INTERNATIONAL SOCIETY OF HEALTH EDUCATION

ALL ABOUT COVID-19

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Chapter one: Properties of Coronavirus and Toxonomy of Virus
Dr.Maitham G.Yousif

1.Introduction

Coronavirus is a serious health problem for humans during 2002 2 2000 5 appear respiratory syndrome called SARS COV. The morbidity of this syndrome was more than 10%.after that MERS COV appeared in Saudi Arabia with a fertility rate is about 36% [1,2].

Coronaviruses can change their genome through mutation and produce a new generation to resist the new environment between the (3,4). So if we understand This strategy of adaptation can control the pandemic of COVID-19. There are viruses cross the species barriers because deadly pneumonia in human starting from this century, severe acute respiratory syndrome coronavirus SARS COV [5].

1.1.Taxonomy of coronavirus

Coronaviruses members of the family Coronaviridae Order Nidovirales Between brackets 1011 .this order include 4 genera Lare you happy; Alpha coronavirus beta coronavirus Gama coronavirus on Delta coronavirus (Figure 1). Mammals infect with Alpha and beta coronavirus while gamma coronavirus infects birds And the Delta corner virus both birds and mammals. Human coronavirus including the following species HCOV - NL 63 TGEV, PEDV, and PRCV.

Coronavirus type porcine epidemic diarrhea virus PEDV was the first diagnosis in the United Kingdom it goes to a small sector of epidemic In Europe during the 1980s. As animals, their developed immunity Coronavirus disappears gradually. Difference (6).As well as coronavirus include beta coronavirus include SARS-COV, MERS COV, bat coronavirus HKU 4, mouse hepatitis coronavirus MHV, cattle coronavirus, and a human being coronavirus OC43.Colonel virus has

envelope was it if stranded RNA. The size of the RNA of this type ranges from 27 to 32 KB. The genetic material of this virus is Beckett inside helical capsid composed by that and you couldn't capture the protein called N and surrounded by envelope this envelope associated with violent envelope or at least three structural proteins including the following the membrane protein call M on the envelope protein E is related a virus assembly. As well as spike protein called S to facilitate virus entry to the host cell. In some strains of coronavirus, they encode an envelope associated with hemagglutinins esterase protein called HE.

1.2.Structure of virus

1.2.1.Spike Proteins

There are many theories about the origin of spike protein, one of these theories is believed to be a member of the class I viral membrane Fusion proteins that includes those from influenza virus, human immunodeficiency virus HIV, as well as ebolavirus (Figure 2).

From these proteins, the hemagglutinins glycoprotein of the influenza virus Was a detailed study (5, 6). Update the cell entity way off SARS COV has led to Noble result first of them SARSCOV spike is not separate by convertase enzyme during maturation of virus and remain dormant state in mature virus (7, 8).

1.2.2.Genomic variation of SARS – COV2

many studies concerned with do norms of COVID-19 and evolutionary relationships dazed on the genetic variation Figure 1 refer that mutation among the majority of COVID-19 genes only 95 violable sites (Figure-1) B the study of phylogenetic trees should suggest two major types of COVID-19, called type 1 and type 2. Further dividing type 1 into type IA and IB The total genomes belonging to type IA, IB is 10 and 18 [(9)].

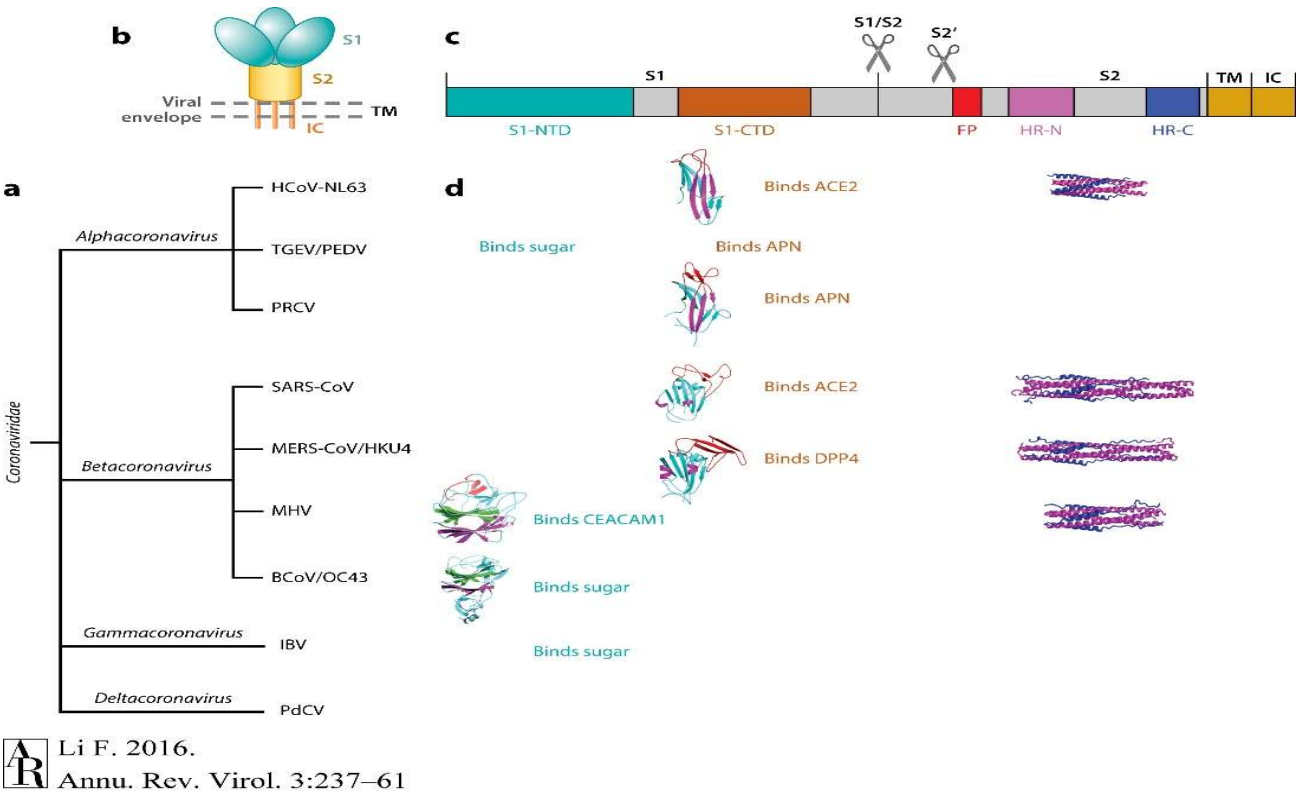


Figure 1 Introduction to coronaviruses and their spike proteins. (a) Classification of coronaviruses. Representative coronaviruses in each genus are human coronavirus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavirus (TGEV), porcine epidemic diarrhea coronavirus (PEDV), and porcine respiratory coronavirus (PRCV) in the genus Alphacoronavirus; severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), bat coronavirus HKU4, mouse hepatitis coronavirus (MHV), bovine coronavirus (BCoV), and human coronavirus OC43 in the genus Betacoronavirus; avian infectious bronchitis coronavirus (IBV) in the genus Gammacoronavirus; and porcine delta coronavirus (PdCV) in the genus Deltacoronavirus. (b) Schematic of the overall structure of prefusion coronavirus spikes. Shown are the receptor-binding subunit S1, the membrane-fusion subunit S2, the transmembrane anchor (TM), the intracellular tail (IC), and the viral envelope. (c) Schematic of the domain structure of coronavirus spikes, including the S1 N-terminal domain (S1-NTD), the S1 C-terminal domain (S1-CTD), the fusion peptide (FP), and heptad repeat regions N and C (HR-N and HR-C). Scissors indicate two proteolysis sites in coronavirus spikes. (d) Summary of the structures and functions of coronavirus spikes. Host receptors recognized by either of the S1 domains are angiotensin-converting enzyme 2 (ACE2), aminopeptidase N (APN), dipeptidyl peptidase 4 (DPP4), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and sugar. The available crystal structures of S1 domains and S2 HRs are shown. Their PDB IDs are 3KBH for HCoV-NL63 S1-CTD, 4F5C for PRCV S1-CTD, 2AJF for SARS-CoV S1-CTD, 4KR0 for MERS-CoV S1-CTD, 3R4D for MHV S1-NTD, 4H14 for BCoV S1-NTD, 2IEQ for HCoV-NL63 HRs, 1WYY for SARS-CoV HRs, 4NJL for MERS-CoV HRs, and 1WDF for MHV HRs.

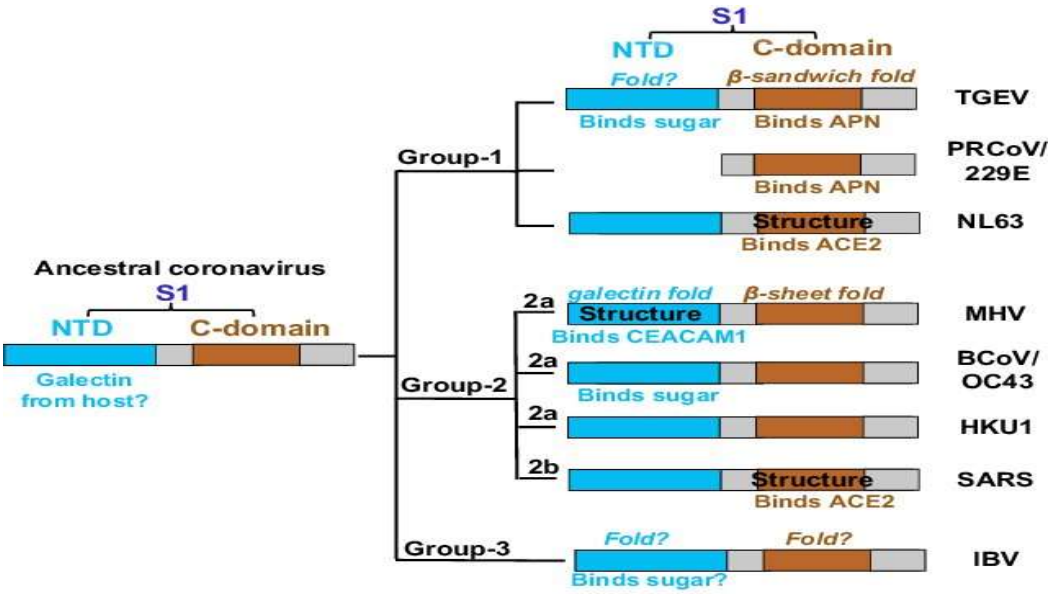
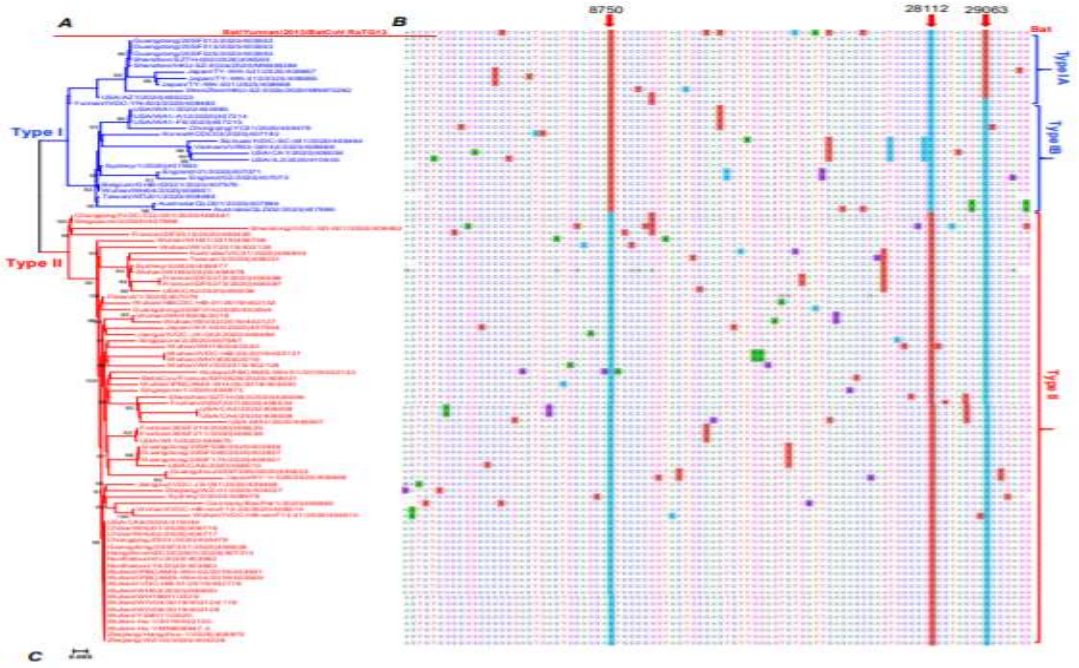


figure 2:Structures, functions, and evolution of S1 subunits of coronavirus spike proteins. The three known crystal structures are indicated by "Structure." Among these structures, MHV NTD has a 13-stranded galectin-like β-sandwich fold, HCoV-NL63 C domain has a six-stranded β-sandwich fold, and the SARS-CoV C domain has a five-stranded β-sheet fold.



	Codon	# Anticodon tRNA genes; tAI*	Codon	# Anticodon tRNA genes; tAI	Codon	# Anticodon tRNA genes; tAI
SNP position	8750		28112		29063	
RaTG13 (bat)	AGT (Ser)	0; 0.16	TCA (Leu)	4; 0.14	TTT (Phe)	0; 0.20
Type I	AGT (Ser)	0; 0.16	TCA (Leu)	4; 0.14	TTT (Phe)	0; 0.20
Type II	AGC (Ser)	8; 0.28	TTA (Ser)	4; 0.14	TTC (Phe)	10; 0.34

a: this column shows the number of tRNA genes in human genome with anticodons matching the considered codons. tAI is a measure of codon's translational efficiency[5], the higher the more efficient.

1.3.References

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, et al. 2003. A novel coronavirus associated with the severe acute respiratory syndrome. *N. Engl. J. Med.* 348:1953–66

2. Peiris JSM, Lai ST, Poon LLM, Guan Y, Yam LYC, et al. 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 361:1319–25

3. Marra MA, Jones SJM, Astell CR, Holt RA, Brooks-Wilson A, et al. 2003. The genome sequence of the SARS-associated coronavirus. *Science* 300:1399–404

4. Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, et al. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300:1394–99.

5. C. Drosten, S. Günther, W. Preiser, S. van der Werf, H.R. Brodt, S. Becker, H. Rabenau, M. Panning, L. Kolesnikova, R. A. Fouchier, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome N. Engl. J. Med., 348 (2003), pp. 1967-1976.

6. Eckert DM, Kim PS. 2001. Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. 70:777–810

7. Skehel JJ, Wiley DC. 2000. Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin. *Annu. Rev. Biochem.* 69:531–69

8. Song HC, Seo MY, Stadler K, Yoo BJ, Choo QL, et al. 2004. Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. J. Virol. 78:10328–35 134.

9. Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS. 2003. The SARS-CoV S glycoprotein: expression and functional characterization. Biochem. Biophys. Res. Commun. 312:1159–64.

10. Zhang L., et al., Origin and evolution of the 2019 novel coronavirus. Clin Infect Dis, 2020.

Chapter two: Lung injury related to COVID-19

2.1.Introduction

Coronavirus disease 2019 (COVID-19) is an emerging pathogen that resulted in a global pandemic. The virus causing the disease is a positive-stranded RNA virus. It is similar to other coronaviruses; these viruses can infect several animals, like other mammals and birds. The origin of the current pandemic has been traced back to a wild animal market in the Wuhan in the Hubei province of China. From there, the virus spread all over the globe, with cases being diagnosed in all continents except Antarctica.

The virus was isolated first from the bronchoalveolar lavage of three patients admitted to a hospital in Wuhan. All three cases reported direct exposure to the Seafood market. The virus showed an 85% shared identity with the previous bat SARS-like coronavirus (SARS-CoV), raising the possibility of animal-to-human transmission [(1)].

2.2. Pathology of lung injury

Angiotensin-converting enzyme 2 (ACE2) receptor is the binding site for S protein of the Covid-19 virus and gains entry into human cells. After binding, host serine protease TMPRSS2 cleaves the S protein and results in the fusion between the viral and cellular membranes. The S protein of Covid-19 and SARS-CoV have the same three-dimensional structures, and, given this, investigators hypothesize that SARS-CoV-2 likely uses the same mechanism [(2)].

Furin, a type 1 membrane-bound protease member of the subtilisin-like proprotein convertase family, also split the site between S1 and S2 subunits of the SARS-CoV-2 S-protein. A furin splitting site is absent in SARS-CoV-1, which is another distinctive aspect in the pathogenesis of COVID-19. Notably, furin is

expressed in many tissues, including the lungs. After SARS-CoV-2 binds to ACE2 with its receptor-binding domain, furin catalyzes the spike protein's splitting (S1/S2), which is otherwise essential for viral entry into the cell.

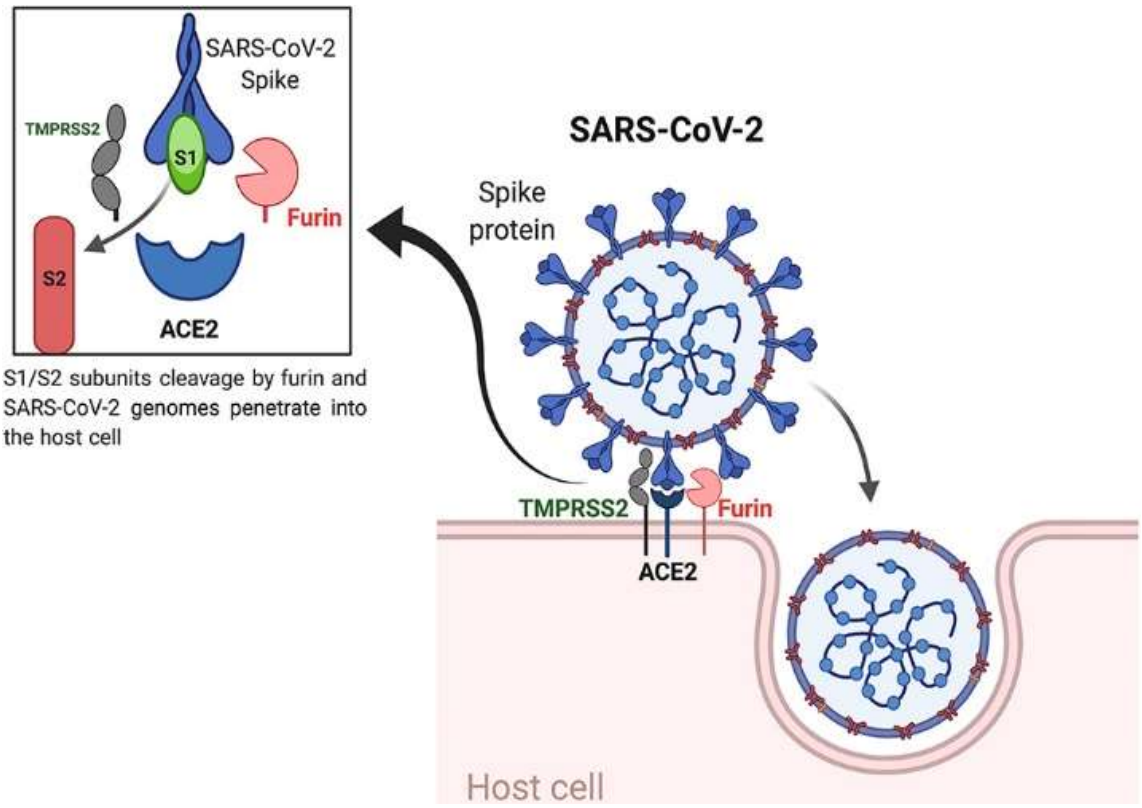


Figure (1): speculated intermolecular interactions between the spike protein of SARS coronavirus 2 (SARS-CoV-2) and the host cellular receptor angiotensin-converting enzyme-2 (ACE2). Significant binding is relying upon spike protein activation by transmembrane serine protease 2 (TMPRSS2) or furin. [3]

This alternative pathway, including furin-mediate activation, would hence allow SARS-CoV-2 to have a lower dependence on TMPRSS2 expressions at the cell surface for infecting them. Thus, SARS-CoV-2 may be able to enter a wide array of cells with less TMPRSS2 expression. It is essential to say here that this crucial pre-activation process that the spike protein must undergo for enabling efficient virus penetration into the host cell may represent an essential immune evasion strategy.

Whereby the antibodies produced against the virus may be unable to efficiently recognize and bind to the "hidden" or "inactivated" receptor binding domain and thus may be unable to neutralize the virus (Figure 1)[3].

The ACE2 receptor is expressed in type-2 pneumocytes, kidney, heart, and gastrointestinal tract. However, the lungs look to be particularly vulnerable to Covid-19 because of their vast surface area and because type 2 pneumocytes seemingly act as a reservoir for virus multiplication. Direct insult to the lung tissue from a viral infection–mediated inflammatory response is one of the speculated mechanisms behind the pulmonary manifestations of COVID-19 [2].

2.3.Cytokine storm and the systemic inflammatory response

Cytokine storm syndrome (CSS) is an exaggerated immune response to stimuli like viral infections. Both macrophage activation syndrome (MAS) and secondary hemophagocytic lymphohistiocytosis (sHLH) are clinically similar CSSs.

Macrophage activation syndrome is a cytokine storm syndrome that is usually seen in the context of rheumatological diseases. Secondary hemophagocytic lymphohistiocytosis can be observed in patients with severe infection. It results from high production of pro-inflammatory and inadequate anti-inflammatory triggers. Some of the pro-inflammatory triggers include foreign antigens, cytokines such as tumor necrosis factor (TNF)–α, interleukin (IL) (like 1β, IL-2, IL-6, IL-7, IL-12, IL-18), granulocyte colony-stimulating factor (GCSF) and interferon (IFN)–γ. Some of the anti-inflammatory triggers include regulatory T cells, cytokines such as IL-10, IL-1ra, and transforming growth factor (TGF)–β. [4]

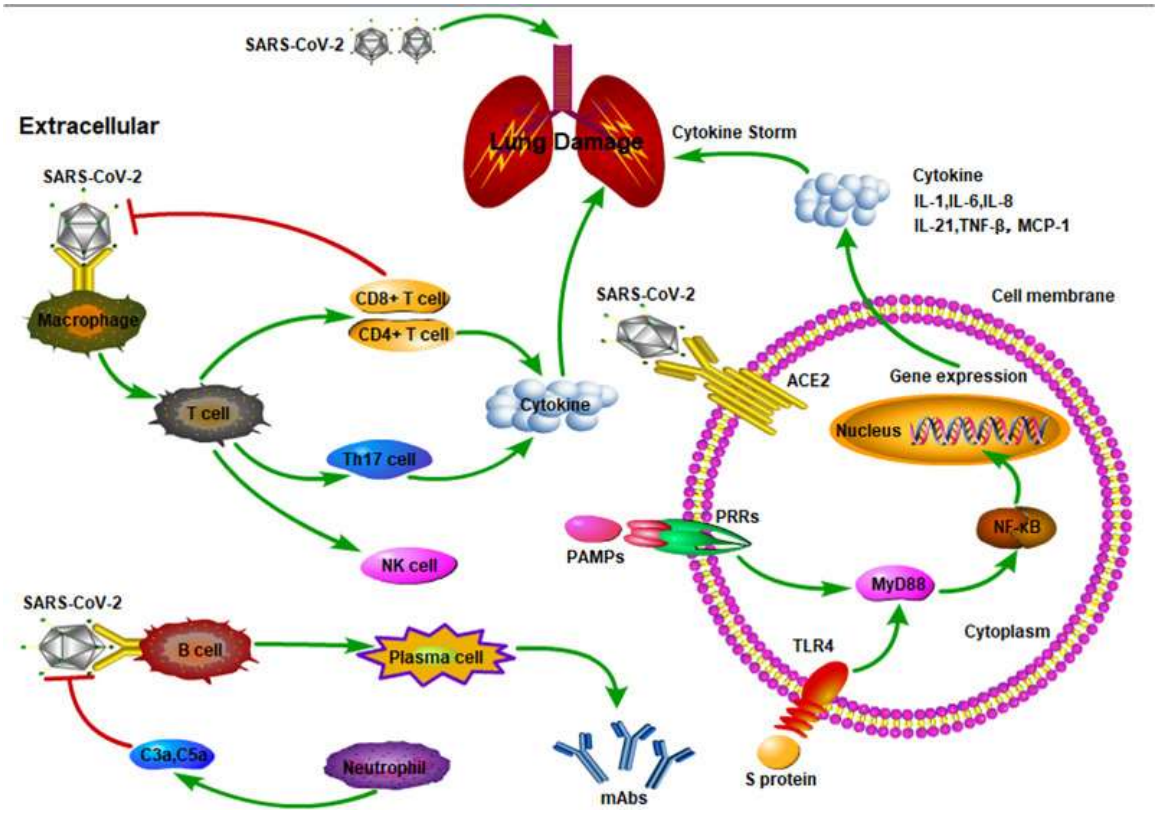


Figure (2): The immune and inflammatory responses in COVID-19 infections. In the immune response, macrophages present COVID-19 antigens to T cells that activate, differentiate, and release cytokines and chemokines like interleukin (IL)-1, IL-6, IL-8, IL-21, monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-β (TNF-β) causing the cytokine storm that causes lymphocytes and leukocytes recruitment to the infection site. In infected cells or immune cells, nuclear factor kappa B (NF-κB) activation may play a vital role in immune response and acute lung injury. ACE2=angiotensin-converting enzyme 2; PAMPs=pathogen-associated molecular patterns; PRRs=pattern recognition receptors; TLR4=Toll-like receptor 4.[5]

Increased IFNγ production by hematopoietic stem cells in response to viral infections is believed to stimulate CSS. CSS is characterized by constant fever and multi-organ insult, including acute respiratory distress syndrome (ARDS) and acute renal and cardiac injury. Laboratory abnormalities may include cytopenias, D-dimer, increased ferritin, and increased pro-inflammatory cytokines serum levels.

Evidence gathered to date revealed that CSS is directly correlated to the severity of the disease process. Laboratory analysis proved that COVID-19 patients showed leukopenia and increased pro-inflammatory cytokines serum levels such as TNFα, IL-2, IL-6, IL-7, IFNγ, and GCSF, similar to that seen in sHLH, suggesting a plausible mechanism for tissue injury [6].

A retrospective multicenter study in Wuhan, China of COVID-19 patients revealed statistically significant increased mortality in patients with an elevated ferritin level (>1200 ng/mL) and high IL-6 levels [7]. Data from China concluded that about 80% of patients with COVID-19 had only a mild infection. Among the rest, 20% of patients, a proportion of the patients developed severe disease with multi-organ failure necessitating ICU admission. The pathogenesis behind this disease mode appears to be correlated to an overwhelming inflammatory response, as observed in sHLH/CSS. The predilection to develop CSS is unknown and is thought to be related to host factors like underlying immunodeficiency or genetic factors.

It has recently been postulated that the inflammation of nucleus tractus solitaries might provoke an aggravation of neurogenic pulmonary edema and microvascular thrombosis in critically ill COVID-19 patients. [8][9] However, in a recent case series, patients with severe COVID-19 have shown features of acute disseminated encephalomyelitis (ADEM) with hemorrhagic changes,[10] which has not been correlated to the severity of lung insult, and it has been partially attributed to diffuse endothelial dysfunction related to the viral binding to the ACE-2 receptors [11,12].

However, neurogenic pulmonary edema could occur in patients with severe COVID-19 pneumonia, although it should not be classified as a form of ARDS, but rather as non-cardiogenic interstitial lung edema with distribution to peripheral lung zone, which could be observed in viral pneumonitis and after brain injury[13].

On clinical grounds, this non-cardiogenic pulmonary edema has been a diagnosis of exclusion. In COVID-19, although the basic distribution pattern of consolidations and ground-glass opacities is peripheral and on the lower lung zones, as has been reported by numerous chest computed tomography (CT) studies, atypical lung involvement patterns may occur [14-17]. In a prospective, longitudinal pulmonary ultrasound study in severe COVID-19 pneumonia, we have recently outlined a diverse lung involvement in several lung zones. [18] Hence, we believe that COVID-19 lung injury could be attributed to multifactorial pathophysiologic mechanisms.

In summary, the COVID-19 infection can lead to an inflammatory cytokine storm in infected patients. In turn, the cytokine storm stimulates ARDS and multi-organ failure and represents a critical factor in COVID-19 exacerbation or even mortality (Figure 3).

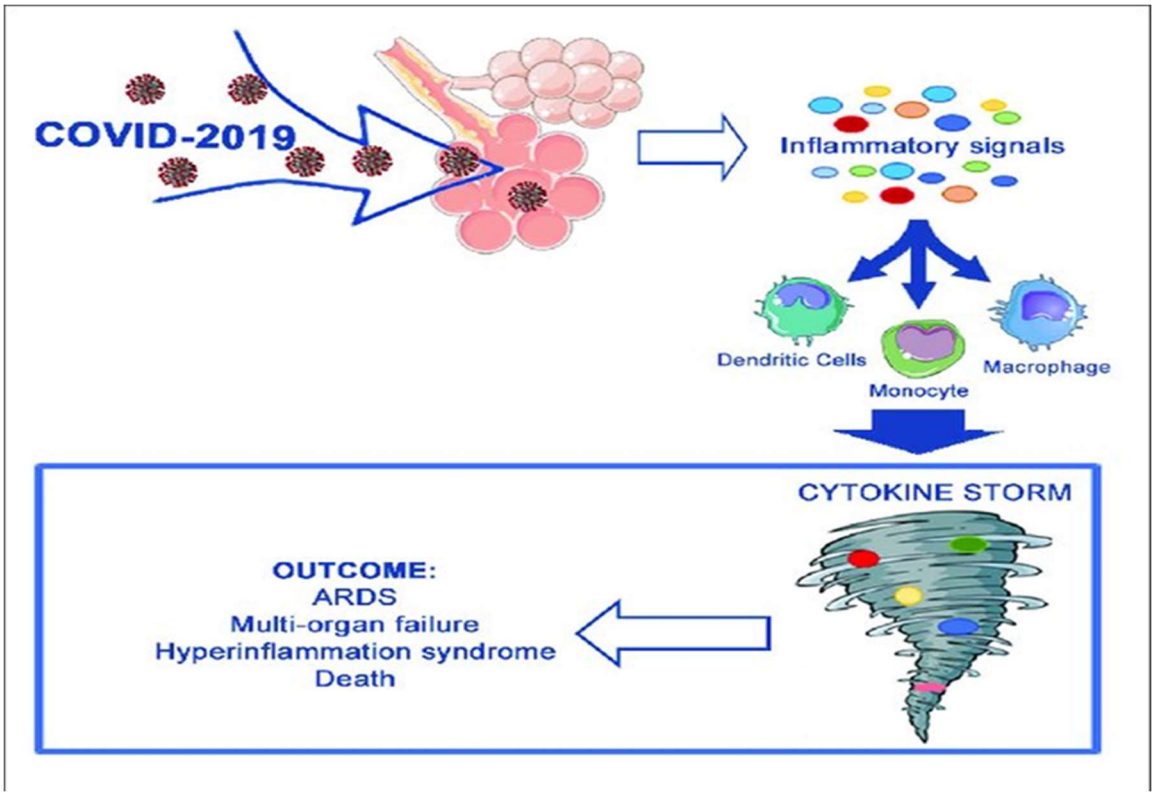


Figure (3): An exaggerated immune host response is occurring during COVID-19 infection[19]

However, the main issue is why some patients are more susceptible to cytokine storms than others. Different genetic mutations may also be regarded as a risk factor for the severe disease with the occurrence of cytokine storm in COVID-19. Data obtained from a global population pointed out that allelic alterations in cytokine genes had a powerful latitudinal impact [20,21]. Geographical latitude is the leading environmental determinant that is affected by our evolutionary history concerning environmental selection. Therefore, the latitude is correlated with various factors comprising genetic background, biometeorological factors, and socio-economic influences.

