vaccines are focused on a viral infection, but these concerns have no scientific evidence. Although newly approved adenoviral-vector use for vaccination, this vaccine platform has been used for decades for gene therapy for cancer and other rare diseases. Inactivated virus and protein subunit vaccine platforms that have been used in transplant recipients for other infections, such as human papilloma virus, pertussis, and hepatitis A and B, are currently under investigation for SARS-CoV-2 (COVID-19) infection in transplant recipients [135].

A previous prospective-Johns Hopkins University-institutional-review-board's-approval cohort study in the US was conducted among 436 transplant recipients (median age 55.9 years (IQR : 41.3-67.4 years), 61 % of women, 89 % of Caucasian transplant recipients, 52 % received the Pfizer/BioNTech (BNT162b2) vaccine and 48 % received the Moderna (mRNA-1273) vaccine, median time since transplant of 6.2 years (IQR : 2.7-12.7 years, maintenance immunosuppression regimen : 2 % of everolimus, 4 % of sirolimus, 9 % of azathioprine, 54 % of corticosteroids, 66 % of mycophenolate, and 83 % of tacrolimus, who underwent COVID-19 vaccination from December 16, 2020 to February 5, 2021. The participants underwent either standard venipuncture or the TAPII-blood-collection- device (Seventh Sense Biosystems)-at-home blood sampling [137], using an enzyme immunoassay (EUROIMMUN) for testing for antibodies to the S1 domain of the SARS-CoV-2 spike protein [138], whereas the anti-SARS-CoV-2 enzyme immunoassay (Roche Elecsys) was used to test for antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein in the venipuncture samples [137]. Both EUROIMMUN (sensitivity of 87.1 %, specificity of 98.9 % [139]) and Roche Elecsys (sensitivity of 84.0 %, specificity of 100 % [140]) tests are mRNA-vaccine-antigens correspondence and semiquantitative and consistent correlation with neutralizing antibodies [139-141].

These two assays are analogous to the antispike antibody assays in mRNA vaccine clinical trials that were used during immunogenicity assessments [137]. The study revealed

that following the first dose of COVID-19 vaccine at 20 days, a median (IQR : 17-24 days), 76 (of 436) participants (17 %, 95 % CI : 14 %-21 %) demonstrated detectable antibody (anti-S1 or anti-receptor-binding domain, 31 in 41 kidney transplant recipients, 28 in 37 liver transplant recipients, 9 in 12 heart transplant recipients, 4 in 5 lung transplant recipients, 1 in 1 pancreas transplant recipient, 2 in 3 multiorgan transplant recipients) [137]. Those participants who received Moderna (mRNA-1273) vaccine developed more antibody response, compared to those receiving Pfizer/BioNTech (BNT162b2) vaccine [137]. Older transplant recipients developed less antibody response (adjusted incidence rate ratio : 0.83 (95 % CI : 0.73-0.93) per 10 years; $p = 0.002$), compared to the younger group [137]. Nevertheless, younger transplant recipients not receiving anti-metabolite maintained immunosuppressives and those younger transplant recipients receiving the Moderna (mRNA-1273) vaccine developed more antibody responses, compared to the older recipients [137]. Transplant recipients who receiving anti-metabolite maintained immunosuppressive therapy developed less antibody response than those participants not receiving immunosuppression therapy (37 % vs 63 %, respective; adjusted IRR : 0.22 (95 % CI : 0.15-0.34); $p < 0.001$) that contrast with the early immunogenicity identified in mRNA vaccine trials [137]. These results also include 100 % antispike seroconversion by the day 15 after mRNA-1273 (Moderna) [142] and the day 21 after BNT162b2 (Pfizer/BioNTech) [143] vaccination that contrast with the early immunogenicity identified in mRNA vaccine trials [137]. Poor antibody-responses-to-spike-protein in transplant recipients following the first-dose-mRNA (Moderna and Pfizer/BioNTech) vaccination indicated that despite COVID-19 vaccination, such organ transplant recipients may still be at higher COVID-19-infection-early risk. Characterization of T-cell responses and memory B-cell and advanced-transplant-recipient immunophenotyping following COVID-19 vaccination will be significant in determining immunological responses and vaccination strategies following the COVID-19-second-dose vaccine.

As of December 31, 2020, when focusing on kidney transplant recipients, there is no evidence of mRNA-vaccine-platform (BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna))-induced off-target immune responses in large phase III clinical trials [144, 145], no replicative potential through homologous recombination demonstrated in AdV-vectored vaccine platform (AZD1222 (Oxford/AstraZeneca), JNJ78436735/Ad26.COV2.S (Janssen),

Convidecia (Ad5-nCoV), Sputnik V (Gamaleya)) [146], does not contain live virus in protein subunit vaccine platform (NVX-CoV2373 (Novavax), SARS-CoV-2 recombinant protein formulation (GSK/Sanofi) (Matrix-M1 contains the same QS21 saponin as the AS01 B adjuvant system contained in the recombinant varicella zoster vaccine) [147] and no association between AS03 exposure and graft rejection (high incidence of anti-HLA antibodies in KTR vaccinated with AS03-adjuvanted influenza vaccines) in protein subunit vaccine platform [145, 148], and does not contain live virus and limited data available in peer-reviewed literature in whole-inactivated (killed) vaccine platform (EpiVacCorona (Vector Institute), BBIBP-CoV (Sinopharm), CoronaVac (SinoVac) [149]. Several transplant organizations, such as American Society of Transplantation, The International Society for Heart and Lung Transplantation, American Association for the Study of Liver Diseases, American Society of Transplant Surgeons, International Transplant Nurses Society, The Transplantation Society, NATCO, UNOS, Leading the Way in Organ Transplantation, Canadian Society of Transplantation, Pediatric Infectious Diseases Society, Association of Organ Procurement Organizations, American Society for Histocompatibility and Immunogenetics, International Liver Transplantation Society, The Alliance, Transplant Infectious Disease, International Pediatric Transplant Association, and International Society of Vascularized Composite Allotransplantation established their statement on COVID-19 vaccination in solid-organ transplant (SOT) recipients as the following : 1) Pre-transplant vaccination of all SOT candidates as a priority whenever feasible, 2) Continued SOT-recipients-SARS-CoV-2 (COVID-19) vaccination and priority for vaccination of their household members and caregivers to decrease exposure risk for these vulnerable patients, 3) Continuation of an at-the-time-of-COVID-19-vaccination-stable-immunosuppression regimen to avoid the organ-rejection risk, and 4) Continued adherence of all transplant recipients to protect measures, including facial-mask wearing and physical distancing regardless of vaccination status [150]. Minimal risk to stable solid-organ-transplant recipients who are more likely to suffer a severe COVID-19 outcome than COVID-19 vaccine will be taken from COVID-19 vaccination.

A previous cohort study among 90 solid-organ-transplant (SOT) recipients with COVID-19 infection, included 17 lung-transplant recipients (LTRs) and kidney-transplant

recipients demonstrated overall mortality of about 24 % [151]. Several risk factors for poor outcomes in LTRs with COVID-19 infection [152-154] are listed in Table 5.

Risk Factors for Poor Outcomes in Lung Transplantation for COVID-19 Patients	Reference
Lower baseline forced-expiratory volume in one second (FEV_1)	[152]
New or worsening respiratory failure requiring high-flow oxygen	[152]
Non-invasive ventilation or intubation	[152, 153]
Presence of lung parenchymal opacities on hospitalization-chest radiograph	[152]
Longer time between symptom onset to beginning the COVID-19 anti-viral therapy	[152]
COVID-19-related pneumonia upon ICU admission	[154]

Table 5: Demonstrating risk factors for poor outcomes in lung transplantation for COVID-19 Patients

In addition to acute allograft rejection, the differential diagnosis of COVID-19-related pneumonia in LTRs includes aspiration pneumonia, acute lung injury, and potential opportunistic infectious pneumonia [155]. Outpatient observation is recommended when COVID-19-infected LTRs present with absence of gastrointestinal symptoms, such as nausea and vomiting, no severe disease symptoms, such as persistent fever (more than 38o C), confusion, hypotension, lethargy, etc., severe cough, absent or minimal dyspnea, pleuritic chest pain, or lung wheezing [156]. The conventionally diagnostic bronchoscopy with bronchoalveolar lavage (BAL) and transbronchial biopsies contributes to the risk of COVID-19 exposure to the healthcare providers [156]. A previous study demonstrated that COVID-19-infected LTRs could develop specific IgG antibody against SARS-CoV-2 (COVID-19) [157]. About 50 % of them who died had worse FEV1 and were diagnosed chronic lung allograft dysfunction (CLAD), compared with 25 % in discharged group [157]. Higher serum levels of interleukin (IL)-6, D-dimer, lactate dehydrogenase, and ferritin and lower lymphocyte counts were found in patients with disease progression [157, 158]. Computed tomography of the chest is useful for the diagnosis of pulmonary embolism, whereas bedside-Doppler ultrasonography is useful for the diagnosis of deep venous thrombosis

and assessment of the vascular volume status and the cardiac function [156]. COVID-19-infected LTRs who do not meet the ambulatory management criteria should be COVID-19-designated-area hospitalized that is similar to the general population [156]. The absence of cytokine release syndrome (CRS) is strongly indicated by a normal or significant reduction of IL-6 or C-reactive protein (CRP) levels [156]. The absence of bacterial superimposition is strongly indicated by a normal or downtrend of procalcitonin levels [156]. The D-dimer levels of at lease 3 µg/mL [159, 160], and presence of thrombocytopenia [161] and antiphospholipid antibodies [162] are related to increased risk of venous thromboembolic events (VTE). ISHLT criteria for reactivation of lung transplantation waitlist [163] are shown in Table 6.

Clinical Scenario	**Criteria for Reactivation**
Positive SARS-CoV-2 RT-PCR Asymptomatic	Two negative SARS-CoV-2 RT-PCR tests, 24-48 hours apart
	Asymptomatic patients with remaining positive RT-PCR more than 28 days
	Diagnosis if high risk mortality without a transplantation
	14 days since the diagnosis unless high risk mortality without a transplantation
Previous symptomatic COVID-19	Two negative SARS-CoV-2 RT-PCR tests, 24-48 hours apart
	Asymptomatic patients with remaining positive RT-PCR more than 28 days since the diagnosis if high risk mortality without a transplantation
	28 days from the onset of symptoms, can be shortened to 14-28 days if high risk mortality without a transplantation
	No other COVID-19-related organ damage
	Clinical resolution
Exposure to suspected or confirmed COVID-19 case within last 14 days	7 days post-exposure
	Two negative SARS-CoV-2 RT-PCR tests, 24-48 hours apart
	Asymptomatic
	High risk of mortality without an organ transplantation

Table 6 : Demonstrating the ISHLT criteria for the reactivation of waitlisted COVID-19 patients

In patients awaiting for transplantation who are unlikely to receive an organ donor within 2-3 weeks, the ISHLT recommends COVID-19 vaccination, whereas the guidelines recommend a waiting period of one month post-transplantation and 3-6 months post-administration of B- or T-cell depleting agents [163] and a protocol recommended 90 days for both post-transplantation and 3-6 months post-administration of B- or T-cell depleting agents [156]. In lung transplantation, the goal of immunosuppression strategy is to achieve a balance between suboptimal immunosuppression and excessive immunosuppression [164]. The ISHLT recommends azathioprine, mammalian target of rapamycin inhibitor (m TOR), or mycophenolate mofetil (MMF) in moderate to severe COVID-19 LTRs [163], whereas an institute keeps calcineurin inhibitors (CNIs) (IL-6 inhibitors) at the baseline dose [156]. Sarilumab or tocilizumab (an IL-6 inhibitor) decreased time to ICU discharge and mortality, preliminarily reported from the REMAP-CAP trial [165]. Lymphopenia that has been related to severe COVID-19 infection, ICU admission, acute respiratory distress syndrome, and mortality can be caused by some immunosuppressants, for examples, lymphocyte-depleting antibodies or antimetabolites [166], whereas m TOR have been related to interstitial pneumonitis development [167]. Most transplant centers will stop usage of these drugs. SpO2/FiO2 ratio < 440 or PaO2/FiO2 < 300 is related to a significant lower risk of intubation, ICU transfer, or death [168]. In severe-COVID-19-hypoxic LTRs, variable-doses glucocorticoids are frequently administered [169].

For the application of this issue, we now have some experience and insight for COVID-19-infected LTRs. We look forward to getting the optimal timing of COVID-19 vaccination adjustment of immunosuppression in LTRs, whereas the investigations of the T-cell response and additional COVID-19-vaccine doses are ongoing [170]. Nevertheless, the best methods to protect LTRs are facial masking, physical distancing, and COVID-19 vaccination. Long-term outcomes of lung transplantation in severe COVID-19 patients are still to be investigated. National and international regulatory transplantation professional bodies should track the transplant-COVID-19 patient for establishing the standard guidelines.

COVID-19 Vaccination in Patients with Cancer

In patients with cancer, SARS-CoV-2 (COVID-19) can contribute to increasing morbidity and mortality [171-173] and decreased survial was found in patients with hematological and intrathoracic malignancies, poor performance status, comorbidities, and increased age [173-175]. Patients with hematological malignancies who were treated with stem cell transplantation and anti-CD-20 antibody demonstrated lower rates of seroconversion, compared to COVID-19-infected-cancer patients [176, 177]. Patients with hematological malignancies might have substantially compromised B-cell and T-cell responses [178]. These study results indicated that following COVID-19 vaccination, overall high seroconversion rates could be anticipated in cancer patients due to different mechanisms and degrees of immune suppression, such as cell therapies (particularly chimeric antigen receptor (CAR)-T cell), anti-CD-20 antibody (B-cell depleting) therapies, stem cell transplantation, immunosuppressive effects of corticosteroid treatment, and cytotoxic-chemotherapy-bone-marrow-suppressive effects in certain subgroups of cancer patients [179]. Currently, there are lacking data in cancer patients in protection following SARS-CoV-2 (COVID-19) infection, reinfection by various SARS-CoV-2 (COVID-19) variants, or COVID-19 vaccination although mucosal surface antigens (e.g. IgA and protective T-cell responses) might be similarly important in protection from natural SARS-CoV-2 (COVID-19) infection [180]. The association of carcinogenesis with genomic information encoding vaccines, particularly with very-transient-intracellular-presence-COVID-19-mRNA vaccines is likely very low [181]. Hypothetically, mRNA-vaccine-encapsulated-small liposomes and lipid carriers may accumulate through the permeation retention and enhanced effect in tumor tissues [182, 183]. The recommendation that have been suggested administering COVID-19 vaccines one to two weeks prior to a chemotherapy dose by many key-professional organizations has not been practical with COVID-19 administration schedules (for examples; two doses of mRNA-1273 (Moderna) are recommended to be given 28 days apart, whereas two doses of BNT162b2 (Pfizer/BioNTech) are given 21 days apart, efficacy > 94 %), variable chemotherapeutic regimens, and limited COVID-19 vaccination slot availability, contributes to allowing the most rapid COVID-19 vaccination of these immunosuppressed cancer patients [184] due to lacking COVID-19-vaccine safety and the information

immunogenicity in the context of immune-system-stimulated immunotherapies (for examples; immune checkpoint inhibitor (ICI) therapy) [185] and general exclusion of malignancy-diagnosed patients in the clinical trials of currently approved COVID-19 vaccines [186]. In convalescent-COVID-19 patients, neutralizing antibody, memory B and memory T cells specific to SARS-CoV-2 (COVID-19) have been identified after six months of infection [187-189] and both antibody production and memory CD4+ T-cells sustained several months after SARS-CoV-2 (COVID-19) infection in rapidly resolved-symptom individuals [190]. Humoral and cell-mediated immunity to SARS-CoV-2 (COVID-19) are the integrated highly-effective-durable-protective key [191]. Antibody-dependent enhancement (ADE) of disease, mediated by virus-binding antibodies and do not neutralize the virus takes two main virus-binding forms (one form in dengue virus infection by virus-binding antibody and the internalization of the antibody-virus complex through interactions with Fc-gamma receptor into replicated macrophages; the other form, non-neutralizing antibodies mediate the formation of incited-inflammation-immune complex) has been proposed as a COVID-19-vaccine-design concern due to high levels of antibodies in in vitro observations and in patients with severe COVID-19 with SARS-CoV-2 (COVID-19)-taken-up macrophages [192]. No compelling evidence of ADE from convalescent plasma has been found [193-195]. Theoretically, ADE is reduced by elicited-antibody-response-on-neutralizing-epitope-COVID-19 vaccines [191]. Inability to integrate into host genome, delivery into cytoplasm, avoidance of anti-vector immunity, eliciting strong humoral and cellular immunity, avoidance of introduction of pathogen, and easier to mass-production are the advantages of mRNA vaccines, whereas the requirement of lipid nanoparticvle for delivery, easy degradation, and freezing storage are their disadvantages [191]. In the USA, a study of 273,00 cancer-diagnosed patients (total of 73 million patients) that 16,570 patients were diagnosed of COVID-19 demonstrated the increased number of COVID-19-infected cancer patients with adjusted OR of 7 [196]. COVID-19 patients with recently diagnosed leukemia, non-Hodgkin lymphoma, and lung cancer were highest odds of COVID-19 infection with adjusted OR of 12.2, 8.5, and 7.7, respectively [196] and demonstrated the greater risk of mortality (14.9 %) among COVID-19-infected-cancer patients, compared to COVID-19 patients without cancer (5.3 %) and cancer patients without COVID-19 infection (4.0 %) [196], whereas hematological-malignancy-diagnosed patients had increased risk of mortality at least 2.5 times, compared to patients with other cancers (at least 1.2 times) [197]. Cancer patients

with COVID-19 vaccination undergoing chemotherapy, with exception of during periods of intensive chemotherapy are expected to generate COVID-19-protective responses that are similar to inactivated influenza [198, 199], pneumococcal polysaccharide, and hepatitis subunit [199, 200] vaccinations. Cancer patients being treated with targeted therapies (tyrosine kinase inhibitors (TKIs) (erlotinib, imatinib, sunitinib, or monoclonal antibodies (trastuzumab, etc.)) are reasonably expected generating protective responses with COVID-19 vaccination [191]. Cancer patients on ICI therapy are expected to produce protective responses following COVID-19 vaccination due to low risk of immune-related adverse events (IRAEs) found in cancer patients with ICI therapy receiving influenza vaccination [191, 201, 202]. In some settings, delaying ICI treatment in cancer patients may be safe from a perspective of cancer treatment [203]. Practically, cancer patients treated with lymphodepleting or anti-B cell or anti-CD19 therapy or plasma-cell-depleting therapy are recommended to receive vaccination or COVID-19 vaccination at least 6 months after therapies [198, 200]. Sometimes, they may be flexible to optimize the timing of COVID-19 vaccinations (for example; COVID-19 vaccination followed by anti-B cell therapy several weeks later) depending on the urgency and phase of a cancer treatment in a patient [191]. Cancer patients on radiation therapy, particularly total body irradiation (TBI) that usually is given to spine and total lymph nodes for bone marrow suppression prior to other rare situations or stem cell transplantation should be COVID-19 vaccinated to produce protective immunity responses [191]. Cancer patients receiving mRNA vaccines are not specifically anticipated safety concerns [191]. Generally, live vaccines, particularly live COVID-19 vaccines are not recommended in cancer patients ongoing cytotoxic, lymphodepleting, or targeted therapies [198-200]. COVID-19 vaccination has been recommended after approval of BNT162b2 (Pfizer) vaccine in cancer patients by the American Association for Cancer Research's (AACR's) COVID-19 and cancer task force [204], American Society of Clinical Oncology (ASCO) [205], European Society for Medical Oncology (ESMO) [206], Infectious Disease Society of America (IDSA) [205], National Comprehensive Cancer Network (NCCN) COVID-19 vaccination advisory committee [191], Society of Imunotherapy of Cancer [207], and the United States Centers for Disease Control and Prevention (US CDC) [191]. COVID-19 vaccination is strongly recommended in cancer patients, including their caregivers, whereas exactly representative data are not available, benefits likely outweight risks of COVID-19 [191]. Immunization or COVID-19 vaccination in cancer patients undergoing

adoptive cell therapy or organ transplantation should be delayed at least 3 months to maximize vaccine efficacy [191, 208]. In patients with breast cancer, sceening examinations of transient lymphadenopathy from COVID-19 vaccination should be performed either before first dose or 4-6 weeks after the second dose of a COVID-19 vaccine [209, 210]. For continuation of the quality of oncological care, cancer patients on clinical trials should be prioritized for COVID-19 vaccination that do not affect the eligibility of the clinical trials.

