

COVID-19: Perspective, Patterns and Evolving strategies

Subject Category: Clinical Virology

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Chapter 1: The Trans-Zoonotic Virome Interface: Measures to Balance, Control and Treat Epidemics

Background

The Global Virome: The viruses have a global distribution, phylogenetic diversity, and host specificity. They are obligate intracellular parasites with single- or double-stranded DNA or RNA genomes, and afflict bacteria, plants, animals, and human population. The infecting virus binds to receptor proteins on the host cell surface, followed by internalisation, replication, and cell lysis. Further, trans-species interactions of viruses with bacteria, small eukaryotes and host are linked with various zoonotic viral diseases and disease progression.

Virome Interface and Transmission: The cross-species transmission from the natural reservoir, usually mammalian or avian, hosts to infect human-being is a rare, but occurs leading to the zoonotic human viral infection. The factors like increased human settlements and encroachments, expanded travel and trade networks, altered wildlife and livestock practices, modernised and mass-farming practices, compromised ecosystems, and global climate change act as drivers of trans-species viral spill-over and human transmission.

Zoonotic Viral Diseases and Epidemics: The zoonotic viruses have caused various deadly pandemics in human history. They are further characterized as newly emerging or re-emerging infectious diseases, caused by pathogens that historically have infected the same host species, but continue to appear in new locations or in drug-resistant forms, or reappear after apparent control or elimination. The prevalence of zoonoses underlines importance of the animal-human-ecosystem interface in disease transmission. The present COVID-19 infection has certain distinct features which suppress the host immune response and promote the disease potential.

Treatment for Epidemics like COVID-19: It appears that certain nutraceuticals may provide relief in clinical symptoms to patients infected with encapsulated RNA viruses such as influenza and coronavirus. The nutraceuticals reduce the inflammation in the lungs and help to boost type 1 interferon response to these viral infections. The human intestinal microbiota acting in tandem with the host's defence and immune system, is vital for homeostasis and preservation of health and protection from disease states including viral infections. Certain probiotics may help in improving the sensitivity and effectivity of immune system against viral infections. The antiviral therapy is available only for a limited number of zoonotic viral infections. The viruses being intracellular parasites, antiviral drugs are not able to deactivate or destroy the virus but can reduce the viral load by inhibiting replication and facilitating the host's innate immune mechanisms to neutralize the virus.

Conclusion: Lessons from Recent Viral Epidemics: Considering that certain nutraceuticals have demonstrated antiviral effects in both clinical and animal studies, further studies are required to establish their therapeutic efficacy. The components of nutraceuticals such as luteolin, apigenin, quercetin and chlorogenic acid may be useful for developing a combo-therapy. The use of probiotics to enhance immunity and immune response against viral infections is a novel possibility. The available antiviral therapy is inefficient in deactivating or destroying the infecting viruses, may help in reducing the viral load by inhibiting replication. The novel efficient antiviral agents are being explored.

The Global Viral Communities

The viruses are the most abundant biological entity on Earth. They have a global distribution, bear phylogenetic diversity, and host specificity, and afflict bacteria, plants, animals and human population [1]. The term Virome refers to the viral metagenomes, which make up all the viral community associated with a particular ecosystem and includes both RNA and DNA viruses [2]. Taken together, the overall virome amounts to about 10³¹ viral particles on Earth. Further, the global virome is widespread, diverse and most of it remains uncharacterized, though broadly classified as plant, zoonotic and human viruses (Figure 1).

In general, characterizing viral communities is more challenging than bacteria, archaea, and eukaryotes because viruses do not possess phylogenetically conserved genes. They are obligate intracellular parasites possessing single- or double-stranded DNA or RNA genomes.

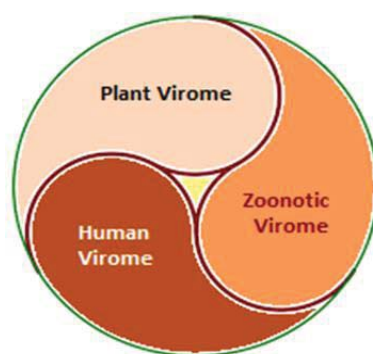


Table 1: Global virome - Plant, Zoonotic and Human Viruses.

Viral infection begins when the viral surface proteins bind to receptor proteins on the host cell surface, followed by internalisation, replication, and lysis. Alternatively, some viruses can remain dormant inside the host cells until conditions are favourable for their replication. While protein-protein interactions between virion and host are species specific in general, some viruses can have a broader host range.

The plant virome is composed of viral nucleic acids, DNA or RNA, and associated with a plant or community of plants. Plant viruses are harmful to the cultivated crop plants negatively affecting host morphology and physiology resulting in disease. Native wild/non-cultivated plants are often latently infected with viruses without any clear symptoms but pose a threat to cultivated crops because they can be transmitted by contact or vectors and cause disease.

The great majority of plant viruses have an RNA genome, which is generally small and single stranded (ss), but some viruses have double-stranded (ds) RNA, ssDNA, or dsDNA genomes. Most plant viruses lack an envelope. The plant viruses can spread by direct transfer of sap by contact of a wounded plant with a healthy one or transmitted by a vector, most often insects. Soil-borne nematodes also have been shown to transmit viruses by feeding on infected roots. Apart from this, a number of virus genera are transmitted, through soil borne zoo-sporic protozoa associated with the plant roots. In addition, plant virus transmission through generations may occur by seeds.