Regarding the impact of biometeorological factors, sunlight has a crucial role in synthesizing Vitamin D, which plays a pivotal role in maintaining immune homeostasis. Genetic factors account for up to 28% of inter-individual variability in serum 25(OH.)D concentrations [22]. Genetic and individual differences in vitamin D status have been observed across various populations [23]. In light of this, we can suppose that there is a possibility that vitamin D status may have some effect on the geographical difference of COVID-19. Furthermore, vitamin D deficiency may lead to exacerbated autoimmunity and increased susceptibility to infections. Vitamin D inhibits the synthesis of pro-inflammatory cytokines (i.e., TNF-a and IFN-g) and induces the release of anti-inflammatory cytokines. Vitamin D mitigates the risk of microbial infection and death via a different mechanism.

A recent review classified those mechanisms into three different groups: physical barriers and innate and adaptative immunity[24]. COVID-19 viruses disrupt junction integrity, increasing the vulnerability to infection by the virus and other microorganisms[25], while vitamin D supports cell junctions integrity[26]. Vitamin D may be valuable in mitigating the cytokine storm and the outcome of COVID-19 patients. Its decreased level leads to higher risk. Vitamin D supplementation could thus be, in theory, useful[27].

However, cytokine regulation relies on different upstream regulators, such as Toll-like Receptors, and these interact with other components of the innate immune system, like complement elements. Toll-like receptors are a family of innate immune sensor proteins exerting a vital function in infection, inflammation, and immunity processes[28]; Toll-like Receptors pathway may be significantly involved in cytokine storms occurring during COVID-19 infection. Up to date, there are no studies regarding the role of Toll-like Receptors signaling in SARS-CoV-2 infection. However, previous studies indicate that genetic variation within Toll-like Receptors or Toll-like Receptors signaling affected SARS-CoV infection [28].

The complement system components interact with Toll-like Receptors, and it is thus implicated in greater vulnerability to the infection and cytokine storm activation[29]. A recent study revealed that the complement system represents a crucial host mediator of SARS-CoV infection. SARS-CoV-infected C3-/- mice exhibited less respiratory impairment and lowered chemokines and cytokines in the organs[30]. Besides, the complement system's hyperactivation was reported in COVID-19 patients, and the highly pathogenic coronavirus N protein exacerbated MASP-2-mediated complement activation[31]. Overall, the complement system is critically implicated in stimulating the cytokine storm and inflammation in SARS-CoV-2 infection.

Histopathologically, Acute lung injury (ALI) is associated with different manifestations such as diffuse alveolar damage (DAD) and acute fibrinous as well as organizing pneumonia (AFOP) and organizing pneumonia (OP)[32].DAD is classified into three histopathological phases that correlate with the time from pulmonary insult: acute (exudative) phase and subacute (organizing) phase as well as chronic (fibrotic) phase. The acute phase of DAD (Figure-4A) occurs within one

week of the initial insult and is featured by intra-alveolar hyaline membranes, edema, and alveolar wall thickening without noticeable inflammation unless it arises in conjunction with acute pneumonia. Vascular thrombosis and micro thrombosis are frequently seen in DAD, even in the absence of a systemic hypercoagulable state, and they are attributed to local inflammation. Angiographic studies also have proved that thrombosis occurs early in ARDS of diverse origins [33]

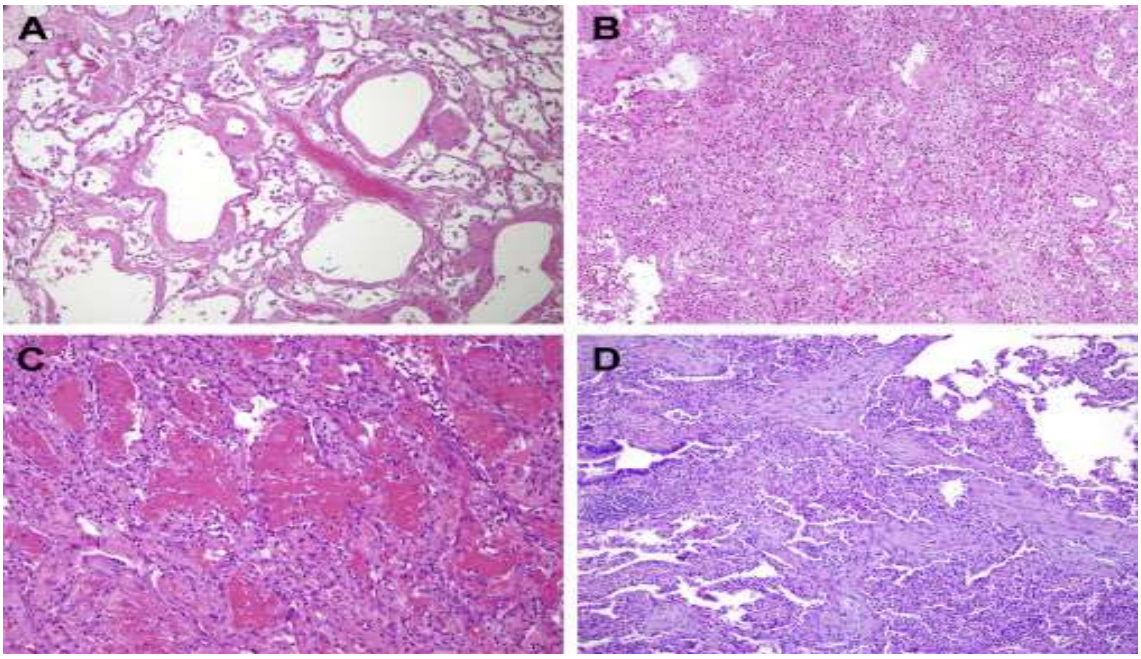


Figure (4): Histopathologic examples of acute lung injury pathology. A- Acute exudative phase. B- Subacute organizing phase of diffuse alveolar damage. C- Acute fibrinous and organizing pneumonia. D- Organizing pneumonia [34]

2.4.Reported Histopathology Findings in COVID-19

2.4.1.Diffuse alveolar damage (DAD) Acute Phase

Of the published autopsy specimens with COVID-19, the acute phase of DAD was the most common pulmonary pathology, seen in 88% of cases. The features include prominent hyaline membranes with edema, mild interstitial inflammatory infiltrates, and desquamated pneumocytes with reactive pneumocyte hyperplasia. [34]

2.4.2.Acute fibrinous and organizing pneumonia (AFOP)

Amongst the COVID-19 cases, (4%) in one paper were described as having AFOP without hyaline membranes on needle biopsy. This study was performed on biopsy-based samples [35]

2.4.3.Organizing Fibrosis

Most of the reported COVID-19 autopsy case series describe the acute phase of DAD as the prominent acute lung injury pattern. However, organizational fibrosis features were seen on histopathologic examination in 52% of the COVID-19 autopsy cases[34]

2.4.4.End-Stage Fibrosis

Progression to fibrosis was rare in all viral ARDS cases reviewed here and only seen in patients with the protracted illness. Among the COVID-19 autopsy cases, late fibrosis was seen in a single patient (1%) diagnosed with chronic myelomonocytic leukemia who died 26 days after symptom onset [36].

2.4.5.Microthrombotic Disease

Of the COVID-19 cases, 57% were reported to have a micro thrombotic disease in capillaries and small and medium-sized vessels[34].

2.4.6.Pulmonary Thrombosis

Thrombosis in large pulmonary vessels was seen in 15% of COVID-19 autopsy cases [34].

2.4.7. Acute Neutrophilic Pneumonia

Secondary bacterial infections have been reported in 32% of reported histologic features suggestive of acute pneumonia in COVID-19 patients. [34]

2.5. Histopathological Abnormalities in Patients With Asymptomatic COVID-19 Infection

Incidental histopathologic findings in 14 asymptomatic patients who have lung nodule resections were subsequently found to have COVID-19 infection. As expected, the histologic findings were less severe than those in patients with symptomatic COVID-19 infection. Most cases have focal edema with proteinaceous exudate, patchy chronic inflammation, pneumocyte hyperplasia, and multinucleated pneumocytes[37,38].

2.6. Clinical Features of lung injury in COVID-19

Similar to SARS-CoV, a clinical feature of COVID-19 may include fever, cough, nasal congestion, sore throat, and even conjunctivitis, which have been reported. Moreover, gastrointestinal symptoms of nausea, vomiting, and diarrhea are also familiar with COVID-19. Reports of anosmia (loss of smell), ageusia (loss of taste), petechiae, red rashes, urticaria, pernio-like purplish-red discoloration of the fingers and toes, and chickenpox-like vesicles have been described.

A wide range has been seen for patients presenting with dyspnea. The initial cohort from Wuhan reported that 55% of patients have dyspnea, while in Washington State, dyspnea was seen as the presenting complaint in 88% of the patients [39]

When comparing severe with other cases, patients in the severe disease cohort were older and more likely to have comorbid conditions[40]. In Washington State, where chronic medical illnesses were common, 58% of the patients had diabetes, another 21% had chronic renal disease, and 14% had asthma [39].

ARDS is a feared sequela of COVID-19. ARDS is diagnosed according to the Berlin criteria by the presence of acute hypoxic respiratory failure with bilateral pulmonary infiltrates without a known etiology in the presence of a known injury

within seven days. The product of the division of the partial pressure of arterial oxygen on the fraction of oxygen inspired (PaO2/FiO2) is used to grade hypoxia in these patients[41]. Decreased lung compliance is a prominent feature and is calculated by dividing the tidal volume on the plateau pressure subtracting the positive end-expiratory pressure from it. However, an atypical form of ARDS in patients with COVID-19 patients was initially reported from Italy [41].

2.7.Diagnosis of Lung injury in COVID-19

2.7.1. Laboratory findings

Several studies showed leukopenia, especially lymphocytopenia and thrombocytopenia, on homography. Patients also had increased C-reactive protein levels, greater than 10 mg/L in 81% of severe patients and 56% of non-severe patients. Procalcitonin, traditionally associated with bacterial pneumonia, remains in the normal range in most COVID-19 cases. Other abnormal laboratory findings include elevated liver enzymes, lactate dehydrogenase (LDH), creatinine, creatinine kinase, and D-dimer. Of these, high LDH may be associated with more severe disease [39].

2.7.2.Criteria for Defining Cytokine Release Syndrome in COVID-19 [42]

- C-reactive protein level >100 mg/L or > 50 mg/L and doubled in the past 48 h
- lymphocyte cell count < 0.6×10^9 cell/L
- serum Interleukin-6 level ≥ 3 times normal upper limit
- ferritin level > 300 ug/L or doubling within 24 h
- ferritin level > 600 ug/L at presentation and LDH > 250 U/L
- elevated D-dimer (>1 μg/mL)

Low risk for developing CRS is the presence of one criterion, the moderate risk is the presence of two to three criteria, and high risk the presence of more than three criteria.

2.7.3. Imaging

2.7.3.1.Chest X-ray

As with other respiratory illnesses, chest radiography is often the first diagnostic test performed. Chest X-rays are an easy bedside imaging modality and can be done by the use of portable devices. The usual CXR findings in COVID-19 are areas with increased hazy opacities, ground-glass opacities, bilateral consolidations, usually with distribution in the lower lobes. Pleural effusion is uncommon. The sensitivity of CXR in the early stage of the disease is about 69%, and the specificity is unknown. Patients who are symptomatic and have severe disease are more likely to have positive findings on chest radiographs (figure 5). (39)(43)

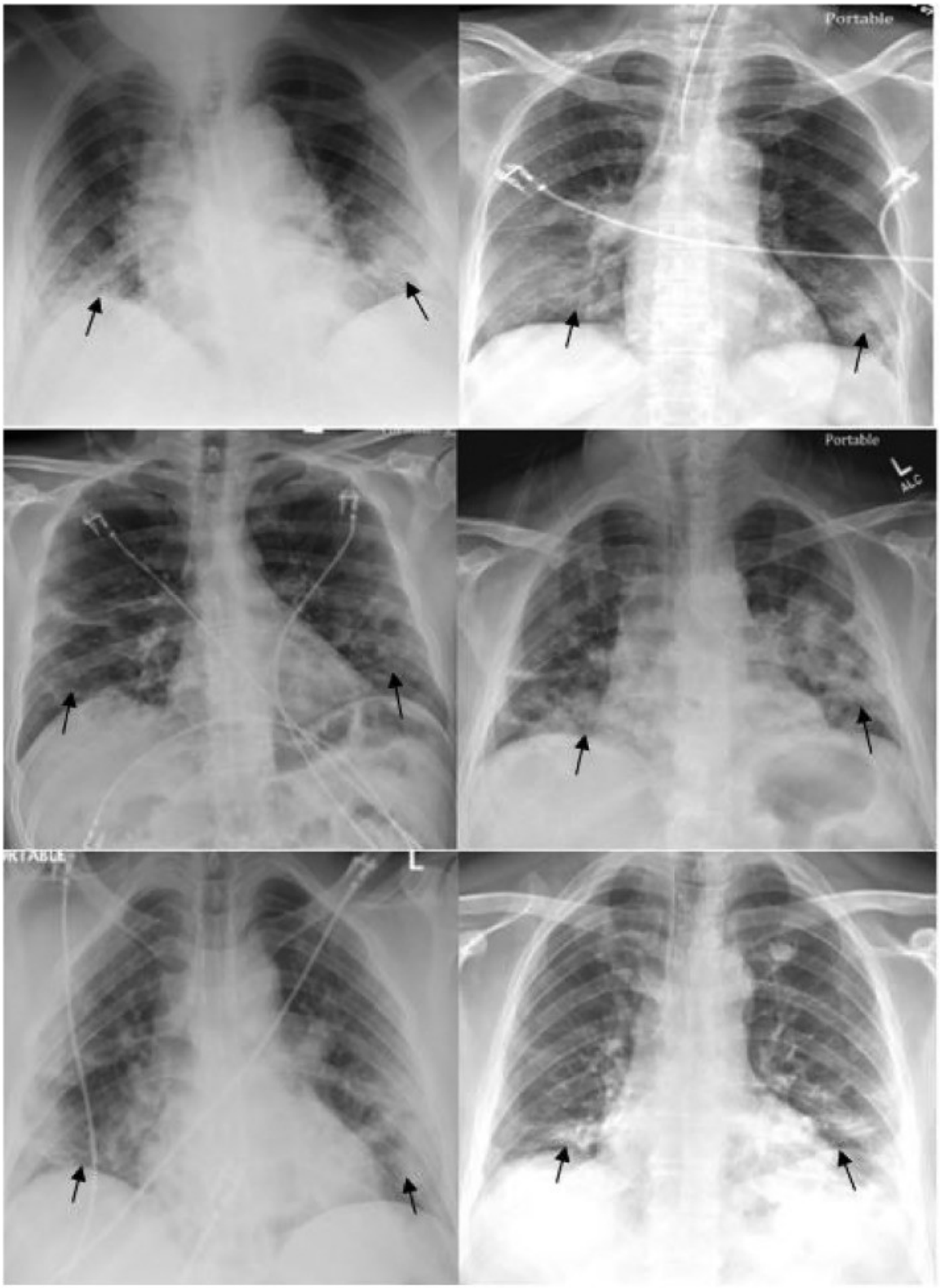


Figure (5) Six different patients with varying degrees of COVID-19 pneumonia predominantly involving the lower lung zones (black arrows) bilaterally on CXR.[44]

2.7.3.2.Computed Tomography

The COVID-19 CT findings are well-reported and include ground-glass opacifications (GGOs), defined as hazy increased lung density preserving vascular and bronchial margins. Other finding include consolidative pulmonary opacities with opacification and obscuration of margins of vessels and airway walls. Bilateral and peripheral distribution are the hallmarks of CT-scan finding in COVID-19 patients (Figure 6) [45]

Bernheim et al. studied the CT findings of 94 positive RT-PCR confirmed COVID-19 patients. They found that 56% of patients had a normal CT scan in the first two days, while only 4% had a normal CT scan after six days. Early findings are bilateral peripheral small GGOs. Over time, there is an increase in opacifications and consolidations; other patterns such as a reversed halo sign, crazy paving, and linear opacities become apparent, and the peripheral predilection and multilobular distribution became clear. [46] Jin et al. reported five temporal phases classified as ultra-early, early, rapid progression, consolidation, then dissipation stages. There may be a reduction in number and extension of lesions in the dissipation stage, with small ill-defined interlobular septal thickening remaining[47].

CT scan is shown to have greater sensitivity than RT-PCR for diagnosing COVID-19 in a highly endemic area with high pre-test probability for the disease. [48] On the other hand, Bernheim showed that in the first few days of infection, more than 50% of patients had a normal CT, and concluded that CT had limited sensitivity early after symptom onset, and because of that, it was not a reliable single tool for ruling out COVID-19 infection. [46] Lomoro et al. reported normal CT studies for almost 5% of confirmed COVID-19 cases [47].

Another critical issue regarding CT is whether it can differentiate between COVID-19 pneumonia and other viral pneumonia. The CT findings in other virus pneumonia have been well documented, but imaging findings and atypical presentation might complicate the picture. [49] Other processes like influenza pneumonia and organizing pneumonia may have bilateral peripheral GGOs [50]. Bai et al. compared the CT-scan findings in COVID-19 to other viral pneumonia.

They observed a tendency toward peripheral rather than central pathology, mainly GGOs, fine reticular opacities, vascular thickening, lower incidence of lymphadenopathy, and pleural effusion in COVID-19 compared to other viral pneumonia. Using CT to differentiate between COVID-19 and other pneumonia did so with a sensitivity of (80.4%) and specificity (96.6%). Thus CT appears to be better suited to exclude COVID-19[51].

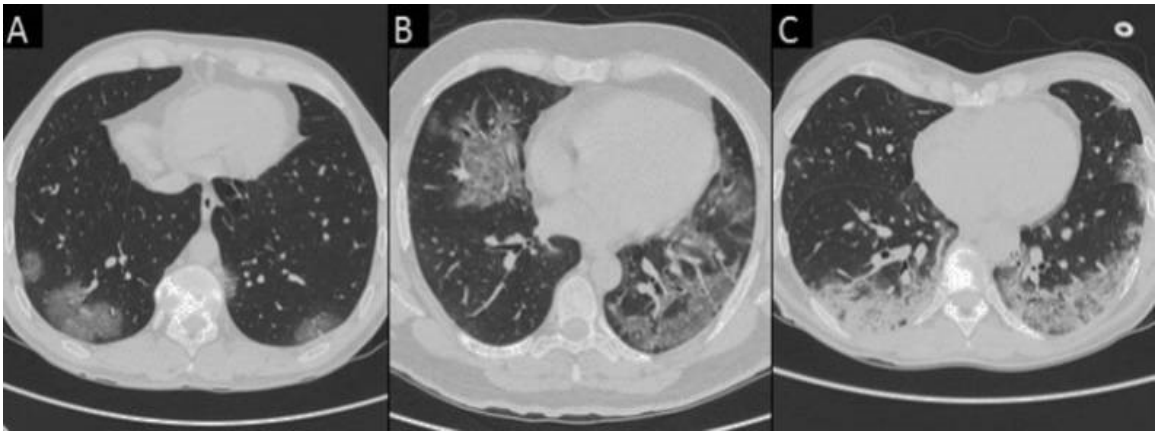


Figure (6) Chest CT images of the various pattern of COVID-19 pneumonia in true positive (TP) patients [45].

2.7.3.3.Ultrasound

Lung and chest ultrasound are already in use by intensive care physicians as a bedside tool for evaluating various conditions, including pneumothorax, pleural effusion, pulmonary edema, pulmonary embolism, pneumonia, interstitial lung diseases, and for patients on mechanical ventilatory support. [52] In the early times of

the COVID-19 outbreak, clinicians were first to perform lung US on infected patients, and because of that, they mastered the technology. Subsequently published their findings [53,54].

Any new-generation US device may be used for lung ultrasound exams: premium machines, small portable devices, and even wireless ones are all suitable for lung and thorax imaging. Different probes are used, basically curvilinear probes for in-depth and comprehensive lung field coverage and linear probes for best visualization of the superficial lesion. Phased array probes allocated for cardiac imaging may also be used [52]

The operator must have adequate training and must be familiar with both the machine and the technique. The operator's objective is to cover the lungs' exam as much as possible in a systematic, quick, and efficient manner. For reproducibility and uniformity of reporting, the lungs are scanned at fixed points. Most of the COVID-19 publications relating to lung US used a focused 12-area approach. The thorax was scanned bilaterally (Right 1–6 and Left 1–6) at the bedside: anterior superior and inferior, lateral superior and inferior, posterior superior and inferior [53-58].

Any lung condition that extends to the pleural surface is amenable to ultrasound. However, ultrasound cannot visualize lesions deep within the lung parenchyma since the lung's air will block ultrasound wave transmission. Usually, chest ultrasound will detect the detailed chest wall structure, including the skin, subcutaneous tissue layer, muscular layer, and ribs. The pleura slides during respiratory movement and is visualized as a sharp continuous echogenic line, posterior and adjacent to the chest wall. The aerated lung looks hypoechoic and homogenous. White A-lines are created due to a reverberation artifact produced by interphase between the superficial layers and the lungs' air. These white A-lines

appear in fixed spacing, parallel to the pleural line and each other, and disappear gradually with depth.

A case series of 20 patients suggests that pleural-line irregularities, a "B-line" pattern, and consolidations are suggestive of COVID-19 (Figure 7). Lung ultrasound has been frequently shown to be more sensitive than chest X-ray, and the significant benefits of lung ultrasonography over CT scanning include portability, no radiation exposure, and expense.

Ultrasound is the only modality of the above that can be used by clinicians. Early reports have revealed promising results and are comparable to CT. With its high availability, lower infection risk, and rapid sterilization, ultrasound may become the fundamental imaging tool for COVID-19 lung injury. Lung ultrasonography training programs are needed to provide clinicians with the skill to implement this technique better [59].

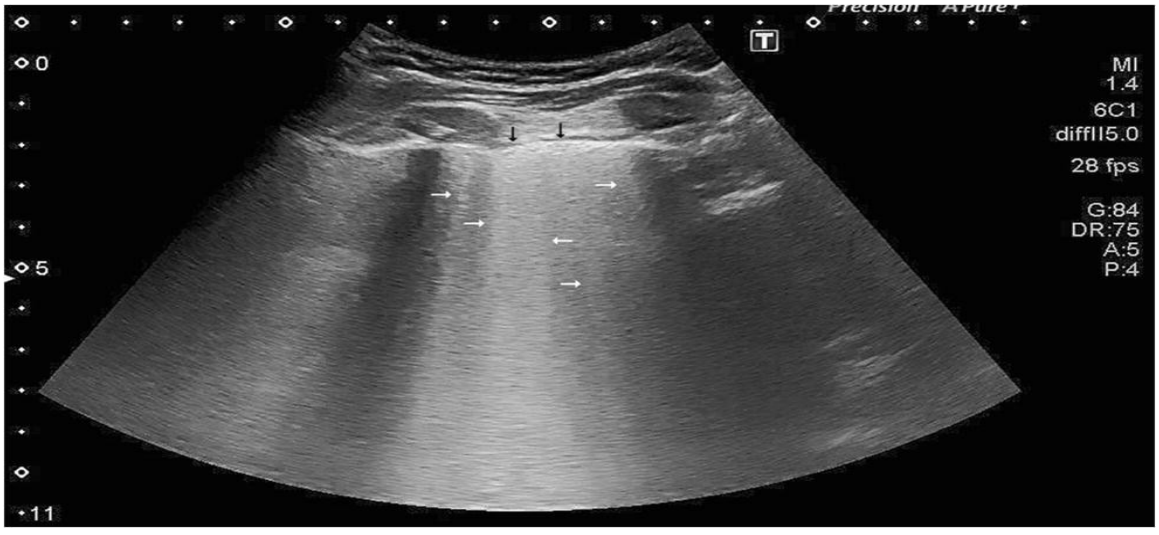


Figure (7): Coalescent B-Lines in a COVID-19 patient: Hyperechoic artifacts (horizontal arrows) arising from the pleural line (black arrows) and extending vertically (regarding the screen) to the bottom of the image, moving with the cycle of respiration. Any horizontal artifacts below the pleura that are usually seen in the healthy lung and represent reverberations of the pleural line (A-Lines) are obliterated by B-Lines, and are absent here [60]

2.7.4.Microbiology

Like other viral respiratory infections, a polymerase chain reaction (PCR) can be obtained from the nasopharyngeal's swap. As with other nasopharyngeal swabs, the results are operator dependent, and obtaining a deep sample is essential.

Several organizations, including the American Association for Bronchoscopy and Interventional Pulmonology, advised against inducing sputum production or bronchoscopy in these infectious patients because of the increased risk of aerosolization [(61)].

2.8.Treatment of lung injury in COVID-19

Like other respiratory viral lung infections, unfortunately, there is no established treatment for COVID-19 lung injury. The initial strategy in the management of this pandemic is advocating personal protection and social distancing. For patients who have been infected already, appropriate triage and supportive care are of great importance. Finally, in very ill patients, the investigation of several experimental therapies is underway.

2.8.1.Oxygen delivery devices

Although no randomized control trial has been done yet, the current paradigm is to support hypoxia up to a level of 92-96% saturation. Several options for oxygen delivery are available. These can include a nasal cannula, which can provide up to 6 L oxygen or approximately 44% FiO2. Further oxygen demand can be supplied by a nonrebreather mask, increasing oxygen flow to 6-10 L while providing almost 100% FiO2.

At this time, the use of noninvasive ventilation in COVID-19 patients is under strong debate. The high-flow nasal cannula (HFNC) and noninvasive positive-pressure ventilation (NIPPV) are currently the standards of care in intensive care for patients with hypoxic respiratory failure and are known to help avoid endotracheal intubation. However, in COVID-19 patients, intubation risk needs to be weighed against the potential risk of aerosolization of the virus particles and potentially increasing the danger to healthcare workers. Currently, no guidelines available to guide management in these cases; however, an initial report from Hong Kong advised against the use of HFNC and NIPPV in COVID-19 patients [62].

2.8.2.Mechanical ventilation

Endotracheal intubation of COVID-19 patients is considered a high-risk procedure. Care must be taken to decrease the aerosolization of the virus and protect the healthcare providers present. First, if possible, all endotracheal intubations should be conducted in negative-pressure rooms. An attempt should be made to decrease bag-mask ventilation, and intubation should be performed by an experienced person using rapid sequence intubation to maximize first-pass intubation. Further, the balloon should be inflated as soon as possible after intubation to prevent the virus's further spread [62].

Like other causes of respiratory failure and ARDS, patients who have intubated secondary to COVID-19 should be managed with lung-protective ventilation with a tidal volume of 6 mL/kg of ideal body weight and maintaining the peek pressure under 30 cm H20. [63] As with ARDS from other etiology, the respiratory rate is then increased to maintain the required minute ventilation. Some groups have also suggested using other less commonly used mechanical ventilation modes like airway pressure release ventilation.

Furthermore, although no specific evidence available, higher positive end-expiratory pressure, the adoption of prone positioning, neuromuscular blockade use, inhaled vasodilators, and maintaining a net negative fluid balance of 0.5-1 L/day might improve respiratory failure [64].

2.8.3.Extracorporeal membrane oxygenation

Extracorporeal membrane oxygenation (ECMO) has a controversial role in ARDS treatment, but there appears to be useful in patients with refractory hypoxemia. At present, there is no substantial evidence to guide the use of ECMO in patients with COVID-19. The use of ECMO treatment is very staff-intensive and has the risk of potentially exposing multiple members of the treating team to SARS-CoV-2 [65]

2.8.4.Antibiotics

Although there is no fundamental role for antibiotics in the treatment of coronavirus infection, 58% of Wuhan patients were started on antibiotics. The use of azithromycin combined with hydroxychloroquine has been described in an open-label trial [39].

2.8.5.Hydroxychloroquine and chloroquine

Antimalarial drugs have been used for years for malaria treatment and prophylaxis, in addition to autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Both have shown particular efficacy against some viruses such as HIV, Zika virus, and even SARS-CoV. Based on this evidence, hydroxychloroquine and chloroquine have been used in COVID-19 infection [66].

Chloroquine was shown to decrease viral replication in vitro as well as block infection by increasing the endosomal pH. It also blocks glycosylation of the cellular receptor of COVID-19[67].

2.8.6.Remdesivir and lopinavir-ritonavir

Remdesivir, a nucleotide analog, has proven particular efficacy against SARS-CoV-2 in vitro. At the current time, several randomized controlled trials are on their way evaluating the therapeutic use of Remdesivir in COVID-19 patients. Adverse effects include nausea, vomiting, and elevated liver enzymes.