COVID-19 Vaccination in Immunocompromised Patients

Preliminary data of phase II/III clinical trials of BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) vaccines those were investigated in more than 50 million COVID-19 cases and 1.35 million COVID-19-related deaths worldwide revealed to be about 95 % effective in COVID-19 prevention and both trials revealed more than 90 % of lowering the severe-COVID-19-illness risk [1, 2 : 211, 212], whereas theses clinical vaccine trials excluded immunocompromised patients, patients on immunosuppressive drugs, or those with immunosuppressive status [3, 4 : 213, 214]. BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) vaccines encode the RBD of SARS-CoV-2 (COVID-19) spike protein and the S-2P antigen, respectively [5, 6 : 215, 216]. A strong humoral response and strong cellular response were elicited by both vaccines through neutralizing-antibody production and a strong cellular response and through inducing Th1-cytokine production and functional and pro-inflammatory CD4+ and CD8+ T cells, respectively [5, 6 : 215, 216]. Methotrexate and rituximab can decrease the neutralizing-antibody-to-neoantigen production, such as SARS-CoV-2 (COVID-19) [7 : 217] that have been demonstrated the reduction of humoral responses to pneumococcal and seasonal influenza vaccines [8 : 218]. Hypothetically, humoral suppression by methotrexate is mediated by increasing regulatory B cells and immunosuppressive adenosine and interaction with the B-cell activation factor (BAF) [9 : 219] and with significant improvement by temporarily discontinuing for 2 weeks post-influenza vaccination, whereas rituximab directly suppresses CD20+ B cells that is significant humoral-response-to-polysaccharide-pneumococcal-vaccination reduction and significant decrease in the immune response to neoantigen [10, 11 : 220, 221]. Rituximab and methotrexate on immune response of a SARS-CoV-2 (COVID-19) vaccine are to be

investigated, yet. Two weeks of holding methotrexate and a few weeks of scheduling after the COVID-19 vaccination are considered until the questions are answered by the further clinical trials.

COVID-19 Vaccination in Pregnant Individuals

A recent study in the USA of a total of 35,691 pregnant participants 16 to 54 years of age from December 14, 2020, to February 28, 2021 [222] was conducted by using data from the v-safe pregnancy registry [223], " v-safe after vaccination health checker " surveillance system [224], and the Vaccine Adverse Event Reporting System (VAERS, a national spontaneous-reporting (passive-surveillance) system established in 1990 and administered by the US CDC and the US FDA) [225], the three US vaccine safety monitoring systems for characterization the initial safety of mRNA COVID-19 vaccines in pregnant participants [222]. Of 3,958 mRNA-vaccine (Pfizer/BioNTech vaccine, Moderna vaccine)-received-pregnant participants registered in the v-safe pregnancy registry, 115 (13.9 %) of 827 completed pregnant participants had a pregnant loss and 712 (86.1 %) had a live birth (mostly among third-trimester-COVID-19-vaccinated pregnant participants) [222]. Preterm birth (9.4 %) and small-size-gestational age (3.2 %) were the adverse neonatal outcomes [222]. The study revealed no neonatal death [222]. Spontaneous abortion (46 cases) was the most frequent event among 221 VAERS-reported-pregnancy-associated adverse events [222]. The study demonstrated although not directly comparable that the calculated proportions of COVID-19-vaccinated-completed- pregnant participants with adverse pregnancy and neonatal outcomes were similar to prior-COVID-19-pandemic-incidence reported in involved-pregnant women [222, 226-237]. Chill, fever, headache, and myalgia were less frequent reported among pregnant individuals than among nonpregnant participants, whereas injection-site pain was more frequent among pregnant participants [222]. Only 14.7 % of the v-safe-surveillance-system-identified-pregnant individuals had been contacted to offer pregnancy-registry enrollment at the time of this study-results analysis [222]. Rare pregnancy outcomes may be detected as the sample size increases contributing to additional reported-pregnancy outcomes due to small-sample-size and mostly-described-third-trimester-vaccination-neonatal-outcome preliminary registry data, thus, adverse pregnancy outcomes that might occur in relation to early-pregnancy exposures

were not evaluated [222]. Follow-up is ongoing due to no early-COVID-19-vaccinated-pregnant individuals who have had live births had been captured in the updated v-safe pregnancy registry [222]. Non-recognized-very-early-pregnancy losses and after-greatest-risk-in-the-first-trimester-of-COVID-19-vaccinated-pregnant participants may contribute to non-reflecting the proportion of the exact postvaccination proportions among the pregnant individuals with spontaneous abortions [222]. Miscarriage was the most common pregnancy-specific condition after COVID-19 vaccination reported to the VAERS [222, 238]. After maternal COVID-19 vaccination during the third trimester of pregnancy, emerging evidence has demonstrated transplacental transfer of SARS-CoV-2 (COVID-19) antibodies that might provide some levels of neonatal-and-maternal-COVID-19 protection [239-242]. Nevertheless, a direct comparison of pregnancy outcomes on the basis of timing of COVID-19 vaccination between some first- and early-second-trimester pregnancies is urgently needed to define the proportion of spontaneous abortion in this study cohort [222]. VAERS do not demonstrate any clear safety signals related to neonatal or pregnancy outcomes and third-trimester-COVID-19 vaccination. Further evaluation of pregnancy, neonatal, maternal, and childhood outcomes related to maternal COVID-19 vaccination, pre-conception period, and earlier stage of pregnancy is needed for continuing monitoring [222], and the US CDC and the US FDA will closely monitor the information involving COVID-19 vaccination (Pfizer/BioNTech and Moderna vaccines) during pregnancy by safety monitoring systems [243]. The preliminary data from these systems via the early dataexternal icon revealed no safety concerns for COVID-19-vaccinated-pregnant individuals or their babies [243]. When compared with non-pregnant individuals, pregnant and recently pregnant individuals are at a greater risk for severe COVID-19 illness although the overall risk of severe COVID-19 illness is low [243]. Increased risk of preterm birth and increased risk of other adverse pregnancy outcomes were found in COVID-19-pregnant people, compared with non-COVID-19-pregnant individuals, whereas there was no safety concerns in pregnant animals receiving a Johnson & Johnson/Janssen, Moderna, or Pfizer/BioNtech vaccine in several studies [243, 244]. Currently, there are no data available on the effects on breastmilk production or excretion, effects of COVID-19 vaccination on the breastfed baby, and safety of COVID-19 vaccines in lactating individuals due to not including pregnant or breastfeeding individuals in the clinical trials for the COVID-19 vaccines currently authorized for use under and Emergency Use Authorization (EUA) in the USA [243, 244].

Nevertheless, recent studies demonstrated that lactating individuals can receive a COVID-19 vaccine and also revealed that COVID-19-mRNA-vaccinated-breastfeeding persons had antibodies against SARS-CoV-2 (COVID-19) in their breastmilk [243, 244]. Further studies are needed to identify what protection to baby provided by these antibodies [243, 244]. Parents can receive a COVID-19 vaccine if currently trying to get pregnant or in the future[243]. As of April 21, 2021, in the interim, the WHO recommends COVID-19 vaccination in pregnant people when the benefits of COVID-19 vaccination to the pregnant people outweigh the potential risks (e.g.; pregnant individuals at high risk of COVID-19 exposures, pregnant individuals with comorbidities that place them in a high-risk group for severe COVID) and continuing breastfeeding [244], https.who.int/groups/strategic-advisory-group-of-experts-on-immunization/covid-19-materials)]. The WHO also recommends both active and passive surveillance approaches to evaluate adverse events following immunization (AEFI) of COVID-19 vaccination, including during pregnancy [244].

COVID-19 Vaccination in Children

How COVID-19 affects children is little yet known in some countries [245-247]. In sub-Saharan Africa, COVID-19 hospitalization and deaths do not break down the cases by age through some official tallies [245, 246]. Thus, pediatricians don't know how outcomes of COVID-19 might be affected by some conditions (e.g.; concurrent tuberculosis, or HIV infection, or malnutrition), and and which deaths were in chidren and young adults, including what will be the burden of co-infection in children, such as massive circulation of SARS-CoV-2 (COVID-19) and respiratory syncytial virus [245, 246]. Currently, China, Israel, and USA are offering vaccines (Moderna-mRNA vaccine, Pfizer/BioNTech-mRNA vaccine, and two Chinese vaccines produced by Sinvac and Sinopharm) to young individuals over the age of 12 [245, 246]. Several studies on COVID-19 vaccines, including two Indian COVID-19 vaccines (Zydus Cadila and Covaxin (inactivated coronavirus) vaccines) are expected to report results in young individuals over the age of 12 very soon [245, 246]. In USA, COVID-19 vaccines for children under the age of 12 might be available in the late 2021, whereas some companies have have started on carrying out clinical vaccine trials in as-young-as-six-months children [245, 246].

A recent study in Malta demonstrated extremely low risk of myocarditis and pericarditis among Pfizer/BioNTech-vaccinated-adolescent individuals aged 12-17 (around 67 and 9 cases per million second doses in adolescent males and females, respectively) [245, 246]. A decrease in SARS-CoV-2 (COVID-19) transmission to vulnerable ageing individuals in Malta may be due to COVID-19 vaccination in adolescents [245, 246]. Currently, Chile is rolling out COVID-19 vaccines to children aged 12 and older, whereas a Chilean pediatric-infectious-disease specialist suggested that countries should probably not move forward with pediatric COVID-19 vaccinations so fast [245, 246]. In May 2021, the WHO chief said that wealthier countries that are vaccinating children are doing so at the expense of high-risk groups and healthcare workers in other countries [245, 246]. Some vaccine experts pointed out that some rich countries bought more than enough vaccine doses to fully vaccinate their populations and the argument for sending COVID-19 vaccines outside the country should not preclude vaccinating children in higher-income countries [245, 246].

Mix-and-Match COVID-19 Vaccination

In low-income and middle income countries, Ebola vaccine (Johnson & Johnson) experience demonstrated that mix-and-match vaccination is feasible, safety, and long-lasting immunization, adopted in phase I and phase II trials and can overcome easily with active community participation and suitable national planning [248, 249]. In addition to Ebola, this concept has been previously implemented for influenza, malaria, and HIV [249]. The prime dose in the most of the current vaccination regimen is at a month interval followed by a second homologous booster dose [250]. Recent interest in COVID-19 mixing vaccination is aimed to simplify countries' facing fluctuation of various vaccine supplies and immunization efforts and increase SARS-CoV-2 (COVID-19) protection by delivery of the similar or same antigens of the disease-causing agent via two different vaccine types [Figure 44] and eliciting a strong and long-lasting immune response as compared to the single vaccine regimen, but has a lack of evidence and a potential risk of increased-mixing-vaccine-adverse side effects [250] that include increased headache, increased fever, increased malaise, increased joint pain, and increased AEFI, particularly in the elderly population [250]. In June 2021, a preliminary study conducted by the University of Oxford scientists demonstrated that mixing the AstraZeneca and Pfizer vaccines produced a robust

immune response against the SARS-CoV-2 (COVID-19) virus and induced higher antibodies than an only two-dose schedule of Astrazeneca vaccine and none of the groups demonstrated decreased neutralizing activity against the Alpha variant (UK variant), but the neutralization titer reduced by 2.5 to 6 times against the Beta variant (South African variant), Gamma variant (Brazilian variant), and Delta variant (Indian variant) [251]. The Comparing COVID-19 Vaccine Schedule Combinations (Com-COV) study (463 cases of the 4-week interval group) revealed that immunization with Astrazeneca vaccine followed by Pfizer vaccine at the 4-week interval demonstrated a better immune response out of the two mixed dosing regimens [251]. Com-COV study demonstrated in the earlier phase that around 30 % to 40 % of those who received mixed doses reported fevers after their second jab, compared to 10 % to 20 % of those who received the same vaccine for both doses. This result could be attributable to the shorter, 4-week interval between doses that was used during the Oxford study, whereas the safety data from a cohort with a 12-week dosing interval is still to appear [251].

The trials in Germany and Spain have also demonstrated that a mixed dosing regimen induced a better immune response than getting two doses of the AstraZeneca vaccine [251]. In South Korea, the study on 100 actually-receiving-mixed-doses cases out of 499 cases conducted by the Korea Disease Control and Prevention Agency on a mixed vaccination, with AstraZeneca vaccine as the first dose and Pfizer vaccine as the second dose revealed the increased neutralizing antibody levels of 6 times higher than those found after two doses of the AstraZeneca jab [251]. Initial phase of Com-COV study or Com-COV1 study has been concentrated on mixing the AstraZeneca and Pfizer jabs, whereas Com-COV2 study or phase 2 of the Com-COV study is assessing the immunogenicity and safety of combining the Moderna and Novavax vaccines with a first dose of either the Pfizer or AstraZeneca jab [251]. Globally, COVID-19-dose-mixing studies on assessing other vaccine combinations are also ongoing, including a Russian trial of an AstraZeneca-Sputnik V combinations and a Philippines-based study mixing Sinovac' s CoronaVac jab with 6 other vaccines [251]. In India, COVID-19 mixing vaccination can assist in scaling up the vaccination drive to a large extent in this world's-largest-COVID-19-vaccination-drive country [250]. As of June 6, 2021, China, UK, and USA have began the COVID-19-vaccine mixing trials, but have not yet officially approved them due to not being designed

to assess actual COVID-19 protection and non-corresponding-COVID-19-real-life protection of the studies' antibody and T-cell measurements, whereas only Canada, Denmark, France, Germany, Norway, and Sweden implemented mixing vaccination to their citizens with rarely reported ChAdOx1 nCoV-19 (AstraZeneca) thromboembolic complications [250, 252]. Whenever it is impossible to provide a second dose of COVID-19 vaccine, the Public Health of England guidelines recommend that it is better to give a different COVID-19 vaccine than not administer the second dose at all [250]. On July 12, 2021, the WHO's chief scientist has suggested individuals against mixing and matching COVID-19 vaccines from different manufacturers [253]. Nevertheless, on July 13, 2021, Thailand defended mixing two different COVID-19 vaccines to fight against a surge in SARS-CoV-2 (COVID-19) infections since COVID-19 outbreak in April 2021 after the WHO's top scientist warned that it was a " dangerous trend " not backed by evidence [252, 254]. Thailand's health authorities will mix a first dose of the Chinese-produced " Sinovac " jab with a second dose of AstraZeneca vaccine to try and achieve a " booster " effect in 6 weeks instead of 12 weeks due to fast spreading disease (more than 353,700 reported- COVID-19-infected cases and 2,847 reported-COVID-19-related deaths in April 2021) [254]. Table 7 [191] and 8 [250] demonstrate advantages and disadvantages of various platforms of COVID-19 vaccines, and recently published clinical trials and ongoing clinical trials on mixing COVID-19 vaccination(as of July 11, 2021), respectively.

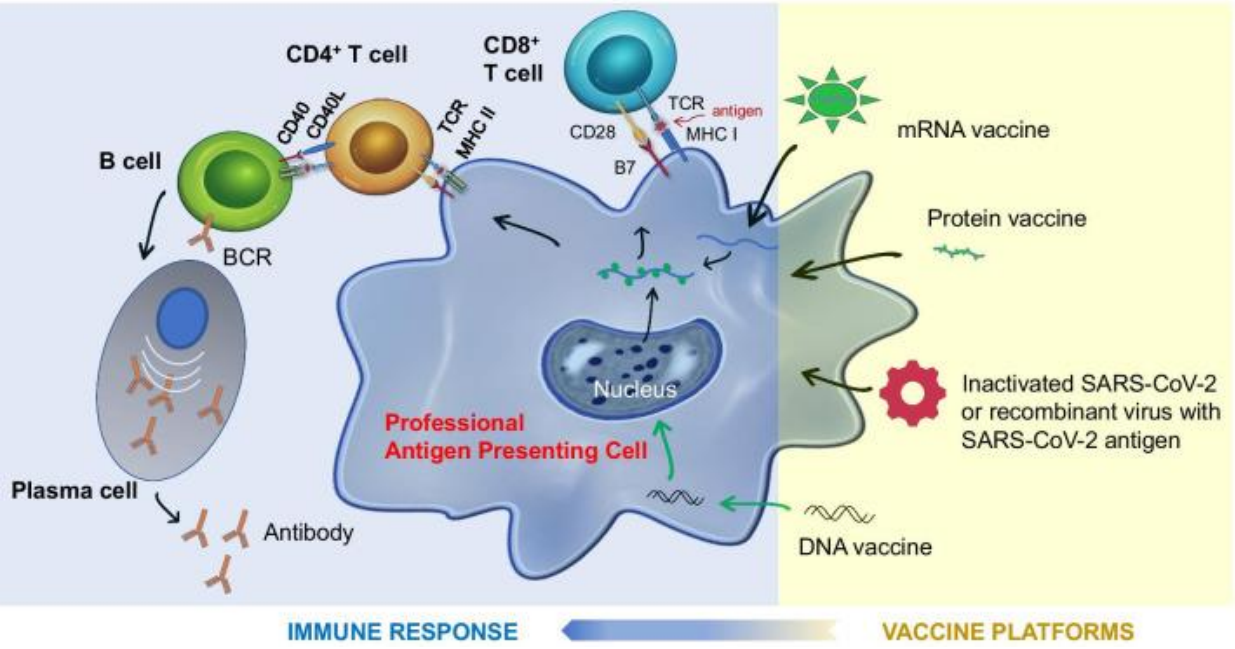

Figure 44 : Different platforms of COVID-19 vaccines and mechanisms of antigen presentation, and protective immunity generation

(Source : Hwang JK, Zhang T, Wang AZ, Li Z. COVID-19 vaccines for patients with cancer : benefits likely outweigh risks. Journal of Hematology and Oncology 2021; 14 : 38. DOI : https://doi.org/10.1186/s13045-021-01046-w)

Vaccine Platform	**COVID-19 Vaccines (approved/in development)**	**Advantages**	**Disadvantages**
Inactivated Virus	SinoVac (CoronaVac + aluminum) Sinipharm (Inactivated whole virus SARS-CoV-2 + aluminum)	Prior experience and technology, e.g., quadrivalent influenza vaccine Easier storage, does not need to be frozen Entire virus, with all antigens presented	Poor inducers of CD8+ T-cell immunity Need adjuvants to boost Large batches of live virus pose biosecurity risk
mRNA	Pfizer/BioNTech (BNT162b2)	Unable to integrate into host genome Delivery into host cytoplasm	Frozen for vaccine storage Needs delivery of lipid nanoparticle

		Avoids introducing pathogen (SARS-CoV-2) Avoids anti-vector immunity Easier mass-production Elicit strong humoral and cellular immunity	
DNA	Inovio (INO-4800)	Avois introducing pathogen (SARS-CoV-2) Easier mass-production Mimics natural infection Elicits strong humoral and cellular immunity	Delivery into nucleus of host cell
Protein subunits	Novavax (NVX-CoV2373) Vector Institute (EpiVacCorona)	Does not introduce pathogen (SARS-CoV-2) Being able to focus on antigens that generate neutralizing antibodies	Lower humoral and cellular immunity response Not efficiently presented Require adjuvants to boost Produced *ex vivo* may not retain post-translational conformation or modifications
Replication incompetent adenoviral vector	AstraZeneca (ChAdOx1 nCoV-19/AZD1222) Johnson and Johnson (Ad26.COV2.S) CanSino Biologics (Ad5-nCoV) Gamaleya (Sputnik V)	Mimics natural infection Avoids pathogen (SARS-CoV-2) Elicits humoral and cellular immunity No new viral particles (Defective Replication)	Lower efficacy if prior anti-vector immunity exists Anti-vector immunity may interfere

Table 7 : Demonstrating advantages and disadvantages of various COVID-19 vaccine platforms

(Source : Hwang JK, Zhang T, Wang AZ, Li Z. COVID-19 vaccines for patients with cancer : benefits likely outweigh risks. Journal of Hematology and Oncology 2021; 14 : 38. DOI : https://doi.org/10.1186/s13045-021-01046-w)

Ongoing Clinical Trials				
Site/Number of Participants	**Current Status (As of July 11, 2021)**	**Type and Clinical Trial Objective**	**Group and Vaccine Type**	**Primary and Secondary Outcomes**
Austria (NCT04907331)/3,000 participants	**Ongoing**	**Randomized, Controlled Trial,phase II; evaluating safety and efficacy of heterologous vaccination with ChAdOx1-S, AZ (Vaxzevria) followed by BNT162b2, Pfizer/BioNTech) (Comirnaty)**	**Clinical Trial Arm :** **Prime :** **ChAdOx1-S or ChAdOx1 nCoV-19 vaccine, AZ (Vaxzevria);** **Boost :** **BNT162b2 vaccine, Pfizer/BioNTech (Comirnaty) 12 weeks apart** **Control Arm :** **Homolog Vaccination with Vaxzerria (Prime/Boost) or Comirnaty (Prime/Boost)**	**Primary Outcomes :** **T-cells response to SARS-CoV-2 (COVID-19) Spike Protein Epitopes in both arms; Neutralizing Antibodies in both arms; Vaccine Failures in both arms**
China (NCT04892459)/300 participants	**Ongoing**	**Randomized, Paralell-Controlled Clinical Trials; evaluating safety and immunogenicity of sequential immunization of a recombinant SARS-CoV-2 (COVID-19) vaccine**	**Clinical Trial Arm :** **Prime :** **Inactive SARS-CoV-2 (COVID-19) (Vero cell) (Sinovac Research & Development Co., Ltd);** **Boost :** **Recombinant SARS-CoV-2 (COVID-19)**	**Primary Outcomes :** **Adverse Reactions within 28 days after the booster dose; Genomic Mean Titer (GMT) of Neutralizing Antibodies against Live SARS-CoV-2 (COVID-19)**