The human microbiome is comprised of communities of commensal, symbiotic and pathogenic bacteria, viruses, archaea, and small eukaryotes that actively interact with each other and the host to maintain homeostasis. The human virome is an essential part of the human microbiome and comprises of endogenous retroviruses, eukaryotic viruses, and bacteriophages. The gut microbial genes are involved in nutrient synthesis, the metabolism of amino acids, carbohydrates and lipids and evolution and maintenance of immune system. The human virome is associated with Type-1 diabetes (T1D), Type-2 diabetes (T2D), Inflammatory Bowel Disease (IBD), Human Immunodeficiency Virus (HIV) infection and malignancy. Further, the trans-species interactions of viruses with bacteria, small eukaryotes and host are associated with various zoonotic viral diseases and disease progression.

Virome Interface and Transmission

Plant viruses affect plants, especially larger plants. Like all other viruses, plant viruses are obligate intracellular parasites. The direct plant-to-human transmission is rare but has been found. There is evidence to suggest that the virus common to peppers, the Pepper Mild Mottle Virus (PMMoV), a member of Tobamovirus family, may infect humans and cause clinical symptoms [3]. The studies indicate that tobamoviruses are highly stable outside living host-cell. Another plant virus, Groundnut bud necrosis virus, one of the commonly occurring tospovirus in India, may have potential for host-switching to human or other animals.

The plant-based food and water are obvious route through plant viruses can get access to human body. The other possible route of access of plant virus directly to human cells is through insects that feed on both plant and human. These findings trigger to re-evaluate the dogmatic concept that plant viruses are safe to human health even though numerous viruses are consumed through various types of fresh foods and food-products [4].

Flora and fauna, plants, and wildlife, especially mammals and birds, are hosts to an enormous number of viruses, which circulate in their specific echo-biospheres. These viruses usually inhabit asymptotically in their natural hosts

and occasionally, spill-over from their reservoir hosts to infect other species and cause disease states (Figure 2). The similar cross-species transmission from their natural reservoir hosts to infect human is also a rare probability leading to the zoonotic human viral infection, which is usually due to the viruses that spread from non-human animals (usually mammalian or avian hosts) to humans [5].

There are about 260 viruses known in humans and a multitude of unknown viruses represent potential cause of zoonoses [6]. Further, it is held that of about 1.6 million mammalian and avian viruses, only about 25 viral families may have potential to cause infections in human [7]. There is, both, an increased recognition of the emergence of zoonotic infections as well as the increased incidence of emerging zoonotic infections in modern times, though such an event may go undetected if there are no significant clinical symptoms or occurs at a small scale [8]. Still, these viruses remain undetected until they cause disease states in humans. Also, some unknown viral agents emerging from the wildlife reservoir causing limited disease outbreak in other animals, including humans, may go unrecognised. The better diagnostic methods and advanced in-depth investigations have led to the prompt recognition and epidemiological action. For example, the Hendra virus (HeV) disease in Australia in 1994 or Nipah virus (NiV) in Southern India in 2019 might have not been identified because of the small scale and random clusters.

The high pathogenicity of the emerging zoonotic viruses and their transmissibility in humans and non-reservoir species is important factor as compared to various indolent viral infections occurring and circulating in the human population. Still, the interspecies transmission of zoonotic agents from their natural reservoir host and its human-to-human transmission is an uncommon event. The increased spill-over events lead to the increased probability of the emergence of a highly plastic and adapted virus capable of trans-human infections. Various factors like increased human settlements and encroachments, expanded travel and trade networks, altered wildlife and livestock practices, modernised and mass-farming practices, compromised ecosystems and habitat destruction, and global climate change are recognised to have impact on the interactions between virome and its hosts and other species and act as drivers of trans-species viral spill-over and human transmission.

Zoonotic Viral Diseases and Epidemics

Zoonotic viral infections and epidemics: The zoonotic viruses have caused various deadly pandemics in human history. The Spanish flu in 1918-19 infected almost one-third of the global human population and caused 50-100 million deaths. More recently, Nipah, Hendra, Hanta and Ebola viruses, several influenza subtypes, and the SARS (severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) coronaviruses have caused limited albeit fatal outbreaks.

The infectious diseases are responsible for about one in five human deaths worldwide. In addition, they impose a heavy societal and economic burden on individuals, families, communities and countries [9]. Jones, et al., describing the emergence of 335 infectious diseases in the global human population between 1940 and 2004, concluded that nearly two-thirds the diseases originated in wildlife and the current global trends indicate that novel viral threats may continue to emerge at an accelerating rate [10]. These can be further characterized as either newly emerging or re-emerging

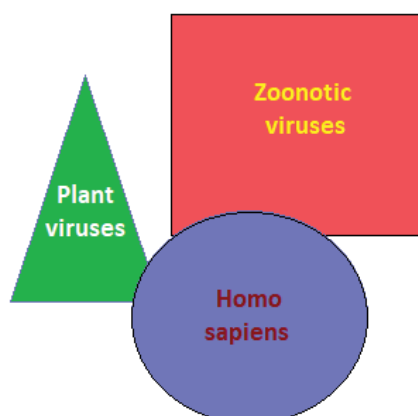


Table 2: Trans-viral Interface and Spill-over.

infectious diseases, caused by pathogens that have infected the same host species earlier, but continue to appear in new locations or in drug-resistant forms, or reappear after their apparent control or elimination [11]. The predominance of zoonoses among the prevalent infectious diseases, underlines importance of the animal–human–ecosystem interface in disease transmission.