Lopinavir, an antiviral drug used in the treatment of HIV infection, was initially shown to have in vitro antiviral activity against SARS in 2003. Ritonavir is added to lopinavir to increase the plasma half-life through the inhibition of cytochrome P-450. Despite initial enthusiasm, a randomized control trial failed to show a mortality benefit [67].

2.8.7. IL-6 inhibitor

Tocilizumab, a recombinant monoclonal antibody designed against the IL-6 receptor, is initially used in rheumatoid arthritis therapy. It was approved in the year 2017 for the treatment of cytokine storm syndrome in patients receiving chimeric antigen receptor-T (CAR-T) cell therapy. As mentioned previously, patients with COVID-19 may develop an exaggerated inflammatory condition that may respond to the inhibition of IL-6–dependent inflammatory pathways. A retrospective study checking the efficacy of tocilizumab in the management of severe COVID-19 is on its way and yet to be published, but it has shown promising results. A phase III clinical trial was also approved by the US Food and Drug Administration [66].

2.8.8.Convalescent plasma

The therapeutic use of plasma from individuals who have recovered recently from COVID-19 infection can provide the patient with passive immunity by the transfer of antibodies.

2.8.9.Corticosteroids

Based on clinical data from SARS-CoV (2002-2003), MERS, influenza, and respiratory syncytial virus, the WHO released a recommendation in January 2020 against the routine use of corticosteroids in managing COVID-19. However, as with ARDS management, there is a possible role for corticosteroid treatment as it suppresses tissue inflammation in the lungs but increases the risk of delaying clearance of COVID-19 [66].

2.8.10.Lung Transplant

When there is complete lung parenchymal damage, lung transplants have been reported and may be a therapeutic option in COVID-19 lung injury [68,69]

2.9. References

1- Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020; 382(8):727-733 (ISSN: 1533-4406).

2- Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. J Virol. 2020; 94(7) (ISSN: 1098-5514).

3- Clinical Chemistry and Laboratory Medicine (CCLM) 58, 9; 10.1515/cclm-2020-0727.

4- Canna SW; Behrens EM. Making sense of the cytokine storm: a conceptual framework for understanding, diagnosing, and treating hemophagocytic syndromes. Pediatr Clin North Am. 2012; 59(2):329-44 (ISSN: 1557-8240).

5- Severe Acute Lung Injury Related to COVID-19 Infection: A Review and the Possible Role for Escin. Luca Gallelli, Leiming Zhang, Tian Wang, MD, and Fenghua Fu. J Clin Pharmacol. 2020 May 22: 10.1002/jcph.1644. DOI: 10.1002/jcph.1644

6- Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020; 395(10229):1033-1034 (ISSN: 1474-547X).

7- Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of 150 patients from Wuhan, China. Intensive Care Med. 2020; 46(5):846-848 (ISSN: 1432-1238).

8- UR A.; Verma K. (2020) Pulmonary Edema in COVID19-A Neural Hypothesis. ACS Chem. Neurosci. 11, 2048.10.1021.

9- UR A.; Verma K. (2020) Happy Hypoxemia in COVID 19- A Neural Hypothesis. ACS Chem. Neurosci. 11 (13), 1865–1867. 10.1021.

10- Paterson R. W.; Brown R. L.; Benjamin L. (2020) The emerging spectrum of COVID-19 neurology: clinical, radiological, and laboratory findings. Brain 10.1093/brain/awaa240.

11- Varga Z. Flammer A. Steiger P. Haberecker M. Andermatt R. Zinkernagel A. Mehra M. Schuepbach R. Ruschitzka F.Moch H. Endothelial cell infection and endotheliitis in COVID-19. Lancet 395 (10234), 1417–1418. 10.1016.

12- Von Weyhern C. H. Kaufmann I. Neff F. Kremer M. Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. Lancet 395, e109.10.1016/S0140-6736(20)31282-4.

13- Khademi S. Frye M. A. Jeckel K. M. Hypoxia mediated pulmonary edema: potential influence of oxidative stress, sympathetic activation, and cerebral blood flow. BMC Physiol. 15, 4.10.1186/s12899-015-0018-4.

14- Bao C. Liu X. Zhang H. Li Y. Liu J.Coronavirus Disease 2019 (COVID-19) CT Findings: A Systematic Review and Meta-analysis. J. Am. Coll Radiol. 17, 701–709. 10.1016/j.jacr.2020.03.006.

15- Anat Ilivitzki, Bar Rinnot, and Luda Glozman. Imaging Manifestations of Lung Injury During the COVID-19 Outbreak: What Have We Learned? Rambam Maimonides Med J. 2020 Jul; 11(3): e0024.

16- Kannan S, Ali PSS, Sheeza A, Hemalatha K. COVID-19 (novel coronavirus 2019) - recent trends. Eur Rev Med Pharmacol Sci. 2020;24:2006–11.

17- Shi H. Han X. Jiang N. Cao Y. Alwalid O. Gu J. Fan Y. Zheng C. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect. Dis. 20, 425–34. 10.1016/S1473-3099(20)30086-4.

18- Xu X.; Yu C.; Qu J.; Jiang S.; Huang D.; Chen B.; Zhang Z.; Guan W.; Ling Z.; Jiang R.; Hu T.; Ding Y.; Lin L.; Gan Q.; Luo L.; Tang X.; Liu J. (2020) Imaging and clinical features of patients with 2019 novel coronavirus SARS-

CoV-2. Eur. J. Nucl. Med. Mol. Imaging 47, 1275–80. 10.1007/s00259-020-04735-9.

19- Cytokine Storm in COVID-19: "When You Come Out of the Storm, You Won't Be the Same Person Who Walked in." Vanessa CastelliAnnamaria CiminiClaudio FerriClaudio Ferri. September 2020Frontiers in Immunology 11:2132 DOI: 10.3389/fimmu.2020.02132

20- Srinivas L, Vellichirammal NN, Alex AM, Nair C, Nair IV, Banerjee M. Pro-inflammatory cytokines and their epistatic interactions in genetic susceptibility to schizophrenia. J Neuroinflammation. (2016) 13:105. DOI: 10.1186/s12974-016-0569-8

21- Debnath M, Banerjee M, Berk M. Genetic gateways to COVID□19 infection: implications for risk, severity, and outcomes. FASEB, J. (2020) 34:8787–95. DOI: 10.1096/fj.202001115R.

22- Shea MK, Benjamin EJ, Dupuis J, Massaro JM, Jacques PF, D'Agostino RB, et al. Genetic and non-genetic correlates of vitamins K and D. Eur J Clin Nutr. (2009) 63:458–64. DOI: 10.1038/sj.ejcn.1602959

23- Lips P, Cashman KD, Lamberg-Allardt C, Bischoff-Ferrari HA, Obermayer-Pietsch B, Bianchi ML, et al. Current vitamin D status in European and Middle East countries and strategies to prevent vitamin D deficiency: a position statement of the European calcified tissue society. Eur J Endocrinol. (2019) 180:23–54. DOI: 10.1530/EJE-18-0736

24- Rondanelli M, Miccono A, Lamborghini S, Avanzato I, Riva A, Allegrini P, et al. Self-care for common colds: the pivotal role of vitamin D, vitamin C, zinc, and Echinacea in three main immune interactive clusters (Physical Barriers, Innate and Adaptive Immunity) involved during an episode of common colds—practical advice on dosages and on time to take these Nutrients/Botanicals in order to prevent or treat common colds. Evid Based

Complement Alternat Med. (2018) 2018:1–36. doi: 10.1155/2018/5813095

25- Rossi GA, Fanous H, Colin AA. Viral strategies predisposing to respiratory bacterial superinfections. Pediatr Pulmonol. (2020) 55:1061–73. doi: 10.1002/ppul.24699

26- Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res. (2011) 55:96–108. DOI: 10.1002/mnfr.201000174

27- Grant WB, Lahore H, McDonnell SL, Baggerly CA, French CB, Aliano JL, et al. Evidence that vitamin D supplementation could reduce risk of influenza and COVID-19 infections and deaths. Nutrients. (2020) 12:988. DOI: 10.3390/nu12040988

28- Sallenave J-M, Guillot L. Innate immune signaling and proteolytic pathways in the resolution or exacerbation of SARS-CoV-2 in Covid-19: key therapeutic targets? Front Immunol. (2020) 11:1229. DOI: 10.3389/fimmu.2020.01229

29- Stoermer KA, Morrison TE. Complement and viral pathogenesis. Virology. (2011) 411:362–73. doi: 10.1016/j.virol.2010.12.045

30- Gralinski LE, Sheahan TP, Morrison TE, Menachery VD, Jensen K, Leist SR, et al. Complement activation contributes to severe acute respiratory syndrome Coronavirus pathogenesis. mBio. (2018) 9:e01753-18. DOI: 10.1128/
mBio.01753-18

31- Gao T, Hu M, Zhang X, Li H, Zhu L, Liu H, et al. Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement over-activation. Infect Dis (except HIV/AIDS). (, 2020). DOI: 10.1101/2020.03.29.20041962 [Epub ahead of print].

32- Beasley M.B.The pathologist's approach to acute lung injury.Arch Pathol Lab Med. 2010; 134: 719-727

33- Greene R.Lind S.Jantsch H.et al. Pulmonary vascular obstruction in severe ARDS: angiographic alterations after i.v. Fibrinolytic therapy.Am J Roentgenol. 1987; 148: 501-508

34- Lida P. Hariri, Crystal M. North, Angela R. Shih, C. Corey Hardin, James R. Stone, Mari Mino-Kenudson Lung Histopathology in Coronavirus Disease 2019 as Compared With Severe Acute Respiratory Syndrome and H1N1 Influenza A Systematic Review. Chest infections: original research. articles in press

35- Copin M.-C. Parmentier E. Duburcq T. Poissy J. Mathieu D.Time to consider a histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. Intensive Care Med. 2020; 46: 1124-1126

36- Schaller T.Hirschbuhl K. Burkhardt K. et al. Postmortem examination of patients with COVID-19. JAMA. 2020; 323: 2518-2520

37- Pernazza A. Mancini M. Rullo E. et al. Early histologic findings of pulmonary SARS-CoV-2 infection detected in a surgical specimen. Virchows Arch. 2020; 477: 743-748

38- Tian S. Hu W. Niu L. Liu H. Xu H. Xiao S.Y. Pulmonary pathology of early-phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. J Thorac Oncol. 2020; 15: 700-704

39- Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020; 382(18):1708-1720 (ISSN: 1533-4406).

40- Ferguson ND, Fan E, Camporota L, Antonelli M, Anzueto A, Beale R, et al. The Berlin definition of ARDS: an expanded rationale, justification, and

supplementary material. Intensive Care Med. 2012; 38(10):1573-82 (ISSN: 1432-1238).

41- Gattinoni L, Coppola S, Cressoni M, Busana M, Rossi S, Chiumello D. COVID-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med. 2020; 201(10):1299-1300 (ISSN: 1535-4970).

42- Abdulrahman Alharthy, Fahad Faqihi, Ziad A. Memish, and Dimitrios Karakitsos. Lung Injury in COVID-19—An Emerging Hypothesis. ACS Chem Neurosci. 2020 Aug 5; 11(15).

43- Anat Ilivitzki, Bar Rinnot, and Luda Glozman. Imaging Manifestations of Lung Injury During the COVID-19 Outbreak: What Have We Learned? Rambam Maimonides Med J. 2020 Jul; 11(3): e0024.

44- Adam Jacobi, Michael Chung, Adam Bernheim, CoreyEber. Portable chest X-ray in coronavirus disease-19 (COVID-19): A pictorial review. Clinical Imaging

Volume 64, August 2020, Pages 35-42.

45- Zeno Falaschi, Pietro Danna, Roberto Arioli, Alessio Pasché, Domenico Zagaria,Ilaria Percivale, Stefano Tricca, Michela Barini, Ferruccio Aquilini, Stefano Andreoni, and Alessandro Carrieroa. Chest CT accuracy in diagnosing COVID-19 during the peak of the Italian epidemic: A retrospective correlation with RT-PCR testing and analysis of discordant cases. Eur J Radiol. 2020 Sep; 130: 109192.

46- Bernheim A, Mei X, Huang M, Yang Y, Fayad ZA, Zhang N, Diao K, Lin B, Zhu X, Li K, Li S, Shan H, Jacobi A, Chung M. Chest CT Findings in Coronavirus Disease-19 (COVID-19): Relationship to Duration of Infection. Radiology. 2020 Jun; 295(3):200463.

47- Lomoro P, Verde F, Zerboni F, Simonetti I, Borghi C, Fachinetti C, Natalizi A, Martegani A. COVID-19 pneumonia manifestations at the admission on chest ultrasound, radiographs, and CT: single-center study and comprehensive radiologic literature review. Eur J Radiol Open. 2020; 7:100231.

48- Ai T, Yang Z, Hou H, Zhan C; Chen C; Lv W et al. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology. 2020; 296(2):E32-E40 (ISSN: 1527-1315).

49- Koo HJ, Lim S, Choe J, Choi S-H, Sung H, Do KH. Radiographic and CT features of viral pneumonia. Radiographics. 2018;38:719–39. DOI: 10.1148/rg.2018170048.

50- Obadina ET, Torrealba JM, Kanne JP. Acute pulmonary injury: high-resolution CT and histopathological spectrum. Br J Radiol. 2013;86:20120614. doi: 10.1259/bjr.20120614. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

51- Bai HX, Hsieh B, Xiong Z, et al. Performance of radiologists in differentiating COVID-19 from viral pneumonia on chest CT. Radiology. 2020 Mar 10;:200823. DOI: 10.1148/Radiol.2020200823.

52- Mayo PH, Copetti R, Feller-Kopman D, et al. Thoracic ultrasonography: a narrative review. Intensive Care Med. 2019;45:1200–11. DOI: 10.1007/s00134-019-05725-8.

53- Buonsenso D, Piano A, Raffaelli F, Bonadia N, de Gaetano Donati K, Franceschi F. Point-of-care lung ultrasound findings in novel coronavirus disease-19 pneumonia: a case report and potential applications during COVID-19 outbreak. Eur Rev Med Pharmacol Sci. 2020;24:2776–80. DOI: 10.26355/eurrev_202003_20549.

54- Peng QY, Wang XT, Zhang LN Chinese Critical Care Ultrasound Study Group (CCUSG) Findings of lung ultrasonography of novel coronavirus pneumonia

during the 2019–2020 epidemic. Intensive Care Med. 2020;46:849–50. DOI: 10.1007/s00134-020-05996-6.

55- Expert Round Table on Ultrasound in ICU. International expert statement on training standards for critical care ultrasonography. Intensive Care Med. 2011;37:1077–83. DOI: 10.1007/s00134-011-2246-9.

56- Mojoli F, Bouhemad B, Mongodi S, Lichtenstein D. Lung ultrasound for critically ill patients. Am J Respir Crit Care Med. 2019;199:701–14. doi: 10.1164/rccm.201802-0236ci.

57- Poggiali E, Dacrema A, Bastoni D, et al. Can lung US help critical care clinicians in the early diagnosis of novel coronavirus (COVID-19) pneumonia? Radiology. 2020;295:E6. DOI: 10.1148/Radiol.2020200847.

58- Yang Y, Huang Y, Gao F, Yuan L, Wang Z. Lung ultrasonography versus chest CT in COVID-19 pneumonia: a two-centered retrospective comparison study from China. Intensive Care Med. 2020 May 25; 1–3. DOI: 10.1007/s00134-020-06096-1.

59- Peng QY, Wang XT, Zhang LN. Findings of lung ultrasonography of novel coronavirus pneumonia during the 2019-2020 epidemic. Intensive Care Med. 2020; 46(5):849-850 (ISSN: 1432-1238).

60- Daniel T. Marggrander, Frauke Borgans, Volkmar Jacobi, Holger Neb & Timo Wolf. Lung Ultrasound Findings in Patients with COVID-19. SN Comprehensive Clinical Medicine volume 2, pages2151–2157(2020)

61- Wahidi MM, Lamb C, Murgu S, Musani A, Shojaee S, Sachdeva A, et al. American Association for Bronchology and Interventional Pulmonology (AABIP) Statement on the Use of Bronchoscopy and Respiratory Specimen Collection in Patients With Suspected or Confirmed COVID-19 Infection. J Bronchology Interv Pulmonol. 2020; 27(4):e52-e54 (ISSN: 1948-8270).

62- Cheung JC, Ho LT, Cheng JV, Cham EYK, Lam KN. Staff safety during emergency airway management for COVID-19 in Hong Kong. Lancet Respir Med. 2020; 8(4):e19 (ISSN: 2213-2619).

63- Brower RG; Matthay MA; Morris A; Schoenfeld D; Thompson BT; Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000; 342(18):1301-8 (ISSN: 0028-4793).

64- Matthay MA; Aldrich JM; Gotts JE. Treatment for severe acute respiratory distress syndrome from COVID-19. Lancet Respir Med. 2020; 8(5):433-434 (ISSN: 2213-2619).

65- Combes A, Hajage D, Capellier G, Demoule A, Lavoué S, Guervilly C, et al.; Extracorporeal Membrane Oxygenation for Severe Acute Respiratory Distress Syndrome. N Engl J Med. 2018; 378(21):1965-1975 (ISSN: 1533-4406).

66- Zhang W; Zhao Y; Zhang F; Wang Q; Li T; Liu Z; Wang J; Qin Y; Zhang X; Yan X; Zeng X; Zhang S. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. Clin Immunol. 2020; 214:108393 (ISSN: 1521-7035).

67- Wang M; Cao R; Zhang L; Yang X; Liu J; Xu M; Shi Z; Hu Z; Zhong W; Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020; 30(3):269-271 (ISSN: 1748-7838).

68- Fei Li, Jie Cai, and Niangua Dong. First cases of COVID-19 in heart transplantation from China. The journal of heart and lung transplant.volume 39, issue 5, p496-497, May 01, 2020.

69- Christian Lang, Peter Jaksch, Mir Alireza Hoda, György Lang, Thomas Staudinger, Edda Tschernko, et al. Lung transplantation for COVID-19-

associated acute respiratory distress syndrome in a PCR-positive patient. Lancet respiratory medicine. case report| volume 8, issue 10, p1057-1060, October 01, 2020

Chapter three:

Dermatologic Manifestations of Covid-19

3.Introduction

Recently, there has been increasing recognition of the dermatologic complications of COVID-19. Cutaneous manifestations are well known to occur in the setting of viral illnesses, and occasionally these manifestations have diagnostic or prognostic value. While much of the focus of Covid-19 has been on the cardiac and pulmonary complications, it is important to be aware of the dermatologic manifestations and skin complications of COVID-19. Knowledge of the components is important to help identify potential COVID-19 patients and properly treat complications.

The cutaneous manifestations most often recorded are morbilliform rash, urticaria, vesicular eruptions, acral lesions, and livedoid eruptions. Some of these skin features arise before the signs and symptoms more commonly associated with COVID-19, suggesting that dermatologic manifestations could be presenting signs of COVID-19. These rashes should trigger consideration of COVID-19, and understanding these manifestations is important to help identify potential COVID-19 patients and properly treat complications.

There are several proposed etiologies for rash in patients with COVID-19. The first is diffuse microvascular vasculitis, resulting from complement system activation. Significant complement protein deposition has been found in the dermal capillaries, as well as interstitial and perivascular neutrophilia with prominent leukocytoclasia, suggesting a vasculitic phenomenon [(1)].

Others have suggested that this occurs as a direct effect of the virus. This has been based on high concentrations of lymphocytes without eosinophils, papillary dermal edema, epidermal spongiosis, and lymphohistiocytic infiltrates [(2,3)]. A rash associated with COVID-19 can involve various body regions, most commonly the

trunk, but extremity involvement may also occur [4]. Pruritus is often minimal but depends on the type of rash, and lesions typically heal quickly, appearing within 3 days and disappearing within 8 days [4, 5].

A challenging aspect of rash associated with COVID-19 is the myriad types of presentation. Many of these rashes have a broad differential diagnosis. However, it is important to consider COVID-19, especially in the patient with upper respiratory or systemic symptoms. Importantly, an individual patient may present with multiple simultaneous cutaneous abnormalities that differ in morphology.

Case series from around the world have identified a range of potential dermatologic manifestations of COVID-19 [6-9]. The frequency (ranging from 0.2 to 20.4 percent of cases) and timing of cutaneous manifestations of COVID-19 are difficult to ascertain [10-12]. Also unclear is the association of certain skin manifestations with the illness severity [13]. Moreover, it cannot be excluded that in some patients the observed skin findings may represent cutaneous reactions to the numerous treatments used for COVID-19 [13, 14].

3.1. Maculopapular (morbilliform) rash

A morbilliform rash predominantly involving the trunk has been reported as the most common cutaneous manifestation of COVID-19. The rash has been noted either at the disease onset or, more frequently, after hospital discharge or recovery. A morbilliform rash is a common morphology seen with viral exanthemas. There are multiple reports of patients presenting with a maculopapular rash, characterized by erythematous macules covered with small papules, or with large plaques [3, 15-17].

The rash may also be perifollicular and associated with scaling and confluence, which may cause it to be mistaken for pityriasis rosea. This type of rash has been suggested to have a mean duration of approximately 9 days [18]. One study of 88 patients in Italy found that a maculopapular rash was present in 14 patients

(16%) [4].

There are several descriptions of the rash in the literature that have identified it most commonly on the limbs and trunk [19-22], the face [23], bilateral heels [24], or as centrifugal in nature, initially starting in the periumbilical or trunk region before spreading distally [3, 21]. Some infants born to mothers with COVID-19 at birth have had transient diffuse, maculopapular eruption that resolved in one day with desquamation, and in others as a diffuse, red, miliaria-like eruption that disappeared within few days without treatment [18, 21].

3.2.Urticaria

Urticaria presents with acute, swollen, red wheals or plaques, typically associated with pruritus. Urticarial eruptions are emerging as a potential COVID-19 skin manifestation, and acute urticaria with or without concomitant fever has been reported as a presenting sign of COVID-19 infection. The temporal onset of urticaria before the more well-known symptoms develop raises the possibility that cutaneous eruptions can be a presenting sign of COVID-19. There have been reports of urticaria affecting various regions of the body in patients of all age groups infected with COVID-19, and describing involvement of the trunk, extremities, and head, as well as rash migration, with sparing of the palms and soles [15, 21-29].

One of the largest series of COVID-19 positive patients with urticaria found that the trunk was most commonly involved, and pruritus occurred in 92% of cases [18]. The mean duration of symptoms was 6.8 days. This study also found that urticaria generally occurred concomitantly with other symptoms in the majority of cases and was associated with more severe disease in this study, with a 2% mortality rate in this population [22-25].

3.3.Vesicular eruption

Varicella-like vesicular eruptions have been described in COVID-19 patients. Vesicular rashes are small, fluid-filled blisters, often on an erythematous base. Numerous case reports have documented vesicular rashes in patients with COVID-19, in 1.1% of patients [4]. Vesicles are more commonly scattered, rather than diffuse in appearance, with one series finding scattered lesions in 16 of 22 patients and diffuse lesions in the remaining six patients [5].

The vesicular rash occurs for a mean duration of 10.4 days, with vesicles appearing mostly on the trunk and extremities. The lesions appear as small and monomorphic as opposed to chickenpox and had hemorrhagic content. In most cases, the vesicular rash precedes other symptoms [18]. Histopathologic features include interface dermatitis with apoptotic keratinocytes, which is similar to findings in many other viral exanthemas [20, 28].

3.4.Petichiae/Purpura

Petichiae are small, subdermal hemorrhages, while purpura are larger variants of this. This rash is less commonly described than some of the other rashes, though there are a few case reports describing this in the literature. One case report described a patient with petichiae who was initially misdiagnosed as dengue fever (in an endemic area), but later discovered to have COVID-19. In this case, the patient was also noted to be significantly thrombocytopenic [30]. Another case described a patient with extensive purpura isolated to flexural areas [31]. Thrombocytopenia is usually not a common complication in COVID-19, so this rash may reflect a less common complication, or the rash may be due to an alternate etiology such as vasculitis.

3.5. Chilblains (Covid Toes)

Acral pernio-like lesions have been reported in patients with COVID-19, and

they may take different forms. Chilblains (also known as pernio or perniosis) is an abnormal response to cold, wherein distal arteries and veins constrict, which can lead to pruritic and tender wounds on the extremities. These lesions have been described across the age spectrum in patients with confirmed or suspected COVID-19, in the absence of cold exposure or underlying conditions associated with pernio. Resolution may occur in two to eight weeks with a mean duration of 12.7 days [18].

Patients can present with erythematous or violaceous papules and macules, bullae, or digital swelling [32]. It typically involves the hands or feet and usually asymmetrical, 32% of cases could be painful and 30% is associated with pruritus. Compared with other rashes, chilblains typically occurred later in the disease course and after other symptoms had presented. Younger patients are more commonly affected with a mean age 32 years [18].

The demonstration by immunohistochemistry and electron microscopy of SARS-CoV-2 in endothelial cells of lesional skin biopsies suggests a virus-induced, vascular injury as a potential pathogenic mechanism [18, 20]. It may be prudent that patients presenting with new-onset, pernio-like lesions that have no other clear cause be PCR tested for SARS-CoV-2. The development of pernio-like lesions in COVID-19 may be associated with a relatively mild disease course, and pernio-like lesions may represent a post viral or delayed-onset process.

3.6. Livedo eruptions

Livedo reticularis-like vascular lesions have been reported in a few patients and seems to be associated with severe cases of COVID-19. Livedo racemosa is a violaceous web or net-like patterning of the skin found more diffusely, compared to livedo reticularis that is usually found in gravity-dependent areas [33].

The rash has a mean duration of 9.4 days. Livedo racemosa is more common in older patients, with a mean age of 63 years. Livedo racemosa is also associated

with more severe disease with 10% mortality rate [18]. Of potential importance is that these lesions are thought to be secondary to COVID- 19-induced thrombotic vasculopathy. Livedoid eruptions are eventually noted to occur in COVID-19 patients with systemic thrombotic vasculopathy, so it will be particularly important to recognize these eruptions clinically, as they may have important prognostic value in these patients.

3.7.Distal ischemia and necrosis

Perhaps one of the most severe complications includes distal ischemia resulting in tissue necrosis. One case series described seven patients with acro-ischemia including finger and toe cyanosis, skin bullae, and dry gangrene [34]. Another report of two patients described the appearance of red and purple papules on the distal fingers due to distal ischemia, which occurred before the appearance of other symptoms [35]. Other case reports describe a 13-year-old with distal toe ischemia presenting with blistering and necrosis [36], as well as one patient with necrotic purpura [15]. Given the coagulopathy impact of coronavirus, these findings may necessitate consideration of intravenous thrombolytic therapy.

3.8.Multisystem inflammatory syndrome in children (MISC)

An erythematous, polymorphic rash, erythema and/or firm induration of hands and feet, oral mucositis, and conjunctivitis, along with systemic, laboratory, and imaging findings of atypical, severe Kawasaki disease, have been described Italian children during the COVID-19 pandemic. Similar cases have been reported in the United Kingdom, the United States, and other countries [36]. Case definitions for MISC have been proposed by the World Health Organization and the United States Centers for Disease Control and Prevention (CDC).

3.9.Miscellaneous cutaneous eruptions

There have been several reports of COVID-19 patients presenting with unusual cutaneous eruptions. A 64-year-old woman in France with COVID-19 developed a rash consistent with Symmetrical Drug-Related Intertriginous and Flexural Exanthema (SDRIFE) 4 days after she became febrile was recently reported [37]. Although SDRIFE is usually a medication-induced eruption, the authors were unable to attribute this case to a culprit medication. Additionally, Joob and colleagues [38] reported a petechial rash in a COVID-19 patient that mimicked the cutaneous eruption seen in Dengue fever. Less frequently reported dermatologic manifestations include papulosquamous eruptions, erythema multiforme-like lesions, dengue-like rashes, petechiae, and gangrene.

3.10.Dermatologic Conditions Related to Covid-19 Pandemic

Skin injury, mechanical/friction dermatitis, and irritant contact dermatitis due to personal protective equipment (PPE) and hand hygiene measures have been reported in the majority of health care workers involved in the direct care of patients with COVID-19.

3.11.Personal protective equipment-induced skin injury:

Health care workers caring for COVID-19 patients or patients potentially infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may spend long hours wearing PPE, cause various occupationally induced dermatologic conditions during the COVID-19 pandemic. PPE-induced skin injury is common, occurring in 43 to 97 percent of Chinese health care workers. Long durations of PPE use (>6 hours per day) increase rates of skin damage. Masks, goggles, face shields, and gloves apply pressure, create abrasion, and retain moisture, and can injure the

nasal bridge, cheek, forehead, and hands.

PPE-induced injuries include desquamation, erythema, maceration, fissuring, papules, and erosions, leading to itching and pain. PPE use can also aggravate underlying skin conditions. Prevention of PPE-related injuries has the potential to reduce PPE protocol breaches due to inadvertent adjustment and touching. The use of barrier films or dressings at pressure points before donning PPE may reduce these types of injuries. However, the effects of these preventive measures on PPE ability to prevent viral spread are not well characterized, and caution is warranted.

3.12.Hand hygiene-related dermatitis

Hand hygiene is considered a key tool against COVID-19. Hand eczema was already an issue among health care workers and is likely to be an even greater problem with higher rates of handwashing and glove use during the pandemic. The frequency of irritant contact dermatitis of the hands may be reduced by frequent usage of emollients, washing with lukewarm water instead of hot water, and usage of alcohol-based cleansers when hands are not visibly dirty. Overzealous hand hygiene may cause hand eczema in the general population as well.

3.13.Prospects

We still have a tremendous amount to learn about the cutaneous manifestations of Covid-19 disease, and there are currently more questions than answers. For one, it is still unclear what percentage of COVID-19 patients develop cutaneous eruptions. Additionally, many of these patients are critically ill and have received numerous medications to help them survive their disease. Thus it can be challenging to determine when the cause of cutaneous eruptions is medication-induced as opposed to truly being COVID-19 manifestations. For example, acral purpura can arise in the setting of treatment with vasopressors, and morbilliform eruptions are common manifestations of adverse reactions to drugs.

Several of the medications most actively being studied and used to treat a patient with COVID-19 are also known to cause various cutaneous eruptions. Furthermore, as many viral illnesses have associated exanthemas and only a few COVID-19 patients with cutaneous abnormalities have been reported, it is not known if any of these are truly specific to infection with SARS-CoV-2. Many more cases are needed to resolve these dilemmas.