		(Adenovirus Type V Vector)	**Ad5 Vectored Vaccine (CanSino Biologics);** **Comparator Arm : Homogeneous Boost Arm with Inactive Vaccine**	**Virus on Day 14 after Booster Dose**
France (NCT04900467)/400 participants	**Ongoing**	**Randomized, Open Label, Non-Inferiority Clinical Trial**	**Arm 1 :** **Prime : Pfizer/BioNTech mRNA Vaccine;** **Boost : Moderna mRNA Vaccine versus Prime : Pfizer/BioNTech mRNA Vaccine;** **Boost :** **Pfizer/BioNTech mRNA Vaccine** **Arm 2 :** **Prime : Moderna mRNA Vaccine;** **Boost : Pfizer/BioNTech mRNA Vaccine versus Prime : Moderna mRNA Vaccine;** **Boost :** **Moderna mRNA Vaccine**	**Primary Outcomes :** **Anti-Spike IgG Titer 28 Days following Vaccination in both arms** **Secondary Outcomes :** **Adverse Events**
Canada (MOSAIC) (NCT04894435)/1,300 participants	**Ongoing**	**Randomized Clinical Trial; evaluating the immune response and safety of two different vaccines for the first and**	**Multiple Groups (13) comparing Various Combination of Moderna mRNA vaccine and ChAdOx1 nCOV-19 (AstraZeneca) vaccine in**	**Primary Outcomes :** **Antibody Response to SARS-CoV-2 (COVID-19) Spike Protein at 28 Days**

		second doses and differing between the first and second doses of the two-dose vaccines	**homologous and heterologous prime-boost regimens**	**following Second Dose of Vaccine;** **Secondary Outcomes :** **Pseudoneutralization Assay, T-Cell Testing, Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody Avidity; Description of Safety Outcomes Over 12 months post-vaccination including serious adverse events (SAEs); Incidence of Grade III solicited local and systemic adverse events, SAEs, AEFIs, within 7 days after vaccination; Durability of Antibody Response to SARS-CoV-2 (COVID-19) Spike Protein over 12 months**
USA (National Institute of Health (NIH) Trial) (NCT04889209)/400 participants	**Ongoing**	**Phase I/II, Open-Label Clinical Trial; evaluating safety, reactogenicity and mmunogenicity of a delayed (> 12 weeks) vaccine boost**	**Vaccines :** **Ad26.COV2.S (Janssen Pharmaceuticals/Johnson & Johnson), BNT162b2 (Pfizer/BioNTech), or mRNA-1273 (ModernaTX);** **Boost :** **Booster shot following complete**	**Primary Outcomes :** **Response Rate of SARS-CoV-2 (COVID-19) Specific Antibody Binding and Neutralization Titers; Occurrence of SAEs after Last Dose on the clinical Trial and after Delayed**

			vaccination will be provided in various combinations via multiple arms of the clinical trial	**Booster Vaccination; Magnitude of SARS-CoV-2 (COVID-19) Specific Antibody Binding and Neutralization Titer**
Published Clinical Trials				
Site/Authors/Clinical Trial Name/Number of Participants	**Type of Clinical Trial**	**Group and Vaccine Type**	**Clinical Trial Outcomes**	**Adverse Events**
Germany/GroB *et al*/26 participants	**Prospective, Observational Clinical Trial**	**First Dose :** **ChAdOx1 nCoV-19 vaccine (AstraZeneca)** **Second Dose :** **BNT162b2 vaccine (Pfizer/BioNTech) following 8-week interval** **No Control Group**	**CD4+ and CD8+ T Cells Reacts to SARS-CoV-2 (COVID-19) Spike Peptide Stimulus 2 Weeks Post-Full Vaccination; Neutralizing Activity Against Prevalent Strain B.1.1.7 (Alpha Strain) with Hetrologous Prime Boost was 3.9 times higher than in persons receiving Homologous BNT162b2 vaccination (Pfizer/BioNtech); Strong Neutralization Titers 2 Weeks Post-BNT162b2 vaccine (Pfizer/BioNTech) boost**	**Prime Dose :** **Mild to Moderate Reaction (88.4 %)** **Boost Dose :** **Mild or Moderate Symptom (80.8 %)** **Common Symptoms :** **Pain at the injection site, Fever, Headache, Chills, Myalgia, Fatigue**
Germany/Hillus *et al*/340 participants	**Prospective, Observational Cohort Study**	**Trial Arm 1 :** **Prime :** **ChAdOx1 nCoV-19**	**T-cell Reactivity : Significantly higher following Heterologous ChAdOx1/BNT162b2 boost compared to Homologous**	**Local eaction : Slight higher frequency following Heterologous ChAdOx1/BNT162b2 booster**

		vacccine (AstraZeneca); **Boost :** **BNT162b2 vaccine (Pfizer/BioNTech 10-12 weeks apart** **Trial Arm 2 :** **Homologous BNT162b2 vaccine (Pfizer/BioNTech) (prime and boost) 3 weeks apart**	**BNT162b2/BNT162b2 boost; S1-IgG Avidity : High following Heterologous ChAdOx1/BNT162b2 boost compared to Homologous BNT162b2/BNT162b2 boost; Neutralizing Antibody Response 3 weeks Post-boost immunization : Homologous BNT162b2 (99.01 %), Heterologous ChAdOx1/BNT162b2 boost (100.0 %); Serum Antibody Response : Strongly Increased following both Homologous and Heterologous boost**	**compared to Homologous BNT162b2/BNT162b2 booster** **Systemic Reactions : Most frequent following prime immunization with ChAdOx1 (86 %) and less frequent following Homologous BNT162b2/BNT162b2 (65 %) or Heterologous ChAdOx1/BNT162b2 booster vaccination (48 %);** **No potential life-threatening reactions following any of the COVID-19 vaccine regimens**
Spain/Borobia *et al*/663 participants	**Randomized, Phase II Trial**	**Trial Arm :** **Prime : ChAdOx1 nCoV-19 vaccine (Vaxzevria, AstraZeneca);** **Boost :** **BNT162b2 vaccine (Comirnaty, Pfizer/BioNTech)** **Control Arm :** **Received only one dose and not received any second**	**Trial Arm :** **Greater Immune Response : 150 times antibody 14 Days following Second Dose; 4 times increase in Cellular Immune Response; Effective in Protecting Against SARS-CoV-2 (COVID-19)** **Control Arm :** **Antibody Titers at 14 Days similar to Baseline Titers**	**Similar in both groups :** **Mild (68.3 %); Moderate (29.9 %)** **Most Common : Headache (44 %); Malaise (41 %); Chills (25 %); Mild Nausea (11 %); Mild Cough (7 %); and Fever (2.5 %)**

		dose of vaccine		
UK/Shaw *et al*/830 participants	**Single-Blind, Randomized, Phase II Trial**	**Trial Arm 1 :** **Prime :** **ChAdOx1 nCoV-19 vaccine (Vaxzevria, AstraZeneca);** **Boost :** **BNT162b2 vaccine (Comirnaty, Pfizer/BioNTech)** **Trial Arm 2 :** **Prime :** **BNT162b2 vaccine (Pfizer/BioNTech);** **Boost :** **ChAdOx1 nCoV-19 vaccine (AstraZeneca)** **Control Arm :** **Homologous Schedule :** **Arm 1 :** **Prime and boost :** **BNT162b2 vaccine (Pfizer/BioNTech);** **Arm 2 :**	**Not yet reported**	**Greater Systemic Ractogenicity in Heterologous prime-boost regimen than their Homologous counterparts;** **Most common symptom : Feverishness** **No hospitalization** **Most of increased Reactogenicity identified within 48 hours following immunization**

		Prime and boost : ChAdOx1 nCoV-19 vaccine (AstraZeneca)		

Table 8 : Demonstrating recently published clinical trials and ongoing clinical trials on mixing COVID-19 vaccination, as of July 11, 2021.

(Source : Adated from : Kunal S, Sakthivel P, Gupta N, et al. Mix and match COVID-19 vaccines : potential benefit and perspective from India. Postgrad Med J 2021. DOI : 10.1136/postgradmedj-2021-140648)

Third (Boosted) Doses of COVID-19 Vaccination

Flaxman et al, a groups of investigators from the University of Oxford, United Kingdom demonstrated the results studied in COVID-19-vaccinated participants in a preprint published on June 28, 2021 that for those who had 8 to 12 week (median age 39), 15 to 25 week (median age 36), and 44 to 45 week (median age 32) intervals between the first and second COVID-19-vaccine doses, the median level of IgG antibody at the day 28 after the second dose were 923, 1,860, and 3,738 tIgG EU, respectively [255]. These results indicated the longer dose intervals the higher IgG-antibody levels [255]. They identified the median 278 and 1,240 tIgG EU in the groups who had a 8 to 12 week and a 15 to 25 week intervals, respectively after 6 months of the second dose, whereas the data for the 44 to 45 week-interval group are not yet available [255]. After the second dose, the IgG binding titers to the four SARS-CoV-2 (COVID-19) variants (alpha, beta, delta, and D614G) tested were significant higher than before the second dose [255]. Among 75 first-two-dose-received participants with an interval of 8 to 16 weeks, the IgG antibody levels were 1,792 and 3,746 tIgG EU at the day 28 after the second dose and after the third (boosted) dose, respectively [255]. They also found that the neutralizing antibody titers after the third dose were higher than those after the second dose against alpha, beta, and delta COVID-19 variants, whereas binding antibody titers to the beta variant significantly increased after the third dose [255]. Hall et al conducted a randomized trial in 120 organ-

transplant recipients (median age 66.6 years, IQR : 63.3 to 71.4, dosing schedule : 0, 1, and 3 months) and revealed that after the third dose of mRNA-1273 (Moderna) vaccination, the median SARS-CoV-2 (COVID-19)-specific T-cell counts were greater in the mRNA-1273 group, compared to the placebo group (432 vs. 67 cells per 106 CD4+ T cells (95 % CI for the between-group difference : 46 to 986)) and there was 71 % and 13 % of the median percent virus neutralization in the mRNA-1273 group and the placebo group, respectively (95 % CI for the between-group difference : 11 to 76 percentage points) [256]. At month 4, 55 % (33 of 66) of the mRNA-1273 group and 18 % of the placebo group (10 of 57) presented an anti-RBD antibody level of at least 100 U/mL (RR : 3.1, 95 % CI : 1.7 to 5.8, $p < 0.001$) [256]. Anti-RBD antibody level was approximately 75 times in mRNA-1273 group compared to the placebo group [256, 257]. One patient in this study (placebo group; pre-infection anti-RBD antibody level : 75 U/mL) and two patients did not provide follow-up blood samples [256]. They suggested a third-dose-booster-COVID-19 vaccine in conjunction with regulatory approval for received-two-dose-of-mRNA-1273-organ-transplant recipients when considering benefit vs. risk [256, 257]. The UK government plans to roll out a third (boosted) vaccination at the beginning of the 2021 autumn for protection of the most vulnerable ahead of 2021 winter [258]. Nevertheless, the director of the Oxford Vaccine Group and the clinical trials lead for the vaccine suggested that there is no indication today for the third (boosted) vaccination [259]. Chile, a Sinovac-shot country will begin a third dose of Pfizer/BioNTech (individuals ageing under 55) and AstraZeneca (individuals with age of 55 and older, Sinovac vaccine received) vaccine on August 11, 2021 and in September 2021, respectively [260]. Recent studies revealed the loss of some efficacy of the Sinovac-vaccine shot over time [260]. Sinovac announced plans in the early August 2021 to open a vaccine plant in Chile that will serve other Latin American countries [260]. Chile, Uruguay, and Israel have announced the third doses of COVID-19 vaccination as the rapid widespread of the delta variant [260]. Currently, as of August 6, 2021, Chile is reinforcing a COVID-19 vaccination campaign of about 65 % of two-dose-delivered population [260]. Israel has announced its plan to start COVID-19-vaccine booster shots to the older adults in early August 2021 against COVID-19-delta variant [261]. A number of other rich countries are also considering the same [261]. Nevertheless, some global health researchers have warned that the current data do not yet demonstrate that third doses are needed to save the world population' lives except the immune-system-

compromised individuals [261]. Each COVID-19-vaccine booster represents a vaccine dose that could go to low- and middle-income countries, where SARS-CoV-2 (COVID-19) variants could emerge [261]. This strategy of the wealthy countries could set back efforts to end the COVID-19 pandemic [261]. Despite the recent pledges, the COVID-19 vaccines will be reached the poorest countries in 2023 [261].

A WHO's internal analysis estimated and briefed by the WHO director-general on July 12, 2021 that the 11 wealthy countries would use up around 440 million doses of the global COVID-19-vaccine supply if they are either considering vaccine boosters or rolling out it in 2021 were to give the vaccine shots to everyone with age above 50 years and the estimates doubles (880 million doses) if all high-income and upper-middle-income countries were to do the same [261]. If these vaccine shots were delivered to low- and lower-middle-income countries (85 % of population (around 3.5 million individuals), having received no doses), they would be more useful for the COVID-19 pandemic curving [261]. As of July 30, 2021, only 2 % of population in the African continent have been vaccinated and the fatality rates are higher than the global average rate [261]. As of July 30, 2021, most high-income-country-authorized-COVID-19 vaccines can decrease more than 90 % of a person's risk of hospitalization and death [261]. Many researchers do not yet know how much an mRNA-based-vaccine-extra jab on top of the standard doses would protect the average individuals [261, 262]. Nevertheless, before giving the third doses of COVID-19 vaccine, all vulnerable individuals around the world should be surely protected from COVID-19. In Thailand, on September 10, 2021, the preliminary report from the Siriraj Institute of Clinical Research (SiCRES), Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand in cooperation with the Department of Medical Sciences, Ministry of Public Health, Thailand demonstrated that the mean anti-SARS-CoV-2 (COVID-19)- RBD-IgG-antibody level to SARS-CoV-2 (COVID-19)-spike protein, measured by chemiluminescent microparticle immunoassay (CMIA; Abbot Laboratories, Ltd.) on the day of the booster vaccination and 14 days after the vaccination was highest following full dose of BNT162b2 (Pfizer) vaccine boosting (5,723 Binding Antibody Units (BAU)/mL, n = 50, 2 weeks following 2 jabs of Sinovac vaccine 8-12 weeks apart in 50 participants, $p < 0.0001$), followed by half-dose of BNT162b2 (Pfizer) vaccine boosting (4,598 BAU/mL, n = 50, 2 weeks following 2 jabs of Sinovac vaccine 8-12 weeks apart in 50 participants,

p < 0.0001), ChAdOx1 (AstraZeneca) vaccine boosting (1,559 BAU/mL, n = 59, 2 weeks following 2 jabs of Sinovac vaccine 8-12 weeks apart in 60 participants, p < 0.0001), and the lowest level when boosting with BBIBP-CorV (Sinopharm) vaccine (218.9 BAU/mL, n = 14, 2 weeks following 2 jabs of Sinovac vaccine 8-12 weeks apart in 14 participants, p < 0.0001) [263, unpublished work], whereas the 50 % plaque reduction neutralization test (PRNT50) against Delta variant (B1.617.2) at 14 days after the booster vaccination revealed the highest reciprocal neutralizing antibody titer (839.9) in the full dose of BNT162b2 (Pfizer) vaccine group, followed by the half-dose of BNT162b2 (Pfizer) vaccine (571.8), ChAdOx1 (AstraZeneca) vaccine (271.2), and BBIBP-CorV (Sinopharm) vaccine (61.3) [263]. Mild to moderate adverse events were found [263].

A most recent preliminary data on third dose (booster) intradermal COVID-19 vaccination among 10 subjects previously received two doses of Sinovac and 10 subjects previously received two doses of AstraZeneca vaccines from SiCRES, Thailand conducted by Chatsiricharoenkul et al in Thailand demonstrated on October 28, 2021 that the anti-RBD IgG levels (measured by chemiluminescent microparticle immunoassay (CMIA; Abbott Laboratories, Ltd.) after intradermally boostering with Pfizer vaccine (5 µg) and AstraZeneca vaccine (1 mL) were 3,209 BAU/mL (compared to intramuscularly half dose boostering of Pfizer vaccine (15 µg) of 3,981 BAU/mL and intramuscularly full dose boostering of Pfizer vaccine (30 µg) of 5,152 BAU/mL) and 2,810 BAU/mL (compared to intramuscularly full dose boostering of AstraZeneca vaccine (5 mL) of 1,358 BAU/mL), respectively among 10 subjects previously received two doses of Sinovac vaccine and 1,490 BAU/mL (compared to intramuscularly half dose boostering of Pfizer vaccine (15 µg) of 1,962 BAU/mL and intramuscularly full dose boostering of Pfizer vaccine (30 µg) of 2,377 BAU/mL) among 10 subjects previously received two doses of AstraZeneca vaccine. This preliminary data from SiCRES indicated that boostering with Pfizer vaccine among people who previously received two doses of Sinovac vaccine and among people who previously received two doses of AstraZeneca vaccine could have anti-RBD IgG levels of around 25-156 times at two weeks following the second dose of Sinovac vaccine, compared to the levels after the second dose of Sinovac vaccine and around 5-8 times at two weeks following the second dose of AstraZeneca vaccine, compared to the levels after the second dose of AstraZeneca vaccine, respectively [264, unpublished work].

Healthy Immune Nutrients

In the face of pathogens, some nutrients have been demonstrated to support immunity. A healthy diet, such as micronutrients, protein, and essential fats will health individuals to be more resilient and healthier. For examples, having sufficient vitamin A is associated with immunity to infections and illness. Vitamin A supplementation provides some protection against complications of life-threatening infections, such as HIV, lung diseases, and malaria [265] and decreases morbidity and mortality in infectious diseases, such as measles-associated pneumonia, measles, HIV infection, diarrheal disease, and malaria [266]. The recommended daily allowance dose is 900 (males) and 700 (females) ug retinol activity equivalents [267]. Vitamin C is currently getting a lot of attention in the time of the COVID-19 pandemic, both positive and negative. Some previous studies demonstrated that vitamin C might assist in reduction of symptoms of colds and shorten their illness duration and might assist in preventing the occurrence of colds in individuals prone to higher levels of stress and athletes when regularly taken [268-270]. In animal studies, vitamin C can protect against coronavirus infections [271, 272]. A previous study in mice with acute pulmonary dysfunction and sepsis demonstrated that intravenous infusion of 200 mg/kg vitamin C significantly decreased pulmonary edema by promoting an improvement in the structure and function of the alveolar epithelial barrier, decreased the pro-inflammatory response (cytokines), decreased infiltration of neutrophil polymorphonuclears in the lung, increased the removal of the alveolar fluid, lowering the severity of tissue damage, preventing changes in the expression of claudin-2, claudin-4, claudin-18, and occludin, preventing rearrangement of actin and the cytoskeletal adaptor protein " zonula occludens " (ZO1), and preserving the paracellular permeability of ions and small molecules [272, 273]. Vitamin C prevents damage to tight junctions associated with endothelial barrier dysfunction that involves the production of the reactive-species-superoxide-anion radical which reacts with nitric oxide, forming peroxynitrite, and with high potential to dephosphorylation of occludins and inducing damage to the endothelial barrier (Figure 45) [273-275]. Vitamin C can inhibit the enzyme NADPH oxidase that is responsible for reactive-species-superoxide-anion radical production, and eliminates reactive-species-superoxide-anion radical and peroxynitrite, therefore, preventing the dephosphorylation of occudins and the consequent loosing of tight junctions (Figure 45) [273-275]. Vitamin C may modulate the cytokine storm in COVID-19

patients [276-279] that characterized by high levels of interleukin (IL)-6, contributing to increased risk of respiratory failure requiring mechanical ventilation [280]. Pretreatment with vitamin C in 12 healthy men in a recent study demonstrated a decrease in the levels of IL-6 that released by the vasoconstrictor " endothelin " (ET-1) [281]. Increased ET-1 expression is associated with the development of acute respiratory distress syndrome (ARDS), pneumonia, interstitial pulmonary fibrosis, and pulmonary hypertension [279]. In a recent study revealed that the levels of some anti-inflammatory biomarkers, such as ferritin and D-dimer are significantly decreased after intravenously 1 g of vitamin C for 3 days in 17 COVID-19 patients with requiring 30 % of oxygen or more and simultaneously receiving hydroxychloroquine, methylprednisolone or tocilizumab as initial treatment [282]. A COVID-19 was reported receiving intravenous administration of 11 g of vitamin C and simultaneously receiving zinc sulphate, azithromycin, hydroxychloroquine, colchicine after developing ARDS and requiring mechanical ventilation and resulted in a decrease in both requiring mechanical ventilation and intensive-care-unit (ICU) stay, including a faster recovery of the patient, in comparison to those who did not receive intravenous vitamin C [283]. In a recent meta-analysis of 18 controlled clinical trials demonstrated that intravenous or oral vitamin C decreased both the duration of mechanical ventilation by 18.2 % (p = 0.001) and the length of ICU stay by 7.8 %-8.6 % (p = or < 0.003) [284]. A recent randomized, controlled, vitamin C unsuccessfully improved invasive mechanical ventilation-free days in 28 days [285]. Nevertheless, this pilot study might reveal a potential benefit in oxygenation for improving PaO_2/FiO_2 in critically ill COVID-19 patients [285]. Due to the frequency of vitamin C deficiency in patients with respiratory infections, including COVID-19 infection, it may be valuable testing vitamin C status among these patients and treating them with intravenous administration within ICUs and oral treatment in hospitalized COVID-19 patient [286]. Several previous studies demonstrated that vitamin C administration decreased the score for respiratory symptoms of pneumonia in critically ill patients [287]. Additionally, vitamin C was used as an adjuvant in two ARDS patients with an effective decrease in pulmonary edema [288, 289], decrease in the deleterious consequences of sepsis associated with acute pulmonary dysfunction, decrease in incidence of pneumonia [290] optimization of the immune defense, decrease in inflammation and organ injuries, and decrease in pathogen infection and virulence [291]. Low levels of vitamin C and older age demonstrated to be co-dependent risk factors for COVID-19 mortality, indicating serum levels of vitamin C as a predictor of mortality among COVID-19-aged patients, in addition to the findings of low levels of vitamin C among 21 critically ill-COVID-19 patients [292].