Further, the emergence of novel zoonotic pathogens is a challenge to global healthcare. Though, the advent of sophisticated diagnostics tools has improved our capacity for early detection, the exposure prophylaxis and post-exposure treatment modalities are insufficient. The emerging zoonoses leading to viral epidemics are potential health threats of modern times. Apart from various biological factors, the ecological, economic, and developmental activities and behavioral practices influence the transmission interface, leading to dissemination and transmission through the human population. In addition, there are complex and dynamic factors such as human population density, mobility, lifestyle, behaviours, and food choices linked to the zoonotic viral spill-over, transmission and spread (Figure 3).

Human Coronavirus Disease: COVID-19

The Interspecies transmission of coronaviruses: There are distinct mechanisms which enable the interspecies transmission of coronaviruses. The coronavirus spike (S) glycoproteins can bind to analogous receptor proteins (receptor orthologs) in species other than their primary host [12]. Further, these viruses are capable of replicating in multiple hosts or a cluster of species. Thus, the SARS-like coronaviruses are capable of binding to angiotensin converting enzyme 2 (ACE2) receptors from multiple species, including humans [13]. Once they are able to infect across species, mutations may arise permitting human-to-human transmission [14]. The coronaviruses with a broader host range possess mutations in the S glycoprotein gene, a phenomenon rendering it capable of binding to a variety of host cell proteins or mutate further during docking and entry to host cells. The coronaviruses recognise various receptor-proteins, including aminopeptidase N, ACE2, and dipeptidyl peptidase 4 (DPP4). Finally, some coronaviruses can use sugar moieties as receptors or co-receptors for entry, availing an alternative strategy for trans-species spill-over and transmission [15].

In addition, the coronavirus structure has some specific features. It has the S-glycoprotein embedded in the lipid bilayer surrounding the nucleocapsid, which mediates viral binding to host receptors. The S glycoproteins of SARS-CoV bind to ACE2 on the surface of host cells for docking and entry, whereas S-glycoproteins of MERS-CoV can bond to DPP4. The small and medium sized Chinese horseshoe bats belonging to Rhinolophidae family are natural reservoirs of SARS-CoV. As the studies have indicated, the intermediate hosts may not be necessary for direct human to human transmission for some SARS-like coronaviruses. The host age has also been identified as a factor in the cross-species transmission of coronaviruses. Further, in the infected persons, the age may influence the disease pathogenesis, clinical course as well as the prognosis.

The pathogenicity of coronaviruses: The coronaviruses have certain pathological features which promote their disease potential. Following SARS infection for the first 12 to 24 hours, there is no measurable interferon-stimulated genes (ISG) response in the infected airway epithelial cells. Later, some ISGs are activated at 24 hours, and most may follow and reach peak titres between 24 and 30 hours. Thus, by the time the cell-intrinsic defence mechanism gets turned on, the viruses have irreversibly damaged the cellular appendages [16]. The MERS infection carries a similar sequence of events.

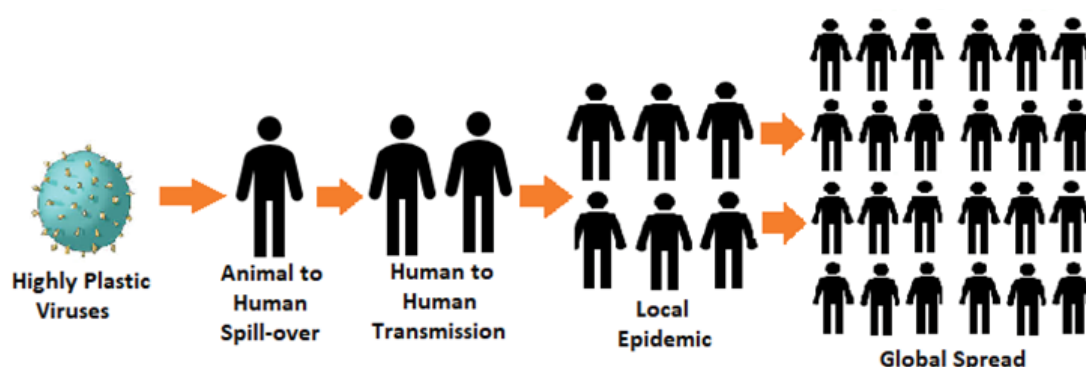


Table 3: Epidemiology of Transmission and Global Spread of Zoonotic Viral Infections.

The significant delay in cell intrinsic immune recognition and ISG induction in SARS, MERS, to some extent in H5N1 and likely in COVID-19, downregulates a subset of the ISGs leading to immune response failure. The mechanism underlying this phenomenon appears to be epigenetically regulated and allows the viruses to manipulate host cell intrinsic response and increases the disease severity.

Over the past few months, COVID-19, a novel RNA coronavirus outbreak has infected more than 90,000 people and caused over 3,000 deaths. The lethality of COVID-19 is about 2.92 percent or more in some instances, being about 30 times or more lethal than the typical influenza. Both influenza and coronavirus (SARS, MERS, and COVID-19) cause severe inflammation in the lungs, leading to viral pneumonia manifesting as acute respiratory distress, multi-organ failure and death.