If there are specific COVID-19 cutaneous manifestations, it will be important to determine if any have clinical value. For example, are there specific early cutaneous abnormalities that may suggest a patient has been infected with SARS-CoV-2? If there are cutaneous manifestations that are pathognomonic for infection, these may be sufficient for diagnosis instead of testing in geographic areas where test availability is problematic. Also, are there cutaneous manifestations that can predict a more severe course and potentially encourage early aggressive intervention? Moreover, if effective medications emerge, are there certain cutaneous manifestations that may support specific treatment algorithms?

To answer such questions, it will be crucial to document the cutaneous abnormalities present at diagnosis and during COVID-19 in as many patients as possible. Although this can be challenging in face of an obvious need to urgently address more critical clinical abnormalities, doing so may eventually reveal clinical clues to help guide diagnosis and treatment. One possible protocol to collect cutaneous abnormality data could involve having nurses and other front-line healthcare workers photograph skin eruptions in COVID-19 patients when present. Dermatologists could examine these and collect clinical data virtually to optimize patient care and research efforts, which would also conserve personal protective equipment and minimize human exposure to SARS-CoV-2.

3.14. References

1. Magro C, Mulvey JJ, Berlin D, et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res. 2020. (https://doi.org/10.1016/j.trsl.2020.04.007).

2. Gianotti R, Veraldi S, Recalcati S, et al. Cutaneous clinicopathological findings in three COVID-19-positive patients observed in the metropolitan area of Milan, Italy. Acta Derm Venereol. 2020. (https://doi.org/10.2340/00015555-3490).

3. Sanchez A, Sohier P, Bingham S, et al. Digitate papulosquamous eruption associated with severe acute respiratory syndrome coronavirus 2 infection. JAMA Dermatol. 2020. (https://doi.org/10.1001/jamadermatol.2020.1704).

4. Recalcati S. Cutaneous manifestations in COVID-19: a first perspective. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16387).

5. Marzano AV, Genovese G, Fabbrocini G, et al. Varicella-like exanthem as a specific COVID-19-associated skin manifestation: multicenter case series of 22 patients. J Am Acad Dermatol. 2020. (https://doi.org/10.1016/j.jaad.2020.04.044).

6. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708–20.

7. Long B, Brady WJ, Koyfman A, Gottlieb M. Cardiovascular complications in COVID-19. Am J Emerg Med. 2020. (https://doi. org/10.1016/j.ajem.2020.04.048).

8. Bridwell R, Long B, Gottlieb M. Neurologic complications of COVID-19. Am J Emerg Med. 2020. (https://doi.org/10.1016/j. ajem.2020.05.024).

9. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in under- standing SARS pathogenesis. J Pathol. 2004;203(2):631–7 Jun.

10. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020;46(4):586–90.

11. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395:497–506.

12. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020; 395(10229):1054–62.

13. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020. (https://doi.org/10.1001/jama.2020.1585).

14. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty. 2020;9(1):45. (https:// doi.org/10.1186/s40249-020-00662-x).

15. Bouaziz JD, Duong T, Jachiet M, et al. Vascular skin symptoms in COVID-19: a french observational study. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/ jdv.16544)

16. Amatore F, Macagno N, Mailhe M, et al. SARS-CoV-2 infection presenting as a febrile rash. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16528).

17. Gisondi P, Piaserico S, Conti A, Naldi L. Dermatologists and SARS-CoV-2: the impact of the pandemic on daily practice. J Eur Acad Dermatol Venereol. 2020. (https://doi. org/10.1111/jdv.16515).

18. Galván Casas C, Català A, Carretero Hernández G, et al. Classification of the cutaneous manifestations of COVID-19: a rapid prospective nationwide consensus study in Spain with 375 cases. Br J Dermatol. 2020. (https://doi.org/10.1111/bjd.19163).

19. Ahouach B, Harant S, Ullmer A, et al. Cutaneous lesions in a patient with COVID-19: are they related? Br J Dermatol. 2020. (https://doi.org/10.1111/bjd.19168).

20. Mahé A, Birckel E, Krieger S, Merklen C, Bottlaender L. A distinctive skin rash associated with Coronavirus Disease 2019? J Eur Acad Dermatol

Venereol. 2020. (https:// doi.org/10.1111/jdv.16471).

21. Morey-Olivé M, Espiau M, Mercadal-Hally M, Lera-Carballo E, García-Patos V. Cutaneous manifestations in the current pandemic of coronavirus infection disease (COVID 2019). An Pediatr (Engl Ed). 2020. (https://doi.org/10.1016/j.anpede.2020.04.002).

22. Rivera-Oyola R, Koschitzky M, Printy R, et al. Dermatologic findings in two patients with COVID-19. JAAD Case Rep. 2020. (https://doi.org/10.1016/j.jdcr.2020.04.027).

23. Hedou M, Carsuzaa F, Chary E, Hainaut E, Cazenave-Roblot F, Masson Regnault M. Comment on "Cutaneous manifestations in COVID-19: a first perspective" by Recalcati S. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16519).

24. Estébanez A, Pérez-Santiago L, Silva E, Guillen-Climent S, García-Vázquez A, Ramón MD. Cutaneous manifestations in COVID-19: a new contribution. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16474).

25. Henry D, Ackerman M, Sancelme E, Finon A, Esteve E. Urticarial eruption in COVID- 19 infection. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16472).

26. Lu S, Lin J, Zhang Z, et al. Alert for non-respiratory symptoms of Coronavirus Disease 2019 (COVID-19) patients in the epidemic period: a case report of familial cluster with three asymptomatic COVID-19 patients. J

Med Virol. 2020. (https://doi.org/10.1002/ jmv.25776).

27. Fernandez-Nieto D, Ortega-Quijano D, Segurado-Miravalles G, Pindado-Ortega C, Prieto-Barrios M, Jimenez-Cauhe J. Comment on: cutaneous manifestations in COVID-19: a first perspective. Safety concerns of clinical images and skin biopsies. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16470).

28. Quintana-Castanedo L, Feito-Rodríguez M, Valero-López I, Chiloeches-Fernández C, Sendagorta-Cudós E, Herranz-Pinto P. Urticarial exanthem as early diagnostic clue for COVID-19 infection. JAAD Case Rep. 2020. (https://doi.org/10.1016/j.jdcr.2020.04.026).

29. Van Damme C, Berlingin E, Saussez S, Accaputo O. Acute urticaria with pyrexia as the first manifestations of a COVID-19 infection. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16523).

30. Joob B, Wiwanitkit V. COVID-19 can present with a rash and be mistaken for dengue. J Am Acad Dermatol. 2020;82(5): e177. (https://doi.org/10.1016/j.jaad.2020.03.036).

31. Jimenez-Cauhe J, Ortega-Quijano D, Prieto-Barrios M, Moreno-Arrones OM, Fernandez-Nieto D. Reply to "COVID-19 can present with a rash and be mistaken for Dengue": petechial rash in a patient with COVID-19 infection. J Am Acad Dermatol, 2020. (https://doi.org/10.1016/j.jaad.2020.04.016).

32. Recalcati S, Barbagallo T, Frasin LA, et al. Acral cutaneous lesions in the time of COVID-19. J Eur Acad Dermatol Venereol. 2020. (https://doi.org/10.1111/jdv.16533).

33. Uthman IW, Khamashta MA. Livedo racemosa: a striking dermatological sign for the antiphospholipid syndrome. J Rheumatol. 2006;33(12):2379–82.

34. Zhang Y, Cao W, Xiao M, et al. Clinical and coagulation characteristics of 7 patients with critical COVID-2019 pneumonia and acro-ischemia. Zhonghua Xue Ye Xue Za Zhi. 2020;41(0):E006. (https://doi.org/10.3760/cma.j.issn.0253-2727.2020.0006).

35. Alramthan A, Aldaraji W. A case of COVID-19 presenting in clinical picture resembling chilblains disease. First report from the Middle East. Clin Exp Dermatol. 2020. (https://doi.org/10.1111/ced.14243).

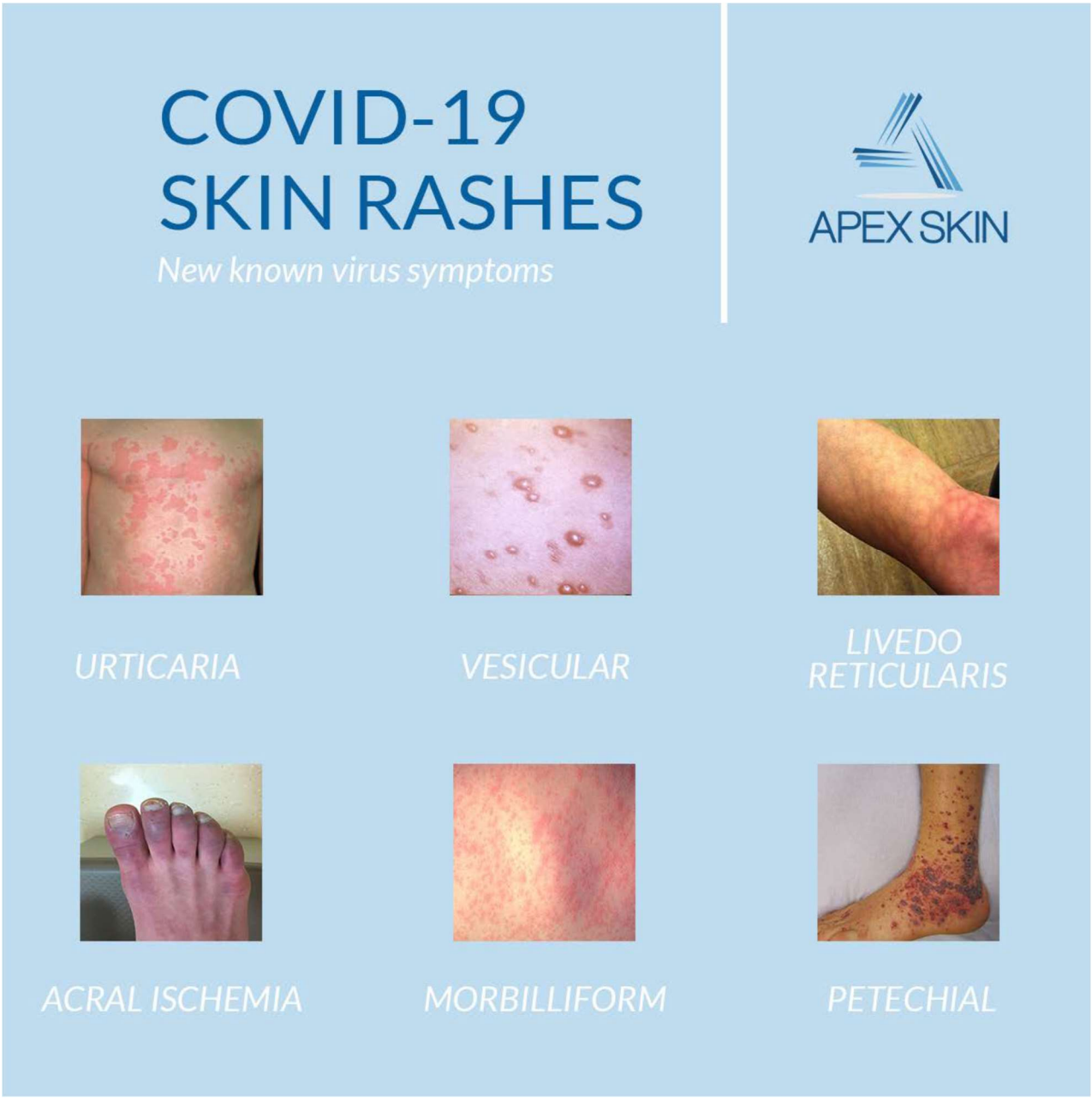
36. Mazzotta F, Troccoli T. Acute Acro-ischemia in the child at the time of COVID-19. Dermatologia Pediatrica. 2020. (https://www.ejpd.com/images/acroischemia-ENG.pdf).

37. Mahé A, Birckel E, Krieger S, Merklen C, Bottlaender L. A distinc- tive skin rash associated with coronavirus disease 2019? J Eur Acad Dermatol Venereol, 2020. (doi:10.1111/jdv.16471).

38. Joob B, Wiwanitkit V. COVID-19 can present with a rash and be mistaken for Dengue. J Am Acad Dermatol 2020 Mar 22. (doi:10.1016/j.jaad.2020.03.036).

39.Photo: Covid-19 Skin Rashes.
(www.apexskin.com20200422covid-19-skin-rashes-linked-to-virus).

40. Photo: Emerging Skin Manifestations of Covid-19.
(www.dawesfretzin.com20200427emerging-skin-manifestations-of-covid-19).



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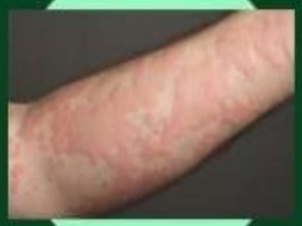
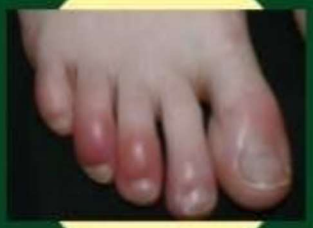


URTICARIA

Hives, commonly seen in viral rashes were reported in confirmed and suspected cases in Italy, France, Finland, Canada and US.

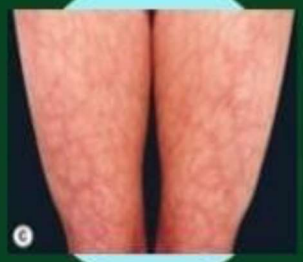


ACRAL ISCHEMIA

COVID-19 causes painful or itchy acral ischemic lesions, possibly from microthrombi, resembling perniosis.

MORBILLIFORM

Diffuse maculopapular eruption, as seen in Dengue, seen in COVID-19 patients in Italy, France and Finland

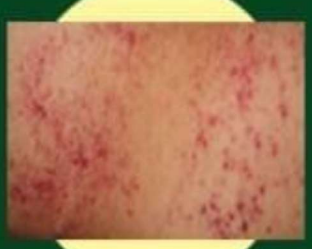


LIVEDO RETICULARIS

Transient blanching or mottling of skin from suspected ischemia of cutaneous blood vessels

VESICULAR

Chicken pox-like vesicles on erythematous base seen in COVID patients in Italy and US



PETECHIAL

Bleeding under the skin resulted in petechial eruption in COVID-19 confirmed patients in Italy and US

Chapter four: Treatment of COVID-19

4.1. Introduction

Viruses are at the top of major biological threats against humanity. Facing a viral pandemic like COVID-19 seems to be like a nightmare, when an aggressive, highly infective, rapidly –spreading and the untreatable new viral agent has attacked the world. We are facing an ambiguous enemy in a battle in which victory requires the best knowledge and the best prospective vision.

The global race for drug discovery started from the first outbreak in Wuhan, China, and has continued till now. Several therapeutic protocols were proposed and adopted including many drugs some of them were proved to be ineffective and some trials are continuing. Many pharmacological controlled randomized trials are being done in different parts of the world and some of their data has clarified the vision about the disease response to treatment.

Therapeutic drug trials of COVID-19 have not been confined to modern synthetic chemotherapeutics but have included some traditional medicines also that may possess potential efficacy (Dong, Hu & Gao, 2019).

Continuous revision of proposed guidelines has been taken place many times. Drug development and efficacy studies included non-stopping in-vitro studies, animal studies, and randomized clinical trials.

In this chapter, we will talk about the obstacles facing the development of radical treatment for COVID-19 disease, drug targets of the proposed drugs, drugs used in the treatment and evaluation of their efficacy, and common therapeutic protocols adopted by different institutions.

4.2. COVID-19 treatment dilemma

Viral diseases in general are difficult to treat. This is related to the hurdles facing antiviral drug design and discovery. The virus is a very delicate microorganism and can exploit the cellular organism to copy itself appears as a part of the cellular physiology. Another obstacle is represented by the high virulence of some viral pandemics which has put the medical workers at risk and a hard challenge of rapidly growing, highly virulent, and slow treatment responding disease. The new viral diseases impose a time interval required to understand the viral behavior at different levels i.e. environmental, intracellular, clinical, epidemiological, etc.

During the COVID-19 attack which started in December 2019 in Wuhan, China till now, no drug treatment has approved a considerable and satisfying efficacy against SARS-CoV-2 virus infection. Nevertheless, scientific attempts are continuing to overcome this pandemic. Ongoing attempts to understand the life cycle of the SARS-CoV-2 virus to open the way toward effective drug discovery (Saxena, 2020).

The prediction of the COVID-19 pandemic is uncertain by nature. The uncertainty is represented by the many unknowns about the virus itself and the perplexity, heterogeneity, and variability of human behaviors, governmental interference, and testing protocols. The vicious and ambiguous nature of this pandemic makes prediction accuracy far less. (Jianxi, 2020)

A comprehensive understanding of the viral structure and the intracellular course remains the cornerstone of any drug treatment whether this drug is repurposed or newly invented. Thus, approaches for antiviral drug discovery against SARS-CoV-2 are continuing based on the molecular biological mechanism of probable effective drugs. Novel drug discovery requires a long time and economic costs which

makes the process of initialization of effective therapeutic protocol for COVID-19 more difficult.

COVID-19 infection in its clinical stages also requires other modalities of treatment apart from antiviral agents including symptomatic, immune restoration, and treatment of associated complications. Hence, therapeutic protocols are continuously updated to reach the optimum management schedule for cases.

4.3.Scope of drug discovery targeting SARS-CoV-2 virus

The previously approved antiviral agents enlighten the road toward SARS-CoV-2 treatment. Systematic specification of virus-host protein-protein interactions (PPIs) gives an effective way toward a better understanding of the viral mechanisms of infection (Yang, Fu, Dong, 2019). Better elucidation of intracellular viral mechanisms means the better ability of antiviral drug design and discovery. Hence, many antiviral drugs were invented based on the interruption of viral cellular mechanisms of entry and replication.

Scientists endeavor to eradicate COVID-19 disease by different means. New antiviral drug design costs much more time and financial burden than trying to use older antivirals as treatment i.e. drug repurposing or ‘’repositioning’’. Hence, the empirical use of different antiviral agents was adopted under the current circumstances of curable drug absence. Drugs like interferon-alpha, lopinavir-ritonavir, remdesivir, favipiravir, and others were used and many randomized controlled trials were conducted. Nevertheless, and despite some promising results, there is no curable drug approved till now (Barlow, 2019).

The drug repurposing process starts with a screening of a huge database of small molecules against antiviral drug targets by use of computational techniques, drugs, or molecules that may be identified to have some antiviral activity (Pizzorno,

2019). Some antiviral drugs known to target specific proteins of viruses like influenza, hepatitis C and Ebola may possess the same effects against viral proteins of the SARS-CoV-2 virus (Bernatchez, 2020).

There are different approaches for the determination of the key target of the antiviral drugs whether the available repurposed or the discovered ones (Saxena, 2020). These approaches include intervention of virus-cellular receptor binding, inhibition of viral endocytosis inside the cell, targeting of viral protein components, and endorsement of host immunity. (Omotade,2019; Tortorici, 2019, Goo, 2020 ,Saha, 2020).

4.4. Therapeutic agents-drugs of choice

4.4.1.Antivirals

REMDESIVIR

Remdesivir (given code GS-5734) was originally manufactured and marketed by Gilead Sciences in 2017 as a therapeutic agent for Ebola virus infection. It is a monophosphoramidate prodrug and an adenosine analog that is metabolized intracellularly into its active form, GS-441524, which further converts to pharmacologically active nucleoside triphosphate form GS-443902. This nucleoside triphosphate GS-443902 acts as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate by incorporating and inhibiting viral RNA-dependent RNA polymerase (RdRp) thereby causing delayed RNA chain termination during the process of viral replication thus terminating viral RNA transcription. Remdesivir has a wide range of antiviral activity against many viral families like filoviruses (e.g., Ebola) and has shown efficacy in in-vitro cell-based assays as well as in the in-vivo rhesus monkey model of Ebola virus disease.

Remdesivir demonstrated antiviral and therapeutic benefits in experimental models of Middle East respiratory syndrome (MERS)-CoV and SARS-CoV-1 infections and has also inhibited all human and animal coronaviruses tested in vitro, including SARS-CoV-2.

United State Food Drug Administration (US FDA) on May 1st, 2020, issued a statement granting emergency use authorization (EUA) to the investigational antiviral drug redeliver to treat adults and children with suspected or laboratory-confirmed COVID-19 and severe disease defined as SpO2 ≤ 94% on room air, requiring supplemental oxygen, mechanical ventilation, or extracorporeal membrane oxygenation (ECMO) in an in-patient hospital setting. While there are inadequate evidence and clinical data about the effectiveness and safety of redelivering in patients hospitalized with COVID-19, this promising drug was detected to curtail the recovery time in some patients.

National Institute of Health (NIH) and EUA –FDA treatment guidelines recommended the use of redelivering only for use in COVID-19 hospitalized patients who require supplemental oxygen but who do not require oxygen remdesivir through a high-flow device, noninvasive and invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO). It should be administered in the dose of 200 mg i.v on the first day followed by a maintenance dose of 100 mg i.v infusion over 30-120 minutes for a minimum duration of 5 days or until hospital discharge whichever is earlier, though some experts recommend to extend it up to 10 days. For patients requiring invasive mechanical ventilation and/or extracorporeal membrane oxygenation (ECMO), the recommended total treatment duration is 10 days.

Adverse reactions associated with remdesivir reported were gastrointestinal symptoms like nausea, vomiting, diarrhea, rash, infusion-related hypersensitivity

reactions, increased liver transaminases, and an increase in prothrombin time. Drug-drug interaction studies of remdesivir have not been conducted though it has the potential to induce CYP enzymes (CYP1A2, CYP2B6, and CYP3A4). Remdesivir may be administered with weak to moderate inducers or with strong inhibitors of CYP450, OATP, or P-glycoprotein but strong induction may substantially reduce remdesivir levels hence is not recommended to be used with strong inducers (e.g., rifampin).

Clinical studies have shown that there was no reduction in remdesivir levels when coadministered with dexamethasone, but there was a decrease in therapeutic efficacy when administered with Chloroquine or hydroxychloroquine, hence coadministration of these drugs is not recommended.

Remdesivir preparation contains sulfobutyletherbeta-cyclodextrin sodium, (SBECD) as a solubilizing agent which is renally cleared. Though the parent compound of remdesivir has minimal renal clearance, SBECD might pose a risk for patients with moderate to severe renal dysfunction. Nonetheless, assuming the risk-benefit ratio in patients with COVID-19, no dose modification is recommended in patients with mild and moderate renal impairment, although it is contraindicated in patients with severe renal impairment (eGFR <30 ml/min). It is also recommended to conduct a Liver function test before starting remdesivir and repeating at regular intervals through contraindicated in patients with alanine transferase (ALT) > 5-times upper limit of normal or in case of severe hepatic dysfunction. Contraindications for REMDESIVIR are if AST/ALT > 5 times Upper limit of normal (ULN), Severe renal impairment (i.e., eGFR <30ml/min/m2 or need for hemodialysis, no dose adjustment for Inj REMDESIVIR if eGFR >30ml/min. Formula to calculate eGFR in Adults

• eGFR, Male: (140 – age in years) × (weight in kg)/ 72 × (serum creatinine in mg/dL);

• eGFR, Female: (140 – age in years) × (weight in kg) × 0.85 / 72 × (serum creatinine in mg/dL)

The use of remdesivir in the pediatric population is also available for compassionate use. For pediatric patients weighing 3.5 kg -40 kg, remdesivir 100 mg injection can be used only in lyophilized powder form in a single loading dose of 5 mg/kg on Day 1 followed by 2.5 mg/kg OD from Day 2 and for children/adolescents of >40 kg, remdesivir lyophilized Powder or injection both can be administered in same doses and duration as adults.

Remdesivir should be used in pregnancy and lactation only if the potential benefit justifies the impending risk to the mother and the fetus as its safety and efficacy has not been evaluated in such clinical population but cannot be withheld if its benefits are indicated though only compassionate use is allowed in pregnant females.

Several clinical trials were conducted to collate this data. The most important one was the Adaptive COVID-19 Treatment Trial (ACTT). It was a randomized, double-blind, multicentre, placebo-controlled trial that incorporated 1,063 patients with lung involvement and having advanced COVID-19. It validated that patients who were given remdesivir had a better average time to recovery as 11 days i.e 31% faster as compared to those who received placebo where average recovery time was 15 days ($P< 0.001$). The outcome also anticipated a survival benefit, less time for recovery, and a lower mortality rate of 8% in the remdesivir group, compared to 11.6% in the placebo group, though it was not statistically significant ($P= 0.059$). (Beigel JH, et al., 2020)

Grein *et al* administered remdesivir on the compassionate-use ground to hospitalized patients of Covid-19. Patients received 200 mg intravenously remdesivir on day 1, followed by 100 mg daily for another 9 days. In this small cohort study, 68% of patients showed clinical progress in oxygen provision status, and 13% of patients showed fatality over an average follow –up of 18 days, but the viral load was not assessed. The most common adverse events reported in the study were hepatotoxicity, diarrhea, maculopapular rash, nephrotoxicity, and hypotension. Serious adverse events reported were multiple organ-dysfunction, septic shock, and acute kidney failure but no new safety signals were discovered in this study. (Grein J, et al., 2020)

Wang *et al* conducted the first randomized, double-blind, multicentric, placebo-controlled clinical study on laboratory-confirmed SARS-CoV-2 infection with pneumonia well-defined by radiological parameters. Patients were randomly allocated in a 2:1 ratio to intravenous remdesivir (200 mg on day 1 followed by 100 mg on days 2–10 in single daily infusions) and the same volume of placebo infusions for 10 days. Though the results evaluated were not statistically significant clinically but the observed hazard ratio of 1.23 indicates that the benefit might be lesser than predicted. Clinical improvement time was drastically reduced in those patients treated earlier with remdesivir and a two-point up-gradation on a 6 point ordinal scale was also observed. (Wang Y, et al., 2020)

Another multinational, randomized, open-labeled study done in hospitalized patients of COVID-19, randomized patients in 1:1 ratio of i.v remdesivir for either 5 or 10 days and demonstrated clinical recovery at 14^{th} day by a 7-point ordinal scale. The results revealed that 5 or 10 days remdesivir had similar clinical benefit though adverse events were more in the 10-day group as compared to the 5-day group. The limitations of the study were the absence of a placebo group and baseline discrepancies in the clinical status of patients. (Goldman JD, et al., 2020)

An open-labeled phase 3 SIMPLE trial with severe COVID-19 hospitalized patients (n = 397) indicated improvement in clinical status with the 5-day remdesivir regimen (improvement for 50% of patients was 10 days in the 5-day treatment group) compared with the 10-day regimen on day 14 (improvement was 11 days in the 10-day treatment group) (OR: 0.75 [95% CI 0.51-1.12]). More than 50% of patients in both therapeutic groups were discharged from the hospital by day 14. The study determined the probable role of a 5-day remdesivir regimen in COVID patients.

FAVIPIRAVIR

Favipiravir, an antiviral agent, earlier known as T-705, is chemically classified as a pyrazinecarboxamide compound. It is a prodrug of a purine nucleotide, favipiravir ribofuranosyl-5′-triphosphate (favipiravirRTP) which potently inhibits the RNA-dependent RNA polymerase (RdRp) of RNA viruses preventing the combination of nucleotides for viral RNA transcription thereby terminating viral replication. Favipiravir displays inhibitory activity against influenza virus, arena-, bunya-, flavi-, filoviruses, and even the Ebola virus. In a preclinical in-vitro study, favipiravir inhibited SARS-CoV-2 in Vero E6 cells at an EC50 of 61.88 μMol/L.

Favipiravir has a half-life of 2–5.5 hours. The favipiravir regimen approved for influenza in Japan constitutes an oral loading dose of 3200 mg on day 1, followed by a maintenance dose of 600 mg BD from 2–5 days. A higher dosage regimen was implemented in phase III i.e. 1,800 mg BD on day 1 followed by 800 mg BD subsequently. There is a definite consensus on the safety and effectiveness of this regimen in influenza. It is indicated in mild to moderate cases of COVID19 in adults >18yrs old in the dose of 1800mg on Day 1 followed by 800mg for 6 days (total 7 days) but can be extended up to a maximum of 14 days.

The major adverse drug reactions of favipiravir comprise of diarrhea, high uric acid levels in the blood, raised transaminases, and a diminution in the neutrophil

counts, hence it is contraindicated in hyperuricemia, severe hepatic & renal impairment, pregnant women and lactating mothers. Favipiravir is metabolized partly by Aldehyde Oxidase (AO) and partly by Xanthine Oxidase (XO) so probable drug interactions by favipiravir occur due to inhibition of aldehyde oxidase (AO), therefore caution should be exercised while co-administering with Pyrazinamide, Repaglinide, Theophylline, Famciclovir, etc. which have to be reconsidered or replaced in therapeutic regimens.