Administration of vitamin C to patients with hypovitaminosis C, severe respiratory infections, and COVID-19 infection, both critically and non-critically appears feasible and practical [293, 294]. Vitamin C could be effective choice as part of the treatment plan for COVID-19-associated lower respiratory tract infection [295]. The recommended daily allowance dose is 90 mg/day and the tolerable upper limit is 2,000 mg/day [267]. Currently, several trials on vitamin C effects and COVID-19 are undergoing in China, such as in Hubei (NCT04264533, ChiCTR2000029768 http://www.chistr.org.cn/showproj.aspx?proj=49131), Shaanxi and Hubei (ChiCTR2000029957 http://www.chictr.org.cn/showproj.aspx?proj=49633), Hubei and Shaanxi (ChiCTR2000030135 http://www.chictr.org.cn/showproj.aspx?proj=50002) [13]. As of December 7, 2020, more than 20 clinical trials with COVID-19 patients were registered and are in progress, seven of them have already began enrolling study participants, five of them intravenously administered vitamin C as an treatment (NCT02735707, NCT04264533, NCT04323514, NCT04357782, NCT04401150) and two of them as an oral treatment (NCT04382040, NCT104468139) [296].

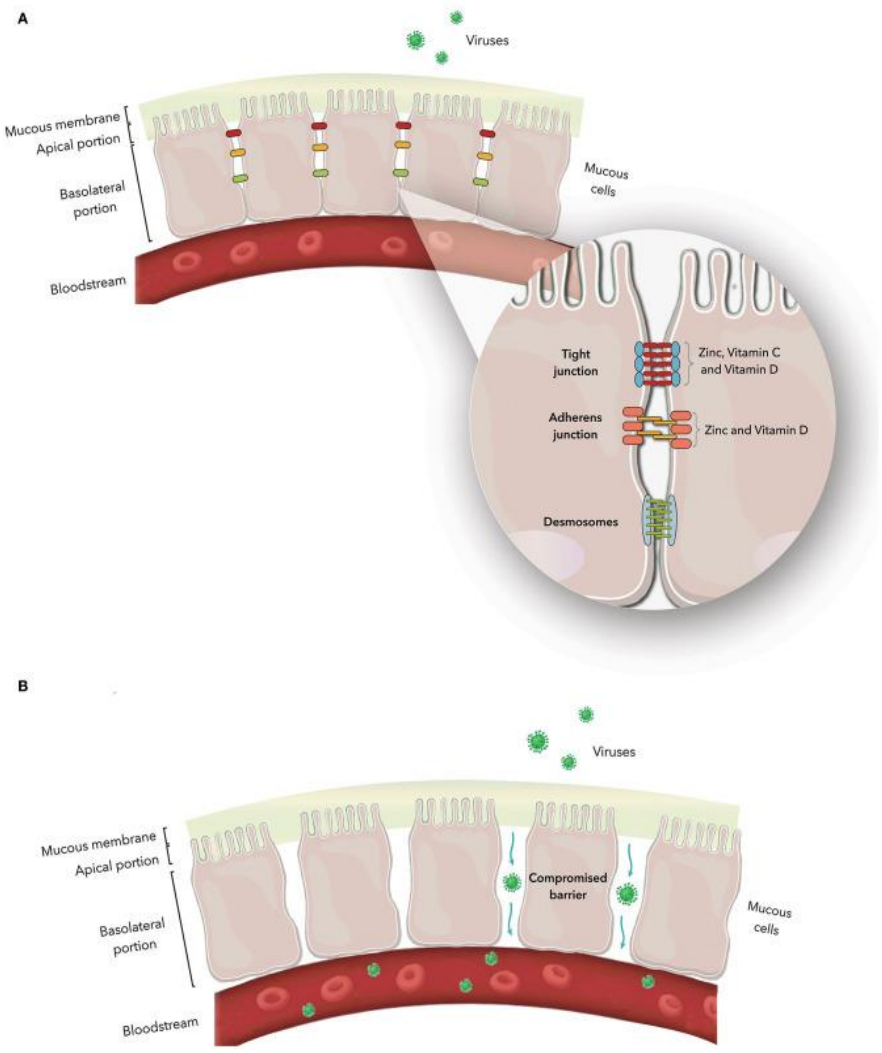

Figure 45 : (A) Junctional complex in epithelial cells. The magnification shows the arrangement of these structures in the paracellular space and the action of zinc and vitamins C and D on tight and adherens junction proteins. (B) Junctional complex dysfunction and its consequences.

(Source : Name JJ, Souza ACR, Vasconcelos AR, Prado PS, Pereira CPM. Zinc, vitamin D and vitamin C : perspectives for COVID-19 with focus on physical tissue barrier integrity. Frontiers in Nutrition 2020; 7. Article Number : 606398. DOI : 10.3389/fnut.2020.606398)

Vitamin D is a principal immune regulator and has demonstrated promise for assisting several auto-immune conditions. Vitamin D and its receptor express an influence on tight junctions, participating in the expression and function of the protein ZO1, claudin, and occludin (Figure 45) [297]. In animal studies, a reduced vitamin D status reported in calves had been demonstrated to cause by the bovine coronavirus [298]. The integrity of tight and adherens

junctions (Figure 45), a function of vitamin D is identified in human colon cancer cell lines [299]. Increased expression of some proteins, such as E-cadherin (a transmembrane protein, for maintaining the polarized epithelial cell phenotype as well as adhesion between cells [294, 295], occluding, vinculin, ZO1 and ZO from adherens junctions (Figure 45) contributing to reduced membrane permeability, as demonstrated by transepithelial electrical resistance throughout vitamin D treatment [299]. Vitamin D supplementation can increase the low levels of T regulatory lymphocytes (Tregs) found in many COVID-19 patients [300]. Vitamin D deficiency and low levels of vitamin D have been associated with an increase in inflammatory cytokines, significantly increased risk of viral upper respiratory tract infections, pneumonia, thrombotic episodes, and occur more frequently in patients with obesity and diabetes [301]. Therefore, vitamin D supplementation would offer a relatively easy option to reduce the COVID-19 impact and safe for reduction of the risk of acute respiratory tract infections and protection against acute respiratory tract infection [302, 303]. Particularly, the 1,25 $(OH)_2$D-vitamin D (25-OHD) receptor complex acts on the cathelicidin gene promotor vitamin D response elements to promote transcription of cathelicidin [304] that is found only in higher primates [305]. Other functions of cathelicidin includes the stimulation of the chemotaxis of neutrophils, monocytes, macrophages, and T cells into the site of infection, promotion of the clearance of respiratory pathogens by inducting apoptosis and autophagy of infected epithelial cells, and the induction of a variety of proinflammatory cytokines [306, 307]. The serum concentrations of 25-OHD are well correlated with the ability of macrophages to produce cathelicidin [308, 309]. These findings support a reasonable explanation for the reported other respiratory diseases or disorders and tuberculosis [310]. The average daily recommended dose is 600 IU and the tolerable upper limit dose is 4,000 IU [267]. Jain and colleagues demonstrated that the inflammatory response was high in COVID-19 patients with vitamin D deficiency [311]. In severe COVID-19 patients, vitamin D level was markedly low [311]. The fatality rate was high in COVID-19 patients with vitamin D deficiency (21 % versus 3.1 %) [311]. This indicated increased mortality in COVID-19 patients with vitamin D deficiency [311]. They recommended mass administration of vitamin D supplements to people at high risk for COVID-19 [311]. More over, treatment of colon cancer cells with vitamin D can re-establish tissue morphology by increasing the expression of tight and adherens junction proteins, such as E-cadherein transmembrane protein, etc. (Figure 45) [312-314]. Vitamin E has immunomodulatory and anti-inflammatory effects. Bioflavonoids from plants can reduce upper-respiratory-tract infections, indicated by previous research. Trial on alpha lipoic acid and COVID-

19 is also undergoing in China (ChiCTR2000029851 http://www.chictr.org.cn/showproj.aspx?proj=49534) [13].

Vitamin B2 and ultraviolet (UV) light effectively decrease the titer of MERS-CoV in the human plasma products [315]. The deprivation of B vitamins may weaken host immune response, therefore, they are recommended to supplement to virus-infected patients to enhance their immune system [310]. Zinc, an essential substance for the homeostasis and integrity of the intestinal barrier (Figure 45) [316-319]. Supplementation with 100 μM zinc can re-establish barrier homeostasis and decreases the permeability of tight junctions (Figure 45) [319], decrease in claudin-2 and claudin-7 protein levels [320-324], and influencing the increased resistance of the epithelial barrier, that is associated with decreased electrolytic permeability [320]. Additionally, zinc deficiency contributes to the delocalization of β-catenin and E-cadherin proteins and delocalization of the cytoskeleton in Caco-2 cells, contributing to increased permeability and consequently, neutrophil infiltration in the paracellular space, and induction of an inflammatory response in the intestinal epithelium [325]. Exposure to cytokines in zinc-deficient lung epithelial cells contributes to increased cell death by apoptosis and barrier dysfunction through β-catenin and E-cadherin proteolysis [326]. The level of intracellular zinc depletion and the time of exposure to zinc depletion associated with acute inflammation are directly proportional to cell apoptosis and barrier dysfunction [327]. Based on the above findings, the mobilization of zinc to epithelial cells at the beginning of an inflammatory response in the lung is an essential innate response both to increase the immune function and to protect other cells from damage caused by inflammation [326]. Zinc supplementation given to zinc-deficient children could decrease measles-associated morbidity and mortality that caused by lower respiratory tract infections [327]. Combination of zinc and pyrithione at low concentration can inhibit the replication of SARS-CoV [328]. The recommended daily allowance dose is 15 mg and the tolerable upper limit dose is 40 mg [267]. Addition of N-acetylcysteine (NAC), a powerful anti-oxidant in the therapy for community-acquired pneumonia can decrease tumor-necrosis-factor (TNF)-alpha and may decrease oxidative and inflammatory in the lungs of the COVID-19-associated pneumonia patients [329].

Other foods that might boost the immune system, based on previous studies are : 1) non-dairy, unsweetened dark chocolate (containing theobromine, an antioxidant); 2) blueberries (containing anthocyanin, a type of flavonoid, an antioxidant); 3) curcumin or turmeric; 4) fish

rich in omega oil (containing omega-3 fatty acids); 4) broccoli; 5) sweet potatoes; 6) spinach (containing carotenoids, flavonoids, vitamin C, vitamin E, antioxidants); 7) ginger (antioxidant); 8) garlic (a common home remedy for the prevention of colds); 9) matcha or green tea (containing flavonoids-may reduce the risk of a viral infection, small amount of caffeine); 10) kefir (an antioxidan); 11) sunflower seeds (containing rich vitamin E, an antioxidant); 12) almonds (containing rich vitamin E, an antioxidant); 13) kiwifruit and orange (containing rich vitamin C-may reduce the duration of common cold and improve immune function); and 14) red bell pepper [330-332].

Conclusion

Currently, there are no finally verified antivirals and vaccine candidates specific to COVID-19. Further preclinical and clinical trials are urgently needed to successfully treat patients with COVID-19 disease and preventing individuals from COVID-19 infection. The intradermal COVID-19 vaccination could provide less volume of vaccine doses, compared to the intramuscular vaccination. *In vitro* and *in vivo* studies are needed before beginning the clinical trials on killing effects of *Andrographis paniculata* and *Boesenbergia rotunda* (Finger Root) on COVID-19 for clinical safety and its efficacy in patients with COVID-19 infection. In addition to taking the various boosting-healthy-immune-system foods mentioned above during the COVID-19 pandemic, the individuals should follow the guidance for maintaining a balance diet : 1) eat fruits and veggies, 2) eat smaller meals more frequently, and 3) drink plenty of fluids.

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CHAPTER 8

Mesenchymal Stem Cell Transplantation in Treating Severe COVID-19

Abbreviations :

ACE 2 : angiotensin-converting enzyme 2

AF : amniotic fluid

ALT : alanine transaminase

Ang-1 : angiopoietin-1

ARDS : acute respiratory distress syndrome

AST : aspartate transaminase

AT : adipose tissue

BALF : bronchoalveolar lavage fluid

BM : bone marrow

CFU-F : colony-forming unit fibroblast

CIK : cytokine-induced killer cells

CM : conditioned media

COVID-19 : coronavirus disease 2019

CRP : C-reactive protein

CT : computed tomography

CTL : cytotoxic T cells

DC : dendritic cell

DP : dental pulp

EVs : extracellular vesicles

FiO_2 : fraction of inspiration oxygen

G-CSF : granulocyte-colony-stimulating factor

GVHD : graft-versus-host disease

HGF : hepatocyte growth factor

HLA : human leukocyte antigen

ICU : intensive care unit

IFITM : interferon-induced transmembrane protein

IFN-α : interferon-alpha

IFN-γ : interferon-gamma

IL : interleukin

IP : interferon gamma-induced protein

ISG : interferon-stimulated gene

KGF : keratinocyte growth factor

MCP : monocyte chemoattractant protein

MIP : macrophage inflammatory protein

MSCs : mesenchymal stem cells

NK : natural killer cells

PaO_2 : arterial partial pressure of oxygen

PB : peripheral blood

PL : placenta

RT-PCR : reverse transcriptase-polymerase chain reaction

SARS-CoV-2 : severe acute respiratory syndrome coronavirus-2

SF-36 : 36 Item Short-Form Health Survey

TGF-β : transforming growth factor-beta

TMPRSS2 : type II transmembrane serine protease

TNF-α : tumor necrosis factor-alpha

UC : umbilical cord

UCB : umbilical cord blood

WJ : Warton jelly

Introduction

In vitro, mesenchymal stem cell (MSC) populations with potentials of similar multi-lineage differentiation have been obtained from several bone marrow (BM) and non-bone marrow tissues [1], including umbilical cord [2-4], placenta [5], amniotic fluid [6, 7], adipose tissue [8, 9], and peripheral blood [10]. The clonogenic BM-human MSCs fraction ranges from 10 to 100 colony-forming unit-fibroblast (CFU-F) per 10^6 marrow mononuclear cells (MNCs) [11]. BM-human MCSs are characterized by lacking CD11b, CD14, CD19, CD34, CD45, CD79α, and human leukocyte antigen (HLA)-DR expression; positive expression of surface antigens CD73, CD90, and CD105; multipotency (i.e., chondrogenic, osteogenic, and adipogenic); and their adherence to plastic [11]. By the year 2000, clinicians increasingly had become interested in intravenously applied MSC therapy [12]. A previous study demonstrated that both human and murine MCSs can induce immune suppression by attracting and killing autoreactive T cells via FasL, therefore stimulating transforming growth factor-beta (TGF-β) production by macrophages and generation of regulatory T cells [13]. The dying T cells that is caused by the interaction involving the MSC-induced Monocyte Chemoattractant Protein-1 (MCP-1) secretion in turn activate macrophages to produce TGF-β, then stimulating regulatory T cells and promoting immune tolerance [14]. The capacity of MSCs for *in vivo* differentiation and engraftment and by their efficacy in promoting wound healing highlighted its clinical relevance [15-21].

In 2006, the International Society for Cellular Therapy came up with the guidelines for MSC characterization for standardization the MSC biology, definition, isolation, and characterization criteria, *in vivo* relevance, and ethical and institutional regulations for its clinical use [11]. Since the COVID-19 pandemic, there are several ongoing trials that have been studied in China, such as the ClinicalTrials.gov identifiers : NCT04252118, NCT04273646, NCT04276987, NCT04293692, NCT04302519, NCT04288102, etc. for fighting against severe COVID-19 or COVID-19 pneumonia [22-27]. MSCs can decrease the overproduction of immune cells caused by a reaction to the COVID-19 and decrease excessive levels of inflammatory substances, contributing to regulating the immune system and recovering to the normal status, particularly of the elderly patients [28].

Published Year	Article Content	Reference
	Clinical trial ID (Registry) : 1) NCT04293692 (ClinicalTrials.gov, umbilical cord MSCs, randomized, triple blinded, withdrawn, China); 2) NCT04273646 (ClinicalTrials.gov, umbilical cord MSCs, randomized, non-blinded, not recruiting, China); 3) NCT04269525 (ClinicsalTrials.gov, umbilical cord MSCs, non-randomized, non-blinded, recruiting, China); 4) ChiCTR2000030138 (ICTPR, umbilical cord MSCs, randomized, double blinded, not recruiting, China); 5) ChiCTR2000030484 (ICTPR, umbilical cord MSCs and derived exosomes, unspecified randomized, unspecified blinded, not recruiting, China); 6) ChiCTR2000030116 (ICTPR, umbilical cord MSCs, randomized, unspecified blinded, recruiting, China); 7) ChiCTR2000029816 (ICTPR, umbilical cord MSCs, randomized, non-blinded, not recruiting, China); 8) NCT04313322 (ClinicalTrials.gov, Wharton jelly MSCs, non-randomized, non-blinded, recruiting, Jordan); and 9) ChiCTR2000030088 (ICTPR, Wharton jelly MSCs, randomized, unspecified blinded, not reruiting, China)	29
	Human umbilical cord MSCs effectively modulated the immune response and repaired injured tissues of critically ill COVID-19 patient with excellent safety by intravenous route three times (5×10^7 cells each time) every three days. After second administration, the vital signs were improved, the tracheal tube was pulled off and the serum C-reactive protein (CRP), serum alanine transaminase/aspartate transaminase (ALT/AST), and serum bilirubin were gradually decreased. MSc significantly improved the pulmonary function and clinical symptoms of patients with COVID-pneumonia.	30