Measures to Deal With Epidemics Like COVID-19

Nutraceuticals and other dietary constituents: It appears that certain nutraceuticals may provide relief in clinical symptoms to patients infected with encapsulated RNA viruses such as influenza and coronavirus (Figure 4).

The nutraceuticals appear to reduce the inflammation in the lungs and help to boost type 1 interferon response to these viral infections [17]. The nutraceuticals that might help in controlling of RNA viruses including influenza and coronavirus include Ferulic acid, Lipoic acid, Spirulina, N-Acetylcysteine, Selenium, glucosamine, Zinc, Yeast Beta-Glucan and Elderberry. They have been found to reduce the duration and severity of infection and reduce mortality in experimental animals infected with influenza [18].

Some recent discoveries have pointed the way to effective use of nutraceuticals for potentiating the type 1 interferon response to RNA viruses. The single-stranded viral RNA trapped within endosomes evokes superoxide production by NOX2-dependent NADPH oxidase complexes, which leads to an oxidation of Cys98 on toll-like receptor 7 (TLR7), blocking its ability to transmit a signal to stimulate type 1 interferon production [19]. These findings point to the possibility that nutraceuticals capable of inhibiting NOX2, promoting clearance of hydrogen peroxide or helping to restore of the native structure of Cys98 in TLR7, may be able to boost the TLR7-mediated induction of type 1 interferon and antiviral antibodies.

Further, it is known that heme oxygenase-1 (HO-1) induction potentiates the type 1 interferon response to influenza virus. Thus, the Phase 2-inductive nutraceuticals – such as ferulic acid, lipoic acid and sulforaphane – which promote induction of HO-1, may be helpful in stimulating the type 1 interferon response. The downstream consequences of hydrogen peroxide may also be mitigated by phase 2-inductive nutraceuticals, which induce peroxidase enzymes and promote the synthesis of glutathione, a cofactor for certain peroxidases and a catalyst in reactions that reconvert oxidized cysteine groups to their native form. Selenium being a cofactor for certain peroxidases, ensuring adequacy of selenium nutrition might also be helpful. The selenium deficiency also increases the rate at which viruses can mutate, promoting

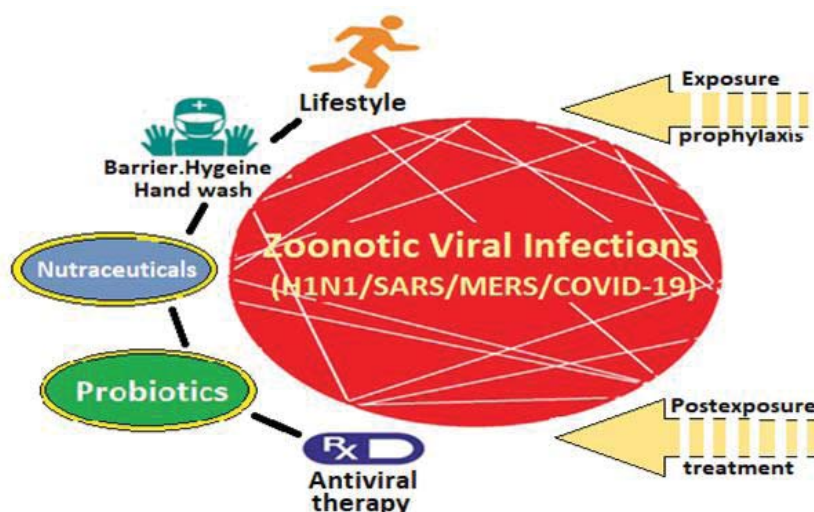


Table 4: Prophylaxis and Treatment Modalities for Viral Infections Like COVID-19.

the evolution of strains that are more pathogenic and capable of evading immune response. Zinc, also, supports function and proliferation of various immune cells.

The phycocyanobilin (PCB) chromophore of cyanobacteria (such as spirulina) and certain types of blue-green algae has been shown to possess NADPH oxidase inhibiting activity and significant antioxidant and anti-inflammatory effects. Hence, the ingestion of spirulina or spirulina extracts enriched in PCB may potentiate the type 1 interferon response in the context of RNA virus infection. In animal experiments, oral administration of spirulina extract rich in phycocyanin has been found to decrease mortality in influenza-infected mice. The antioxidants can also protect lung parenchyma by quelling excessive inflammatory reaction. They can also decrease the inflammatory response, in general, both by suppressing viral spread and by reducing pro-inflammatory signaling in endothelial cells. The nutraceuticals like Black Currant, Jamaican Sorrel, bee pollen, Echinacea purpurea, Siberian Ginseng, honey, bee propolis, and Goldenseal, and components of these nutraceuticals such as luteolin, apigenin, quercetin and chlorogenic acid may hold promise.