A trial of Favipiravir was done in an open-labeled, controlled study on 80 positive COVID-19 patients. Thirty-five patients received oral FPV 1600 mg twice daily on day 1 and 600 mg twice daily from days 2-14 in addition to aerosol inhalation of 5 million U twice daily interferon (IFN)-a and 45 patients in the control arm received LPV/RTV 400 mg/100 mg twice daily plus 5 million U twice daily interferon (IFN)-a by aerosol inhalation. Results concluded a shorter viral negativity time and significantly recovered chest radiograph, 91.43% in the favipiravir arm versus 62.22% in the control arm. (Cai Q, et al., 2020)

In another randomized controlled clinical trial, on patients of mild-moderate COVID-19, favipiravir was compared with umifenovir (arbidol) which demonstrated 55.86%, as 7 day's clinical recovery rate in the arbidol group as compared to 71.43% in the favipiravir group (P = 0.0199). Also, the fever reduction time and cough improvement in the favipiravir group was considerably lesser than in the arbidol group. The adverse events were more in the favipiravir group 13.79% as compared to 2.50 % in the arbidol group. (Chen C, et al., 2020)

Russian health authorities have temporarily approved Avifavir (Favipiravir) as its first antiviral COVID-19 drug which has shown efficacy in patients with coronavirus during clinical trials. The interim results of Phase II/III Multicenter Randomized controlled Clinical Trial conducted in Russia where moderate COVID

patients were randomized in a 1:1:1 ratio to receive either AVIFAVIR 1600 mg BD on Day 1 followed by 600 mg BD on Days 2-14 (1600/600 mg, n=20) or AVIFAVIR 1800 mg BD on Day 1 followed by 800 mg BD on Days 2-14 (1800/800 mg, n=20) and the third group received standard of care (SOC) according to the Russian guidelines of COVID-19 (n=20) received either, hydroxychloroquine or chloroquine, lopinavir/ritonavir or no treatment. The results demonstrated that both dosing regimens of Avifavir showed similar virologic response as compared to the SOC group, thus concluding that Favipiravir is clinically efficacious in patients of moderate COVID-19. (Ivashchenko AA, et al., 2020)

Another significant phase 3 trial was conducted by Glenmark on mild-moderate patients of COVID-19 who received Favipiravir tablets 3,600 mg (1,800 mg BD) Day 1 followed by 1,600 mg (800 mg BID) Day 2 onward for a maximum of 14 days, along with standard supportive care. Patients were randomized based on disease severity into mild (n=90) and moderate (n=60). Results concluded that there were quicker viral clearance and a shorter median time to clinical cure in the favipiravir arm as compared to the control arm. Adverse events were 35.6 % in favipiravir as compared to 8 % in the control arm, however, they were mild to moderate and none led to drug discontinuation or dosing adjustments.

LOPINAVIR/RITONAVIR

Lopinavir and Ritonavir are selective, competitive, and reversible inhibitors of HIV-1 protease also found effective against SARS-CoV. Since these viruses are also RNA viruses just like HIV, they encode a protease enzyme involved in the production of structural proteins and enzymes from a large polyprotein synthesized in the infected cell. This polyprotein is cleaved into functional components like RNA dependent RNA polymerase and helicase by the protease enzyme. The two main protease enzymes are papain-like protease (PLpro) and 3-chymotrypsin like protease

(3CLpro). Lopinavir/Ritonavir inhibits 3CLpro thereby preventing protease hydrolysis and impeding the viral replication process. Thus they block the infectivity of the nascent virions and prevent subsequent waves of infection.

The doses recommended in adults is lopinavir 400 mg/Ritonavir 100 mg combination given orally twice daily for 10–14 days and in children < 18 yrs of age, Lopinavir 300 mg/m^2 plus Ritonavir 75 mg/m^2 (maximum: Lopinavir 400 mg/Ritonavir 100 mg per dose) orally twice daily for 7 days. The oral bioavailability of these drugs is variable. The half-life of Lopinavir is 5-6 hrs and Ritonavir is 3-5 hrs. They are metabolized by hepatic CYP3A4 and also inhibit CYP3A4, the most potent inhibitor being Ritonavir. Thus Ritonavir is usually given in combination with lopinavir as it allows the dose reduction of the latter thereby improving patient compliance, but co-administering with other drugs is not recommended as it may lead to toxicity.

Adverse reactions include nausea, vomiting, diarrhea, QTc prolongation, and paraesthesia. Hyperglycemia, insulin resistance, dyslipidemia, lipodystrophy, pancreatitis, and hepatotoxicity are the watchable ones. Drug-induced hepatoxicity especially raised alanine transaminase levels warrants cautiousness because it not only exacerbates liver damage resulting from COVID-19 but also affects the metabolism of concomitant drugs. The use of Lopinavir/Ritonavir in pregnant females has a good safety profile and no evidence of teratogenicity and less placental transfer to the fetus. Use in infants, children, and adolescents is also safe.

One of the studies of lopinavir/ritonavir showed that if lopinavir/ritonavir were added to standard treatment practices for the severe acute respiratory syndrome, it was associated with improved clinical outcomes mainly focusing on the fact that early initiation of therapy during the peak viral replication phase was more effective.

But the results of this may be inconclusive as it was a retrospective study and patients were not randomly assigned to treatment and control groups (Chan KS, et al., 2003)

IVERMECTIN

Ivermectin is an approved antiparasitic drug obtained from *Streptomyces avermitilis* found in soil. It activates the glutamate-gated chloride channel and causes hyperpolarization by increasing the intracellular chloride concentration in nerve and muscle cells of the organism, leading to tonic paralysis of the organism which is then phagocytized by reticuloendothelial cells. Studies highlighted that SARS-Cov protein Importin α/β1 (IMP) has a role in infection during signal-dependent shutting of nucleocapsid protein and ORF6 isolates IMP α/β1 on the ER or Golgi membrane.

Ivermectin inhibits IMP α/β1 mediated nuclear transport activity of viral proteins of SARS-CoV-2, thereby having antiviral activity. In a recent study ivermectin showed in-vitro activity and in a dose of 5 μM showed a 5,000-fold reduction in SARS-CoV-2 RNA levels in infected Vero/ hSLAM cells incubated for 48 hours in contrast to the control group.

It is an FDA-authorized anti-parasitic drug that is used for the treatment and prophylaxis of several tropical diseases like helminthiasis, onchocerciasis, and scabies. The replication of a few single-stranded RNA viruses, like Zika virus, dengue virus (DNV), yellow fever virus, and other viruses are also inhibited by this drug.

The dose given in mild cases of COVID-19 is 12mg once daily for 3 days. The prophylactic dose is 200μg/kg body weight or 12 mg. In high-risk individuals who came in close contact with COVID-19 patients should be administered 12mg on day 1 and day 7 and the dose in health care workers is 12mg on day 1, day 7, and day 30 to be followed by once a month regimen. The tablet is to be taken 2 hours post-dinner.

It is well absorbed orally, peak plasma is achieved in 4-5 hrs, and highly plasma protein-bound. More than 97% is metabolized in the liver and eliminated in faeces and half-life is 12 hours. Ivermectin is a safe drug, well tolerated but may cause itching, skin edema, orthostatic hypotension, arthralgia, lymphadenopathy, sore throat, cough, headache ocular irritation, fever, and hepatitis. It is contraindicated in pregnancy, lactation, and in children <5 years of age.

CYP3A4 inhibitors (eg, ritonavir) may lead to drug interactions that warrant careful consideration.

Several clinical trials of ivermectin are underway in many countries. An observational study from 169 hospitals across several countries evaluated critically ill hospitalized patients diagnosed with COVID-19. Fifty-two patients who received ivermectin (150 mcg/Kg) after mechanical ventilation showed a survival benefit for ivermectin and the hospital length of the stay was also reduced (Patel A, et al., 2020).

Another study from the Dominican Republic administered ivermectin in 1,300 early stage COVID-19 patients in a standard dose of 100-200 mcg/kg and escalated it to 400 mcg/kg. Results concluded that 99% of patients were cured and the average duration of infection came down from 21 days to 10 days. The only side effects reported in this study were mild heartburn and diarrhea.

(https://www.trialsitenews.com/president-of-dominican-republics-largest-private-healthgroup-discusses-the-success-of-ivermectin-as-a-treatmentfor-early-stage-covid-19/; 2020 Jun.)

A retrospective cohort study in Florida hospitals with confirmed SARS-CoV-2 infection (n=280) showed significantly lesser mortality rates in those who received ivermectin compared with standard care (15% vs 25.2%; P = 0.03). The mortality rate was also lower among 75 patients with the severe pulmonary disease treated with ivermectin (38.8% vs 80.7%; P = 0.001) (Rajter JC, et al., 2020).

A randomized controlled trial from Bangladesh involving mild to moderate degree of COVID-19 patients, divided into two study groups, the first group with n = 60, prescribed Ivermectin 200 mcg/kg single dose combined with Doxycycline 100mg BD for 10 days and the second group with n =56, prescribed HCQ 400 mg 1st day, then 200mg BD for 9 days along with Azithromycin 500mg daily for 5 Days. The recovery rate was 100% in the first group as compared to 96.36% in the second group and also had a better clinical response, reduced recovery duration, lesser side effects, and better patient compliance concluding that Ivermectin may have a clinically beneficial role in treating COVID-19 (Chowdhury AT, et al., 2020). Nearly 40 clinical trials are ongoing globally for measuring the outcome of Ivermectin treatment in COVID-19 as it is safe and well-tolerated, gives hope for its activity against SARS-CoV-2, and permits further exploration for potential benefits in humans.

RIBAVIRIN

Ribavirin is a guanine nucleotide analogue that has a broad-spectrum antiviral activity. It's mono and triphosphate derivatives generated intracellularly alters cellular nucleotide pool and inhibits GTP and viral RNA synthesis. Ribavirin inhibits inosine monophosphate dehydrogenase thereby inhibiting guanosine production from its precursor. Ribavirin's wide-ranging antiviral properties may prevent viral replication, reduce the patient's viral load, decrease the subsequent tissue damage, and breaks the chain of transmission.

The Chinese government recommended the use of ribavirin in Treatment Plan Edition 5, in diagnosed patients of COVID pneumonia. A 4-g oral loading dose should be given, followed by a 1.2-g oral dose every 8 hours. This guidance was later modified to 500 mg IV BID or TID in revised protocols. (Treatment Plan Edition 5. 2020. http://www.gov.cn/zhengce/zhengceku/2020-

02/05/5474791/files/de44557832ad4be1929091dcbcfca891.pdf. Accessed Aug 30, 2020.)

Ribavirin is given by oral or inhalation route, which is well tolerated having low toxicity because of poor systemic absorption. The plasma half-life is 9-10 hrs and eliminated by the renal route. Adverse effects include dose-dependent anemia, hemolytic anemia, bone marrow suppression, cardiac toxicity, neurological disorders, flu-like symptoms, gastrointestinal symptoms, pancreatitis, liver dysfunction, elevated transaminase levels, pulmonary embolism, and interstitial pneumonitis. It is a known teratogenic, mutagenic, embryotoxic, and gonadotoxic drug hence contraindicated in pregnancy. Hence, if used it can be tested in low doses and combination or adjuvant therapy in COVID-19 patients.

UMIFENOVIR (ARBIDOL)

Arbidol, an indole-derivative, has been licensed for decades in Russia and China against influenza. This broad-spectrum antiviral drug has shown efficiency against influenza viruses as it targets the hemagglutinin (HA) envelope glycoprotein. It acts as a fusion inhibitor by forming the drug–HA complex. Arbidol creates hydrophobic interactions and induces some conformational reorganizations to form salt bridges across the binding site thus stabilizing the membrane and inhibiting fusion.

In coronavirus it aims at the S protein/ACE2 alliance, blocks its trimerization, and hinders membrane fusion of virus, preventing its entry. It also has immunomodulatory property via induction of serum interferon and activation of phagocytes, increasing the body's immunity to fight infection thus decreasing the frequency of complications associated with a viral illness. Experimental studies of arbidol and arbidol mesylate on the infected cultured cells GMK-AH-1 (D) was

effective in suppressing the transcription of the SARS virus, but arbidol mesylate was 5 times more effective than arbidol.

For SARS prophylaxis (during contact with SARS patients), the dosage for children >12 and adults is 200 mg once a day; for children from 7 -12 yrs is 100 mg, taken before food on empty stomach, once a day for 12 – 14 days. For the treatment of SARS, the dose in adults and children >12 is 200 mg BD for 8 – 10 days.

Arbidol is metabolized in the liver. The half-life of the drug in the body is 17-21 hours. About 40% is excreted in unchanged form, mostly through bile (38.9%) and an insignificant amount through the kidneys (0.12%). Possible adverse reactions may be allergic reaction or rash. No significant drug interactions observed when administered with other medications. It is contraindicated in children < 2 years and those having enhanced sensitivity to the medication.

A nonrandomized study of umifenovir in patients of COVID-19 demonstrated a lower mortality rate (0% vs 16%) and higher discharge rate with 9 days of treatment as compared to the control group (Wang Z, et al., 2020). Another retrospective nonrandomized cohort study suggested that a combination of arbidol in a dose of 200 mg thrice a day and Liponavir/ritonavir (400 mg/100 mg) orally twice a day for 5–21 days was associated with a much superior negative conversion rate of coronavirus test in 7 and 14-days and a suggestively clear chest CT scan in 7-days as compared to Lopinavir/ritonavir alone. Thus this combination might give a favorable clinical response by delaying the advancement of lung lesions and diminishing the viral load of SARs-CoV-2. (Deng L, et al., 2020)

OSELTAMIVIR

Oseltamivir, a prodrug of oseltamivir carboxylate is a specific inhibitor of influenza neuraminidase enzyme. This enzyme is responsible for cleaving sialic acid residues on freshly synthesized virus particles which helps them release from the cell

and facilitates the spread of infection to another cell. Inhibition of neuraminidase prevents the release of the newly infected virus particles thus breaking the chain of infectivity. It is efficacious against avian H5N1, H1N1, and H9N2 influenza strains.

It can be given orally in mild, moderate, and severe cases of COVID-19 in a dose of 75mg BD for 5 days, recommended due to the possibility of H1N1 co-infection along with COVID-19 disease. The plasma half-life is 1-3 hrs. Common side effects include nausea, vomiting, diarrhea, headache, and abdominal pain. Rarely allergic rash, toxic epidermal necrolysis, hepatitis, and seizures may occur. It is not recommended in children <1yr and pregnant females.

In a study conducted in Paris on patients positive for influenza virus, 52% received oseltamivir and it was determined that empirical oseltamivir can be given in suspected MERS-CoV and SARS-CoV infection.

NITAZOXANIDE

Nitazoxanide is a nitrothiazoly-salicylamide broad-spectrum drug having antiprotozoal and antiviral activities. It is a prodrug and is rapidly converted to the active form tizoxanide and tizoxanide conjugates which are inhibitors of pyruvate ferredoxin oxidoreductase (PFOR) enzyme that is an essential pathway of electron transport energy metabolism in anaerobic organisms. It is effective against various protozoa, helminths and also used as antidiarrheal in children and AIDS patients. Nitazoxanide has also validated in vitro efficacy against MERS-CoV and other coronaviruses by potentiating interferon α and interferon β formation.

Its absorption is increased by food and is highly (99%) plasma protein bound, hence can displace other drugs from protein binding sites causing their toxicity. Though it is well tolerated, may have mild side effects like GI upset, headache, and increased creatinine levels. In a study on influenza patients nitazoxanide given in the

dose of 600 mg BD for 5 days, reduced the symptomatic period and morbidity with minor side effects.

Similarly, another study endorses that nitazoxanide should be administered with hydroxychloroquine for COVID-19 as this regimen could diminish the severity of illness by dropping viral titers and salvaged the innate-immune system which was disrupted by the virus. (Padmanabhan S, 2020)

Based on the above studies and evidence regarding the antiviral activity and immunomodulatory effects of nitazoxanide and its good safety profile, nitazoxanide/azithromycin and HCQ combination might be considered to decrease the disease severity and should be potentially used as a treatment possibility for SARS-CoV-2.

HYDROXYCHLOROQUINE/CHLOROQUINE

Chloroquine (CQ) and its analogue hydroxychloroquine (HCQ) prescribed routinely as an anti-malarial drug, was initially found to be effective in the treatment and prophylaxis of COVID. They accumulate in the acidic vacuole of Plasmodium and raise the vacuolar pH since it is basic and prevents degradation of hemoglobin by parasitic lysosomes. The chloroquine-haeme complex then damages the plasmodial membrane killing the parasite. Similar to its anti-malarial action, it was demonstrated that in vitro chloroquine and HCQ increases endosomal pH thereby preventing entry of the virus, and post-entry, it obstructs the glycosylation of cellular receptor of SARS-CoV, thus limiting viral replication and subsequent pathogenicity.

Another proposed mechanism is that CQ and HCQ additionally blocked the quinone reductase-2, which is required for sialic acid biosynthesis, a prerequisite for receptor-ligand recognition of human coronavirus (HCoV), that makes it efficacious against the virus. Furthermore, by changing the lysosomal pH, they check cathepsins, which causes the development of autophagosomes that splits the spike proteins of

SARS-CoV-2. Moreover, chloroquine besides inhibiting the MAP-kinase and restricting SARS-CoV-2 molecular signaling also modifies the viral organization, growth, replication, and impedes the catalytic modulation of the M protein.

Chloroquine also led to impairment in the glycosylation of viral surface receptors, so that it cannot cohere to ACE2 on the target cell thus having a potent anti-SARS-CoV efficacy in vitro. Chloroquine and HCQ are also proposed to hinder the post-translational adaptation of viral proteins involving proteases and glycosyltransferases, which requires a low pH and occurs within the endoplasmic reticulum or Golgi apparatus, thereby inhibiting virus assembly. These drugs may also impede the accumulation and maturation of membrane M protein in the Golgi complex which further inhibits the budding of the viral particles from the cell as defined in MERS-CoV.

Additional to its antiviral action, CQ and HCQ also have an immune-modulating and anti-inflammatory activity which may synergistically augment its antiviral effect in vivo, thus having a significant efficacy in the treatment of COVID-19 patients. Identification of viral antigen via a toll-like receptor-dependent pathway on antigen-presenting cells entails endosomal acidification and alteration of pH by chloroquine also hinders this recognition of viral antigen by dendritic cells, thus demonstrating immune inhibitory activity.

It further inhibits interleukin-1 beta (IL-1β) and IL-6 cytokines in monocytes/macrophages and tumor necrosis factor-alpha (TNFα) generation by immune cells. Apart from inhibition of SARS-CoV2, HCQ is also an efficacious anti-inflammatory agent that decreases the manufacture of inflammatory cytokines and pro-inflammatory factors, suppresses T- lymphocyte response to mitogens, stabilizes lysosomes and decreases its chemotaxis, and also traps the free radicals, hence used in various autoimmune diseases like systemic lupus erythematosus,

rheumatoid arthritis, and Sjogren's syndrome. In patients of COVID-19, cytokine storm was found to be associated with disease severity, hence it may be proposed that in such patients, HCQ may attenuate the inflammatory response giving a better outcome.

Chloroquine is quickly and completely absorbed orally. Effective plasma concentration is reached in 2-3 hrs by oral route and in 15 mins by intramuscular route. Approximately 55% of the drug is plasma protein bound and gets highly concentrated in the liver, spleen, lung, and kidney. Metabolized in the liver to the active form 4- hydroxychloroquine. A loading dose is necessary at the starting of treatment as it has a high volume of distribution. It persists for a long time in the body even after discontinuation due to a high affinity for tissue proteins.

Emergency use authorization (EUA) recommended Dose of CQ in adults and adolescents ≥50 kg for mild-moderate cases of COVID pneumonia is 1 g orally once on Day 1, followed by CQ 500 mg orally once daily for 4–7 days and HCQ 800 mg once on Day 1, followed by HCQ 400 mg orally once daily for 4–7 days total. Remdesivir should not be coadministered with CQ or HCQ. National Health Commission of the People's Republic of China, suggested the use of chloroquine at an adult dose of 500 mg twice per day for 10 days in patients with COVID-19. But later due to the toxicity of chloroquine, the treatment guidelines were revised, and the duration was shortened to 7 days while endorsing a lower dose for patients less than 50 kg weight.

The National Task Force for COVID 19 created by the Indian Council of Medical Research (ICMR) under the Ministry of Health and Family Welfare in India recommends the usage of Hydroxychloroquine for prophylaxis of asymptomatic health care personnel involved in the care of suspected or established cases of COVID-19 and asymptomatic frontline workers: 400 mg twice daily on day 1

followed by 400 mg once weekly for next 7 weeks to be taken with meals and in asymptomatic household contacts of laboratory-confirmed cases: 400 mg twice daily on day 1 followed by 400 mg once weekly for next 3 weeks to be taken with meals. For the treatment of mild-moderate cases, the dose given is 400 mg BD on day 1 followed by 200mg BD for 4 days in COVID care centers or home isolation.

Chloroquine and hydroxychloroquine though well tolerated but can give rise to serious and rare adverse effects (<10%) in susceptible patients like QTc prolongation and cardiomyopathy.

Neuropsychiatric symptoms and neuromyopathy, hypoglycemia, GI disturbances, photosensitivity, retinopathy and precipitates hemolysis in patients with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. Thus it is advisable to take electrocardiography preceding to and following initiation of these drugs to evaluate for prolonged QTc because of the chances of ventricular tachycardias, particularly in sick patients and those taking simultaneous QT-interval prolonging medications such as azithromycin and fluoroquinolones. Thus US-FDA alerts against the use of chloroquine or hydroxychloroquine for COVID-19 barring the hospital settings or a clinical trial due to threat of arrhythmias.

Irreversible retinopathy with retinal pigmentation changes, visual field defects, maculopathies, color vision abnormalities, corneal edema, and opacities including the corneal deposition of the drug were adverse reactions of concern. Due to the extra hydroxyl molecule, HCQ is less penetrable to the blood-retinal barrier and has a brisk removal from retinal pigment cell, thus having a reduced probability of retinal harmfulness as compared to chloroquine. Besides, chloroquine having a narrow therapeutic and safety margin, HCQ is a non-toxic alternative to chloroquine which permits higher daily dosing with less drug-drug interactions. According to the American Academy of Ophthalmology, the crucial hazards for CQ and HCQ retinal

pathology are high doses, the maximum recommendation being 5.0 mg/kg for HCQ, and 2.3 mg/kg for CQ for a duration less than 5 years. CQ and HCQ are considered safe in pregnancy.

An open-labeled, non-randomized trial conducted on 36 patients by Gautret *et al.* in France established that HCQ solo and on combining with azithromycin remarkably improved virological clearance in nasopharyngeal swab in six days in COVID-19 subjects as compared to control. The virological negativity at day 6 with HCQ was 70.0% versus 12.5% for HCQ and control groups respectively. Besides, the virological negativity in 6 patients at day-6 in HCQ plus azithromycin group, was 100% suggesting an augmenting effect of azithromycin with HCQ. (Gautret P, et al., 2020)

Gautret P, *et al* conducted another prospective observational study on 80 patients who had SARS-CoV-2 presence in the nasopharyngeal sample. They were administered HCQ 200 mg thrice daily for 10 days with azithromycin (500 mg on day 1 followed by 250 mg from days 2–5) and observed that 97.5% of patients showed clinical improvement, 93% exhibited decreased viral load by 8^{th} day, and hospital stay dropped to 5 days, thus further establishing the role of HCQ. (Gautret P, et al., 2020)

To date, these antimalarials drugs have been one of the commonly used medications in this pandemic with epidemiological and in vitro evidence, however, limited clinical evidence still warrants their use in COVID-19 treatment protocols. While surplus clinical trials continue to assess the potential effectiveness of these drugs in treating or preventing COVID-19, the results and scientific data from these studies will soon be available corroborating their efficacy and safety.

4.4.2.Antiinflammatory/immunomodulator

Corticosteroids

ARDS in COVID pneumonia occurs due to extensive immune response from the host. The uncontrolled viral replication, increased number of infected epithelial cells promoting inflammatory cells to produces cytokines and inflammatory mediators generates a massive 'cytokine storm' along with hyperinflammation. Corticosteroids have potent anti-inflammatory effects by regulating the signaling pathways, preventing the production of inflammatory factors like cytokines, chemokines, increase the production of anti-inflammatory mediators such as interleukin 10 (IL-10) thus corticosteroids are being used as adjuvant therapy in SARS-CoV to preventing host inflammatory responses and cytokine storm in COVID-19 pneumonia patients.

The corticosteroids used in COVID pneumonia are either Methyprednisolone or Dexamethasone. In moderate cases, methylprednisolone injection is given in the dose of 0.5-1mg/kg and in severe cases 1-2mg/kg. It is an intermediate-acting steroid with an extended half-life of 12-36 hrs together with good penetration into lung tissue. Dexamethasone is a very potent and long-acting glucocorticoid given in moderate cases in a dose of 0.1-0.2mg/kg and in severe cases 0.2-0.4mg/kg, both for 5-7 days.

However, the use of corticosteroids is associated with adverse effects, involving deferred viral clearance and a greater threat of secondary infection due to an immunocompromised state, psychosis, hyperglycemia, avascular necrosis, and inhibition of the Hypothalamic–Pituitary–Adrenal (HPA) axis.

A retrospective study of COVID-19 patients in China established that methylprednisolone use in ARDS patients was related to curtailed death risk which was 46% with steroids vs 62% in the control group. (Wu C, et al., 2020)

The recovery trial, launched in March, was one of the world's biggest randomized, controlled trials for coronavirus treatments which enrolled 2,100 participants who received dexamethasone 6 mg/day for 10 days and compared against 4,300 people who received standard care for coronavirus infection.

The study announced its findings in a press release on 16 June concluding that dexamethasone was most effective among critically ill patients on ventilators. Those who were receiving oxygen therapy but were not on ventilators also showed improvement: their risk of dying was reduced by 20%. The steroid did not affect patients of mild COVID-19 — those not receiving oxygen or ventilation. Shortly after the results were released, the UK government announced that it had immediately authorized the use of dexamethasone for hospitalized COVID-19 patients who required oxygen, including those on ventilators. (Ledford H., 2020)

The use of corticosteroid for viral pneumonia therapy in COVID-19 was restricted previously due to uncertain efficacy and potential harms as was cautioned by WHO unless an associated indication like refractory shock or acute respiratory distress syndrome was present, but after results of recovery trials, it is being used in various clinical settings in different countries.

MONOCLONAL ANTIBODIES

Monoclonal antibodies (mAbs), being antigen-specific, directed against immune response can target viral proteins at various stages of infectivity, hence can be a budding class of adjunctive therapies for COVID-19. The counteracting monoclonal antibodies target either the receptor-binding domain (RBD) on the spike protein or ACE2 on the host cell which ultimately may block the virus entry. The

foremost reason for death in COVID-19 is ARDS and organ failures. The pathophysiology behind this is a hyper inflammation syndrome and a fatal hypercytokinemia or cytokine storm leading to multiorgan failure. There is a likely increase of IL-2, 6, 7, IF-gamma, TNF-α, etc. due to enhanced immune response. Thus, monoclonal antibodies against IL-2,6,7, IF-gamma, and TNF-α could weaken the immune response and lead to better clinical outcomes.

Tocilizumab, a monoclonal antibody antagonizing IL-6 receptor, is accepted by the FDA to treat rheumatoid arthritis and cytokine release syndrome after T-cell therapy. Itolizumab, an anti-CD6 IgG1 monoclonal antibody that was launched for treating chronic plaque psoriasis, is being repurposed and approved in emergency use for the treatment of cytokine release syndrome in moderate to severe ARDS patients of COVID-19. Tocilizumab is an Off Label therapy in COVID-19, may be considered in patients with severe disease with progressively increasing oxygen requirements and mechanically ventilated patients not improving despite the use of steroids.

Tocilizumab/itolizumab is indicated if IL-6 levels are 50-100 fold higher than normal (Normal range 0 - 9.5pg/ml), the worsening trend of the inflammatory markers (Ferritin, LDH, CRP) is there and clinical condition is deteriorating with worsening of PaO2/Fio2 ratio (more than 25% deterioration from the immediately previous value). In severe cases Tociluzumab inj is given in the dose of 8mg/kg (maximum 800 mg at one time) given slowly in 100 ml normal saline over 1 hr and the dose can be repeated once every 12-24 hrs if needed. Itolizumab injection is given in the first dose of 1.6mg/kg by i.v infusion and subsequently, 0.8mg/kg infusion over 4 hrs if required.

Contraindications to therapy are those patients with active infections (systemic bacterial/fungal), high serum procalcitonin, Tuberculosis, active hepatitis,

Absolute Neutrophil Count < 2000/mm3 and Platelet count < 1,00,000/mm3, hepatic and renal impairment; patients on chronic steroid therapy, Paediatric patients <18 years old, pregnant and lactating mothers. Adverse effects reported are Infusion reactions, Diahorea, and Pruritus.

A study on serious COVID-19 patients from China comparing tocilizumab to standard therapeutic regimen recognized that tocilizumab was effective in improving respiratory functions, decreasing the temperature, improving the discharge rate, and decreasing mortality. They identified that interleukin 6, had a major role in inciting cytokine storm which was responsible for compromised lung functions and increased mortality, thus monoclonal antibody targeting the IL-6 pathway may potentially restrict this inflammatory storm. Hence Tocilizumab which blocks IL-6 receptors showed inspiring clinical results. (Xu X, et al., 2020)

Another single-center study on grave COVID-19 patients of pneumonia with the hyperinflammatory disorder and ARDS in Brescia, Italy identified that tocilizumab in the dose of 8 mg/kg by two successive intravenous infusions 12 hrs apart was associated with speedy, sustained response i.e 77% of patients showed significant clinical improvement. (Toniati P, et al., 2020)

One more study based on clinical data highlighted that cytokine storms in serious patients were an important reason for mortality and interleukin-6 (IL-6) played an imperative role in cytokine release syndrome (CRS). Therefore, therapy obstructing the signal transduction pathway of IL-6, directed at treating cytokine storms may help rescue severe patients. Tocilizumab, an effective blocker of the IL-6 signal transduction channel is likely to become a potential remedy for patients with severe COVID-19. (Zhang C, et al., 2020)

A prospective pilot open-labeled, single-arm study conducted in multiple centers on off-label use of tocilizumab on 63 adults hospitalized patients diagnosed

with severe COVID-19 assessed laboratory and clinical parameters at day 0, 1, 2, 7, and 14. There was a substantial improvement in the laboratory factors like ferritin, C-reactive protein, and D-dimer levels. Survival rate improved with tocilizumab use within 6 days from hospitalization, thus they concluded that it could be a safe alternative. (Sciascia S, et al., 2020)

Guaraldi G, *et al* conducted an observational retrospective, cohort study on patients with severe COVID-19 pneumonia in Italy, administered standard therapy with tocilizumab 8mg/kg i.v or 324 mg subcutaneously in a nonrandom group of patients. Evaluation of invasive mechanical ventilation or death by Sequential Organ Failure Assessment (SOFA) score established that it may lessen the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia. (Guaraldi G, et al., 2020)

Sarilumab, another IL-6 receptor antagonist granted for Rheumatoid Arthritis, is being evaluated in a multicenter, double-blind, placebo-controlled, phase 2/3 trial for hospitalized patients of severe COVID-19 (NCT04315298). Other monoclonal antibodies being explored in clinical trials include bevacizumab (anti-vascular endothelial growth factor medication; NCT04275414), fingolimod (sphingosine-1-phosphate receptor immunomodulator approved for multiple sclerosis; NCT04280588), eculizumab (anti-C5 antibody obstructing terminal complement; NCT04288713), adalimumab (anti-TNF), and ixekizumab (anti-17A). A study on Meplazumab (anti-CD147) indicated improved clinical and virological outcomes in 17 COVID-19 patients by inhibiting T cell chemotaxis and also virus cell entry.