2020	MSC transplantation in patients with influenza A (H_7N_9) infection induced ARDS were conducted in a single center and open-label clinical trial. Several parameters, such as computed tomography of the chest (1 week, 1 months, 6 months, and 12 months), pulmonary ventilatory function (6 months and 12 months), 36 Item Short-Form Health Survey (SF-36) (Chinese version) of the Medical Outcome Study (Health-Related Quality of Life (HRQoL) (6 months and 12 months) after MSC transplantation. MSCs have ability to decrease inflammatory effects and defend against cytokine storm. MSCs probably decrease the secretions of the inflammatory factors. MScs significantly decreased the mortality (16.7 % in MSC group versus 54.5 % in control group). No serious adverse effects are identified after MSC transplantation during the period of 5 years. Nevertheless, long-term pulmonary dysfunction is still a problem after 2 years of hospital discharge.	31
	In animal models, H_9N_2 viral infection increases serum and pulmonary chemokines responsible for pulmonary leukocyte infiltration. Antiviral protein members of the IFITM family members are unique as they prevent infection before a virus can traverse the lipid bilayer of the cell contributing the limitation of infection in cultured cells by many viruses, such as SARS coronavirus, influenza A virus, Ebola virus, dengue virus, etc., whereas knockdown of IFITM3 rendered MSC susceptible to infection by a variety of viruses, such as Zika virus, Yellow Fever virus, etc. Pro-inflammatory cytokines including IFN-γ induced non-constitutive ISGs including CD74, IFNAR2, MT1G, MT1X, SAT1, SERPING1, whereas significantly increasing the expression of constitutive antiviral genes, such as CCL2, IFI6, IFITM1, ISG15, PMAIP1, and SAT1. In a respiratory viral infection including COVID-19, MSCs	

	might present two distinct antiviral mechanisms : 1) constitutively elevated the levels of MSC-specific ISGs to function as mediators of an antiviral protection, and 2) a secondary response to IFN, contributing to ISG induction and broad viral resistance. MSCs could present a mix of inducible and intrinsic innate antiviral defenses that could contribute to treatment benefits in severe COVID-19 patients. BM-MSCs are permissive to avian influenza A (H_5N_1) infection, losing immunoregulatory activities, and viability. Virus-infected MSCs may not be functionally effective at stopping virus replication and pulmonary injury. BM-MSCs and UC-MSCs were more effective than adipose tissue-derived MSCs in decreasing mortality in pre-clinical acute lung injury models (rodents, pig, sheep, and explanted human lungs). Neither synergistic or xenogeneic MSC administration, either alone or as an adjuvant therapy with oseltamivir was effective either when administered prophylactically, prior to H_1N_1 virus inoculation, or when therapeutically administered, similar to the results of both systemic and intratracheal administration of human and mouse MSCs. MSCs did not improve influenza-mediated lung injury regardless of administration route. Avian influenza-virus infection can trigger a very intense pro-inflammatory response compared to other influenza virus, thus the beneficial effects might be a specific consequence of different pathogenic features as compared to swine-origin H_1N_1 infection. *In vitro* airway epithelial cell models and experimental lung injury induced by Influenza A (H_5N_1) infection in female mice, UC-MSCs were more effective than human BM-MSCs at restoring impaired alveolar fluid clearance and permeability. MSCs-derived EVs were more effective than MSCs themselves in some H_5N_1 cases inflammation and injury of the lungs in pre-clinical lung injury	32

	models, particularly EVs isolated from pig BM-derived MSCs in reducing virus shedding in nasal swabs, influenza replication in the lungs, BALF pro-inflammatory cytokines and chemokines, and histopathologic changes in a mixed swine (H_3N_2, H_1N_1) and avian (H_9N_5, H_7N_2) influenza-induced pig lung injury model after 12 hours of administration. Clinical improvement within 2-4 days after MSC administration was observed in a patient with critically severe COVID-19. The intravenous dosing range of MSCs varies between 0.4 and 42 X 10^6 cells/kg. The highest dose of MSCs used in non-viral ARDS was 10 X 10^6 cells/kg (START trial). The MSC dosing strategy ranged between a single and 5 doses with an average frequency of every 2 days.	
	Seven patients with COVID-19 pneumonia were enrolled and evaluation of the outcomes for MSC transplantation after 14 days of MSCs injection (January 23, 2020-February 16, 2020). The pulmonary function (one million MSCs per kilogram body weight) and symptoms of all 7 patients were significantly improved without observed adverse effects within 2 days after MSC injection. One severe and two common COVID-19 patients were recovered and discharged in 10 days after MSC treatment. After MSC transplantation, the CRP decreased, peripheral lymphocytes increased, and the overactivated cytokine-secreting immune cells (CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells) disappeared in 3-6 days. CD14+CD11c+CD11b^{mid} regulatory DC cell population increased. TNF-α level was significantly reduced. IL-10 level increased. The cytokine storm was dramatically improved in one patient with critically severe COVID-19 in 2-4 days after MSCs injection. MSCs were free from COVID-	33

	19 infection by demonstration of ACE2 and TMPRSS2 in gene expression profile.	
	Much superiority in using MSC treatment in comparison with other treatment modalities includes : 1) easily accessible and can be isolated from various tissues (BM, AT, UC, buccal fat pad, fetal liver, menstrual blood, etc.), 2) are multipotent stem cells, 3) MSCs can be stored for repetitive therapeutic usage, 4) MSCs can easily expand to clinical volume in a proper period of time, 5) clinical trials of MSCs have not demonstrated adverse side effects or reactions to allogeneic MSC, and 6) several clinical trials demonstrated safety and effectiveness of MSCs. MSC treatment probably can prevent the cytokine storm releasing by the immune system and promote endogenous repair by reparative properties of the MSCs. MSCs could protect pulmonary alveolar epithelial cells, recover the pulmonary microenvironment, intercept pulmonary fibrosis, and cure pulmonary dysfunction and COVID-19 pneumonia. MSCs can be isolated from BM, PB, AT, PL, UC, WJ, AF, UCB, and neonatal birth-associated tissues. MSC transplantation is also widely used in the treatment of graft-versus-host disease (GVHD), spinal cord injury, autoimmune disease, type 2 diabetes mellitus, and other high immunity diseases. Second and third intravenous injections of UC-MSCs in combination with lopinavir/ritonavir, α1-thymosin, IFN-α, and oseltamivir as well as intravenous injection of immunoglobulin, methylprednisolone, Xuebijing, and moxifloxacin in a 65-year-old female patient with critically ill COVID-19 demonstrated the improvement of COVID-19 pneumonia 2 days after the third injection of MSCs.	34

Table 9 : Demonstrating article contents of mesenchymal stem cell transplantation in treating COVID-19 and references published between January 2020 and April 2020.

Currently, there are no approved therapeutic options for either the prevention or treatment of COVID-19 [29]. MSCs act via a paracrine mechanism [30]. They release biological active substances " secretome " that is made of both growth factors and extracellular vesicles (EVs) [32] and soluble proteins, including a broad spectrum of chemokines and cytokines. EVs are also described as mediating the protective effects of MSCs in pre-clinical models of bacteria and non-infectious acute pulmonary injury [32]. Additionally, MSCs can secrete angiopoietin-1 (Ang-1), keratinocyte growth factor (KGF), and hepatocyte growth factor (HGF) that contribute to the restoration of alveolar-capillary barriers disrupted as part of ARDS pathogenesis [32]. Releasing soluble proteins and EVs interact with the target cells by internalization or ligand-receptor interaction. MSC-secretome acts on several cytokines potentially, simultaneously, and synergistically [30]. MSC-secretome can activate endogenous stem cells, and progenitor cells, regulate the inflammatory response, stimulate angiogenesis and remodeling of the extracellular matrix, suppress apoptosis, mediate chemoattraction, and reduce fibrosis [30]. Nevertheless, mediators responsible for ameliorating respiratory viral-induced lung injury remain unclear [32]. One of the two distinct antiviral mechanisms of the MSCs is the constitutively elevated levels of MSC-specific interferon-stimulated genes (ISGs) to function as mediators of an antiviral protection [32]. The effectiveness of MSC-secretome on autoimmune diseases and ARDS is evidenced, both *in vivo* and *ex vivo*. Secretome with highly stability in the blood circulation spread into tissues, particularly lungs and provide immune modulation, restoration of capillary barrier function, resolution of inflammation, and enhance bacterial clearance [30]. Generally, secretome is considered safer than MSCs due to low immunogenicity [30], low emboli formation [30, 31], and lacking the potential for endogenous tumor formation [30] in treating COVID-19, virus-induced ARDS and other viral disease, such as H_7N_9-severe lung disease [30, 31]. With fewer costs and ready-to-use product, MSC-secretome treatment seems to be technological advantages [30].

Very recently, MSC-secretome can be formulated as both injectable dosage forms and inhalable dosage form. MSC-secretome therapy emerges as a promising cell-free treatment modality for both acute and chronic pulmonary diseases [30]. Recently, two Chinese clinical trials, NCT04276987 (inhaled secretome for the treatment of critically ill COVID-19 pneumonia) and NCT04313647 (secretome tolerance in healthy volunteers)

appeared on the URL : http//www.clinicaltrials.gov [30]. A previous report from China revealed that the levels of serum IL-2, IL-7, G-SCF, IP-10, MCP-1, MIP-1 A, and TNF-α in ICU-COVID-19 patients were higher than those of non-ICU-COVID-19 patients [33]. Nevertheless, there are only a small number of pre-clinical investigations on effects of MSC administration in pre-clinical models of respiratory virus infections and there yet no pre-clinical data investigating the effects of MSC administration in the models of coronavirus respiratory infection, mostly due to lacking an established animal model. [32].

A previous study on both human and mouse MSCs administration demonstrated that MSCs did not improve influenza (H_1N_1)- mediated pulmonary injury regardless of administration route [32]. Nevertheless, there are evidence-based studies that MSC therapy can inhibit the overactivation of the immune system and promote endogenous repair by improving the microenvironment [33]. At least 4 of the trials will utilize either MSC-derived conditioned media (CM) or EVs. Two of these propose aerosol inhalation of MSC-derived EVs, one from adipose-derived MSCs, for which there is no pre-clinical supporting data. Six studies will utilize other cells including UCB-derived mononuclear cells, cytotoxic T cells (CTL), dendritic cells (DC), natural killer cells (NK), umbilical cord blood stem cells (UCB-SC), or cytokine-induced killer cells (CIK). Only the latter study describes dosing and frequency of MSC injections [32]. Apparent pre-clinical data are not available to support the rationale for any of these therapeutic interventions. More pre-clinical data involving the models of coronavirus-induced pulmonary injuries are needed to initiate trials of MSC-based studies with highest standards for rationale and properly designed investigations. These are the only ways that a rationale evidence-based framework for potential cell-based therapies can be developed [32]. A recent study of 7 COVID-19 patients in China (one with critical severe type, 4 with severe type, and the other 2 with common type of COVID-19 syndrome) were received 1 million MSCs per kilogram body weight and were closely observed their symptoms for 14 days. This study revealed that all symptoms disappeared by 2-4 days after MSCs intravenous administration with no apparent adverse effects [32-34]. The majority of patients demonstrated negative results of the reverse transcriptase polymerase chain reaction (RT-PCR) tests for COVID-19 or SARS-CoV-2 or novel coronavirus-2019 nucleic acid over a week or two weeks as well as the significant resolution of pneumonic infiltration in the chest computed tomographic (CT)

imaging after MSC intravenous administration [32-34]. The National Health Commission of China classifies the clinical grading of the COVID-19 as the following : 1) Mild type-mild clinical manifestation, none imaging performance, 2) Common type-fever, respiratory symptoms, pneumonia performance on chest X-ray or CT, 3) Severe type-meet any of the followings : 3.1) respiratory distress, respiration rate at least 30/minute, 3.2) oxygen saturation not higher than 93 % at rest state, and 3.3) arterial partial pressure of oxygen (PaO_2)/fraction of inspiration oxygen (FiO_2) not higher than 300 mmHg (1 mmHg = 0.133kpa), and 4) critically severe-meet any of the followings : 4.1) respiratory failure needs mechanical ventilation, 4.2) shock, and 4.3) combined with other organ failure, patients need ICU monitoring and treatment [33]. Anti-inflammatory and immunomodulatory properties of MSCs in the treatment of respiratory diseases were confirmed by at least 17 clinical studies and more than 70 clinical trials are registered in this issue that are available at : https://www.clinicaltrials.gov [34]. MSC transplantation improves the treatment outcome of COVID-19 patients may be due to controlling inflammatory response and promoting tissue regeneration and repair [33].

Conclusion

Human MSCs are currently being evaluated as a stem cell treatment for a number of diseases, particularly severe COVID-19 and have been demonstrated to be safe in clinical trials. There are some promising reports to apply MSCs therapy to treat COVID-19. MSCs may possibly be one of the most ideal therapeutics, or a combination of treatment to treat patients with COVID-19. Nevertheless, further studies are urgently needed to investigate and optimize a number of variables in the human MSC culture environment by developing a bioprocess that can be operated in accordance with the Good Manufacturing Product (GMP).

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CHAPTER 9

Management of Critically Ill-Adult Patients with Severe COVID-19 in Intensive Care Unit

Abbreviations:

AKI : Acute Kidney Injury

ARDS : Acute Respiratory Distress Syndrome

COPD : Chronic Obstructive Pulmonary Disease

CPAP : Continuous Positive Airway Pressure

CT : Computed Tomography

ECMO : Extracorporeal Membrane Oxygenation

FiO_2 : Fraction of Inspired Oxygen

GI : Gastrointestinal

HFNO : High-Flow Nasal Oxygen

ICU : Intensive Care Unit

IPC : Infection Prevention and Control

IQR : Interquatile Range

MAP : Mean Arterial Pressure

NIV : Non-Invasive Ventilator

PaO_2 : Partial Pressure of Oxygen

PBW : Predicted Body Weight

PEEP : Positive End-Expiratory Pressure

RCT : Randomized Controlled Trial

RM : Recruitment Manoeuvres

RNA : Ribonucleic Acid

RT-PCR : Reverse Transcriptase-Polymerase Chain Reaction

SAR-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus 2

SARI : Severe Acute Respiratory Infection

SOFA : Sequential Organ Failure Assessment

SpO_2 : Saturated Partial Pressure of Oxygen

Immunological Reactions

Previous studies demonstrated an increased severity towards the closely related SARS-CoV diseases in persons with HLA-B*46 : 01 [1]. Differences in HLA haplotype may influence the persons' response to SARS-CoV-2 (COVID-19) infection and some haplotypes may be associated with increased disease severity. Thus, HLA genotyping may help in identifying persons at risk. COVID-19 testing along with HLA genotyping is highly recommended to predict susceptibility to disease severity and assisting in future vaccination strategy plan.

In acute respiratory distress syndrome (ARDS), IL-1β and its family (IL-18, IL-33) are significant players to increase the recruitment of immune cells subsequent production of cytokines [2]. IL-1β and TNF-α are required to develop Th17 cells and assist in Th17 mediate immune response and increased vascular permeability [2]. IL-17 and GM-CSF, cytokines of the Th17 pathway increasing in patients with severe COVID-19 [3] have researchers urgently investigate the role of Th17 in severe COVID-19 cases [4]. Th17 cell-increased expression in the peripheral blood of patients with COVID-19 indicates a player in the COVID-19 cytokine storm as reported in the patients with MERS and SARS [5]. Some Th17 pathway-specific cytokines, such as GM-CSF, IL-1β, IL-17, and TNF-α are elevated in severe COVID-19 patients [3] A case study on severe COVID-19 demonstrated an elevated count of Th17 cells, activated CD4+, and CD8+ T cells [6], whereas another previous study revealed a decrease in Th17 subset indicated by low IL-17 secretion urges the need to investigate the role of Th17 specific response in COVID-19 [7]. An increased IFN-γ, IL-1β, IP-10, and MCP-1 serum concentrations contribute to the activation of Th1 cell response and further aggravation the cytokine storm like the occurrence in MERS-CoV and SARS-CoV [8, 9].

IL-6, a multifunctional cytokine involving the formation of follicular helper T cells, generation of plasma cells, and differentiation of Th 17 cell subsets plays a primary role in cytokine storm that occurs in patients with COVID-19 [10]. IL-6 also inhibit IFN-α, therefore, suppress CD8+ cytotoxic T cells [10]. As demonstrated by *PD-1* and *Tim-3* expressions, IL-6 induces T cell exhaustion, thus, T cell-mediated immune response might be suppressed during cytokine storm [11]. Role of IL-6 in COVID-19 disease severity has been demonstrated by increased IL-6 levels and its positive association with disease severity [11-18]. A recent report from Germany revealed that COVID-19 cases with IL-6 levels of at least 80 pg/ml had a 22-fold increased risk of respiratory failure with median time to mechanical ventilation of 1.5 days [19] and higher serum IL-6 levels were also reported even 24 hours before death [20, 21]. Thus, IL-6 could be used for early detection of COVID-19 patients at risk for respiratory failure as a single parameter or in association with other parameters.

Single cell RNA-sequencing-based characterization of bronchoalveolar lavage fluid (BALF) from three severely ill, three mild COVID-19 patients and eight healthy subjects demonstrated that a monocyte-derived *FCN-1*$^{+}$ macrophages were the predominant macrophage in the BALF [22]. An elevated level of CD14^{+} and CD16^{+} monocyte subset was revealed in patients with COVID-19 when compared to healthy subjects and also demonstrated higher level in COVID-19 patients requiring ICU admission [23]

A previous study on immunophenotyping the antiviral response in 4 patients with COVID-19 (male young, male elderly, female young, female elderly) by using PBMCs collected pre-ICU, during ICU, and post-ICU stays demonstrated a significant increase in monocytes and plasmacytoid dendritic cells (pDC) populations and a gene signature of elevated expression of DDX58, IRF8, TLR7, and ISGs like *IFITM1* in the ICU patients' specimens, when compared to pre- and post-ICU specimens [7]. Therefore, there is evidence of delayed or dampened type-1 IFN response in the initial phases of the infection with subsequent increase with active viral replication, a part of pathogenesis of SARS-CoV [5, 24]. A previously subsequent study in 50 COVID-19 patients of varying severity involving profiling of cytokine levels, whole blood transcriptome, and immune cells demonstrated a significant impaired type-1 IFN response in the critical patients, characterized by reduced levels of IFN-α and IFN-β accompanying with high levels of IL-6 and TNF-α. This study

also revealed a significant downregulation of 6 ISGs that specify type-1 IFN response in severe COVID-19 patients and reduction of pDC population compared to healthy subjects [25].

Roles of Corticosteroids in Treating Critical, Severe and Non-Severe COVID-19 Patients

Glucocorticoids or corticosteroids have main anti-inflammatory effects to inhibit a vast number of pro-inflammatory genes that involve encoding of cell adhesion molecules, chemokines, cytokines, inflammatory receptors and enzymes to restore homeostasis and address the inflammatory process [26]. A previous systematic review and meta-analysis revealed that severe COVID-19 patients were more likely to require corticosteroids treatment (RR = 1.56, 95 % CI = 1.28-1.90, $p < 0.001$) [27]. The length of stay (LOS) was longer in the corticosteroid group (WMD = 6.31, 95 % CI = 5.26-7.37, $p < 0.001$, $I^2 = 1.8$ %, $p = 0.361$ as well as the same results in the subgroup analysis of SARS-CoV-infected patients (WMD = 6.34, 95 % CI = 5.24-7.44, $p < 0.001$, $I^2 = 50.3$ %, $p = 0.156$) [27]. Nine studies in this systematic review and meta-analysis by pooled relative risk demonstrated higher mortality in COVID-19 patients receiving corticosteroid treatment (RR = 2.11, 95 % = 1.13-3.94, $p = 0.019$, $I^2 = 80.9$ %, $p < 0.001$), whereas mortality of neither SARS-CoV (RR = 2.56, 95 % CI = 0.99-6.63, $p = 0.053$, $I^2 = 77.4$ %, $p < 0.001$) nor MERS-CoV (RR = 2.06, 95 % CI = 0.66-6.44, $p = 0.213$, $I^2 = 89.4$ %, $p = 0.002$) [27].

Corticosteroid-treated COVID-19 patients were more likely to develop adverse reactions, such as hypokalemia (RR = 2.21, 95 % CI = 1.07-4.55, $p = 0.032$, $I^2 = 53.1$ %, $p = 0.104$) and bacterial infection (RR = 2.08, 95 % CI = 1.54-2.81, $p < 0.001$, $I^2 = 0.0$ %, $p = 0.926$) [27]. The study revealed no association between corticosteroid treatment and the development of hypocalcemia (RR = 1.35, 95 % CI = 0.77-2.37, $p = 0.302$, $I^2 = 80.4$ %, $p = 0.024$) and or hyperglycemia (RR = 1.37, 95 % CI = 0.68-2.76, $p = 0.376$, $I^2 = 74.2$ %, $p = 0.049$) [27]. Nevertheless, funnel plots in this study demonstrated no publication bias on the corticosteroid usage in critical and non-critical COVID-19 patients, whereas mortality might be included in the publication bias [2]. Different previous studies have demonstrated corticosteroid effects varying from harmful to beneficial [28]. Wu *et al* concluded that use

of steroids was not statistically different between COVID-19-related-acute-respiratory-distress-syndrome (ARDS) survivors and COVID-19-related-ARDS non-survivors [29].

Inhaled Corticosteroids for Treatment or Prevention of COVID-19

X *et al* demonstrated that COVID-19 patients treated with corticosteroids were more likely to be associated with harm [30], whereas Russell *et al* concluded that neither inhaled or systemic corticosteroids was distinguished [31]. In a previous *in vitro* study, inhaled corticosteroids (ICS) can inhibit SARS-CoV-2 (COVID-19) replication in infected epithelial cells [32]. COVID-19 patients with ICS treatment has been demonstrated reduction of inflammatory biomarkers [33] and improvement of pulmonary physiology [34]. Patients with stable asthma and stable chronic obstructive pulmonary disease (COPD) while using ICS should continue ICS treatment. At the onset of an exacerbation of asthma, there is no evidence to suggest increasing the dose of ICS. Ciclesonide, a proposed candidate ICS has been demonstrated suppression of SARS-CoV-2 (COVID-19) replication in cultured cells and suggested direct inhibition of acting anti-viral activity [35].

The World Health Organization's Recommendation in Corticosteroids for COVID-19 Treatment

Eight randomized control trials (RCTs) (7,184 COVID-19 Patients) were reviewed by the World Health Organization's panel on July 17, 2020 [36]. The largest of the seven trials, " RECOVERY " reported the mortality among subgroup of 6,425 hospitalized patients with severe and non-severe COVID-19 (2,104 were randomized to dexamethasone and 4,321 were randomized to usual care) in the United Kingdom by evaluating the effects of oral or intravenous dexamethasone 6 mg prescribed once daily for up to 10 days. Sixteen percent of patients were receiving invasive mechanical ventilation or extracorporeal membrane oxygenation, 60 % of patients were receiving oxygen only (with or without non-invasive ventilation), and 24 % were receiving neither at the time of randomization [37]. The seven other smaller trials included approximately 700 critically ill patients (critically illness definition varied across the studies, enrollment was up to June 9, 2020) and 63

non-critically ill patients [36]. Approximately four-fifths were invasively mechanically ventilated, approximately 50 % of patients were randomized to receive corticosteroid treatment, and approximately 50 % were randomized to no corticosteroid treatment [36]. All trials reported the mortality at 28 day after randomization, whereas one trial reported at 21 days and the other reported at 30 days [36].