Glucosamine administration may up-regulate the mitochondrial antiviral-signaling (MAVS) protein activation, which is a key mediator for type 1 interferon response, leading to activation of cytosolic RNA virus detectors RIG-1 and MDA5, and subsequently, the activation of the transcription factor interferon regulatory factor 3 (IRF3). The mice fed on a glucosamine-enriched diet markedly enhances the survival of wild-type mice infected with influenza virus. The high-dose glucosamine supplementation might aid prevention and control of RNA virus infections. The yeast beta-glucan also has immunostimulant effects by amplifying the dendritic cell activation via dectin-1 and CR3 receptors. It has been shown to enhance immune response in mice challenged with influenza virus [20]. Certain herbal preparations such as extracts of elderberry, a rich source of anthocyanins and their metabolite, ferulic acid, have shown potential for improving the symptoms of infections with influenza and other RNA viruses [21].

Gut Microbiota and Probiotic Therapy

Human Gut Biosphere and Immunity: The gastro-intestinal tract, skin and genitals, and various other body organs such as upper and lower respiratory tract and lungs, harbour large and diverse communities of bacteria, viruses, and other microscopic life. The microbial ecosystems throughout the body interact with the molecular processes, which have been linked to various aspects of human physiology including immunity, and a disturbed microbiota is associated with deranged immune response.

The gut epithelium actively senses microbes, playing an essential role in maintaining host-microbial homeostasis at the mucosal interface. There has been documented a causal relationship between altered microbial communities, i.e. dysbiosis, immune response and disease. There is a crosstalk between the gut microbiota and host immune system (Figure 5). The human intestinal microbiota acting in tandem with the host's defence and immune system is vital for preservation of health and the integrity and the activity of the gut microbes are responsible for the protection from disease states including viral infections. Certain metabolites or antigens presented by members of the microbiome may help raise the immune system's sensitivity and effectivity to viral infections. Further, the gut microbes harbour enzymes and secrete molecules that can influence drug absorption, metabolism, efficacy, and toxicity.

Gut microbiome and viral infections: During the in course of infection process, various viruses encounter the commensal microbiota of the hosts. It is possible that there are robust interactions between these viruses and the commensal microbiota. Thus, in the regulation of viral infection, commensal microbiota appears to play a variable but critical role. It may promote viral infectivity through diverse mechanisms but can also exert substantial inhibitory effects on viral infection. In addition to fostering the generation of immunoregulatory Treg cells, the commensal microbiota has antiviral effect by suppressing the activation of effector immune cells and by inhibiting the production of various inflammatory cytokines that are pivotal for virus elimination. On the downside, the commensal microbiota may facilitate genetic recombination of viruses and enhance their infectivity.



Table 5: The Basis of Probiotic Treatment in Viral Infections.

There is evidence of modulation of virus infectivity by the commensal microbiota of the host. During the infection process, viruses may have substantial and intimate interactions with the commensal microbiota. There is evidence that the commensal microbiota regulates and is in turn regulated by invading viruses through diverse mechanisms, thereby having stimulatory or suppressive roles in viral infections. Further, the commensal microbiota may modulate the efficiency of viral replication, transmission and persistence, and the outcome of the viral infection. The integrity of the commensal microbiota can be disturbed by invading viruses, causing dysbiosis in the host and further influencing virus infectivity [22]. On the other hand, the microbial dysbiosis may interfere with the absorption, metabolism and therapeutic efficacy of the antiviral and other supportive drugs given for treatment.

The evidence from experimental studies: There is a potential for microbe-based probiotic adjunctive therapeutic approach to critical respiratory viral infections [23]. In general, the use of probiotics is related to the process through which the viruses activate the recognition and bonding of receptors and domains on the host cells and direct the replication of virions and alter the host immune response. Various animal and in vitro studies have identified mechanisms underlying the immunomodulatory capacity of specific probiotics, which appears to be strain-specific and results from a combination of signaling pathways activated as a result of a specific microbe-derived ligands interacting with the corresponding pattern recognition receptors and domains on host cells. The probiotics induce changes in dendritic cell phenotype and function, T- cells, natural killer cells and alveolar macrophages forming the basis of the protective effect of probiotics.

The beneficial probiotic bacteria are demonstrated to promote the host defence and to modulate immune response in various viral infections [24]. The probiotics frequently include *Lactobacillus* or *Bifidobacterium* species, apart from other bacteria, including non-pathogenic forms of *Escherichia coli* and *Bacteroides*, as well as certain yeasts, such as *Saccharomyces*. The lactic acid bacteria (LAB) has shown functional antibacterial and antiviral activity against diverse human and animal viruses [25]. In the highly contagious, coronavirus induced transmissible gastroenteritis (TGE) causing severe diarrhoea and other symptoms leading to death in young piglets, the probiotic *Lactobacillus plantarum* strain N4(Lp) has been demonstrated to have protective effects. The in vitro Lp supplementation led to dose-dependent rescue of viability of infected cells and pre-treatment of cells with probiotic metabolic products reduced viral proliferation [26]. Another *Lactobacillus* strain, *Lactobacillus rhamnosus* GG protects mice from H1N1 influenza virus infection by regulating respiratory immune responses [27]. In a recent study, *Lactococcus lactis* strain Plasma (LC-Plasma) was shown to possess strong stimulatory activity for plasmacytoid dendritic cells via the TLR9-pathway to promote viral replication control [28].