4.4.3.Anticoagulants /Antithrombotics

COVID-19 predisposes a hypercoagulable state in moderate to severe patients and has led to thrombotic disease which could be fatal. The probable pathophysiology behind hypercoagulability is attributed to disseminated

intravascular coagulation (DIC), antiphospholipid syndrome, activation of the complement cascade, and endothelial dysfunction induced by the virus. SARS-CoV-2 mortality has been linked to cytokine storm or macrophage activation syndrome (MAS), leading to the dysregulated inflammatory reaction. Studies have shown that pneumonia and acute respiratory distress syndrome (ARDS) in COVID-19 are associated with fibrin collection and thrombin generation within the bronchoalveolar system. Hence mortality in COVID-19 patients may be due to a devastating inflammatory response that damages the lungs. Thus the use of Heparin or Low Molecular Weight Heparin (LMWH) was recommended.

The indication for use is marked abnormality or elevation of D-Dimer (if D-dimer is more than 1000ng/ml) but without a parallel fall in platelet or prolongation of clotting time, suggesting that a local and not disseminated thrombin generation and fibrinolysis is taking place.

The European Society of Cardiology lately recommended an anticoagulation algorithm. For high thrombotic risk patients, in ICU, typically having dyspnea, respiratory rate > 24, oxygen saturation < 90%, raised C reactive protein, elevated D-dimer levels, and higher fibrinogen levels, parenteral heparin should be started with a close watch on APTT. For non-ICU patients, they endorsed the use of subcutaneous enoxaparin 1 mg/kg BD or the same heparin regimen. If deep venous thrombosis is diagnosed, continue anticoagulants as recommended otherwise phasedown to subcutaneous enoxaparin 40 mg BD. (Atallah B, et al., 2020)

Indian guidelines also suggest Inj. Enoxaparin 40 mg s.c once daily for 7 days in mild and moderate cases if D-dimer levels are >1000ng/ml or X-ray shows ground-glass opacity. In severe cases Inj. Enoxaparin 1mg/kg s.c for 7 days is to be administered. Alternately Inj Fondaparinux 2.5mg OD administered by s.c injection or Unfractionated Heparin 5000 Units s.c twice daily. Contraindications to the use

of anticoagulants are End-Stage Renal Disease, active bleeding, emergency surgery, platelets < 20,000/mm3, and BP >200/120 mmHg.

A study by Lin *et al*. affirms that low molecular weight heparin (LMWH) could serve as a therapeutic strategy to control the escalation of D-dimer on days 7–14 of the disease and reduce the risk of sepsis-induced disseminated intravascular coagulation (DIC). They recommended a subcutaneous dose of 100 IU/kg of LMWH twice a day, for at least 3–5 days (Lin L, et al., 2020)

Existing literature shows encouraging results with the use of therapeutic anticoagulation in high-risk individuals. Further prospective studies are needed to better analyze the risks and benefits of anticoagulation in COVID-19 patients.

4.4.4.Antimicrobials

AZITHROMYCIN

Azithromycin is a macrolide antibiotic used for skin, respiratory and other infections. It has significant broad-spectrum antiviral properties against Ebola, Zika, respiratory syncytial virus, H1N1 influenza virus, enterovirus, and rhinovirus in vitro as well as in vivo. Azithromycin has also reported a significant antiviral effect on SARS-CoV-2 alone and synergistically in combination with HCQ in vitro and clinical scenario. Azithromycin inhibits the virus entry into cells and augments the immune response against the virus. It has an antibacterial effect by combining with 50S ribosome subunits and interferes with translocation thereby prematurely terminating bacterial protein synthesis

Azithromycin has remarkable pharmacokinetic properties such as rapid oral absorption, acid stability, extensive distribution into tissues, especially the lungs where average concentrations in most tissues are much higher than in plasma. Slow-release from intracellular sites contributes to its long half-life of >50 hrs hence once

a day dosing is preferred which enhances the compliance. Azithromycin is approved in both adults and children aged ≥ 6 months. The treatment regimen is 500 mg on day one followed by 250 mg for the remaining 4 days.

Adverse drug reactions include gastric upset, abdominal cramps, headache, and dizziness. Serious side effects though are uncommon, however, they may cause cardiac arrhythmias, especially in the elderly and in those with preexisting QT interval prolongation, bradycardia, low serum potassium or magnesium, and in individuals who are taking certain antiarrhythmic drugs or terfenadine, astemizole, cisapride, theophylline, and HCQ, due to inhibition of CYP3A4 resulting in high levels of concurrently administered drugs.

Azithromycin is a safe and effective drug to be used in combination with other antivirals for individuals with early mild or moderate COVID-19.

DOXYCYCLINE

Doxycycline is a semisynthetic derivative of tetracycline, a broad-spectrum antibiotic. It acts by inhibiting protein synthesis in bacteria by binding to the 30s ribosome. Apart from this in-vitro studies have also demonstrated the antiviral activity of Doxycycline against SARS-CoV-2. The antiviral effect is due to the upregulation of transcription of intracellular zinc-finger antiviral protein (ZAP), an encoding gene in host cells that binds to specific target viral mRNAs and blocks the RNA translation of the virus. (https://www.mediterranee-infection.com/invitro-antiviral-activity-of-doxycycline-against-sars-cov-2).

Doxycycline is a highly lipophilic agent that chelates zinc compounds on matrix metalloproteinases (MMPs) of host cells by which coronavirus undergo cell fusion and replication thereby proposing that doxycycline's inhibition of MMPs may

prevent viral fusion and replication. Inhibiting MMPs may also help repair the damaged lung tissue in COVID-19 and improve recovery.

Doxycycline also possesses anti-inflammatory effects at a low dose (20-40 mg/day) and high (100 or 200 mg/day) doses by inhibitory action on mediators of inflammation and modulating effects on pro-inflammatory cytokines IL-6, IL-8, and tumor necrosis factor-alpha which are involved in the pathogenesis of cytokine storm. (Di Caprio R, et al., 2015)

Doxycycline is well absorbed orally, metabolized in the liver, and has slow excretion in faeces as an inactive conjugate, hence elimination is independent of renal function. It has a longer half-life of 16 hrs permitting less frequent dosing. The dose of doxycycline considered is 100 mg BD orally for 5 days along with other drugs in COVID-19. Adverse drug reactions include allergy, photosensitivity, GI intolerance though the incidence of diarrhea is lesser, hepatic dysfunction, and deformed bones and teeth hence it is contraindicated in pregnant females and children less than 12 years of age.

Doxycycline, being cost-effective, safe, efficacious, and easily available drug may serve as a rational and realistic combination drug to be used along with other antivirals in COVID-19.

4.5.Role Of Symptomatic Treatment Agents

The signs and symptoms of COVID-19 vary, but most patients experience fever with or without chills, cough, sore throat, running congested nose, shortness of breath, fatigue and body aches, headache, myalgia. Loss of smell (anosmia) or taste (ageusia) may precede the onset of respiratory symptoms in COVID-19 especially among women and young or middle-aged patients. Some people may also experience gastrointestinal symptoms such as nausea, vomiting, or diarrhea. Symptoms may vary with disease severity and atypical appearances are reported. In the geriatric age

group and persons with medical comorbidities may have delayed presentation of fever and respiratory symptoms. While many of the symptoms of COVID-19 are common to other respiratory or viral illnesses, anosmia appears to be more specific to COVID-19.

Several studies have reported that the signs and symptoms of COVID-19 in children are similar to adults but are usually milder. Symptomatic management remains the mainstay of treatment in mild and moderate patients of COVID-19. Mild COVID-19 cases may be given symptomatic treatment such as antipyretic-Paracetamol 500-650mg sos for fever and pain, adequate nutrition, and appropriate rehydration. If the cough is present, N Acetylcysteine tab 600mg TDS administered. Sp02 should be assessed in the patient and if it is 92-96% (88-92% in patients with COPD) in moderate COVID-19, supportive oxygen therapy to be started.

Oxygen administration – 2L/min with nasal prong or 5L/min with a face mask. The device for administering oxygen (nasal prongs, mask, or masks with breathing / non-rebreathing reservoir bag) depends upon the increasing requirement of oxygen therapy. If a high flow nasal cannula (HFNC) or simple nasal cannula is used, an N95 mask should be applied over it. Awake proning may be used as salvage therapy.

In severe COVID-19 and acute respiratory distress syndrome or hypoxemia if SpO2 $<$ 90% and respiratory rate $\geq$ 30/min, supplemental oxygen therapy to be started immediately. Initiate oxygen therapy with a face mask with the non-rebreathing bag at 8-10 L/min. Based on SpO2/FiO2 ratio, High – Flow Nasal Cannula oxygenation (HFNO) or non – invasive mechanical ventilation should be done. Patients receiving HFNO should be in a monitored setting and cared for by experienced personals. If the patient acutely deteriorates or does not improve after a short trial (about 1 hr), tracheal intubation and invasive mechanical ventilation should be used on time.

The signs of septic shock to be recognized early and begin standard care within 1 hour of identification: antimicrobial therapy and fluid loading and ionotropic support –Noradrenaline, which should be titrated according to mean arterial pressure. Fluids given at least 30 ml/kg of isotonic crystalloid in adults in the first 3 hours and children give 20 ml/kg as a rapid bolus and up to 40-60 ml/kg in the first 1 hr. Do not use hypotonic crystalloids, starches, or gelatins for resuscitation. Fluid resuscitation may lead to volume overload, including respiratory failure, and hence should be carefully modulated. Use conservative fluid management in patients with severe COVID when there is no evidence of shock.

4.6. Immune Boosters

Augmenting the body's natural defense mechanism plays a vital role in preserving optimum health. In times of COVID-19 pandemic, to date, since no drug or vaccine has been developed, it will be worthy to take preventive measures that boost our immunity. It is known that viral clearance from the body after infection requires a strong host immune response, which could be enhanced by nutrition or immune boosters. It is a well-known fact that nutrition is an influential factor in modulating immune homeostasis. The compromised immune defense may occur due to malnutrition, micronutrient deficiencies commonly by age-related or lifestyle factors which could exacerbate the infection and enhance morbidity and mortality in patients.

VITAMIN C: Vitamin C, a water-soluble vitamin, is well known to support the function and proliferation of lymphocyte cells and augments phagocytic activity and oxidative killing by neutrophils. It has additional antioxidant properties so it can scavenge out harmful reactive oxygen species (ROS) which cause damage to lung tissues, hence this property may be helpful in symptomatic COVID-19 patients. Vitamin C can reduce ARDS, cytokine storms, neutrophils damage, oxidative stress,

alveolar damage, acute respiratory failure, and helps reduce mortality caused due to SARS-CoV-2. It is beneficial in supporting normal neutrophils function and motility, activation of pro-inflammatory transcription factor nuclear factor κB (NFκB) signal cascade, regulation of inflammatory mediators, phagocytosis, gene regulation, and signaling pathways in T-cells. This nutrient helps maintain epithelial barrier function against microbes, support respiratory defense mechanisms, modulates the adaptive and innate immune functions, and protects against oxidative stress thereby conferring a protective benefit in infectious diseases especially viral infections, and reducing their duration and severity. (Galmés S, et al., 2020)

It has been reported that administrating a high intravenous dose of 200mg/kg body weight can reduce clinical symptoms in viral infections. Oral dosage of vitamin C up to 6g per day can reduce the risk of many viral infections and helps to improve health status. According to the study, an i.v infusion of 15 g/day of Vitamin C for 4 days reduces the mortality of patients with sepsis and acute respiratory failure. Thus it was recommended to be used in mild-moderate cases of COVID -19 in a dose of 500mg TDS for 7 days and in severe cases injection of 1.5gm I.V can be administered 6 hourly for 5 days.

VITAMIN D: Vitamin D, a fat-soluble vitamin is synthesized with help of ultraviolet B (UVB) radiation exposure in the epidermis of the skin. Vitamin D plays a dynamic role in innate and adaptive immune responses as vitamin D receptors are expressed by T and B lymphocytes, macrophages and monocytes, and other immune cells. Vitamin D modulates innate cellular immunity by promoting gap junctions, tight and adherent junctions by enhancing the expression of peptides like defensins, adherents, E-cadherin, etc. It helps to maintain the integrity of these junctions and promotes phagocytosis of the bacterial cell. Also, it moderates the adaptive immune response by decreasing the production of pro-inflammatory cytokines, IL-2 and interferon-

gamma (INF-γ) and enhancing the production of anti-inflammatory cytokines by Th2 cells and indirectly suppress the Th1 cell functions.

Many studies have proved that viruses significantly damage the integrity of epithelial tight junctions increasing the risk of infection and spread hence sufficient levels may help to protect the respiratory epithelium from pathogenic invasion, decreasing the risk of infection. In COVID-19 also deficiency of vitamin D has been suggested to increase incidence and severity of infection, therefore, supplementation of vitamin D is suggested to enhance immunity against COVID-19 and reduce acute respiratory distress syndrome induced mortality (Shakoor H, et al.).

According to the European Food Safety Authority (EFSA), the adequate intake of Vitamin D should be 15 µg/day, and the Upper limit not more than 100 µg/day which will cover most nutritional needs.

ZINC: Zinc is a trace element, having dual immunomodulatory and anti-viral properties. It possesses a broad-spectrum antiviral activity by inhibiting RNA-dependent RNA polymerase thus inhibiting the synthesis, replication, and transcription of respiratory viruses and also preventing viral attachment and uncoating by blocking viral proteases. Zinc plays a significant role in augmenting innate and adaptive immunity, including enhancing the recruitment of neutrophils, granulocytes and improving the chemotactic activity and phagocytosis, increasing the number of CD4+ and CD8 + T cells, and develops cell's resistance to apoptosis. It also stabilizes the cell membrane which could inhibit viral entry and fusion. It was demonstrated that Zn deficiency was associated with lower immune functions and deficient antibody production, increased proinflammatory cytokines, remodeling of lung tissue, and modification of cell barrier function in lung epithelial tissues.

Studies have shown that zinc supplementation decreases COVID-19 symptoms and prevents the severity of infection thus it may be of benefit for

prophylaxis and treatment of COVID-19. Certain studies of COVID-19 have confirmed that there is a synergistic action with hydroxychloroquine (HCQ) and chloroquine, thereby increasing the uptake of zinc into infected cells and exerting its action. The recommended dose of zinc in COVID-19 is 50mg OD for 7 days.

4.7. Other nutrients with immunomodulatory action

The anti-oxidant agent's Vitamin E, and trace element selenium, also affect the immune response and viral pathogenicity. Vitamin E and selenium both augment anti-oxidant defense pathways to increase the cytokines secretions IL-2, upsurges the T cells, and enhances its natural killer cell activity, boosts the lymphocytic response thus diminishing the risk of infection. Magnesium also boosts the immune defense mechanism and deters inflammation and prevents the release of inflammatory mediators, thus may be beneficial in preventing cytokine storm in COVID-19. Similarly Vitamin A is known to have promising role in preventing respiratory infections and may be of potential benefit in COVID-19.

Supplementation of diet with these nutrients in COVID-19 infection, have shown favorable consequences and has the propensity to decrease the risk of complications leading to morbidity and mortality.

4.8. References

- Dong L, Hu S and Gao J 2020 Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov. Ther.14 58–60
- Ambrish Saxena. Drug targets for COVID-19 therapeutics: Ongoing global efforts
- Journal of Biosciences volume 45, Article number: 87 (2020)
- Jianxi Luo. Predictive Monitoring of COVID-19. Data-Driven Innovation Lab Singapore University of Technology and Design Updated on May 14, 2020
- Yang S, Fu C, Lian X, Dong X, Zhang Z Understanding Human-Virus Protein-Protein Interactions Using a Human Protein Complex-Based Analysis Framework. mSystems. 2019 Mar-Apr; 4(2):
- Barlow A, Landolf KM, Barlow B, Yeung SYA, Heavner JJ et al. 2020 Review of emerging pharmacotherapy for the treatment of coronavirus disease 2019. Pharmacotherapy
- Pizzorno A, Padey B, Terrier O and Rosa-Calatrava M 2019 Drug repurposing approaches for the treatment of influenza viral infection: reviving old drugs to fight against a long-lived enemy. Front. Immunol. 10 531
- Bernatchez JA, Tran LT, Li J, Luan Y, Siqueira-Neto JL et al.(2020) Drugs for the treatment of Zika virus infection. J. Med. Chem.63 470–489
- Omotade TO and Roy CR 2019 Manipulation of host cell organelles by intracellular pathogens. Microbiol. Spectr.7
- Tortorici MA and Veesler D 2019 Structural insights into coronavirus entry. Adv. Virus Res.105 93–116

- Goo J, Jeong Y, Park YS, Yang E, Jung DI, et al. 2020 Characterization of novel monoclonal antibodies against MERS-coronavirus spike protein. Virus Res.278 197863
- Saha P, Banerjee AK, Tripathi PP, Srivastava AK and Ray U 2020 A virus that has gone viral: amino acid mutation in S protein of Indian isolate of Coronavirus COVID-19 might impact receptor binding, and thus, infectivity. Biosci. Rep.40
- Beigel JH, Tomashek TM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 — Preliminary Report. N Engl J Med. 2020; DOI: 10.1056/NEJMoa2007764.
- Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med. 2020; DOI: 10.1056/NEJMoa2007016.
- Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2020; 395(10236):1569-1578.
- Goldman JD, Lye DCB, Hui DS, et al. Remdesivir for 5 or 10 days in patients with severe COVID-19. N Engl J Med. 2020. Available at: https://www.ncbi.nlm.nih.gov/pubmed/32459919.
- Gilead announces results from phase 3 trial of investigational antiviral remdesivir in patients with severe COVID-19. https://www.gilead.com/news-and-press/press-room/press-releases/2020/4/gilead-announces-results-from-phase-3-trial-of-investigational-antiviral-remdesivir-in-patients-with-severe-covid-19.

- Cai Q, Yang M, Liu D, et al., Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering. 2020;https://doi.org/10.1016/j.eng.2020.03.007.
- Chen C, Huang J, Cheng Z, et al. Favipiravir versus arbidol for COVID-19: a randomized clinical trial. 2020.Website;https://www.medrxiv.org/content/medrxiv/early/2020/04/08/2020.03.17.20037432.full.pdf. Accessed 12 May 2020.
- Ivashchenko AA, Dmitriev KA, Vostokova NV, et al. AVIFAVIR for Treatment of Patients with Moderate COVID-19:Interim Results of a Phase II/III Multicenter Randomized Clinical Trial. https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1176/5890024. Accessed on 10 September 20
- Chan KS, Lai ST, Chu CM, et al. Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicentre retrospective matched cohort study. Hong Kong Med J. 2003;9(6):399-406.
- Patel A, Desai S, Grainger D, Mehra M. Usefulness of Ivermectin in COVID-19 Illness. 2020 Apr 19. Available at: SSRN 3580524.
- India e Trial Site News [Internet]. President of Dominican Republic's largest private health group discusses the success of ivermectin as a treatment for early stage COVID-19 [cited 19 July 2020]. Available from: https://www.trialsitenews.com/ president-of-dominican-republics-largest-private-healthgroup-discusses-

the-success-of-ivermectin-as-a-treatmentfor-early-stage-covid-19/; 2020 Jun.

- Rajter JC, Sherman M, Fatteh N, Vogel F, Sacks J, Rajter JJ. ICON (Ivermectin in COvid Nineteen) study: Use of ivermectin is associated with lower mortality in hospitalized patients with COVID-19. medRxiv. 2020 Jun 09.
- Chowdhury AT, Shahbaz M, Karim MR, Islam J, Dan G, He S. A comparative observational study on Ivermectin-Doxycycline and Hydroxychloroquine-Azithromycin therapy on COVID19 patients [Preprint] [cited 2020 July 19]. Available from: https:// www.researchgate.net/publication/342159343; 2020
- Treatment Plan Edition 5. 2020. http://www.gov.cn/zhengce/zhengceku/2020-02/05/5474791/files/de44557832ad4be1929091dcbcfca891.pdf. Accessed Aug 30, 2020
- Wang Z, Yang B, Li Q, et al. Clinical Features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin Infect Dis. 2020; doi:10.1093/cid/ciaa272
- Deng L, Li C, Zeng Q, et al. Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: A retrospective cohort study. J Infect. 2020;https://doi.org/10.1016/j.jinf.2020.03.002
- Padmanabhan S. Potential dual therapeutic approach against SARS-CoV-2/COVID-19 with Nitazoxanide and Hydroxychloroquine.2020;

https://www.researchgate.net/profile/Srivatsan_Padmanabhan/publication/339941717.

- Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open label non-randomized clinical trial. Int J Antimicrob Agents. 2020; https://doi.org/10.1016/j.ijantimicag.2020.105949.
- Gautret P, Lagier J-C, Parola P, et al. Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: a pilot observational study. Travel Med Infect Dis. 2020;34:101663.doi: 10.1016/j.tmaid.2020.101663.
- Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020; doi:10.1001/jamainternmed.2020.0994
- Ledford H. Coronavirus breakthrough: dexamethasone is first drug shown to save lives. Nature News. 2020;https://www.nature.com/articles/d41586-020-01824-5. Accessed June 22, 2020
- Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A. 2020;117(20):10970-75
- Toniati P, Piva S, Cattalini M, et al. Tocilizumab for the treatment of severe COVID-19 pneumonia with hyperinflammatory syndrome and acute respiratory failure: A single center study of

100 patients in Brescia, Italy. Autoimmun Rev. 2020;19(7):102568.

- Zhang C, Wu Z , Li J-W, et al. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. Int J Antimicrob Agents. 2020;55(5):105954.
- Sciascia S, Aprà F, Baffa A. Pilot Prospective Open, Single-Arm Multicentre Study on Off-Label Use of Tocilizumab in Patients With Severe COVID-19. Clin Exp Rheumatol. 2020;38(3):529-32.
- Guaraldi G, Meschiari M, Cozzi-Lepri A, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol. 2020;https://doi.org/10.1016/S2665-9913(20)30173-9.
- Atallah B, Mallah SI, AlMahmeed W. Anticoagulation in COVID-19. European Heart Journal - Cardiovascular Pharmacotherapy. 2020;(pvaa036). 10.1093/ehjcvp/pvaa036.
- Lin L, Lu L, Cao W, Li T. Hypothesis for potential pathogenesis of SARS-CoV-2 infection–a review of immune changes in patients with viral pneumonia. Emerging Microbes & Infections. 2020;9(1):727–732. doi: 10.1080/22221751.2020.1746199.
- In vitro antiviral activity of doxycycline against SARS-CoV-2 – IHU. Available from:https://www.mediterranee-infection.com/invitro-antiviral-activity-of-doxycycline-against-sars-cov-2.

- Di Caprio R, Lembo S, Di Costanzo L, Balato A, Monfrecola G. Anti-inflammatory properties of low and high doxycycline doses: an in vitro study. Mediators Inflamm. 2015;2015:329418.https://doi.org/10.1155/2015/329418.
- Galmés S, Serra F, Palou A. Current State of Evidence: Influence of Nutritional and Nutrigenetic Factors on Immunity in the COVID-19 Pandemic Framework. Nutrients 2020, 12, 2738; doi:10.3390/nu12092738.
- Shakoor H, Feehan J, Dhaheri ASA et al. Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19? Maturitas 143 (2021) 1–9. https://doi.org/10.1016/j.maturitas.2020.08.

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Chapter five: Personality between Normality and Abnormality under Corona Pandemic

5.1.Normality and Abnormality

Psychologists see that the comprehensive definition of personality is All Port's definition as the dynamic organization within the individual of all psychophysical and physical systems that determine the person's adjustment with his/her environment. (Atta, 29, Alaulddin, 710).

Situations of appropriate adjustment refer to psychological health while inappropriate adjustment situations refer to disorder of psychological health. (Abdulaziz, 3). Normal behavior is the familiar and dominant behavior of people who encounter a situation that conforms to the most common behavior within the limits of accepted behaviour. For example, if someone is infected with Corona virus, the situation will be sad for the family members. But, when a family member laughs instead of being sad, we will, no doubt, be astonished and the behaviour will be considered shameful and unfamiliar. (Al-Rifay: 56)

The identification of this behavior is not everything since encountering the situation is something related to person, his experiences, and the environment conditions surrounding people. Moreover, the situation is related to the way used to encounter the situation. (Attallah: 100). Also, superstitious behaviours that deal with the pandemic is a kind of abnormal behavior according to the international health standard. Some people resort to accept false information about the pandemic, which motivates people to underestimate the seriousness of pandemic.

To specify the behavior as being abnormal faces many difficulties due to the factors and elements interference within behavior in addition to the proximity of normality to some degrees of abnormality. There is an urgent need to identify the criteria of normal and abnormal behavior. (Al-rifay: 57)

5.2.Criteria of Normality and Abnormality

5.2.1.Self- criterion

When we watch people's behavior and if the behavior conforms with our views, ideas, and self-reflection, we will describe it as a normal one. Otherwise, we consider it abnormal. For instance, when we see a person sneezing in front of a pharmacy, from the outset, we will fear, worry, and judge him as being infected with Coronavirus. Moreover, the person's ideas and beliefs create his/ her knowledge system that controls his/her self-evaluations of what he/ she sees or observes. These observations create an objective vision, which confirms the health standards. But, when a knowledge system about the pandemic provides him/her with distorted information, it leads him/ her to underestimate the prevention measures, which will be the worst. There is no doubt that attending a party is normal behavior that conforms to the social values on the contrary to not attending the party which will be a violation of social norms.

5.2.2.Statistical Criterion

It depends on the repetition of behavior and prevalence among people. The behavior of the majority group is a normal one while the behavior of a minority group is abnormal. Normality is the average or the common behavior but deviation of this average is the abnormality. For example, when I see most

people wear a face mask and I try to follow their behavior, it is called normal behavior but fewer people do not wear masks and this is abnormal behavior.

5.2.3. Ideal Criterion:

It is the positive trend of identifying the manifestations of normal behavior. When someone takes all precautionary measures of dealing with the Corona pandemic whether it is health or psychological ones, we say that his/ her behavior is normal. Another person is careless of all preventive measures; we will say that his/ her behavior is abnormal.

5.3. University Students' Psychological Situation under Corona Pandemic

In all ages, humanity passes through a fearsome and unknown disease. For example, leprosy in the old ages, plague in the medieval ages, tuberculosis during the industrial revolution, HIV, pneumonia, and Covid 19 as known by Corona is the last one.

Covid 19 dominates all aspects of life to the extent that it invades streets, surfaces, and walls. It surrounds man everywhere, who stands powerless and panic. Here, the problem begins. When there is a crisis, there should be a scientific evaluation. We should question ourselves; what does affect us more? We should identify the type of feelings we feel. Is it fear? Of what? Or is it an anonymous source of psychological anxiety that intensifies our nerves?

The individual's psychological situation is closely related to the immunity system. As the psychological situation worsens, the immunity system is affected negatively.

- Carelessness: Accumulative frustration of people turns them to be careless of the virus. Moreover, Iraqi society co-exists with crises, wars, embargo, and other economic and social changes. All these dramatic situations convince society that they are immune to plights and risks. This kind of carelessness becomes a collective behavior that includes most of the society classes, young or old.
- Shock: When a virus spreads all over the world like wildfire, the health systems of super nations collapsed. At that moment, ideas are falling apart and groundless allegations appear. But, they are all fall before this gigantic killer, which reaps people, young, baby or old. The symptoms appear as (headache, fever, dry cough, shortness of breath, muscle pain, throat infection, diarrhea, smell, and taste loss).

  The peoples' awareness is not developed to the level of a pandemic. Instead of staying at home, the suspects visit hospitals, which spreads virus among the infected, toucher, and suspect. Many people still live in illusion and stultify facts to the extent the infected person does not know that he/ she is infected and starts to spread the virus among his/ her family, relatives, and friends though he/ she has an internal feeling that he/ she is infected. Therefore, health facilities face this great wave of infected people without the minimum requirement of prevention or necessary infrastructure until they reach the stage of the collapse of individuals or government, especially in the developing countries. This leads to a situation of chaos, confusion, and panic especially in this stage of the pandemic since it is out of expectation and represents a threat to man's existence.
- Adjustment and recovery stage: Crises management is to adapt and adjust to various factors that initiated the crisis. The individual who is

unable to control or handle his/ her crises whether by amplifying the crisis value or accept it, / will live in tension. What happens can be used for our side if we think positively; to accept what happens and move forward to the internal peace that enhances resistance against this virus and improves psychological health.

- Frustration: The individual lives in his environment and interacts with it. He adjusts with it and adapts it for him. Since the man's motives are many, he feels happy when he's able to satisfy his motives. If the motif is urgent and the impediment is tough, the distress, pain, and tension will be severe due to the inability to achieve goals and the feeling of failure and surrender.

Frustration is an emotional expression that includes an individual's realization of the impediment that prevents him to satisfy his need or expect this impediment in the future as the case of a threat, struggle, oppression, or confusion.

Under Corona pandemic, in the midst of exceptional conditions, and the influence of audio-visual media, which affects individual's knowledge system as the doctor's statements about Corona spread that refer to the mortality of the pandemic of all ages who fall like leaves in Autumn, fear escalates among health sector employees in addition to politician's declaration to be ready to leave your beloved ones. Moreover, there are so many cases of health quarantine and isolation that contradict human nature, the imposition of curfew to limit virus infection, and the suspension of study at universities and schools. All these situations create a horrible atmosphere of anxiety, tension, and psychological traumas. As a result, the expectation of pain becomes more painful than the pain itself since everyone is afraid of death.

Based on what is mentioned earlier, the pandemic severely affects us whether we are aware of it or not, and it turns us to be less ambitious for life and more desire to erupt out of rage or isolate. These contradictory feelings lead to despair, surrender, and loss of faith, which will threaten the infected psychological immunity to tolerate pain, adjustment process, and psychological health.

5.4. Classification of Frustration

Primary and Secondary Frustration:

Corona infected person may find himself seeking to satisfy a need but the topic of satisfying it is unavailable so it is called primary frustration. For example, when an infected person goes to a hospital to receive treatment but he is surprised to find that the medication is not available or inactive. This kind of frustration creates a feeling of fear, panic, and weak immunity.