The corticosteroid regimens in the trials included methylprednisolone 40 mg every 12 hours for 3 days and then 20 mg every 12 hours for 3 days (GLUCOCOVID trial) [38], dexamethasone 20 mg daily for 5 days followed by 10 mg daily for 5 days (DEXA-COVID and CoDEX trials) [39], hydrocortisone 200 mg daily for 4 to 7 days followed by 100 mg daily for 2 to 4 days and then 50 mg daily for 2 to 3 days (CAPE-COVID trial) [40], hydrocortisone 200 mg daily for 7 days (REMAP-CAP) [41], methylprednisolone 40 mg every 12 hours for 5 days (Steroids-SARI) [42, 43]. Seven of these studies were conducted in Brazil, China, Denmark, France, and Spain, whereas REMAP-CAP was recruited in 14 European countries, Australia, Canada, New Zealand, Saudi Arabia, and the United Kingdom [36]. The WHO's panel reviewed the data from the GLUCOCOVID (n = 63) trial [38] only the data involving the outcome of mechanical ventilation due to the mortality data being not reported by subgroup [38]. The WHO recommends systemic corticosteroids rather than no systemic corticosteroids for the treatment of critical and severe COVID-19 patients (strong recommendation, based on moderate certainty evidence) [36]. Even the WHO's strong recommendations, these recommendations should not be applied to patients in whom the intervention is contraindicated as determined by the treating clinician. These recommendations are applied to critical and severe COVID-19 patients regardless of hospitalization status [36]. The WHO suggests not to use corticosteroids in treating non-severe COVID-19 patients (conditional recommendation, based on low certainty evidence) [36].

The WHO defines the exclusive categories of the COVID-19 illness severity as the following [36] :

1) Critical COVID-19 is defined by the criteria for ARDS, sepsis, septic shock or other conditions that normally would require the life-sustaining therapy provision, such as vasopressin therapy or mechanical ventilation (invasive or non-invasive)

2) Severe COVID-19 is defined by any of :

2.1) signs of severe respiratory distress (for examples; inability to complete full sentences, accessory muscle use; and in children, grunting, central cyanosis, severe chest wall indrawing, or presence of any other general danger signs).

2.2) respiratory rate > 30 breaths per minute in adults and children > 5 years old; at least 60 breaths per minute in children less than 2 months; at least 50 breaths per minute in children 2-11 months; and at least 40 breaths per minute in children 1-5 years old.

2.3) oxygen saturation < 90 % on room air.

3) Non-severe COVID-19 is defined by absence of any signs sever or critical COVID-19.

Management of Critically Ill Adult Patients with COVID-19

Approximately 14 % to 20 % of COVID-19-infected cases develop severe COVID-19 requiring hospital admission and oxygen support and around 5 % to 10 % of infected cases require admission to the intensive care unit (ICU). A recent multivariable analytic study demonstrated that D-dimer > 1 µg/L, higher Sequential Organ Failure Assessment (SOFA) score (ranges from 0 to 24 points related to six organ systems : 1) respiratory (hypoxemia defined by low partial pressure of oxygen (PaO_2)/fraction of inspired oxygen(FiO_2)); 2) coagulation (low platelets); 3) liver (high bilirubin); 4) cardiovascular (hypotension); 5) central nervous system (low level of consciousness defined by Glasgow Coma Scale); and 6) renal (low urine output or high creatinine), and older age on hospital or ICU admission were related to higher mortality. This study also revealed the longest duration of viral shedding in COVID-19 survivors of 37 days and a median duration of viral ribonucleic acid (RNA) detection of 20.0 days (Interquatile range (IQR) : 17.0-24.0) in survivors. COVID-19 virus was detectable until death in non-survivors. Currently, there is no known difference the clinical manifestations of COVID-19 COVID-19 pregnant and

non-pregnant women or adults of reproductive age. Pregnant and recently pregnant women with suspected or confirmed COVID-19 should be treated with supportive therapies related to immunologic and physiologic adaptations during and after pregnancy [44].

The frequency of repeated specimen collection (upper respiratory tract : nasopharyngeal and oropharyngeal; lower respiratory tract : expectorated sputum, endotracheal aspirate, or bronchoalveolar lavage in ventilated patients) for COVID-19 testing by reverse transcriptase-polymerase chain reaction (RT-PCR) in hospitalized patients will depend on local epidemic characteristics and resources (a positive rapid diagnostic test for dengue does not exclude the COVID-19 testing). Two negative RT-PCR tests at least 24 hours apart in a clinically recovered patients is recommended for hospital discharge. Patients with severe acute respiratory infection (SARI) and respiratory distress, hypoxemia or shock that indicates severe COVID-19 is managed by supplemental oxygen therapy targeting SpO_2> 94 %. Patients with severe COVID-19 should be closely monitored for signs of clinical deterioration, such as rapidly progressive respiratory failure and sepsis. Timely, effective, and safe supportive therapies is the cornerstone for patients with severe COVID-19. Empiric antimicrobials, based on the basis of microbiology results, the clinical diagnosis (community-acquired pneumonia, health care-associated pneumonia or sepsis), local epidemiology and susceptibility data, and national treatment guidelines are given to treat all possible pathogens causing SARI and sepsis as soon as possible within one hour of initial assessment for severe COVID-19 patients with sepsis. A COVID-19 patient with respiratory distress who is failing to respond to standard oxygen therapy is indicated severe hypoxemic respiratory failure in acute respiratory distress syndrome (ARDS) requiring advanced oxygen or ventilatory support (minimum flow rates of 10-15 L/min is required to maintain bag inflation at FiO_2 = 0.06-0.95) [44].

Endotracheal intubation with airborne precaution should be performed by a trained and experienced health provider. Patients with ARDS should be pre-oxygenated with 100 % FiO_2 for 5 minutes via a face mask with reservoir bag, bag-valve mask, high-flow nasal oxygen (HFNO) or non-invasive ventilator (NIV) due to rapidly oxygen desaturation during intubation. After an airway assessment that reveals no signs of difficult intubation, rapid-sequence intubation is appropriate. Adult ARDS patients should be strongly implemented with mechanical ventilation using lower tidal volumes (4-8 ml/kg predicted body weight

(PBW)) and lower inspiratory pressures (plateau pressure < 30 cmH_2O). This recommendation is also suggested for patients with sepsis-induced respiratory failure who do not meet ARDS criteria by using the initial tidal volume of 6 ml/kg PBW. If undesirable side effects (e.g. blood pH < 7.15, dyssynchrony) occur, the tidal volume up to 8 ml/kg PBW is allowed. Permissive hypercapnia is also permitted. For controlling respiratory drive and achieving tidal volume targets, the use of deep sedation may be required. For pregnant women, being placed in the lateral decubitus position may benefit, whereas there is little evidence on prone position in pregnant women. Higher positive end-expiratory pressure (PEEP) (avoiding disconnecting the patient from the ventilator, that results in loss of PEEP and atelectasis) instead of lower PEEP and recruitment manoeuvres (RM) is advised in COVID-19 patients with moderate or severe ARDS. PEEP titration requires consideration of benefits to reduce atelectrauma and improve alveolar recruitment. Monitoring of patient with moderate or severe ARDS to identify those who respond to initial application of a different RM protocol or higher PEEP are advised to stop these interventions in non-responders. A previous randomized controlled trial (RCT) demonstrated that high PEEP and prolonged high pressure RMs demonstrated harm. Neuromuscular blockage by continuous infusion, including systemic corticosteroids should not be routinely used in COVID-19 patients with moderate-severe ARDS (PaO_2/FiO_2 < 150). Nevertheless, continuous neuromuscular blockage may still be considered in ARDS patients with dyssynchrony despite sedation or refractory hypoxemia or hypercapnia (rare finding) [44].

Either HFNO or NIV should be used only in selected COVID-19 patients with hypoxemic respiratory failure from ARDS (dominant finding and most common reasons for ICU admission leading to mechanical ventilation and/or hypotension requiring vasopressor treatment with high mortality rate) and should be closely monitored for clinical deterioration. Adult HFNO systems can deliver 60 L/min of O_2 flow and FiO_2 up to 1.0. HFNO, NIV, and bubble CPAP with monitoring should be used with airborne precautions due to uncertainty around the potential for aerosolization until further completion of evaluation of safety. HFNO decreases the need for intubation. Nevertheless, HFNO is not recommended in COVID-19 patients with some comorbidities, such as abnormal mental status, multiorgan failure, hemodynamic instability, cardiogenic pulmonary edema, or exacerbation of chronic obstructive pulmonary disease (COPD), although some data indicate

that HFNO may be safe in patients with mild-moderate and non-worsening hypercapnia. Referral of COVID-19 patients with refractory hypoxemia despite pulmonary protective ventilation is required extracorporeal membrane oxygenation (ECMO) [44].

Common complications of COVID-19-related ARDS include acute kidney injury (AKI, approximately 29 %), elevated hepatic enzymes (approximately 29 %), and cardiac injury (cardiomyopathy, pericarditis, pericardial effusion, arrhythmia, and sudden cardiac death, approximately 23 %, 33 % in a United States cohort)). Encephalitis is rare. Prevention of complications in COVID-19 patients with critical illness anticipates several outcomes, such as reduction of days of invasive mechanical ventilation by using weaning protocols and minimizing continuous or intermittent sedation; reduction of incidence of ventilator-associated pneumonia by using oral intubation, semi-recumbent position of the patients, closed suctioning system, a new ventilator circuit for each patient, and changing heat moisture exchanger; and reduction of incidence of pressure sores-stress ulcers and gastrointestinal (GI) bleeding by patient turning every 2 hours, giving early enteral nutrition (within 24-48 hours of hospital admission), administering proton-pump inhibitors or histamine-2 receptor blockers; reduction of incidence of catheter-related bloodstream infection by using checklist with completion verified by a real-time observer as a daily reminder to remove catheter if no longer needed; reduction of venous thromboembolism by using low molecular-weight heparin (preferable if available) or heparin 5,000 units subcutaneously twice daily, or by using intermittent pneumatic compression devices, and reduction of incidence of ICU-related weakness by actively mobilizing the patients early in the course of illness when it is safe to do [44].

Vasopressors are needed in COVID-19 patients with septic shock to maintain mean arterial pressure (MAP) at least 65 mmHg and serum lactate level at least 2 mmol/L in absence of hypovolemia. Fluid resuscitation with 250-500 ml of crystalloid fluid, including normal saline and Ringer's lactate solutions as rapid bolus in first 15-30 minutes and reassess for signs of fluid overload after each bolus. Determining need for additional fluid boluses (250-500 ml) based on clinical response and improvement of perfusion target (MAP > 65 mmHg, urine output > 0.5 mL/kg/hour, improvement of skin mottling and extremity perfusion, capillary refill, level of consciousness, serum lactate level, and heart rate. Dynamic indices of volume responsiveness to guide volume administration beyond initial resuscitation

based on local experience and local resource should include intrathoracic pressure during mechanical ventilation, stroke volume, inferior vena cava size, pulse pressure, variations in systolic pressure or fluid challenges with serial stroke volume measurements, and passive leg raises. Vasopressors, such as norepinephrine (first-line treatment, dopamine is not recommended if norepinephrine is not available), epinephrine, vasopressin, and dobutamine (low risk of tachyarrhymia) can be administered via a peripheral intravenous route if central venous catheters are not available [44].

Currently, there are limited evidences on clinical manifestations and perinatal outcomes after COVID-19 during pregnancy or puerperium. Nevertheless, all recently pregnant women with COVID-19, including pregnant women with covering from COVID-19 should be provided with information and counselling on safe infant feeding and appropriate infection prevention and control (IPC) for preventing COVID-19 virus transmission. Infants born to mothers with suspected, probable, or confirmed COVID-19 should be breastfed according to standard infant feeding guidelines [44].

Corticosteroid Practical Treatment in COVID-19

Clinically, corticosteroids are widely used in severe pneumonia. Russell *et al* suggested that corticosteroids should not be administered in COVID-19-induced shock or lung injury outside of a clinical trial [31]. Nevertheless, Chinese investigators suggested short course of low-to-moderate doses of corticosteroids for critical COVID-19-pneumonia patients [45]. Results from systematic reviews and meta-analyses indicated that severe COVID-19 patients were more likely to require corticosteroid treatment. In the early stage of inflammation, glucocorticoids reduce phagocytosis, leukocyte infiltration, inflammatory cell exudation, and capillary dilatation, whereas in the late stage of inflammation, glucocorticoids inhibit the excessive proliferation of fibroblasts and capillaries [26]. Additionally, glucocorticoids can inhibit nuclear transcription factor-kB (NF-kB) signaling and further inhibit the transcription and translation of inflammatory factors [26]. These can explain why corticosteroid treatment is more needed in critically and severely ill patients with COVID-19 infection. Nevertheless, there are number of limitations as the following : 1) most of systematic reviews and meta-analyses are retrospective cohort studies, historical

control studies, etc. that lack of randomized controlled trials with suitable design, 2) there is no uniform standard for the dosage and time of corticosteroids used in the studies, and 3) other therapeutic options may influence the corticosteroid effects. Finally, the publication bias of corticosteroid treatment in COVID-19 patients may be due to other currently non-reported developments [27].

ICS could reduce the inflammatory ARDS-like response affecting a minority of COVID-19 patients and may directly inhibit viral replication. Urge caution before using corticosteroids for ARDS-associated COVID-19. Corticosteroids are not recommended for mild COVID-19 patients. Moderate corticosteroids can be used in critical and severe COVID-19 patients. Currently, there has been no enough clinical trials or observational studies to examine the use of ICS in COVID-19. A rigorous blinded randomized multicentric clinical trials are urgently needed to further conclusion verification for the harm or benefit of corticosteroid treatment with confidence. Only a small proportion of COVID-19-infected patients progress to severe COVID-19 requiring critical care. In the absence of proper cure of COVID-19, it is necessary to identify the factors that may assist in assessment of the COVID-19 disease severity before rapid progression of the disease. Currently, there is no specific anti-COVID-19 therapy. There are several ongoing clinical trials on various potential antivirals both in China, United States, and many other countries.

Lung Transplantation for Severe COVID-19

A previous study between May 1, 2020 and September 30, 2020 in 12 patients with underwent-bilateral-lung-transplant-COVID-19-related ARDS at six high-volume transplant centers in 4 countries (USA-eight recipients at three centers, Italy-two recipients at one center, Austria-one recipient, and India-one recipient) [46]. Nine of the 12 recipients were male with the median age of 48 years (IQR : 41-51) [46]. Despite extracorporeal membrane oxygenation and prolonged mechanical ventilation, severe lung damage did not improve [46, Figure 46]. Increased intraoperative transfusion requirements, hilar lymphadenopathy, and severe pleural adhesions were the technically lung transplant procedure challenging

[46]. The explanted lungs demonstrated extensive continuing acute lung injury [46, Figure 47, 43] . The lung allografts demonstrated no recurrence of SARS-CoV-2 infection or pathology and similarity with short-term survival to that of transplant recipients without COVID-19 that all 12 patients could demonstrate the ability of weaning off extracorporeal support [46]. The characteristics of the lung donors in this study 8 males (67 %), 4 females (33 %), median age of 34 years (29-43), 173 (170-179) cm. of height, 84 (76-92) kg of weight, 6.1 (5.1-7.2) liters of the predicted total lung capacity, 5 (42 %) with having smoking history (current or past smoker), 1 (8 %) of subarachnoid bleeding cause of death, 6 (50 %) traumatic brain injury cause of death, 3 (25 %) drug overdose cause of death, 1 (8 %) of intracerebral bleeding cause of death, 1 (8 %) ischemic brain injury cause of death, 7 (58 %) of normal chest roentgenogram, 5 (42 %) of abnormal chest roentgenogram, 84 (66-105) hours of the median intubation time, 417 (362-489) of the PaO_2/FiO_2 at the time of offer, 39 (35-45) mmHg of $PaCO_2$ at the time of offer, 9 (75 %) of normal bronchoscopy, 5 (25 %) of abnormal bronchoscopy, 3 (25 %) of ideal type of lung donor, 9 (75 %) of marginal type of lung donor, and 5 (3-7) of median Oto score [46]. Due to uncertainty of the severe pulmonary-SARS-CoV-2 (COVID-19)-infection duration, the major concern was ongoing SARS-CoV-2 (COVID-19) infection at the time of lung transplantation and feasible reinfection of the lung allograft [47]. Several recent studies indicated rarely detecting replicating virus more than 10 days after SARS-CoV-2 (COVID-19) infection [48-50]. Currently, the Program of Vienna Lung Transplantation, takes a major role in a world consortium that comprises transplantation experts from Asia, Europe, and USA [51]. They established the medical guidelines for the potential-transplantation-global criteria as the following : 1) all conservative therapeutic option exhaustion, 2) COVID-19-related-damaged lungs un-recovery despite ventilation/ECMO of four weeks of duration, 3) evidence of irreversible and advanced lung damage in several consecutive CT scans, 4) age below 65, and 5) no relevant comorbidities [51]. Additionally, having good physical condition and a good chance of complete physical rehabilitation after transplantation of the lung transplant candidates are strictly requested [51]. Lung-transplant-long-term outcomes of severe COVID-19 patients are still to be investigated. National and international regulatory transplantation professional bodies should track the transplant-COVID-19 patient for establishing the standard guidelines.

Conclusion

Corticosteroid administration, both intravenously and inhaled routes in severely or critically ill COVID-19 is controversial, depending on physicians' clinical justment. Management of critically-ill-adult-COVID-19 patients should follow the WHO's criteria. Either HFNO or NIV should be used only in selected COVID-19 cases with ARDS-related hypoxemic respiratory failure. ECMO is required in refractory-hypoxemia-COVID-19 patients. Clinicians should be aware of the common complications of ARDS-related COVID-19, particularly cardiac injury and acute kidney injury.

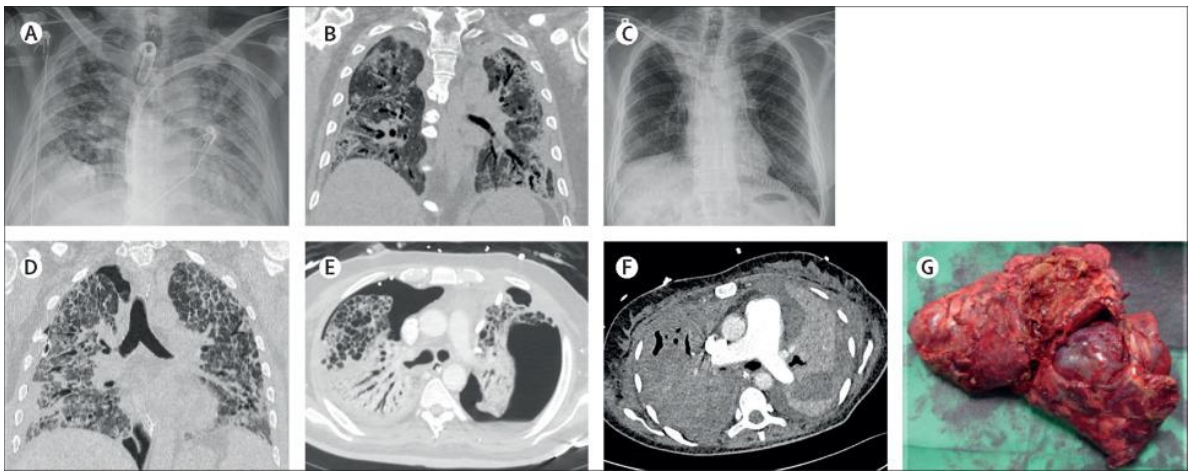

Figure 46 : Imaging and gross pathology of transplant recipients

Typical chest radiograph (A) and CT (B) of a recipient undergoing lung transplantation for COVID-19-associated acute respiratory distress syndrome at the time of listing, showing honeycombing, consolidation, and bronchiectasis. (C) A chest radiograph of a representative recipient at the time of hospital discharge is given for comparison. (D–G) Typical radiological and gross pathological features seen in our patients at the time of listing: diffuse fibrosis in all lobes (D), pneumothoraces and shrinking lungs (E), parenchymal necrosis (F), and cavernous changes (G).