Apart from the probiotic bacteria, their components are also able to induce potentially beneficial effects for host cells. The compounds, such as lactic acid, acetic acid and γ -aminobutyric acid produced by probiotic bacteria are capable of enhancing body immunity and controlling sepsis. The exopolysaccharides (EPSs) are biological high-molecular long-chain polysaccharides that are secreted by microorganisms. The EPS secreted by *Lactobacillus acidophilus* were found to inhibit TGE viral infection and improved levels of IFN- α , IL-6, IL-8 [29,30]. Further, the probiotic therapy has been shown to facilitate CD4 recovery in HIV-1 infected patients [31].

Specific antiviral therapy

The aim of antiviral therapy is to minimize symptoms and infectivity as well as to shorten the duration of illness. Currently, antiviral therapy is available only for a limited number of infections [32]. Because viruses are obligate, intracellular parasites, it is difficult to find drug targets that interfere with viral replication without harming the host cells. Unlike other antimicrobials, antiviral drugs do not deactivate or destroy the virus but aim reduce the viral load by inhibiting replication and facilitating the host's innate immune mechanisms to neutralize the virus. The most dreaded complication of an uncontrolled virus infection is sepsis, which is often underdiagnosed and has downhill course [33]. The virulent viruses causing viral sepsis, on one hand display the capacity to evade the immune system, whereas on the other, induce powerful inflammatory responses, often characterised by high levels of TNF- α and IL-6 expression along with low IFN- γ expression that can damage the host tissues.

The influenza viruses cause upper-respiratory infections in their hosts with symptoms such as severe lassitude, headache, chills, muscle aches and delayed mild cough with signs of fever. These systemic symptoms are due to the release of cytokines by the bronchial epithelial and macrophage cells. There are drugs which can inhibit influenza virus from entering or being effective inside the bronchial epithelial cells. The most commonly used drug for H1N1 is

a neuraminidase inhibitor, oseltamivir, which prevents newly formed viruses from finalizing their budding from an infected cell.

Amantadine is an M2 ion channel blocker, whereas rimantadine is a weak NMDA receptor antagonist, Oseltamivir, zanamivir and peramivir are potent neuraminidase inhibitor and are recommended for both influenza treatment as well as prophylaxis. Baloxavir marboxil is a new influenza antiviral drug, approved recently. Baloxavir targets the cap-dependent endonuclease activity of influenza virus [34].

Conclusion: Lessons from Viral Epidemics

Considering that certain nutraceuticals have demonstrated antiviral effects in both clinical and animal studies, further studies are required to establish their therapeutic efficacy in the encapsulated RNA viral infections like H1N1, SARS, MERS and COVID-19, where definitive treatment modalities are being envisioned and explored.

Certain nutraceuticals (and herbs) decrease the cytokines levels to reduce inflammation and tissue injury. The components of various nutraceuticals such as luteolin, apigenin, quercetin and chlorogenic acid may be useful for developing a combo-therapy. By combining these compounds with other drugs showing efficacy against viral infection, more effective drug combinations may be made to treat the potentially deadly viral diseases.

Understanding how the commensal microbiota enhances viral infection, especially the molecular requirements for the microbiota-mediated promotion of viral infections, may lead to the development of novel, feasible antiviral strategies. The probiotic therapy is expected to be a rational adjunctive therapeutic modality for options for critical viral respiratory disease. The use of probiotics to enhance immunity and immune response against viral infections is a novel possibility. Apart from probiotic bacteria, their components can also induce potentially beneficial effects for host cells. The compounds, such as lactic acid, acetic acid and γ -aminobutyric acid, and EPSs produced by probiotic bacteria are capable of improving immune response body, limiting cytokine storms, and regulating septicaemia. The available antiviral therapy is inefficient in deactivating or destroying the infecting viruses, though it may help in reducing the viral load by suppressing replication. The novel efficient antiviral agents are being explored.

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Chapter 2: Exploring Pathophysiology of COVID-19 Infection: Faux Espoir and Dormant Therapeutic Options

Background

COVID-19 virus structural components: The 2019-nCoV, also called SARS-CoV-2, was first reported in Wuhan, China in December 2019. The disease was named Coronavirus Disease 2019 (COVID-19) and the virus responsible for it as the COVID-19 virus, respectively, by WHO. The 2019-nCoV has a round, elliptic or pleomorphic form with a diameter of 60–140 nm. It has single-stranded RNA genome containing 29891 nucleotides, a lipid shell, and spike, envelope, membrane, and hemagglutinin-esterase (HE) proteins.

Steps in progression of COVID-19 illness: Once inside the airways, the S protein on the viral surface recognizes and mediates the attachment to host ACE-2 receptors and gains access to endoplasmic reticulum. The HE protein facilitates the S protein-mediated cell entry and virus spread through the mucosa, helping the virus to attack the ACE2-bearing cells lining the airways and infecting upper as well as lower respiratory tracts. With the dying cells sloughing down and filling the airways, the virus is carried deeper into the lungs. In addition, the virus is able to infect ACE2-bearing cells in other organs, including the blood vessels, gut and kidneys. With the viral infestation, the activated immune system leads to inflammation, pyrexia and pulmonary edema. The hyperactivated immune response, called cytokine storm in extreme cases, can damage various organs apart from lungs and increases susceptibility to infectious bacteria especially in those suffering from chronic diseases.

The current therapeutics for COVID-19: At present, there is no specific antiviral treatment available for the disease. The milder cases may need no treatment. In moderate to severe cases, the clinical management includes infection prevention and control measures, and symptomatic and supportive care, including supplementary oxygen therapy. In the critically ill patients, mechanical ventilation is required for respiratory failure and hemodynamic support is imperative for managing circulatory failure and septic shock.