If the need is found and the topic required to accomplish it is available but there is an impediment that prevents it, such frustration is called a secondary one. For example, when the patient goes to the doctor but is unable to pay the lab. fees, MRI, or prescription.

Internal and External Frustration:

Frustration may inflict Corona infected person due to internal factors like internal indigence, inability to work, internal disability, loss of self-confidence, anxiety, panic, and fear especially when one of the body internal organs like stomach, lung, throat, respiratory system, and disorder of smell and taste. All these situations are considered internal frustration that affects infected life.

Frustration may be external due to factors like external indigence as a shortage of house needs, loss of a job to satisfy daily needs of the family, death of beloved one because of Corona, hearing pessimistic news via social media or quarantine and never goes out of the home. All of these are examples of external frustration.

Positive and Negative Frustration:

When there is a tough impediment accompanied by a threat that precludes satisfying an urgent need, it will leave an effect on the person. For example, someone is unable to meet his friend due to a virus infection. If the need is available and there is something that impedes it; there is an obstacle without threat, this will be called negative frustration. For instance, someone attempts to exercise at home to minimize the activity of the virus, but he feels tired and fatigue in the first 15 minutes. We understand from this example that the reasons for frustration may be internal or external. Also, the obstacle might be private or public conditions for a great group of people.

Frustration situation is different due to the individual's ability to tolerate. Therefore, the individual's realization of frustration depends on his trust in the environmental conditions that surround him. Through observing the reaction of Corona infected persons and their behaviors, we have noticed that some of them, and out of frustration, to be aggressive against nurses in hospitals or against other members of the family as disclosed by some of these families. The aggression may be verbal or physical against one of the family members or break things in front of him.

Studies have shown that the frustrated person has symptoms of severe gloom, the melancholy of his life and future, absence of desire, fluctuation of

mood, degradation of the self, and fatigue. Moreover, there are other symptoms like loss of appetite, sleep disorder, and continuous worry.

Therefore, psychological therapy is more important than medications. A lady in her 60's is surprised to catch the virus through her contact with one of her relatives, who died. The lady moved to the quarantine hospital and she receives there psychological support. She reveals that the psychological situation is more important than medication besides in her faith in God that this is her fate. After 10 days, she conquers the virus and sent home. (We fight two enemies)

As there are physiological symptoms, any one of us has psychological symptoms especially students including:

- Psychological anxiety (expecting pain, sometimes, is more painful than the pain itself)
- Tension
- Fear of future
- Death anxiety
- The anxiety of separation from others
- Confusion and exam phobia.
- Study anxiety
- Fear of infecting family members
- Expecting pain, sometimes, is more painful than the pain itself.
- Depression
- Compulsive obsession with using sanitizers.
- Use defensive mechanisms
- Loss self- confidence

- Weak will
- Panic and horror
- Knowledge distortion of ideas and subjects.
- Generalization and exaggeration
- Sadness, despair, hopelessness, and negative tendency towards (self, others, and future).

Psychological Conflict:

The individual in his reaction and attempt to adjust with his environment whether it is social, metaphysical, or natural, faces many conflicts and contradictory motives. He stands confused because he does not accept conflicts or they are not accepted by society and its moral system. The process of satisfying motives may lead to a kind of anxiety and disorder. The adjustment process may be accomplished rapidly and the conflict could be ended or developed to the maximum degree, and the conflict may be emotional.

A conflict is a situation when the individual is unable to satisfy two urgent or more motives that cannot be satisfied at the same time. It is natural that the infected person will face certain contradictory situations before, during the infection, and maybe after recovery.

Approach- Approach Conflict:

It happens when the individual has two positive motives or desires that one contradicts with the other, and getting one may lead to the loss of the other. For example, when the infected person, as a preventive measure, has two choices, either to go to the pharmacy to buy medications or herbs shop for the same purpose. Decision- taking in such a situation of conflict will be

easy and has no impact since the decision maker will figure out the solution depending on the benefits of the two medications.

Avoidance- Avoidance Conflict:

The infected person with Corona virus finds himself in front of two negative motives, where both of them cause harm for him. If he attempts to avoid one of them, he will fall in the second. For example, an infected person oscillates between his desire to work in his office though he knows or suspects of his infection and his other desire to stay at home but he knows the risk on his family members.

Approach- Avoidance Conflict:

The infected person finds himself in the midst of psychological conflict when he encounters two-faced motives, one of the two faces is a positive and the other is negative one. For instance, the doctor writes the prescription that treats his lung function but it may harm the function of liver. This kind of conflict is the most dangerous one since it leads to depression and anxiety.

Approach- Avoidance Double Conflict:

The infected finds himself in front of two desirable goals but each one of them includes negative elements that make their achievement is risky. The individual is confused and hesitant to make a decision. For example, the sick person conflicts with going to the hospital for free treatment and there is the possibility of infection or going to a certain expensive doctor to receive treatment.

Unconscious Conflict:

In most of the above-mentioned cases of conflict, they happen consciously but others are kept in unconscious levels that are embedded deep in the personality between, ID the source of desires and instincts, and superego represented by consciousness and the ideal picture the person aspires to achieve in addition to the sovereign ego that takes the decision. When the ego weakens and the moral restraints are absent in front of the strength of desires and instincts, the conflict prevails and the ego worries about encountering reality. So, the person resorts to adopt adjustment mechanisms in his environment.

5.5.Methods of Abnormal Adjustment:

When the person faces certain pressure whether it is a conflict, frustration or imminent danger that is happened or expected to happen. When he fails to deal with it, he will resort to unconscious defensive mechanisms to mitigate the pressure and adapt with his social environment. Under Corona pandemic, we have found many infected people resort to these tricks as:

Denial: the infected attempts to convince himself and others that Corona is a conspiracy and it manipulates with economy in favour of certain categories and countries.

Projection: the infected attributes his defects to others, luck, and invisible powers. This kind of behavior is a confession more than accusations that he attempts to mitigate the severity of frustration or the negative expectations.

Repression: Corona infected person tries to eliminate fruitless and sick ideas that hurt his feelings and moves his thinking from conscious into

unconscious zone. He tries to bury his negative ideas that he feels like fear, anxiety, and tension. But they appear in form of emotions and unexpected behaviours.

Reaction Formation: the infected person tries to hide the motives that are not acceptable in society through repressing them in the unconscious. He expresses himself through exaggerated anti- motives. For example, when the sick person is controlled by the infection and fear, we will find him clap as if he is happy.

Displacement: when the situation of corona virus infected person accentuated, he tries to move his rage and reactions to another person as a defensive mechanism. For instance, he freed the beast all over his sons or wife in an attempt to release the internal tension.

Sublimation: due to the conflict, frustration, and pressure the infected person suffered from, he attempts to find more acceptable ways for the self and others to get rid of these pressures and avoid confusion.

Regression: when the infected person faces such psychological and physical pressures, he resorts to or regresses to an earlier stage of his age to get comfort.

Compensation: a set of reactions by the infected person, which are intended to appear in certain situation or cover another feature. The sick person covers his situation and emotions using more acceptable behavior like laugh or different activity to compensate the shortage he has.

Rationalization: it is the infected person attempt to rationalize his behaviours and actions using reasons that seem logical and reasonable

but the real ones are emotional. It is an attempt to support his self to end the conflicts and mitigate frustrations.

Generalization: the sick person generalizes his experience as bad one over similar experiences as a trick to decrease tension and avoid pains.

The aforementioned defensive mechanisms are methods of satisfying the self and temporary solutions to avoid failure. They work unconsciously and can forge reality. There is no harm to use properly but if it is used excessively, it may affect the individual's behavior and turns to be dangerous. Defensive mechanisms of infected persons are similar to tranquilizers that do not treat disease but reduce pain. We realize that anxiety of the infected person weakens his immune system and this is the appropriate time to employ defensive mechanisms to protect the self and the immunity system. But, it may lead to the reverse when it debilitates his immunity system and his ability to adapt to the disease.

5.6. Individual's Psychological and Social Adaptation with Corona Pandemic

The comprehensive definition of psychological health is a set of fundamentals that work together to help the person adapts and satisfies himself in addition to his adaptation with the environment that leads to the person's happiness and society development. All living organisms tend to change their responses when environmental factors change and adopt new and suitable ways of living.

I. Psychological Adaptation: it is the person's ability to harmonize with his mental, emotional, physiological, and social components of his character, which leads to the feeling of health and psychological tranqulity.

II. Social Adaptation: it is the ability to change and deal with the different social conditions and the response to the requirement and social development. It is the ability to co-exist with the new society that he will live with his family, traditions and customs. Co- existence and adaptation with Covid 19 becomes a de facto. It seems that the world is convinced that this pandemic will take long time and the return to normal life is a necessity now. So, the responsibility is on the shoulder of:

A. The government, which has to enact laws and legislations that limit the spread of the pandemic.

B. The individual has to bear responsibility to protect himself.

All humanity has undergone the trauma and it is over. Now, it is the time of psychological adaptation. The most prominent signs of co-existence with Corona is that several countries that witnessed spread of Covid 19 (Germany, France, Spain, Italy, and UAE) have reduced closures and open shops, parks, stadiums, and beaches. They have to learn how to co-exist with the virus and protect themselves.

1- Stage of carelessness and feeling of illusion.
2- Trauma (shock) stage.
3- Stage of co-existence and adaptation.

Prophet Mohammed (All Prayers and Blessings of Allah be upon him and his pure progeny) has said that: "If you get wind of the outbreak of plague in a land, do not enter it; and if it breaks out in a land in which you are, do not leave it" Almighty Allah Has said that: "Say: "Nothing will happen to us

except what Allah has decreed for us: He is our protector": and on Allah let the Believers put their trust.” (At-tauba: 15)

Allah’s fate is inescapable.

- The greatest psychological challenge that faces the infected is the feeling of boredom, loneliness, depression, and inability. So, it is important not to abandon the infected alone and try to communicate them electronically.
- Some people underestimate the disease or careless about it. Whenever the man becomes mature, he accepts the responsibility to maintain social safety. If he is reckless and indifferent, it reflects his lack of aware and narcissism.
- There is a Japanese proverb says that crises are chances. This crisis is a chance to learn the important values of society. It turns man and society to be stronger and show the good characteristics of people as volunteering and social responsibility.
- The pleasure of warm drink and relaxation on the couch or read a book is what is needed to recharge his energy and reach internal equilibrium.
- Do any work like cooking, drawing, designing or learning another language.
- Take care of your body and exercise regularly.
- Follow a reliable source of information and make your readings and watching limited.
- Eat your meals with sufficient water.
- Never approach smoking.
- Try to face hard times wisely. It is ok to know that there are things out of your hands but you can concentrate on things that can be controlled.

- Do things that give you interest and enjoyment.
- Focus on the present time and remember this thing is a temporary one.
- Ensure to get enough sleep daily.
- Have faith, hope, and try to take lessons through reading inspiring stories and others' experiences to overcome difficulties.
- Additional help as talking to a close friend or one of the family members you trust.
- Acclimatize yourself with sadness and loss. (Al-Ajoz, 2020).
- Enhance self-trust and psychological ability to endurance and confrontation.

2-Introduce information and psychological and health education when dealing with crises.

3-provide basic needs for individuals including (psychological and social) like physiological, security, social status, self-esteem, and self-respect.

4- When the person feels panic, horror, and fear, he can improve his psychological situation by reciting the Noble Quran verse or Prophet's hadith.
5- Awareness: the way the individuals interpret the events to have a direct effect on their psychological situation, where negative realizations disturb the mind but the positive one improves the person's mind and increases his sense of safety.
6- Most people are improved by themselves.
7- Keep in touch with your friends and your family members through any communication means.
8- Talk about the reasons for your concern.

Studies have shown that individuals are subject to more psychological disorders than a physical ones. So, it is necessary to support them to reinforce personality and accept the reality to co-exist with it. They have to work to overcome the problem with steadfastness. Individual's levels (cognitive dimension, affective dimension, and behavioral dimension)

5.7.Advices based on the data with the continuity of the pandemic

1- Do not sit watching media for hours.
2- Turn to Almighty Allah and do your religious customs since they create a secure psychological environment.
3- Practice sport that results in psychological and physical relaxation.
4- Speak positively and increase positive energy among your family members. Remind them that his crisis does not last forever and it is a temporary one.
5- Perform hobbies and read what you like.
6- Reduce receiving negative news that will lead to horror.
7- Do house tasks that we do not have time to perform in the past.
8- Attempt to learn new hobbies or create a new routine until this crisis is over.
9- Pay attention to the value of the present moment and the value of health.
10- Ask others' help whether they are close or ask for psychological help through behavioral and cognitive therapy via the internet.
11- Express your feelings and psychological pains because repression leads to depression or other psychological disorders that the effects appear later.

5.8.Advices for Adaptation

A- Diversify home activities to fight routine and boredom.

B- Change clothes: people believe that since they are at home, he will wear sleep clothes and does not try to change. It is a negative indicator and we should change clothes to look at life optimistically.

C- Positive talk inside the home: the talks should not focus on viruses but on positive aspects of life, which improves psychological health.

- Build up a social positive relationship with others through social media and discuss positive subjects and how to encounter this virus and give advice about it.
- Depend on the health system to reinforce the immunity system.
- Sleep should be natural, neither too much nor very little.

1-Accepting Quarantine:

Look at the quarantine as natural behavior the person follows to secure his psychological safety and security. It is not a punishment but a positive behavior. It is a chance to discover the self and its ability to endure the situation and find alternatives.

2-Avoid Addiction to Virus News:

It leads to anxiety, tension, and depression. We should not focus on the negative news instead of a positive one as the number of recovered people who overcome the dangerous stage.

1- Avoid believing rumors:

This situation is prevalent in social media and spreads like wild fire because it leads to negative frustration rather than a positive one.

4-Focus on well-informed information from official sources, in reverse, frustration comes from unreliable sources.

5-avoid excessive use of social media: it leads to frustration, despair, surrender, and inability to think clearly.

1- Safe shake hands as waving or nodding.
2- Never go to a restaurant, café, parties, and social occasions.
3- Keep students away from their grandfathers for a specific period.
4- Keep two meters distance in trains and gatherings.
5- Put a tissue on the mouth and nose during sneezing and coughing.
6- Avoid going to surgeon's clinics, pharmacies, and hospitals.
7- Avoid gathering and crowd.
8- Man's behavior has to be flexible to find alternatives to communicate with life requirements so as not to be a victim of anxiety and tension.
9- People are not intensified because of their fear of death or physical harm only, but they are afraid of inability to work, isolation, and social elimination. If it is heard that he is sick or in quarantine, he will be afraid of losing his job.

- Corona crisis is an opportunity to learn and reinforce social ties.
- If psychological health is improved, physical health will improve and the reverse is correct.
- Tension, anxiety, fear, panic, and confusion may turn psychological pressure into feelings of rage and threat.
- Whenever a person's responsibility increases, he follows the instruction accurately.
- Man, by nature, is a social creature.
- Home quarantine may turn out to be an unforgettable vacation.

Chapter sex: Immunology in pregnancy

Pregnancy comprises a specific immunological condition, that help to protect the fetus from possible maternal rejection, allowing normal fetal development and protecting against microorganisms (1, 2).The maternal immune system is triggerd by paternal alloantigens expressed by the fetus and the placenta. However, the mother does not develop a classic response to this allograft (3).

During pregnancy, fetal microquimerism occurs, where some fetal cells, including nucleated erythrocytes, trophoblastic cells, and leukocytes (3), cross the fetoplacental barrier and expose the mother to fetal alloantigens. These cells can remain in the maternal bloodstream and tissues for many years after delivery (4, 5). During normal pregnancy, hormonal variations can modulate immunological responses, generating a decline in the number of DCs and monocytes, and a reduction in the activation of macrophages, T, and B cells (6).

During the antenatal period there is increases monocytes, granulocytes, pDCs, mDCs in the blood, the maximum changes seen during second trimesters in comparison to postnatal period. also during pregnancy there is a reduction in CD3, CD4, and CD8 T cells in comparison with post-natal period. B cells are also decreased during the third trimesters. NK cells CD56 dim may be reduced in the second and third trimester of pregnancy vs the first trimester and post-partum period. During the second half of pregnancy, NK and CD4 T cells present a reduction in the production of IFN-γ, TNF, IL-6 cells, compared with post-partum (7)

6.1.Viral infection in pregnancy:

Perinatal outcomes after viral infections during pregnancy may range from no adverse effect to pregnancy loss by abortion to fetal infection which may lead to congenital viral syndromes. The importance of understanding the possible role of viral infection during pregnancy is becoming more obvious as growing risks of pandemics, which may significantly affect both the pregnant mother and the fetus[8]. some epidemiologic evidence suggest that pregnant women are at higher risk of severe illness and mortality from viral infections[9,10], specially during pandemics such as influenza, EBOLA and Lassa fever[11,12]. Furthermore, viral infection may predispose to preterm labor and preterm delivery[13,15]

Many factors can affect the incidence, and severity of viral infection at the maternal-fetal interface. Viruses can gain access to the cells within the decidua and placenta by ascending from the female lower reproductive tract or through hematogenous transmission Following access to the upper reproductive tract, viral tropism for either the decidua or placenta will depend on both viral entry receptor expression by the cellular component of tissues and the specific maternal immune response to the virus. These factors vary by cell type and gestational age at which infection start and can be affected by changes to the in utero environment and maternal immunity. Therefore, the virus-host interaction is complex and highly variable during the pregnancy.(16, 17). Placenta may play a role in immune response, Pregnant women represent an immunologically unique person because their immune system is affected by different signals originating from the placenta[18]. However, this modulation exerted by placenta is not suppressive, but protective to the fetus. so the maternal immune system is well prepared to

control infections and ensure the survival of the fetus. But sometimes the placenta is also a target for viral infections. Recent researches suggest that although the placenta can be infected by different viruses it has a unique capacity to prevent extention of the virus and transmission to the growing fetus, [19,20,18].

Changes in maternal hormonal levels and immune system function may increase women's susceptability to infections. Pregnant women show higher mortality rates and complications associated with viral infections if compared to the general population (21,22). For example, varicella disease in children is mild, but primary infections during pregnancy can progress to varicella pneumonia and even death (23).

6.2. Fetal and neonatal immunity and viral infections:

The World Health Organization (WHO) states that about 2.5 million infants died within the first month of life in 2018(24). The majority of deaths are due to preterm birth, intrapartum-related complications like birth asphyxia , infections and congenital birth defects. Regarding the infection observed in early-life, it is generally due to an immature immune system of infant during the transitional post-natal period (25).

Around 5 weeks of pregnancy, neutrophils are present in human fetal liver parenchyma (26), , neonatal neutrophils have qualitative and quantitative impairments in the response during stressful conditions, like reduced chemotaxis, respiratory burst, and extracellular traps formation when compared to the adult response (27). The cytokine production by antigen-presenting cells (APCs) monocyte andmacrophage in newborn differs from those produced by adults. Typically, antigen-presenting cells from neonates

can produce less pro-inflammatory cytokines like IL-1β, TNF-α, IL-12p70, and type I IFN (28). Otherwise, it produces great amounts of Th17-promoting cytokines (IL-6 and IL-23) when compared with adult response (29). Also great amount of IL-10 produced by newborn monocyte/conventional DC (cDC) compared to adults (30).

Neonates display an immature immune response, the first exposure to an environmental life can trigger the lung's immune response (31). Furthermore, there is a predominant type 2 immune response in the lungs (32), these characteristics make infants susceptible to respiratory viral infections, a common cause of infant's death (33)

6.3.Covid 19: fetal and maternal immue response

In pregnancy, pneumonia may be the cause of an increased mortality to the mother and fetus (34), which can also lead to serious complications like preterm birth and small for gestational age infant (35). Placental syncytiotrophoblast cells highly express the ACE2 receptor in the first half of pregnancy. Both placental immaturity and the early ACE2 expression can make the first trimester the most likely time for SARS-CoV-2-infection (36)

Pregnancy has been one of risk factor for increased illness and death for both pandemic and seasonal influenza[9]. Mortality rates among pregnant women in the pandemics of 1918 and 1957 appeared to be abnormally very high[37,38]. Among 1,350 reported cases of influenza infection among pregnant women during the pandemic of 1918, the proportion of deaths was 27%. Similarly, among a small case series of 86 pregnant women in Chicago for influenza in 1918 hospitalized, 45% death rate[37]. Deaths in Minnesota during the 1957 pandemic among pregnant women, influenza was the leading cause

of death, nearly 20% of deaths associated with pregnancy during the pandemic; half of women who died were pregnant[38]

During the influenza pandemic of 1918, high rates of spontaneous miscarrige and preterm labor were reported[37, 39], especially among women with pneumonia (in one study, >50% of pregnancies in which the pregnant woman had influenza complicated by pneumonia were not reach successfully to term)[37]. In 1957 pandemic the Asian influenza, studies suggested a possible increase in the central nervous system abnormalities[40,41–]and several other adverse pregnancy outcomes, including spontaneous abortion[41], fetal death, and preterm delivery[42]. Studies of the deliterious effects of seasonal influenza infection on the fetus have been contraversy. A small increased risk for birth defects have been observed in some but not all studies[43].

Many reports stated that symptomatic infected-mothers did not transmit the virus to their fetus during pregnancy. seven cases in case report study, showed that three babies were tested to SARS-CoV-2 and only 1 baby was positive within 36 hours post-partum (44). On the other hand, another study shows increase in inflammatory cytokines and virus SARS-CoV-2-specific IgM levels in newborns, from infected-mothers, in first few hours after birth (45), and Zeng H and Xu C study report that newborns presented virus-specific IgM and IgG, but no SARS-CoV-2-infection (46). This may be due to the possibility of the activation of the maternal immune system by SARS-CoV-2 may have some implication of the infant's health and immune system development later on.

there is no conclusive report of vertical transmission(47,48) and A recent case report, was described two cases of rashes and one with facial ulcerations (49). COVID-19 infection can progress to a severe lung

inflammation that can progress to life-threatening illness at the late stage (50). This inflammatory process is associated with markedly increase plasma levels of cytokines (cytokines storm) (51).

This might play an important role in pregnancy as IL-2 has been founded to be upregulated in pre-eclampsia (50) and abortion (52) and IL-7/IL-7R signaling pathway in abortion (53), due to the upregulation in the ratio of Th17/Treg cells (54). Also, cytokines polymorphisms, such as TNF-α 308G/A (rs1800629) polymorphism may be associated with recurrent miscarriage (55).

In fact, TNF-α and TNF-α receptor proved to play an important role in the development of the fetus in utero, being present in the ovary, , placenta, endometrium, and fetus, and in different concentration in the amniotic fluid according to gestational age (56) and This changes in TNF-α during pregnancy may influence different health outcomes depending on the stage of pregnancy (57), that may result to tissue necrosis in the placenta and fetal hypoxia (58). On the other hand, The use of antiviral drugs can permanently affect immune response of the newborn (59) and till now no standard protocol regarding the use of antibiotics or antivirals (35).

6.4.Physiological changes in pregnancy:

Many physiological changes and anatomical changes of pregnancy may affect the respiratory system and increase pregnant women susceptibility to various infections, which may interfere with diagnosis of COVID-19 or the clinical course of the disease. The pregnant women may develop pregnancy-associated rhinitis and physiologic dyspnea which may delay the diagnosis of COVID-19 (60). Also the lung functional residual capacity was decreased

in pregnancy which may result in a relative inability to clear respiratory secretions (60). pregnant are not necessarily more prone to viral illness; however, immunosuppression changes during pregnancy may affect severity of symptoms, particularly towards in last trimester[61, 62.] the major hormone of pregnancy including estrogen and progesterone increase angiotensinogen and renin, leading to an increase in ACE-2 levels that may facilitate COVID-19 infection (63, 64).

6.5.Antenatal and postnatal Clinical manifestations of covid-19:

systematic review of 86 studies evaluating clinical manifestations and pregnancy outcomes of SARS-CoV-2 infection in pregnancy showed that

common maternal characteristics include:

- maternal age > 35 years
- Black, Asian, or minority ethnicity in 50.8%
- obesity (body mass index > 30 kg/m^2)
- presence of comorbidity in the pregnant women like diabetes, asthma and hypertension (65)

Pregnancy may be associated with increased risk of COVID-19-related hospitalization, intensive care unit admission, and mechanical ventilation compared to nonpregnant status in women of reproductive age (66). Screening for COVID-19 in pregnant women is similar to that in the general population.

- COVID-19 may range from mild disease to severe illness. The symptoms may include fever, cough, and sore throat. In United States studies reports that pregnant women with COVID-19 may have lower rates of pyraxia, headache, muscle pain, chills, and diarrhea than

nonpregnant women with COVID-19, but similar rates of coughing, dyspnea, loss of taste or smell (67)

6.6.Vertical transmission:

most transmission of SARS-CoV-2 thought to occur between close contacts. Maternal infection with COVID-19 is thought to be associated with low risk of vertical transmission to the fetus but most observations include women infected with SARS-CoV-2 in late pregnancy; outcomes of SARS-CoV-2 infection on intrauterine transmission in the first and second trimesters are still unclear (68). A systemic review done by Alexander M Kotlyar et al conclude that vertical transmission of SARS-CoV-2 diagnosed by nasopharyngeal swab reported in 3.2% of neonates(69) .

initial manegment:

- universal testing can also be considered, particularly in high prevalence areas, due to the potential of asymptomatic pregnant women presenting to the labor and delivery unit

- American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM) recommendations for outpatient care for pregnant women with suspected or confirmed infection with COVID-19
    - fever ≥ 38 degrees C (100.4 degrees F) or ≥ 1 of the following
        - cough
        - dyspnea or shortness of breath
        - chills
        - headache

- sore throat
- new loss ofsmelling or taste
- fatigue
- muscle aches

- runny nose

.gastrointestinal symptoms like nausea, vomiting, or diarrhea

- Contact with COVID-19-positive individual

- if absent, contiue with routine prenatal care
- if present, recommend testing for SARS-CoV-2 infection and proceed with illness severity assessment including presence of any of the following
  - shortness of breath
  - difficulty completing a sentence without gasping for air or dyspnea on exertion
  - hemoptyesis
  - pressure on chest other than pain with coughing
  - any signs of dehydration
  - confusion or decreased concentration
- if yes to any questions on illness severity assessment (above), patient is considered to be at increased risk of severe disease
-
  - recommend immediate admission in emergency department
  - notify the staff that a woman under investigation is being referred to minimize chance of spreading infection
  - adhere to local infection control practices specially personal protective equipment

- if no sign of severe disease is present, assess other clinical risks for COVID-19 infection including
    - presence of comorbidities, such as hypertension, diabetes, asthma, HIV, heart disease, liver disease, chronic lung disease, chronic renal disease, blood disease, and long term use of immunosuppressive therapy
    - obstetric complications, such as preterm labor
- if there is any clinical risk mentioned above:
    - examine patient as soon as possible to determine severity of illness, with isolation of patient
    - clinical assessment for respiratory compromise includes
        - physical exam
        - pulse oximetry
        - chest x-ray with shield
        - arterial blood gas as clinically indicated
        - chest computed tomography with abdominal shielding if clinically recommended
- if patient is determined to have respiratory compromise or complications, admit for further evaluation and testing
- if patient does not have any identified clinical risks or patient does not have respiratory compromise refer for symptomatic care at home, including adequate hydration and rest
- monitoring for development of any new symptoms and take routine obstetric precautions (70).

1.-Mor G, Cardenas I. the immune system in pregnancy: a unique complexity. *Am J Reprod Immunol.* (2010) 63:425–33. doi: 10.1111/j.1600-0897.2010.00836.x

PubMed Abstract | CrossRef Full Text | Google Scholar

2. PrabhuDas M, Bonney E, Caron K, Dey S, Erlebacher A, Fazleabas A, et al. Immune mechanisms at the maternal-fetal interface: perspectives and challenges. *Nat Immunol.* (2015) 16:328–34. doi: 10.1038/ni.3131

PubMed Abstract | CrossRef Full Text | Google Scholar

3. Ando T, Davies FT. Self-recognition and the role of fetal microchimerism. *Best Pract Res Clin Endocrinol Metab.* (2004) 18:197–211. doi: 10.1016/j.beem.2004.03.002

PubMed Abstract | CrossRef Full Text | Google Scholar

4. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria AM. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA.* (1996) 93:705–8. doi: 10.1073/pnas.93.2.705

PubMed Abstract | CrossRef Full Text | Google Scholar

5. Koopmans M, Kremer Hovinga IC, Baelde HJ, Harvey MS, de Heer E, Bruijn JA, et al. Chimerism occurs in thyroid, lung, skin and lymph nodes of women with sons. *J Reprod Immunol.* (2008) 78:68–75. doi: 10.1016/j.jri.2008.01.002

PubMed Abstract | CrossRef Full Text | Google Scholar

6. Schumacher A, Costa SD, Zenclussen CA. Endocrine factors modulating immune responses in pregnancy. *Front Immunol.* (2014) 5:196. doi: 10.3389/fimmu.2014.00196

PubMed Abstract | CrossRef Full Text | Google Scholar

7. Kraus TA, Engel SM, Sperling RS, Kellerman L, Lo Y, Wallenstein S, et al. Characterizing the pregnancy immune phenotype: results of the viral immunity and pregnancy (VIP) study. *J Clin Immunol.* (2012) 32:300–11. doi: 10.1007/s10875-011-9627-2

PubMed Abstract | CrossRef Full Text | Google Scholar

8. Gervasi MT, Romero R, Bracalente G, Chaiworapongsa T, Erez O, Dong Z, Hassan SS, Yeo L, Yoon BH, Mor G, Barzon L, Franchin E, Militello V, Palu G. Viral invasion of the amniotic cavity (VIAC) in the midtrimester of pregnancy. J Matern Fetal Neona. 2012 [PMC free article] [PubMed] [Google Scholar]

9. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med. 2014;370:2211–2218. [PMC free article] [PubMed] [Google Scholar]

10. Kwon JY, Romero R, Mor G. New insights into the relationship between viral infection and pregnancy complications. Am J Reprod Immunol. 2014;71:387–390. [PMC free article] [PubMed] [Google Scholar]

11. Price ME, Fisher-Hoch SP, Craven RB, McCormick JB. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. BMJ. 1988;297:584–587. [PMC free article] [PubMed] [Google Scholar]

12. Jamieson DJ, Uyeki TM, Callaghan WM, Meaney-Delman D, Rasmussen SA. What Obstetrician-Gynecologists Should Know About Ebola: A Perspective From the Centers for Disease Control and Prevention. Obstet Gynecol. 2014 [PubMed] [Google Scholar]

13. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS, Kim CJ, Yeo L. Prevalence and Clinical Significance of Sterile Intra-amniotic Inflammation in Patients with Preterm Labor and Intact Membranes. Am J Reprod Immunol. 2014 [PMC free article] [PubMed] [Google Scholar]

14. Kwon JY, Romero R, Mor G. New Insights into the Relationship between Viral Infection and Pregnancy Complications. Am J Reprod Immunol. 2014 [PMC free article] [PubMed] [Google Scholar]

15. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007;25:21–39. [PubMed] [Goo

16. Salzberger B, Myerson D, Boeckh M. Circulating cytomegalovirus (CMV)-infected endothelial cells in marrow transplant patients with CMV disease and CMV infection. *J Infect Dis*. 1997;176(3):778–781.