(Source : Bharat A, Machuca TN, Querrey M, Kurihara C, Za-Castillon R, Kim S, et al. Early outcomes after lung transplantation for severe COVID-19 : a series of the first consecutive

cases from four countries. Lancet Respiratory Medicine 2021; 9 : 487-497. Available at : www.thelancet.com/respiratory (accessed on May 28, 2021))

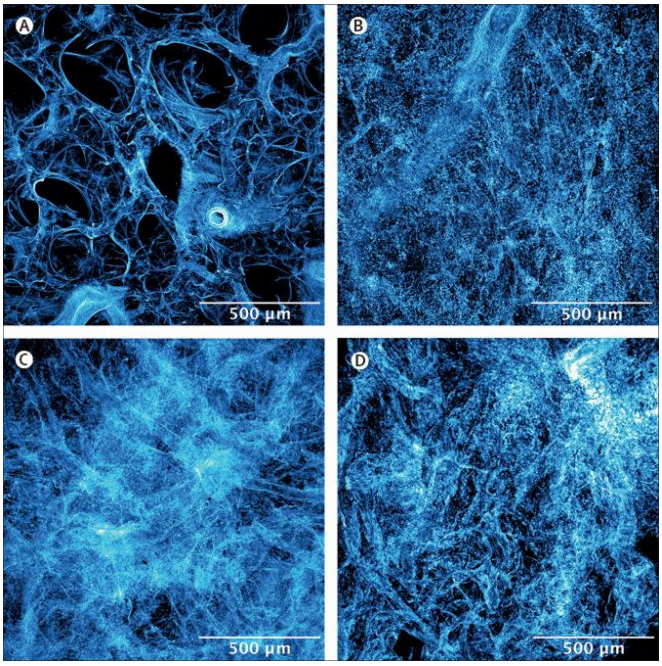

Figure 47: SHIELD tissue-cleared imaging of human lungs in late-stage severe COVID-19

Cleared lung tissue allowed visualization of the collagen structure and matrix of the lung tissue (cyan; original magnification 10×). (A) Normal collagen matrix of human lungs. (B) Destroyed matrix with inflammatory cells in explanted lungs from a lung transplant recipient with late-stage severe COVID-19. (C) Explanted lungs from a lung transplant recipient with end-stage emphysema. (D) Explanted lungs from a lung transplant recipient with end-stage α1-antitrypsin deficiency.

(Source : Bharat A, Machuca TN, Querrey M, Kurihara C, Za-Castillon R, Kim S, et al. Early outcomes after lung transplantation for severe COVID-19 : a series of the first consecutive cases from four countries. Lancet Respiratory Medicine 2021; 9 : 487-497. Available at : www.thelancet.com/respiratory (accessed on May 28, 2021))

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Chapter 10

Impact on Food Supply Chain and Lifestyle and Tips of Daily Healthy Food and Nutrition Intake During COVID-19 City Lockdown and Self-Quarantine

Abbreviations :

BMI : Body Mass Index

CAP : Common Agricultural Policy

CPS : Cyber Physical System

CREA : The Council for Agricultural Research and Economics of Italy

EU : European Union

GDP : Gross Domestic Product

MD : Mediterranean Diet

OR : Odds Ratio

p : Probability

PA : Physical Activity

SCM : Supply Chain Management

UAE : United Arab Emirates

US CDC : United States Centers for Disease Control and Prevention

US FDA : United States Food and Drug Administration

WHO : World Health Organization

Food Supply Chain Impacted By COVID-19 Pandemic

Five stages of food supply chain include agricultural production, postharvest handling, processing, distribution/retail/service, and consumption [1]. The two used-food-supply-chain systems include system-based on regulations and laws that use mandatory standards which

are under the state agency inspection and voluntary standards which are under the market laws or international associations [2]. The World Health Organization (WHO)'s the strategic preparedness and response plan that includes the health measures with eight priority steps and actions : 1) Coordination, planning, and monitoring at the country level, 2) Risk communication and community participation, 3) Surveillance, rapid response teams, and case investigation, 4) Entry points, 5) National laboratories, 6) Prevention and control of infection, 7) Situation management, and 8) Operational support and logistics [3]. Due to rapidly spread globally of the COVID-19, it eventually forced countries to apply lockdowns and strict social (physical) distancing measures. Currently, online meeting and flexible work from home have become standard practices [4-5]. The United States Centers for Disease Control and Prevention (US CDC) developed response plans to provide guidance for continuity of operations in the food processing facilities and manage COVID-19 in the food industry , particularly, meat and poultry processing industries [6]. COVID-19 pandemic might contribute to a US $ 80 billion loss in tourism sector and US $ 113 billion loss in aviation [7, 8].

The European Food Safety Authority stated that there is no evidence of association between risk of COVID-19 and food consumption [9], whereas the Norwegian officials stated that there is no association between the transmission of SARS-CoV-2 (COVID-19) via imported food and the origin of the salmon outbreak is still unclear [10, 11]. Nevertheless, some eating and cooking habits may contribute to the emergence of the coronavirus from animals to humans [9]. During the COVID-19 pandemic era, four major issues involving the food supply chain and the food industry have been raised, 1) People tend to have follow a healthy diet for protecting their immune system [12], therefore , the demand in bioactive-ingredient-containing functional food is increased, 2) Producers, retailers, and consumers has payed more attention on food safety, 3) Due to people on lockdown restrictions, food security concerns have been arisen, and 4) During the COVID-19 pandemic era, the food sustainability problems have emerged [13]. In Germany and France, some of the markets limited the number of items, such as pork and beef products that a customer can buy and some restaurants stopped serving beef hamburgers. Close-down of the food plants contributed to the ripple effect in the food supply chain [14-17].

Governments are facing financial pressures because of the economic shrinkage and reallocating their resources focusing on financial incentives and social assistance programs [18, 19]. Seasonal or temporary employment, particularly for planting, sorting, harvesting, processing, or transporting crops to markets is common in the developing and developed countries that significantly affects the food supply chain as a result of the shortage of local or migrant workers because of travel restrictions imposed by city lockdown or sickness [20]. The objective of " Pick for Britain " campaign in Britain was to identify 70,000 British to work during the harvest and in the field, whereas a call has been performed to the unemployed individuals to work in fields in France [21]. In India, tea plants were being lost due to logistical challenges, whereas the British chair of dairy farmers demonstrated that around 5 million liters of milk are at risk in one week [22].

Impact of COVID-19 Pandemic on Consumer Behavior

The consumers' food demand varies depending on income level of consumers, consumption, the price of foodstuffs, sociodemographic situation, time constraints, and shopping preferences, in addition to spending money on food in per visit changed and number of visits to food store [23, 24]. Interruption of the daily-routine life by COVID-19 pandemic resulted in boredom with high consumption of carbohydrate, fat, and protein, in addition to quarantine-caused stress that pushed the people to sugary food consumption for feeling positive [25]. Due to ability of carbohydrate-rich foods to encourage serotonin production, they can be used as self-medicating components [25]. These unhealthy eating habits may lead to obesity-associated-COVID-19 serious complications and chronic inflammation [25]. An unusual demand shift from food service to retail due to the closure of restaurants and limited service eating places that affected the eating or purchasing habits [23, 26]. Using food service and purchasing food from supermarkets had the same ratio as 50 % before the COVID-19 outbreak, nevertheless, it is almost 100 % for supermarkets [23, 26]. During the COVID-19 lockdown, consumers experienced decreased availability of certain types of foods [23, 26]. The spending money on food was raised per visit, whereas the number of visits to food store was decreased [23, 26]. Flour, a staple product was not found on food store shelves because of the more interest in home-baking, as a family activity in European countries [23, 26]. Individuals have focused on the products

with long shelf life, such as canned or dried foods, milk, milk substitutes, pasta, and frozen foods because of convenience and daily cooking at home [23, 26]. Interestingly, the shortage of eggs was not only due to lack of packing for retail but also increased demand [27, 28].

In the United States, sales for eggs rose by 44 % compared to last year (2019), whereas household egg consumption increased 40 % since march 20, 2020 in Argentina [4, 27, 28]. During COVID-19 pandemic, the flexibility associated with the packing and labelling of eggs because of the insufficient availability of suitably labelled retail packages to facilitate the egg distribution and fulfill the demand was provided by the United States Food and Drug Administration (US FDA) [4, 27, 28]. In the European countries, the demand for frozen vegetables increased by 52 % and fresh bread by 76 % in the week when the COVID-19 pandemic was announced, whereas the demand for alcoholic beverages increased twice, one month after pandemic announcement [29]. A study on 18 countries revealed that food buying behavior has changed due to willingness of healthy food consumption without exceeding normal budget. Most customers adopted a basic approach of returning to ingredient-containing beverage products, such as olive oil, whole grains, legumes, fruit, and vegetables and natural food. They are also looking for food products that improve their COVID-19-related mood [25, 30].

The behavior of the Italian population on food choices and behavior was assessed under COVID-19 quarantine in a recent poll performed by the Italy's the Council of Agricultural Research and Economics (CREA) among approximately responded 2,900 individuals from all regions of Italy demonstrated that healthy food and beverage consumption increased for 33 % of vegetables, 29 % of fruit, 26.5 % of legumes, and 21.5 % of extra-virgin olive oil. Nevertheless, 44.5 % and 16 % of them consumed more sweet food and drank more wine, respectively, whereas 44 % and 37 % of them reported body weight gain because of low-level physical activity and intake of higher calories and needed weight loss by adjusting their diet, respectively [31].

During COVID-19 pandemic in the United States, a survey of 630 consumers in May 2020 revealed that 70 % of consumers decreased the frequency of food shopping and preferred online shopping, 56 % of them were worried about not finding particular

foods they would like to buy in the store or forgetting to buy something, 70 % of them consumed more food while staying at home, 43 % of them consumed more fruits, 42 % of them consumed more vegetables, 30 % of them consumed more protein-containing foods (fish, chicken, or meat), 39 % of them made their breakfast more balanced, 47 % of them consumed more sweet foods, 24 % of them consumed less vegetables, 21 % of them consumed less fruits, and 19 % of them consumed less protein-containing foods [32].

A survey on 1,005 over-18-year-old-male and -female French people demonstrated that during the 8-week quarantine, they changed their views on the ecological, economic, and social value of food production [33]. They would only buy " essential " foods, spend more time cooking, and pay more attention to food spending when they return to " normal " after COVID-19 pandemic measures have been relieved [33]. Approximately, one-third of them wasted less food, 29 % of them bought more local food, and 20 % of them went online shopping [33]. Another survey in France conducted on April 6th and 7th, 2020 among 1,000 above-18-year-old adults revealed that 82 % of them believed in safety of foods they bought, 7 % of them believed in unsafety of foods they bought, 42 % of them preferred the packaged foods more than normal, 42 % of them stated that the COVID-19 pandemic did not change their attitudes towards packaged foods, 77 % of them believed in enough food production to meet the consumers' needs, and 16 % of them did not believed in enough food production [34].

Impact of COVID-19 Pandemic on Global Food Trade

Before COVID-19 crisis, the vulnerability of food systems to problems-associated with diseases and climate has been confronted long, including the SARS and Ebola outbreaks, the oil crisis in the 1970s, and the food crisis in 2006-2008 [35]. Due to current COVID-19 crisis, some governments changed the food trade policies by moving towards facilitating imports and restricting exports for ensuring the maintenance of the number of products in the domestic markets. The export restriction has some negative effects as the following : 1) Countries will lose their competitive advantage by losing their place in international markets, 2) Dropping domestic prices that will decrease crop production and incentives in the industry, and 3) Undermining exporters' reputation and decreasing

importers' confidence in the international markets that contribute to destroying future business opportunities and trust for exporters [36, 37]. Due to COVID-19 pandemic, a total of 19 countries have taken to export restrictions for 27 food products. Currently, a total of 8 countries are going on their measures on 11 food products. For considering the assessment of the effects of the import restrictions in term of kilocalorie unit, Tajikistan, Uzbekistan, Afghanistan, and Azerbaijan were negatively affected by 79 %, 70 %, 61 %, and 54 %, respectively [38]. The world prices of stable food commodities, such as rice, wheat, and maize were pushed up by export-restricted policies and contributed to decreasing the quantity and quality of food products [39]. Due to export restrictive policies and negatively effects of the capacity utilization of food-manufacturing plants to respond demand, foods that are not locally grown but needed for processing were not available, including inability of local sellers to find buyers that resulted in excess supply and waste accompanying economic losses and transportation challenges for air and sea cargo [40-43].

Minimizing The Impact of COVID-19 Pandemic

COVID-19 disruptions may contribute to hunger, malnutrition, and increasing number of individuals facing extreme hunger to 265 million in 2020 [44, 45]. Among children who are younger than 5 years old, COVID-19 pandemic contributed to 14.3 % increase in prevalence of malnutrition wasting or health and social-protection interruption in low- and middle-income countries [46].

Food Supply Chain Strategies

At the household level, COVID-19 pandemic resulted in 12 % of increase in food waste [47]. Approximately, one-third of all food productions was wasted across the food supply chain stages (production, postharvest handling, processing, distribution, and consumption). Some bioactive compounds can be gained from food wastes to re-utilize them in food chain, such as carotenoids, essential oils, flavonoids, glucosinolates, isothiocyanates, phenols, and whey protein by conventional or innovative techniques (extraction, fractionation, and isolation stages) [13, 48, 49]. Robot systems assist individuals

to serve the foods to consumers in food-serving industry, in addition to monitoring the unsafe or low-quality food products in food supply chain by the Cyber Physical System (CPS) [50, 51]. Approximately, 25 % increase of productivity by automation to complete the work more efficiently than humans, indicating an important role by making data-driven autonomous decision in production in the fourth industrial revolution [50, 51]. The COVID-19 pandemic resulted in challenges that include adopting new workplace policies, actions to decrease human contact, and change of working conditions [52].

To respond to these challenges, organizations should establish some measures as the following : 1) Monitoring COVID-19 symptoms of the workers, suppliers, contractors, and visitors before entering the facilities, monitoring all staff to wear face protection equipment and gloves, and performing body temperature screening of all staff at the entrance of the facilities; 2) Should consider employees' work rotation, working hour reduction, dividing number of workers in each work shift into 3 or 4 groups, and adjusting their break time to avoid overcrowding; and 3) Should redesign warehouses and processing to allow social (physical) distancing, build barriers or dividers that cover the upper part of the body of the workers to maintain social distance, and use diagonal arrangement in case of using two side engagement in food processing [53]. All countries should maintain the balance between workers' safety and food product quantity [54]. Decentralization of the food manufacture provides reduction of the transportation and storage costs, minimizing the environmental impacts, shortening the food supply chain, reduction of the emission and energy consumption during storage and transportation, flexibility in food supply chain, and simplifying the administrative procedures [55-57]. During COVID-19 pandemic, changes in food demands should be determined by using simulations, statistical models and forecasts, particularly, the daily-life products, such as food items and sanitizers to propose optimal decision for demand disruptions and tackling supply by the manufacturers [58]. Storage centers should be invested by the government or private centers. Web-based-food-distribution system should be established to strengthen the relationship between buyer and seller [20, 54, 59-60]. Digital commerce services, an important role in interaction and trading activities among the actors of the food supply chain allow small farmers to reach more consumers in a direct effective way and collaboration between the largest e-commerce companies and government to encourage rural markets to be part of e-commerce economy and offer

mostly organic fertilizers at a reasonable cost [61], in addition to " Supply Chain Management (SCM) Data Science " [62].

Agricultural production collection centers with high capacity storage at the location comfortably reached by small-scale farmers should be built by countries [63]. Maintaining the activities of small- and medium-size agricultural enterprises requiring additional capital injection by using the capital injections from donor or government through improved technologies or modern facilities that entail higher production costs [64]. Contractual agricultural arrangement can be made by the horizontal and vertical coordination mechanisms between food banks and farmer associations as the following : 1) Assist farmers to create new markets [65], 2) Countries can deploy warehouse receipt systems that allow small-scale food producers to easily access to financial loans and receive the best price for their agricultural products [66], 3) Countries should develop e-commerce for small shareholders to commercialize agricultural products to wider scale of consumers [67], and 4) Small-scale agricultural producers should have easy access to credit for involving the financial problems [63]. Additionally, confidence in financial organizations can be promoted by the temporary liquidity guarantee program (TIGP) that allows a limited term guarantee for newly issued debt of financial companies and affiliates and non-interest bearing transaction accounts [54, 68-70].

Recommendations for Government

To focus on the impact of COVID-19 pandemic on agricultural products and food supply cuts by observation of the progress and recommended actions without waiting too long for the implementation of certain interventional strategies, a COVID-19 crisis committee should be appointed during food value chain in collaboration with the private sectors [20]. An Agriculture Response Program was designed by the government of Canada for 50-70 % funding assistance without paying back regarding health protocol, strategic projects, product distribution, product movement, marketing, development, and abattoir efficiency [71]. The government of Canada also implemented a US $ 50 million financial aid program for small farmers who hired temporary foreign employees through the COVID-19 outbreak by allowing employers to get US $ 1,500 per foreign worker with 14-day-

self-isolation upon entry into Canada [72]. Additionally, The government of Canada and Belgium allowed postponing the recruitment or offer long-term contracts for employers [73]. The Logistic Sub-Group of the United Kingdom developed crisis management, shore base logistics and freight management, accommodation and transportation, and safe passage programs to provide safe passage (health issue) and assurance to health personnel and their families, including guidelines and raising awareness to logistic sector [74]. The " green lanes " for vehicles carrying agri-food products for ensuring the fast and free movement on the borders was implemented by the Commission of the European Union (EU), in addition to highlighting the free movement of seasonal workers and agri-foods for easy reaching their workplaces. Common Agricultural Policy (CAP) payments and temporary framework for state aid measures were also introduced by the Commission of the EU to extend the farmers' application deadline to get income support and supported farmers and agri-food business for ensuring liquidity [75]. To facilitate connections between the local residents and agriculture sectors, online platforms should be implemented [68]. The best way to solve the labor shortage over the medium to longer term during COVID-19 pandemic is " labor-replacing mechanization policy " [76]. Additionally, the employed agricultural-production individuals were importantly considered as " critical infrastructure workers " by the United States government [77, 78]. To take the recommendations and measures in food and agriculture during the COVID-19 pandemic, the COVID-19 Commission that consisted of two members of from the Ministry of Agriculture and Forestry and seven academicians in Turkey was established [79].

Many countries like Saudi Arabia, United Arab Emirates, Bahrain, Egypt and Sudan did not begin easing restrictions until the end of June 2020, whereas Yemen declared easing lockdown restrictions in Mid-July 2020 [80]. The lockdown restrictions included closure of the borders, closure of non-essential businesses, local movement and travel restrictions, nightly travel curfew, cancelling prayers to avoid mass gathering events, and facilitating remote working and online learning [80]. Numerous financial plans were implemented by many countries to cope with the COVID-19 curfew. The Ministry of Finance of Saudi Arabia has supported the private sectors and individuals who lost their income by funding during COVID-19 crisis [81] as well as Canada and some countries [82, 83]. A recent study in Saudi Arabia revealed that approximately 52 % of employed

study participants who might usually not have adequate free time to cook demonstrated changes in their eating habits due to having more time to cook [84]. Nevertheless, increased prevalence of food insecurity due to COVID-19 pandemic lockdown among negatively affected individuals was demonstrated despite this efforts [85]. The health organizations in Saudi Arabia should focus on the importance of promoting positive eating habits, avoiding overconsumption of foods, and increasing physical activity during curfew for maintaining health and preventing body weight gain [84].

ALMughamis *et al* conducted a recent study in Kuwait and demonstrated that 41.6 % of the valid respondents reported that their body weight would increase, 63.8 % of them felt anxious sometimes during day, 25.5 % of them always felt anxious, and 10.7 % of them never felt anxious [86]. More than 69 % of them revealed that their physical activity had decreased than before and the mean number of hours spent being sedentary at home were 9.56 [86]. When using the logistic regression model for predictors of body weight increase among the Kuwaitis in this study, the study revealed that those respondents who reported eating unhealthy diets were 4.5 times (95 % Confidential Interval = 2.45 to 8.23) more likely to report an increase in body weight, compared with the respondents with diet changes [86]. Those respondents demonstrating having anxiety throughout the day were 2.45 times more likely to have an increase in body weight than those who never confronting it [86]. There was also association of 3.27 times higher odds of increase in body weight among respondents who consumed snacks excessively (> 3 times per day) than those who did not consume it, whereas consuming moderate amounts of snacks (1-3 times per day) did not differ than the respondents who never consumed snacks throughout the day [86]. Considering Post-COVID-19 era in Kuwait, an increase in body weight gain and unhealthy eating habits will be a challenge that the Kuwait government's policies strengthen health systems to tackle it [86]. Kuwait National Program for Healthy Living (2013-2017) had previously been developed, therefore, the authorities can develop a strategic plan to fight against harmful effects of this pandemic on health originated from unhealthy eating behaviors, psychological issues, and sedentary lifestyle [86].