Conclusion: Confusion, despair and hopes: There is no vaccine for preexposure prophylaxis or postexposure management. There are no specific approved drugs for the treatment for the disease. A number of drugs approved for other conditions as well as several investigational drugs are being canned and studied in several clinical trials for their likely role in COVID-19 prophylaxis or treatment. The future seems afflicted with dormant therapeutic options as well as faux Espoir or false hopes. As obvious, not all clinical trials will be successful, but having so many efforts in progress, some may succeed and provide a positive solution. Right now, though, confusion and despair prevail.

Exploring Pathophysiology of COVID-19

Witnessing and Defining the Disease

The coronaviruses (CoVs) are positive-stranded RNA viruses with a crown-like appearance due to the presence of large spike glycoproteins on the envelope. There are seven CoVs capable of infecting humans (HCoVs), out of which HCoV-OC43 and HCoV-HKU1 (betaCoVs of the A lineage), and HCoV-229E and HCoV-NL63 (alphaCoVs) were identified in the 1960s and responsible for about 5% to 10% cases of common cold and short-term upper respiratory infections, with about 2% of the human population being healthy carriers of a CoV. The remaining three HCoVs belonging to betaCoVs of the B and C lineage, have been discovered only in recent years and include SARS-CoV, MERS-CoV and SARS-CoV-2. The newer HCoVs have caused epidemics with variable clinical severity featuring respiratory and extra-respiratory manifestations with the mortality rates up to 10% or more.

The 2019-nCoV, also called SARS-CoV-2, was first reported in Wuhan, China in December 2019. The disease was later named Coronavirus Disease 2019 (COVID-19) and the virus responsible for it as the COVID-19 virus, respectively, by World Health Organization (WHO) [1]. COVID-19 is an acute respiratory disease caused by novel coronavirus SARS-CoV-2 or 2019-nCoV. Recently, on March 11, 2020, COVID-19 was declared by the WHO as a virus pandemic disease.

COVID-19 Virus Structural Components

The SARS-CoV-2 or 2019-nCoV has a round, elliptic or pleomorphic form with a diameter of 60–140 nm. Its single-stranded RNA genome contains 29891 nucleotides, encoding for 9860 amino acids and bears a sequence identity

approximately 50% to MERS-CoV and 79% to SARS-CoV [2]. Like other CoVs, it is sensitive to ultraviolet rays and heat and can be effectively inactivated by lipid solvents including ether and ethanol.

The 2019-nCoV has a lipid shell, an RNA genome, spike, envelope and membrane and hemagglutinin-esterase dimer proteins (Figure 1). The world-wide research is going on to determine the structural components of the 2019-nCoV as related to the pathogenetic mechanisms.

1. The glycosylated Spike protein (S) is a large component making the distinct spikes on the surface of the virus. The S protein containing two functional domains, a receptor binding domain and a second one to mediate fusion of the viral and cell membranes, is cleaved by a host cell furin-like protease into two separate polypeptides S1 and S2. The SARS-CoV S1-protein contains a conserved Receptor Binding Domain (RBD), which recognises the angiotensin-converting enzyme 2 (ACE2). The S glycoprotein cleavage is a precondition for the host cell entry. The nature of the cell protease that cleaves the S glycoprotein varies according to the coronavirus and the proteolytic cleavage of the S glycoprotein is supposed to determine whether the virus can cross species, for example, from bats to humans. It has been observed that the S protein from a MERS-like CoV from Ugandan bats bind to human cells and fails to mediate viral entry, but on adding the protease trypsin, the cleavage followed by entry occurs. In relation to the SARS-CoV-2, the S protein has a furin cleavage sequence (PRRARS[V]), and the furin proteases being abundant in the respiratory tract, the SARS-CoV-2 S glycoprotein is cleaved in the exiting VLPs from epithelial cells, which readily infect new cells [3].

2. The RNA genome of the virus is bound by the phosphorylated nucleocapsid protein (N) in a beads-on-a-string conformation. On entering the host cells, the N protein potentially tethers the viral genome to replicase-transcriptase complex (RTC), and helps in packaging the encapsulated genome into viral particles [4].

3. The Envelope protein (E) is found in small quantities in the virus and is appears to be a transmembrane protein with ion channel activity. The protein facilitates assembly and release of the new virions. It is related to the disease pathogenesis and important for the disease progression.

4. The Membrane protein (M) is the most abundant structural component of the virus. It exists as a dimer and enables to maintain membrane curvature on one end and bound to nucleocapsid proteins on the other.

5. The Hemagglutinin-esterase (HE) is also a dimer protein and binds to sialic acids on surface glycoproteins. It is responsible for facilitating and enhanceing S protein-mediated cell entry and virus spread through the mucosa.

The Viral Transmission and Infection

As with other respiratory pathogens, including flu and rhinovirus, the transmission is believed to occur through respiratory droplets from coughing and sneezing or by being carried to oral or nasal mucosa by hands from the virus-infested surfaces. It appears that all COVID-19 patients – asymptomatic, mild or severe have a massive throat/mucus titre of virus, shedding it in the surroundings. Aerosol transmission may also be possible in closed and confined spaces.