View this article via: PubMed CrossRef Google Scholar

17.Fisher S, Genbacev O, Maidji E, Pereira L. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. *J Virol*. 2000;74(15):6808–6820.

18. Cardenas I, Mor G, Aldo P, Lang SM, Stabach P, Sharp A, Romero R, Mazaki-Tovi S, Gervasi M, Means RE. Placental viral infection sensitizes to

endotoxin-induced pre-term labor: a double hit hypothesis. Am J Reprod Immunol. 2011;65:110–117. [PMC free article] [PubMed] [G

19. Bayer A, Delorme-Axford E, Sleigher C, Frey TK, Trobaugh DW, Klimstra WB, Emert-Sedlak LA, Smithgall TE, Kinchington PR, Vadia S, Seveau S, Boyle JP, Coyne CB, Sadovsky Y. Human trophoblasts confer resistance to viruses implicated in perinatal infection. Am J Obstet Gynecol. 2014 [PMC free article] [PubMed] [Google Scholar]

20. Ouyang Y, Mouillet JF, Coyne CB, Sadovsky Y. Review: placenta-specific microRNAs in exosomes - good things come in nano-packages. Placenta. 2014;35(Suppl):S69–S73. [PMC free article] [PubMed] [Google Scholar]

21. Silasi M, Cardenas I, Kwon JY, Racicot K, Aldo P, Mor G. Viral infections during pregnancy. *Am J Reprod Immunol.* (2015) 73:199–213. doi: 10.1111/aji.12355

PubMed Abstract | CrossRef Full Text | Google Scholar

22. Neggers Y. The association between viral infections, maternal and fetal mortality/morbidity. *Glob J Reprod Med.* (2018) 4:GJORM.2018.04.555638. doi: 10.19080/GJORM.2018.04.555638

CrossRef Full Text

23. Paryani SG, Arvin MA. Intrauterine infection with varicella-zoster virus after maternal varicella. *N Engl J Med.* (1986) 314:1542–6. doi: 10.1056/NEJM198606123142403

PubMed Abstract | CrossRef Full Text | Google Scholar

24. World Health Organization. *Newborns: Reducing Mortality*. Geneva: World Health Organization (2019).

Google Scholar

25. Kollmann TR, Kampmann B, Mazmanian SK, Marchant A, Levy O. Protecting the Newborn and Young Infant from infectious diseases: lessons from immune ontogeny. *Immunity.* (2017) 46:350–63. doi: 10.1016/j.immuni.2017.03.009

PubMed Abstract | CrossRef Full Text | Google Scholar

26.De Kleer Willems F, Lambrecht B, Goriely S. Ontogeny of myeloid cells. *Front Immunol.* (2014) 5:423. doi: 10.3389/fimmu.2014.00423

PubMed Abstract | CrossRef Full Text | Google Scholar

27.. Carr R. Neutrophil production and function in newborn infants. *Br J Haematol.* (2000) 110:18–28. doi: 10.1046/j.1365-2141.2000.01992.x

PubMed Abstract | CrossRef Full Text | Google Scholar

28. Maródi L. Innate cellular immune responses in newborns. *Clin Immunol.* (2006) 118:137–44. doi: 10.1016/j.clim.2005.10.012

PubMed Abstract | CrossRef Full Text | Google Scholar

29. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol.* (2007) 7:379–90. doi: 10.1038/nri2075

PubMed Abstract | CrossRef Full Text | Google Scholar

30. Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G. Regulatory activity of autocrine IL-10 on dendritic cell functions. *J Immunol.* (2001) 166:4312–8. doi: 10.4049/jimmunol.166.7.4312

PubMed Abstract | CrossRef Full Text | Google Scholar

31. Gagnon A, Acosta E, Miller SM. Age-specific incidence of influenza A responds to change in virus subtype dominance. *Clin Infect Dis.* (2020) 27:ciaa075. doi: 10.1093/cid/ciaa075

PubMed Abstract | CrossRef Full Text | Google Scholar

32. Drajac C, Laubreton D, Riffault S, Descamps D. Pulmonary susceptibility of neonates to respiratory syncytial virus infection: a problem of innate immunity? *J Immunol Res.* (2017) 2017:8734504. doi: 10.1155/2017/8734504

PubMed Abstract | CrossRef Full Text | Google Scholar

33. Williams AL, Uren EC, Bretherton L. Respiratory viruses and sudden infant death. *Br Med J (Clin Res Ed).* (1984) 288:1491–3. doi: 10.1136/bmj.288.6429.1491

PubMed Abstract | CrossRef Full Text | Google Scholar

34. Goodnight WH, Soper ED. Pneumonia in pregnancy. *Crit Care Med.* (2005) 33:S390–7. doi: 10.1097/01.ccm.0000182483.24836.66

PubMed Abstract | CrossRef Full Text | Google Scholar

35. Chen YH, Keller J, Wang IT, Lin CC, Lin CH. Pneumonia and pregnancy outcomes: a nationwide population-based study. *Am J Obstet Gynecol.* (2012) 207:288.e1–7. doi: 10.1016/j.ajog.2012.08.023

PubMed Abstract | CrossRef Full Text | Google Scholar

36. Pringle KG, Tadros MA, Callister RJ, Lumbers RE. The expression and localization of the human placental prorenin/renin-angiotensin system throughout pregnancy: roles in trophoblast invasion and angiogenesis? *Placenta.* (2011) 32:956–62. doi: 10.1016/j.placenta.2011.09.020

PubMed Abstract | CrossRef Full Text | Google Scholar

37. . JW H. Influenza occurring in pregnant women. JAMA. 1919;72:978–980. [Google Scholar]

38. Freeman DWBA. Deaths from Asian influenza associated with pregnancy. Am J Obstet Gynecol. 1959;78:1172–1177. [PubMed] [Google Scholar]

39. Nuzum JWPI, Stangl FH, Bonar BE. Pandemic influenza and pneumonia in a large civilian hospital. JAMA. 1918;71:1562–1567. [Google

40. Coffey VPJW. Maternal influenza and congenital deformities. A follow-up study. Lancet. 1963;1:748–751. [PubMed] [Google Scholar

41. Wilson MGSA. Teratogenic effects of Asian influenza. An extended study. JAMA. 1969;210:336–344. [PubMed] [Go

42. Hardy JMAE, Mannini A, Medearis DN, Jr, Cooke RE. The effect of Asian influenza on the outcome of pregnancy, Baltimore, 1957–1958. Am J Public Health. 1961;51:1182–1190. [PMC free article] [PubMed] [Google Scholar]

43. Acs NBF, Puho E, Czeizel AE. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. Birth Defects Res A Clin Mol Teratol. 2005;73:989–996. [PubMed] [Google Scholar]

44. Yu N, Li W, Kang Q, Xiong Z, Wang S, Lin X, et al. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. *Lancet Infect Dis.* (2020) 20:559–64. doi: 10.1016/S1473-3099(20)30176-6

PubMed Abstract | CrossRef Full Text | Google Scholar

45. Dong L, Tian J, He S, Zhu C, Wang J, Liu C, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA.* (2020) 323:1846–8. doi: 10.1001/jama.2020.4621

PubMed Abstract | CrossRef Full Text | Google Scholar

46. Zeng H, Xu C, Fan J, Tang Y, Deng Q, Zhang W, et al. Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA.* (2020) 323:1848–9. doi: 10.1001/jama.2020.4861

PubMed Abstract | CrossRef Full Text | Google Scholar

47. Li Y, Zhao R, Zheng S, Chen X, Wang J, Sheng X, et al. Lack of vertical transmission of severe acute respiratory syndrome coronavirus 2, China. *Emerg Infect Dis.* (2020) 26:1335–6. doi: 10.3201/eid2606.200287

CrossRef Full Text | Google Scholar

48. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet.* (2020) 395:809–15. doi: 10.1016/S0140-6736(20)30360-3

PubMed Abstract | CrossRef Full Text | Google Scholar

49. Chen Y, Peng H, Wang L, Zhao Y, Zeng L, Gao H, et al. Infants born to mothers with a new coronavirus (COVID-19). *Front Pediatr.* (2020) 8:104.

doi: 10.3389/fped.2020.00104PubMed Abstract | CrossRef Full Text | Google Scholar

140.

50. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* (2020) 8:420–2. doi: 10.1016/S2213-2600(20)30076-X

PubMed Abstract | CrossRef Full Text | Google Scholar

51. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

PubMed Abstract | CrossRef Full Text | Google Scholar

52. Giannubilo SR, Landi B, Pozzi V, Sartini D, Cecati M, Stortoni P, et al. The involvement of inflammatory cytokines in the pathogenesis of recurrent miscarriage. *Cytokine.* (2012) 58:50–6. doi: 10.1016/j.cyto.2011.12.019

PubMed Abstract | CrossRef Full Text | Google Scholar

53. Wilson R, Moore J, Jenkins C, Miller H, Maclean MA, McInnes IB, et al. Abnormal IL-2 receptor levels in non-pregnant women with a history of recurrent miscarriage. *Hum Reprod.* (2003) 18:1529–30. doi: 10.1093/humrep/deg287

PubMed Abstract | CrossRef Full Text | Google Scholar

54. Wu L, Li J, Xu HL, Xu B, Tong XH, Kwak-Kim J, et al. IL-7/IL-7R signaling pathway might play a role in recurrent pregnancy losses by increasing

inflammatory Th17 cells and decreasing Treg cells. *Am J Reprod Immunol.* (2016) 76:454–64. doi: 10.1111/aji.12588

PubMed Abstract | CrossRef Full Text | Google Scholar

55. Sudhir N, Badaruddoza Beri A, Kaur A. Association of tumor necrosis factor-alpha 308G/A polymorphism with recurrent miscarriages in women. *J Hum Reprod Sci.* (2016) 9:86–9. doi: 10.4103/0974-1208.183516

PubMed Abstract | CrossRef Full Text | Google Scholar

56. Haider S, Knöfler M. Human tumour necrosis factor: physiological and pathological roles in placenta and endometrium. *Placenta.* (2009) 30:111–23. doi: 10.1016/j.placenta.2008.10.012

PubMed Abstract | CrossRef Full Text | Google Scholar

57. Azizieh FY, Raghupathy GR. Tumor necrosis factor-α and pregnancy complications: a prospective study. *Med Princ Pract.* (2015) 24:165–70. doi: 10.1159/000369363

PubMed Abstract | CrossRef Full Text | Google Scholar

58. Carpentier PA, Dingman AL, Palmer DT. Placental TNF-α signaling in illness-induced complications of pregnancy. *Am J Pathol.* (2011) 178:2802–10. doi: 10.1016/j.ajpath.2011.02.042

PubMed Abstract | CrossRef Full Text | Google Scholar

59. Nihi F, Moreira D, Santos Lourenço AC, Gomes C, Araujo SL, Zaia RM, et al. Testicular effects following *in utero* exposure to the antivirals acyclovir and ganciclovir in rats. *Toxicol Sci.* (2014) 139:220–33. doi: 10.1093/toxsci/kfu024

PubMed Abstract | CrossRef Full Text | Google Scholar

60. Antonella LoMauro[1], Andrea Aliverti. Respiratory physiology of pregnancy: Physiology masterclass. Breathe (Sheff) 2015 Dec;11(4):297-301.

61. Royal College of Obstetricians and Gynaecologists (RCOG) and The Royal College of Midwives. Coronavirus (COVID-19) Infection in Pregnancy. RCOG 2020 Jul 24 PDF

62. Society of Obstetricians and Gynaecologists of Canada (SOGC). Committee Opinion on COVID-19 in Pregnancy.SOGC 2020 May 14

63. K B Brosnihan[1], L A A Neves, L Anton, J Joyner, G Valdes, D C Merrill. Enhanced expression of Ang-(1-7) during pregnancy. Braz J Med Biol Res. 2004 Aug;37(8):1255-62

64. Lei Fang[1], George Karakiulakis[2], Michael Roth. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection?. Lancet Respir Med 2020 Apr;8(4):e21.

65. Asma Khalil, Erkan Kalafat, Can Benlioglu, Pat O'Brien, Edward Morris,Tim Draycott. SARS-CoV-2 infection in pregnancy: A systematic review and meta-analysis of clinical features and pregnancy outcomes. E clinical medicine, VOLUME 25, 100446, AUGUST 01, 2020

66. Sascha Ellington[1], Penelope Strid[1], Van T Tong[1], Kate Woodworth[1], Romeo R Galang[1], Laura D Zambrano[1], John Nahabedian[1], Kayla Anderson[1], Suzanne M GilboaCharacteristics of Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status - United States, January 22-June 7, 2020. MMWR Morb Mortal Wkly Rep. 2020 Jun 26;69(25):769-775

67. Reem Matar , Layan Alrahmani , Nasser Monzer , Labib G Debiane , Elie Berbari, Jawad Fares , Fidelma Fitzpatrick , Mohammad H Murad. Clinical Presentation and Outcomes of Pregnant Women with COVID-19: A Systematic Review and Meta-Analysis

68. W Joost Wiersinga , Andrew Rhodes , Allen C Cheng , Sharon J Peacock , Hallie C Prescott. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19): A Review. JAMA. 2020 Aug 25;324(8):782-793.

69. Alexander M Kotlyar 1, Olga Grechukhina 2, Alice Chen 3, Shota Popkhadze 2, Alyssa Grimshaw 4, Oded Tal 5, Hugh S Taylor 3, Reshef Tal. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. Am J Obstet Gynecol,2020 Jul 31;S0002-9378(20)30823-1

70. Outpatient Assessment and Management for Pregnant Women With Suspected or Confirmed Novel Coronavirus (COVID-19). Revised July 14, 2020. Copyright 2020 American College of Obstetricians and Gynecologists

Chapter seven: SARS-CoV-2 Vaccines

In December 2019, a novel strain of coronavirus emerged in Wuhan, China which later caused the most sever pandemic in the last century.

Coronaviruses have been isolated from infected humans since the mid-sixties of the 20th century. [1] The 2019 emerging coronavirus showed high virulence and high transmissibility which let it be considered a disease of high concern to global health authorities.

This is the third time that a high impact coronavirus has emerged during the twenty-first century and showed very dangerous characteristics. In 2002 SARS- coronavirus emerged in China and in 2012 MERS-coronavirus emerged in Saudi Arabia-Jordan area, causing severe pneumonia with very high morbidity and mortality rates. The new 2019 coronavirus was named SARS-CoV-2 and the disease it causes was named Covid-19 [2]

The complete genome sequence of the virus was published on January 11, 2020, by a Chinese scientist called Yong Zhan Zhang.The first infection with SARS-COV2 in the United States of America was reported on the twenty-first of January 2020, and the declaration of this highly transmissible disease as a global pandemic by the World Health Organization did not occur until March 11, 2020.

It was remarkably observed that two days after publishing the genetic sequencing of the SARS-COV2 virus and before any infection was reported in America, an American drug maker called MODERNA (Name derived from modern RNA) designed a vaccine using a two-decade-old technology called “genetic vaccines”, that was on January 13, 2020.[3]

This technology uses messenger RNA (mRNA) to encode a pre-designed genetic material inside the human body so that the body can use its cellular machinery (Ribosomes) to build up an epitope which was identified to be the "spike protein" in corona viruses. (The immune-dominant antigen of the virus).

Without causing COVID-19 symptoms, the injected epitope will provoke an immune response which will help to initiate a secondary immune response when the person is exposed to the actual virus.This two decades old technology has been used to successfully treat some cancers and in many vaccine research projects. [4]

Given that this is a novel virus causing severe respiratory infection, the accumulated global experience coming from coronavirus experts who managed Severe Acute Respiratory Syndrome before directed their efforts to developing a vaccine. Scientists, medical doctors and pharmaceutical companies started developing a number of effective and safe vaccines. The only quick solution to control this type of disease is through mass vaccination programs to achieve community immunity. This did not stop research and clinical trials to develop and test various treatments for this disease.

Data from the first week of September 2021when this chapter was written indicated that the number of vaccines being developed for Covid-19 disease that are still in multiple clinical trial in different phases of research has reached 102 vaccines, of which 33 are in the final stage of these experiments, and in addition to that, there are 75 vaccines in the pre-clinical (animal-testing) phase.

So far, eight vaccines have been fully approved in some countries, and there are 13 vaccines that were approved on an emergency basis. Research on five types of vaccines was suspended and related vaccines were abandoned due to ineffectiveness or other reasons that emerged during clinical trials. This happened despite the huge amount of money that were spent on them. [5]

Most of the vaccines that are currently in use, aim at neutralizing the spike protein covering the virus that has been found to be the main immune antigen of corona viruses, this was considered after evaluation of patients with SARS-CoV-2 revealed that binding and neutralizing antibodies primarily target the receptor-binding domain of the S1 subunit.*

Vaccines research end points are to be efficacious in preventing COVID-19–related hospitalization and death, but have varying efficacy in preventing clinical disease, particularly disease caused by the novel SARS-CoV-2 variants.

7.1.Vaccine manufacturers aim to have their vaccine to:

1. Be capable of producing neutralizing antibodies to the main virus antigen.
2. Generate an immune response in T cells
3. Avoid the condition of antibody-dependent enhancement (ADE) which is described as inducing the disease through the vaccine. This occurs when the antibodies act in a way that contradicts the desired action from them

7.2.Types of vaccines

There are four main categories of vaccines based on the way they are manufactured, and hence the mode of action.

1. **Genetic Vaccines**

In these vaccines, lipid nanoparticles are used to protect the perfusion-stabilized S protein–encoding mRNA, en route to the intracellular space. The host uses the mRNA to make the target protein (S protein in this case), which induces a coordinated immune response. The vaccine nanoparticles enters the inside of the cell, but remains outside the nucleus, which means it does not mix with the genetic material of the vaccinated individual. [6]

Examples are Pfizer-Biontech and Moderna vaccines (Approved by the WHO)

1. Viral vector vaccines

Viral vector vaccines uses recombinant technology to produce replication-deficient viruses engineered to express the genetic sequence of the antigen of interest in host cells.Adenovirus have been used as the main vector for those vaccines. [6]

Examples: Johnson and Johnson, AstraZeneca (Approved by the WHO)

2. Protein subunit based vaccine

Vaccines that contain coronavirus proteins but no genetic material. Some vaccines contain whole proteins, and some contain fragments of them. Some pack many of these molecules on nanoparticles.

More than 60% of vaccines currently in development use a protein subunit approach, although none are authorized for use. [6]

3. Inactivated Coronavirus Vaccine

Creation of inactivated vaccines derived from virus grown in culture and then chemically inactivated, which may deliver stably expressed, multiple antigenic epitopes. [6]

Multi-epitope vaccines are supposed to be more capable to resisting vaccine avoidance by virus variants because the whole virus epitopes are present in the vaccine. This has not been demonstrated with the current multiple antigenic epitope vaccine.

Examples: Sinopharm and Sinovac vaccines (Approved by the WHO)

7.3.SARS-COV-2 variants and Vaccines

Viral variants of concern may emerge with dangerous resistance to the immunity generated by the current vaccines to prevent coronavirus disease 2019 (Covid-19).

Moreover, if some variants of concern have increased transmissibility or virulence, the importance of efficient public health measures and vaccination programs will increase. The global response must be both timely and science based.

References:

1. National foundation for Infectious Diseases, https://www.nfid.org/infectious-diseases/coronaviruses/ Accessed on September, 9, 2021
2. World Health Organization
3. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it
   Accessed on September 10, 2021
4. Center for Disease Control and Prevention (CDC)
   https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/mRNA.html?s_cid=11344:mrna%20vaccine%20technology:sem.ga:p:RG:GM:gen:PTN:FY21
   Accessed on September 9, 2021
5. The New York Times Vaccine Tracker
   https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html
   Accessed on September 9, 2021
6. Journal of American Medical Association (JAMA) Network
   https://jamanetwork.com/journals/jama/fullarticle/2777059
   Accessed on September 7, 2021

Chapter eight: Epidemiology of COVID-19

Coronavirus is is a major virus caused serious and fatal disease, is a zoonotic virus can infect human and animals, this new strain of virus name as COVID-19 standing for coronavirus disease 2019& Cov-SARS2 [(1)]. Previously in 2002 &2012, there are also outbreaks reported caused by a coronavirus and, including severe acute respiratory syndrome (SARS-CoV) and the Middle East respiratory syndrome (MERS-CoV), which is described as a significant public health threat [(3&4)].

In 2002, coronavirus infections (SARS-CoVs) spread in Guangdong, south China, and the infected patient suffer from high fever, breathlessness, and pneumonia, and rapidly spread in 26 countries, resulting in about 8096 cases and 774 deaths [(5,6)]. Whereas MERS-CoV infection was first detected in Saudi Arabia in 2012. The disease has mild respiratory symptoms that can lead to acute respiratory syndrome and death. The infection cases reports about 2494 cases were infected by the virus and 858 died in more than 25 countries [(7–9)].

The infection caused by new coronavirus COVID19 range from mild to severe disease and can infect multi organs and caused disease such as gastroenteritis, pneumonia, septic shock, metabolic acidosis and bleeding [(2&3)]. The incubation period has been estimated from 5 - 14 days and may vary from patient to patient according to age and infection history [(14)].

The COVID-19 patients may show varying degrees of laboratory abnormalities e.g. leukopenia, leukocytosis, lymphopenia … etc [(23),] in the early stage another diagnostic character like chest x-ray (CXR) and CT scan are usually normal; however, it may show bilateral infiltrates and ground-glass opacity in late stages and more severe forms [(23,24)]

Once transmitted from an infected to a healthy person, it will attach lung and caused Atypical unknown pneumonia, which was first recorded in Wuhan city, Hubei province in December 2019[1-3]. people who are infected with this virus suffer from high fever (more than 38 C°), dry cough, malaise, and breathing difficulties[3]. The story of infection begins and is linked to the seafood market of Wuhan, China, and named COVID-19 [10–12], then it was spreading to other Far East Asian nations, then to the Middle East and Europe[4].

The pandemic started from China then rapidly crossed the international borders to involve, Italy, Iran, Spain, France, Germany, Turkey, the United Kingdom, and the United States of America[15]. On April 9, the total cases in this group of countries accounted for more than 75% of total globally reported cases[15]. A less dramatic scale of cases was witnessed in Belgium, Switzerland, Netherland, and Brazil. Other countries are experiencing either less severe epidemics or a very small number of cases. Countries which are south of the equator are less affected in general. The responses to the pandemic among various countries were variable but all those countries with high incidence rates faced substantial difficulties to deal with the daily flow of cases[16].

In Iraq, the first case was reported on February 24th, 2020, and in Basrah on March 9th. While the number of newly reported cases started to build up, the total number remains relatively low. It is worth noting that the Iraqi Foreign Ministry was working with Chinese authorities to evacuate the 30th Iraqi students with their families from the city of Wuhan, China to the capital Baghdad by February 5, 2020, the foreign ministry statement at that time noted that the Iraqi embassy in China had not recorded cases of infection

among the students. They were quarantined for 14 days then declared free of COVID-19 infection and released by February 19, 2020[18].

The surveillance cases data from February 24 to April 30, 2020, officially reported by the Iraqi health authorities were used to estimate the rapidly cumulated incidence rates of SARS-CoV-2 confirmed infections. During the first 37 days of epidemics, 694 cases of COVID-19 were reported in the country, with death cases reaching up to 50 deaths during March 31, 2020. An Iraqi family of four who returned from Iran tested positive for the coronavirus in Kirkuk governorate[19].

They were the first Iraqis known to have caught the disease, a day after an Iranian student in the Najaf governorate became Iraq's first confirmed case on February 24, 2020. After two days another 2 tested positives for coronavirus cases in Baghdad were returning from Iran. The Iraqi government, which has already banned all travel from Iran and China, added Italy, Thailand, South Korea, Singapore, and Japan to its travel ban list, nevertheless, the returning Iraqi citizens are exempt[20].

The government urged Iraqis to avoid all public gatherings. Gatherings were banned in Iraqi governorates. Schools and universities were shut; the northern Kurdish region and the capital Baghdad canceled all education until after a March 20 holiday [18&19].

Large numbers of Iraqis, including religious pilgrims and merchants, had been in Qom as the crisis escalated and were gradually brought back by bus and plane to Baghdad, where only small numbers of patients from their communities are being treated in public hospitals[19].

Health authorities have reported 13 deaths and 164 infections in Iraq from the novel coronavirus by that time and reached up to 50 deaths and 694

after 2 weeks. However, many suspect the number of cases could be higher, as fewer than 4000 people have been tested in a country of 40 million[20].

In the north of Iraq areas like Sulaimani, Duhok, and Irbil also people infected by COVID19 [22]. The last data from WHO on 24 August 2021weekly reported shows there are declining trends this week and for the first time since the end of May, the Region reported a 10% decrease in cases, with just over 450 000 new cases this week. These declines were largely due to decreases in the number of new cases reported in the Islamic Republic of Iran, Morocco, Pakistan, and Iraq, although it is important to note that there is still ongoing transmission in all countries in the Region and case numbers while declining, remain high in most countries. Following seven weeks of increasing death incidence, this week over 7100 new deaths were reported in the Region, a number s similar to that of the previous week. Eight out of the twenty-two countries reported increases in deaths over the past seven days[24].

The highest numbers of new cases were reported from the Islamic Republic of Iran (251 610 new cases; 299.6 new cases per 100 000; a 7% decrease), Morocco (54 212 new cases; 146.9 new cases per 100 000; a 16% decrease), and Iraq (50 702 new cases; 126.1 new cases per 100 000; a 21% decrease). The highest numbers of new deaths were reported from the Islamic Republic of Iran (4146 new deaths; 4.9 new deaths per 100 000; an 11% increase), Morocco (744 new deaths; 2.0 new deaths per 100 000; a 10% increase), and Tunisia (630 new deaths; 5.3 new deaths per 100 000; a 30% decrease) [24].

Reference

1. Chen Y, Liu Q, Guo D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. *J Med Virol* (2020) **92**:418–423. doi:10.1002/jmv.25681
2. Hoek L, Pyrc K, Jebbink MF, Vermeulen-oost W, Berkhout RJM, Wolthers KC, Dillen PMEW, Kaandorp J, Spaargaren J, Berkhout B. Identification of a new human coronavirus. *Nat Med* (2004) **10**:368–373. doi:10.1038/nm1024
3. Gralinski LE, Menachery VD. Return of the coronavirus: 2019-nCoV. *Viruses* (2020) **12**: doi:10.3390/v12020135
4. 4. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun* (2020)1–4. doi:10.1016/j.jaut.2020.102433
5. WHO | Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. *WHO* (2015)
6. SARS (severe acute respiratory syndrome) - NHS. Available at: https://www.nhs.uk/conditions/sars/ [Accessed June 7, 2020]
7. Padron-regalado E. Vaccines for SARS-CoV-2□: Lessons from Other Coronavirus Strains. *Infect Dis Ther* (2020) **9**:255–274. doi:10.1007/s40121-020-00300-x
8. Contini C, Nuzzo M, Barp N, Bonazza A, Giorgio R, Tognon M, Rubino S. The novel zoonotic COVID-19 pandemic□: An expected global health concern. *J Infect Dev Ctries* (2020) **14**:254–264. doi:10.3855/jidc.12671

9. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* (2012)
10. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* (2020)**382**:727–733.
11. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, Hu Y, Tao ZW, Tian JH, Pei YY, et al. A new coronavirus associated with human respiratory disease in China. *Nature* (2020) **579**:265–269.
12. Naming the coronavirus disease (COVID-19) and the virus that causes it. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technicalguidance/ naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it[Accessed June 8, 2020]
13. Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 Pandemic : A Comprehensive Review of Taxonomy, Genetics , Epidemiology , Diagnosis , Treatment , and Control. *J Clin Med* (2020) **9**:1–29.
14. Xiao Z, Xie X, Guo W, Luo Z, Liao J, Wen F, Zhou Q, Han L, Zheng T. Examining the incubation period distributions of COVID-19 on Chinese patients with different travel histories. *J Infect Dev Ctries* (2020) **14**:323–327. doi:10.3855/jidc.12718
15. www.worldometers.info/coronavirus/ Accessed daily from March 20 to April 10, 2020
16. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020 Feb 28. doi: 10.1056/NEJMoa2002032. [Epub ahead of print] The

Medical Journal of Basrah University Epidemiology of COVID19 Infection (2020);38(1): 7-18

17. European Centre for Disease Prevention and Control (ECDC). Novel coronavirus disease 2019 (COVID-19) pandemic: increased transmission in the EU/EEA and the UK – sixth update 12 March 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/RRA-sixth-update-Outbreak-of-novel-coronavirus-disease-2019-COVID-19.pdf Accessed on April 8.
18. Bogoch II, Watts A, Thomas-Bachli A, Huber C, Kraemer MU, Khan K. Potential for global spread of a novel coronavirus from China. J Travel Med 2020. Cited by reference 21
19. Rodriguez-Morales AJ, MacGregor K, Kanagarajah S, Patel D, Schlagenhauf P. Going global - travel and the 2019 novel coronavirus. Trav Med Infect Dis 2020;33:101578. Cited by reference 21
20. Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020. Cited by reference 21
21. Al-Malkey, M. K., & Al-Sammak, M. A. (2020). Incidence of the COVID-19 in Iraq–Implications for travellers. *Travel medicine and infectious disease*, *38*, 101739.
22. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, vol. 43; 2020
23. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507e13.