A recent study in United Arab Emirates (UAE) revealed that during COVID-19 pandemic, the participants reported lost body weight 20.9 %, gained body weight 31.0 %, maintained body weight 40.1 %, and they did not know their body weight change 7.9 %

[87]. The study participants perceived health state during COVID-19 pandemic as the following : 21.4 % excellent, 39.7 % very good, 28.1 % good, 10.1 % fair, and 0.7 % poor [87]. The most common source of information for health and nutrition updates that the participants relied on were 69.1 % and 67.8 %, respectively [86]. The second source of information for health information and nutrition updates were 65.4 % and 48.7 %, respectively [87]. The study results demonstrated a significant increase in the percentage of participants consuming mostly homemade meals during COVID-1 pandemic and a significant decrease in those consuming fast-food ($p < 0.001$) [87]. Additionally, the percentage of participants consuming five or more meals per day increased from 2.1 % before the COVID-19 pandemic to 7 % during the COVID-19 pandemic ($p < 0.001$), whereas the percentage of participants consuming breakfast increased from 66 % to 74.2 % ($p < 0.001$) [87]. The percentage of those skipping meals reduced from 64.5 % (mainly due to lack of time before the COVID-19 pandemic (62.3 %), 36 % of participants was lack of appetite) to 46.2 % during the COVID-19 pandemic ($p < 0.001$) [87]. The percentage of participants intaking water increased from 24.1 % (consuming eight or more cups per day) before the COVID-19 pandemic to 27.8 % during the COVID-19 pandemic ($p = 0.003$) [87]. More than half (51.2 %) of the study participants did not consume fruits daily, 46.2 % of the participants did not consume milk and dairy products daily, 37 % of the participants did not consume vegetables daily, 46.1 % of the participants consumed sweets and desserts at least once daily, 37.1 % of the participants consumed salty snacks (nuts, crackers, and chips) daily, 86.5 % of the participants never consumed energy drinks during COVID-19 pandemic, 69.2 % of the participants consumed tea or coffee at least once daily, and 44.2 % of the participants never consumed tea or coffee [87]. When considering the physical activity, 32.1 % of the participants did not engage in any physical activity before the COVID-19 pandemic, the percentage increased to 38.5 % during the pandemic ($p < 0.001$) [87]. Interestingly, there was significant association between the reported change in body weight and the frequency of performing physical activity among the participants during COVID-19 pandemic ($p < 0.001$) [87]. There was notification of a significant higher percentage (47.6 %) of study participants spent more than five hours per day on the computer for work or study compared to before the pandemic (32 %) ($p < 0.001$), and spent more than five hours on screens for fun increased from 12.9 % of the participants before the city lockdown to 36.2 % of the participants during the lockdown ($p < 0.001$) [87]. The

study results also demonstrated a significant increase in the percentage of the participants of the all four stress parameters (physical exhaustion, emotional exhaustion, irritability, and tension) "all the time" during the COVID-19 pandemic compared to before the pandemic : 13.3 % versus 7.7 % for physical exhaustion; 14.1 % versus 6.3 % for emotional exhaustion; 13.5 % versus 6.9 % for irritability; and 17.8 % versus 6.3 % for tension (all $p < 0.001$) [87]. When considering the poor sleep quality during the COVID-19 pandemic and before the pandemic, the results demonstrated a significant decrease in the sleeping hour that was less than seven hours per night from 51.7 % of the participants to 39 % of the participants during COVID-19 pandemic ($p < 0.001$), 28.1 % of the participants increase in poor sleep quality compared to 17.3 % of the participants before the pandemic, 60.8 % of the participants demonstrated sleep disturbance compared to 52.9 % of the participants before the pandemic, and 30.9 % of them felt lazy and less energized during the COVID-19 pandemic, compared to 4.7 % of the participants before the pandemic ($p < 0.001$) [87]. More male reported significantly reduced engagement in physical activity (50 % versus 39.3 %; $p = 0.013$) and increased screen time (54.5 % versus 51 %; $p = 0.002$) [87]. Female participants demonstrated significantly higher sleep disturbances ($p = 0.011$) [87]. Weight gain and an increase in the number of meals consumed daily were reported ($p = 0.042$ and $p = 0.024$, respectively) [87]. Participants aged 18-35 were mostly affected in sleep duration and quality ($p < 0.001$) [87]. Association between lifestyle changes and difference of the education levels was not demonstrated [87]. The realized Mediterranean diet is benefit as an anti-inflammatory dietary pattern that emphasizes on low consumption of red meat and dairy, moderate consumption of monounsaturated fat source such as olive oil, and high consumption of plant foods that associate with increased immunity, and lower risk of inflammation and chronic diseases [88-91], in addition to having a lower environmental impact than the characteristic Western diet and a favorable effect on chronically inflammatory diseases, such as type 2 diabetes mellitus, metabolic syndrome, and visceral obesity [92-97]. Several previous studies indicated a transformation of the diet in Eastern Mediterranean countries from a traditional Mediterranean diet to a more Westernized diet that is low in fruits, vegetables, fiber, and polyunsaturated fat and high in refined carbohydrate, salt, cholesterol, saturated fat, and energy [98-101]. Thus, current UAE dietary behaviors may be ineffective against the SARS-CoV-2 (COVID-19), whereas it was questionable that these dietary patterns were due to city lockdown following the COVID-

19 pandemic [102, 103]. By considering an adequate supply of macro- and micro-nutrients are necessary for optimal immune function and response can be detrimental implications [102, 103]. For delivering mental health service, the use of telehealth has been demonstrated to be useful in providing support to the patients [104]. An increased compliance with the Mediterranean diet can be associated with better sleep, higher scoring for self-perceived health status, and lesser mental distress [105-107], in addition to having a protective effect on the risk of cardiovascular diseases and some types of malignancies [96, 108].

In Zimbabwe, there were crucial policy implications for the national government centred around the need to stop the COVID-19 spread using city lockdowns, in addition to the glaring need to deal with negative impacts of such policy decisions on food security and livelihood that were greatly affected due to mobility restrictions [109]. Naja and Hamadeh provided the recommendations the government of Zimbabwe on how to provide nutrition demands during COVID-19 pandemic by using a multilevel framework for action adapted from the ecological model of health behavior [110]. The government of Zimbabwe was also encouraged to produce evidence and informed decisions for ensuring responsible lockdown exit strategies [109]. A previous study in Zimbabwe demonstrated that there was decrease in immunizations and growth monitoring (37.8 %), access to medical doctors (58.6 %), and access to drugs (59.9 %) during the COVID-19-city-lockdown period, [109], in addition to disruptions in drug- and vaccine- supply chains-associated with defaulters on immunization schedules among children [111].

A recent study in Poland during COVID-19 pandemic with city lockdown demonstrated that 43 % of participants ate more, 52 % of the participants ate snack more, more than 18 % experienced body weight gain, 18 % of the participants confronted body weight loss, whereas older (aged 36-45 and > 45), obese, and overweight participants tended to gain body weight more frequently [112]. Those participants with underweight tended to lose their body weight further [112]. Participants with increased body mass index (BMI) frequently consumed less vegetables, fruits, and legumes with higher adherence to higher adherence to fast-foods, dairy, and meat during quarantine [112]. Additionally, 14.6 % of the participants demonstrated an increase in alcohol consumption, particularly in participants addicted to alcohol, and more than 45 % of the participants with cigarette smoking revealed an increase in smoking frequency during quarantine [112]. City lockdown during COVID-

19 pandemic in Poland may affect dietary habits and eating behaviors and advocates for organized nutritional support during future disease-epidemic-related quarantines, especially for the most vulnerable people [112]. Proactive development of strategies must be stressed to mitigate the increase in alcohol consumption among alcohol-addicted Poles during COVID-19 pandemic or during future disease-epidemic-related quarantines or city lockdowns [112]. Another recent study among the Poles revealed that 34 % of the participants revealed an increase in food consumption, 43 % of them demonstrated a decrease in physical activity (PA), and 49 % of them revealed an increase in screen time [113]. Before the COVID-19 pandemic in Poland, a low percentage of the Polish people met the WHO's recommendations for PA, and lower percentage of people were actively engaged in sports than those in other European Union countries [114]. Adult Poles over 40 years old, those living with unemployed, children, those not consuming homemade meals, and those living in a region with a higher Gross Domestic product (GDP) could be more exposed to unhealth behaviors [113]. In maintaining health by giving the importance of PA, its promotion during COVID-19 pandemic should take a new meaning [113]. The issue of PA in Poland was little publicized although there were appeals for PA at home on the websites of the National Health Fund and the Ministry of Health, and fitness instructors also posted the videos with exercises for the duration of the COVID-19 pandemic on several websites [113]. Thus, working on special programs of gymnastics education and enhancing the message " to be active " in the situations of the mandatory home isolation or quarantine is essential [113]. It is critical to create effective, targeted recommendations and tools to maintain health and to prevent chronic diseases that exist among the Polish population [113]. A recent Italian survey demonstrated that 17.7 % of the participants had less appetite, 34.4 % of the participants had more appetite, 33.9 % of the participants felt hungry before the main meals, 22.8 % felt hungry in between the main meals, 11.2 % of the participants felt hungry after dinner [115]. The study revealed that after-dinner hungry was associated with the habit of having a break before bedtime (OR= 4.067, $p < 0.001$) [115]. During the COVID-19-city lockdown in Italy, 37.4 % of the participants ate more healthy food (vegetables, fruit, legumes, and nuts), and 35.8 % of the participants ate less healthy food [115]. Age and BMI were positively and inversely associated with the increased appetite and night snacks, respectively (OR = 1.073, $p < 0.001$; OR = 0.972, $p < 0.001$) [115]. North and Center Italy were both inversely associated with appetite increase,

compared to the South and Islands (OR = 0.527, $p < 0.001$; OR = 0.582, $p < 0.001$), whereas living in Southern and Islands and Center Italy was associated with the after dinner snack in comparison to the Northern Italy (OR = 1.843, $p = 0.009$; OR = 2.128, $p = 0.002$) [115]. The participants with training during the COVID-19-city lockdown and the participants from Northern and Center Italy had a minor perception of body weight gain, compared to those from the Southern and Islands (OR = 0.660, $p < 0.001$; OR = 0.786, $p = 0.024$; OR = 0.747, $p < 0.001$) [115]. Lower age, higher BMI, enhanced appetite, and after dinner hunger were associated with an increase of junk food consumption (sweet beverages, dressing sauces, savory snacks, and packaged sweets and baked products) (For higher BMI : OR = 1.025, $p = 0.005$; For lower age : OR = 0.979, $p < 0.001$; For enhanced appetite : OR = 4.044, $p < 0.001$; For after dinner hunger : OR = 1.558, $p < 0.001$) [115]. Participants with having suspended their usual job or started smart working had a greater perception of having increased their body weight in comparison to the participants not changing their routine job (OR = 1.250, $p = 0.037$) [115]. The majority of the participants purchased food at the supermarket (75.8 %), 26.0 % of them purchased at the grocery shops, 14.8 % of them purchased at farmers, organic or local markets or using " Solidal Purchasing Groups ", and 9.0 % used online delivery [115]. Nevertheless, 11.8 % of the participants declared not to purchase food and to delegate shopping to the third parties and 54.0 % of them used the leftover food more than 30 % of times [115]. Using " Solidal Purchasing Groups " or shopping at farmers, organic markets, or local markets was associated with the habit of recycling the leftover food (OR = 1.468, $p < 0.001$) that the participants from the North and Center of Italy appeared to be more prone to this behavior in comparison to Southern and islands participants (OR = 2.109, $p < 0.001$; OR = 1.735, $p < 0.001$) [115]. In consideration of the physical activity during COVID-19-city lockdown, there was no significant difference between the percentage of participants who did not train before (37.7 %) nor during (37.4 %) the COVID-19-city lockdown ($p = 0.430$) [115]. Conversely, a higher frequency of training during the emergency was identified in comparison to the previous period (McNemar value = 259.529, $p < 0.001$) [115]. In consideration of the highest adherence to the Mediterranean diet (MD), 93.7 % of the participants consumed vegetables, 80.9 % of them consumed legumes, 75.9 % of them consumed nuts, 63.3 % of them consumed fish, and 58.7 % of them consumed fruit [115]. MD could be one of the best nutritional models to construct innate and adaptive immunity during COVID-19 pandemic and might be a

supplemental therapeutics of the COVID-19 [115]. Decreasing the consumption of junk food to reduce " obesogenic environment " that contributes to body weight gain and susceptibility to COVID-19 was strongly recommended [116, 117]. Consumption of junk and ultra-processed food in the postprandial period contributes to a greater susceptibility significantly to the development of inflammation, oxidative stress or damage, and chronic diseases, while the consumption of foods rich in antioxidants and seasonal foods is highly protective [118].

Review of personnel occupational health and safety practices, human resource planning in the face of absenteeism or increasing demand, travel limitations, business continuity planning, progressive investment and resource plans of the next 3 years, alternative input source channels, establishing COVID-19 positive-reporting system, and promoting understanding COVID-19 transmission are important to change the business models of the agricultural firms [119, 120]. More organized by using the COVID-19 crisis as a driving force are needed by the small companies [121]. COVID-19 pandemic, panic buying, food- supply-chain disruptions, and city-lockdown restrictions caused a significant risen-food price [122], whereas some consumers will pay more attention to decrease food waste for improving food security [123]. Movement restrictions both national and international contributed to challenges, including consumers' changes in demand. These restrictions caused consumers prepared cooking at their home, in addition to preventing them from getting COVID-19 infection at the stores, restaurants, markets, and supermarkets [44].

Tips of Daily Healthy Nutrition Intake

While no foods or dietary supplements can prevent COVID-19 infection, maintaining a healthy diet is an critical part supporting a strong immune system [124]. Maintaining a healthy immune diet during the COVID-19 pandemic, strict city lockdown, or self-quarantine is causing much changes in individuals' daily lives around the globe. Nevertheless, everyone is encouraged to follow the WHO and the governmental advice to protect against infection and transmission of COVID-19, such as good hygiene and physical (social) distancing, including good food hygiene. Good nutrition is very critical before, during, and after an

infection, particularly COVID-19 infection. Maintaining a healthy diet is a critical part of supporting a healthy immune system whereas no dietary supplements can prevent COVID-19 infection. Some countries that have implemented strict city lockdown and physical distancing regulations have not so much experiences in widespread food-supply disruptions [125].

The general tips for daily dietary intake during COVID-19 pandemic, strict city lockdown, or self-quarantine are recommended as the following : 1) Taking the foods only what they need. Individuals might feel the need to purchase large amounts of foods, but ensure that what is already in their pantry, as well as foods with shorter shelf life [124, 125]. 2) Spending longer periods of time at home may offer the possibility to make those recipes individuals previously did not have time to make. Nevertheless, various healthy and delicious recipes can be found online [125]. 3) Some cities and countries have rather advanced delivery systems for ingredients and ready meals. Individuals should take the advantage of these delivery options. It is critical to keep food at the safe temperatures (below 5^{o} C or above 60^{o} C). Nevertheless, individuals should keep in mind that these services might be overwhelmed [125]. 4) Individuals should be aware of portion sizes of foods. Young children will need smaller portions, compared to adults' portions [124, 125]. 5) Following safe food handling practices (keep individuals' hands, kitchen and utensils clean; separate raw and cooked food, particularly raw meat and fresh produce; cook individuals' food thoroughly; keep individuals' food at the safe temperatures; and use safe water and raw material) [124, 125]. 6) Limiting individuals' salt intake (less than 5 g of daily salt). 7) Limiting individuals' sugar intake (less than 5 % of the total energy intake for adults should come from free sugar-around 6 teaspoons) [124, 125]. 7) Limiting individuals' fat intake (less than 30 % of the total energy intake, of which no more than 10 % should come from saturated fat, intake healthy fats, such as in olive, sesame, peanut or other oils rich in unsaturated fatty acids). Individuals should avoid foods that commonly contain trans fat, such as fried and processed foods [124, 125]. 8) Consuming enough fiber (vegetables, fruit, pulses, nuts, and wholegrain foods in all meals). Wholegrain foods include oats, brown pasta and rice, quinoa and whole-wheat bread and wraps [124, 125]. 9) Staying home with well hydration. Well and healthy processing-tap water is the healthiest and cheapest drink (6-8 glasses a day for most adults). Individuals should avoid drinking large

amounts of strong tea, strong coffee, and particularly energy drinks and caffeinated soft drinks [124, 125]. 10) Avoiding alcohol beverages or at least reduce individuals' alcohol consumption. Alcohol weakens the individuals' immune system [124, 125]. Nevertheless, additional strategies for healthy lifestyle include not smoking, regularly exercising, getting adequate sleep, and minimizing and coping with stress [124].

Conclusion

During strict city lockdown or self-quarantine period, many consumers were concerned that food might be running out from the supermarket shelves. Nevertheless, many retailers in countries around the world are working to overcome this challenge by hiring extra-workers to rapidly restore shelves, decreasing store hours to allow more time to restock, and re-deploying workers from other tasks to assist with restocking shelves. Many families are spending more time at home that provide new opportunities to share meals together. Family meals are a critical opportunity for parents to be role models for strengthening family relationship, and for healthy eating.

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CHAPTER 11

COVID-19 Recovery : Duration of Isolation and Precautions for Adults and Potential Treatment for Post-Intensive Care Syndrome

Abbreviations :

CDC : United States Centers for Disease Control and Prevention

COVID-19 : Coronavirus Disease 2019

RNA : Ribonucleic Acid

SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus type 2

Introduction

Several previous studies demonstrated that after onset of symptoms, concentrations of SARS-CoV-2 (COVID-19) ribonucleic acid (RNA) in upper respiratory tract specimens decline [1-5, unpublished CDC's data-2020]. Replication-competent virus has not been found in COVID-19 patients with mild to moderate symptoms after 10 days following initial onset of symptoms [2, 6-9, unpublished CDC's data-2020]. Recovery of replication-component virus after 10 days and 20 days following initial onset of symptoms was demonstrated approximately, 88 % and 95 % of the severe COVID-19 patients' specimens [5]. A previous study conducted by Chen *et al* demonstrated that if their SARS-CoV-2 (COVID-19) exposure to a case SARS-CoV-2 (COVID-19) patient began at least 6 days after the initial symptom onset of a case patient, the high-risk household and hospital contacts did not get infection [10]. SARS-CoV-2 (COVID-19) RNA can be continuously detected in upper respiratory samples of the recovered COVID-19 patients for 12 weeks or more although replication-competent virus was not isolated 3 weeks after symptom onset [8, 11, 12].

A previous study revealed that there was no secondary infections among 790 contacts who contact with 285 persistently positive SARS-CoV-2 (COVID-19)-RNA tested

individuals, including 126 individuals with recurrent symptoms [8]. Recovered patients with subsequent development of new symptoms and their specimens retested positive by reverse-transcriptase polymerase-chain reaction (RT-PCR) had no replication-competent virus detected [8, 9]. Based on limited evidence from another betacoronavirus (HCoV-OC43), the genus to which SARS-CoV-2 belongs, the risk of reinfection with SARS-CoV-2 (COVID-19) may be lower in the first three months after initial infection [13].

The current reinfection reports have been infrequent is expected to increased with time after recovery from infection due to possibly genetic drift and immunity waning. A recent study demonstrated that one of 48 SARS-CoV-2 (COVID-19)-infected skilled nursing facility workers had weakly positive-nasopharyngeal swab more than 20 days after the first diagnosis of COVID-19 [14]. Nevertheless, the specimen of this patient was not subjected to serial passage to reveal the presence of replication-competent virus [14]. The robustness and duration of immunity to SARS-CoV-2 (COVID-19) remains under investigation [15]. Individuals with mild to moderate COVID-19 and individuals with more severe to critical illness or severe immunocompromise likely remain infectious no longer than 10 days and 20 days, respectively [15]. Several previous studies have not demonstrated evidence that clinically recovered individuals with persistent SARS-CoV-2 (COVID-19) detection have transmitted SARS-CoV-2 (COVID-19) to others [15]. Relying on a symptom-based, rather than test-based strategy for ending isolation justification according to this strengthening findings should be followed [15]. Hence, individuals with current evidence of no longer SARS-CoV-2 (COVID-19) infection are not kept unnecessarily isolated and excluded from responsibilities [15]. Currently, SARS-CoV-2 (COVID-19) reinfection is likely to be infrequent during the first 90 days after initial onset of symptoms [15]. A positive RT-PCR without new symptoms among individuals recovered from SARS-CoV-2 (COVID-19) infection during the 90 days after initial onset of symptoms is prone to be persistent viral RNA shedding [15]. Currently, equivalent data from children and infants are not available. Most of the current available data are derived from adults [15].

If COVID-19 critical illness is prolonged, the patients will develop chronic inflammation, fibrosis, and thrombosis (Long COVID-19). Evidence of low-grade inflammation in patients with major cardiac events, anemia, and lung cancer were reduced when treated for secondary prevention with interleukin-1β (IL-1β) monoclonal antibody

canakinumab, suggested in the CANTOS trial [16]. Interestingly, the COLCOT trial demonstrated that reducing IL-1β-related inflammation increased risk of infection, a significant consideration in the functionally immunosuppressive post-intensive-care-syndrome population [17].

Conclusion

Presently, there are some questions urgently needed to be answered whether recovered individuals are definitely immune to SARS-CoV-2 (C)OVID-19) reinfection due to uncorrelated biomarkers of immunity with human-infection protection. Nevertheless, most recovered persons would have a level of immunity for 3 months or more after the first diagnosis. The intensive care unit (ICU) community should conduct large internationally multicentric trials for ICU survivors to investigate the efficacy of these drugs for COVID-19-ICU survivors, including investigation on immune biomarker profiles based on disease prognosis.

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Subject Index

Notes

Different methods of imaging, except in unusual circumstances, have been omitted to simplify the index.

To save space in the index, the abbreviations have been used same as in each book chapter.

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