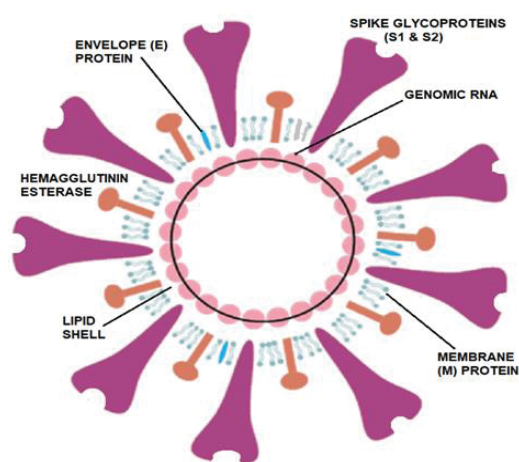


Figure 1: The structural diagram and components of COVID-19 Virus.
Modified from - <https://d1gk4lfg3bst7c.cloudfront.net/media/wysiwyg/2019-nCoV-figure-ad.png>

The incubation period is from 3 to 7 days, 14 days being considered as the longest possible time from infection to appearance of symptoms [5]. The research supports that in addition to the respiratory droplets and direct contact, fecal-oral transmission might also be the route of transmission of 2019-nCoV. It is to be noted that the information is derived from the early research and reports. Further studies are warranted to understand the mechanisms of transmission, the incubation period, duration of infectivity and the clinical course of COVID-19 [6].

The WHO recommends collecting specimens from both the upper respiratory tract (nasopharyngeal and oropharyngeal samples) and lower respiratory tract such as expectorated sputum, endotracheal aspirate, or bronchoalveolar lavage for a diagnostic test. The samples are needed to be stored at 4°C. The amplification of the genetic material extracted from the sample is done through a reverse polymerase chain reaction (RT-PCR). If the test result is positive, it is recommended that the test is to be repeated for verification. In patients with confirmed COVID-19 diagnosis, the laboratory evaluation should be repeated at intervals to evaluate for viral clearance prior to discharge from observation. In a positive case, lymphopenia as well as raised liver enzymes, LDH, muscle enzymes and C-reactive are negative prognostic factors.

Steps in the COVID-19 Illness

Once inside the airways, the S protein on the viral surface recognize and stick to the receptor protein called ACE2 and the virus attacks the ACE2-bearing cells lining the airways. It can infect upper as well as lower respiratory tracts and with the dying cells sloughing down and filling the airways the virus is carried deeper into the lungs. The thin layer of surfactant coating the airways becomes even thinner and the brush border less efficient to evict viruses and other foreign particles with a colder temperature and dry air, which may also dampen the immune response to the invading viruses. It appears that the virus is able to transmit while still confined to the upper airways, before invading the lower respiratory tract and lungs and causing severe symptoms. In addition, the virus is able to infect ACE2-bearing cells in other organs, including the blood vessels, gut and kidneys.

With the viral infestation, the activated immune system leads to macrophages to be recruited to the alveolar space, which increase cytokine production and attract additional immune cells to the affected area such as T-helper cells CD4 and CD8 to combat the virus. The pattern recognition receptors (PRRs) of the immune cells recognize the virus and signal release of the pro-inflammatory cytokines such as interferon gamma (IFN- γ), tumor necrosis factors (TNFs), interleukins (ILs), and chemokines. IFN- γ activates macrophages which produce IL-6, TNF- α , and IL-10. There are triggered additional pathways associated with PRRs, including cyclo-oxygenase (COX)-2 and c-Jun N-terminal kinase (JNK) [7]. With recovery, once the virus is cleared, the immune pathways shut down. In a cytokine storm, however, the process goes into overdrive, initiating vascular leakage, coagulation cascades, and disseminated intravascular coagulation (DIC). Further, it leads to an increased susceptibility to infectious bacteria. Furthermore, the process affect other organs besides the lungs, especially in those suffering chronic diseases, leading to multi-organ failure (MOF) [8].

Age is an important epidemiological factor. The elderly people are at risk of severe infections possibly because the ineffective initial anti-viral immune response. It appears that children may be less severely affected because their immune system is unlikely to progress to a cytokine storm. Further, there are other factors like individual genetic make-up, the amount of virus load, the other microbes in the body including gut microbiota which may play a role in acquirement of the infection and its progression.

Directed by the viral RNA, the synthesis of polyprotein 1a/1ab (pp1a/pp1ab) in the host cells is brought about with the transcription occurring through the replication-transcription (RT) complex organized in double-membrane vesicles (Figure 2). The open reading frames (ORFs) guide the production of both pp1a and pp1ab polypeptides which are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or more papain-like proteases for producing 16 non-structural proteins (nsps) [9]. The ORFs also encode for structural proteins, including spike, membrane, envelope and nucleocapsid proteins. The pathophysiology and virulence mechanisms of COVID-19 virus has been related to the function of the nsps and structural proteins. It has been documented that the nsps are able to block the host innate immune response, as well [10]. The E protein also plays a crucial role in promoting viral assembly and release of nascent virions.

ACE2 Receptors and their Expression

It appears that ACE2 is the main host cell receptors for 2019-nCoV, playing a crucial role in the invasion of virus into the cell through the receptor binding domain (RBD) and infectivity and affliction of upper respiratory tract, lower

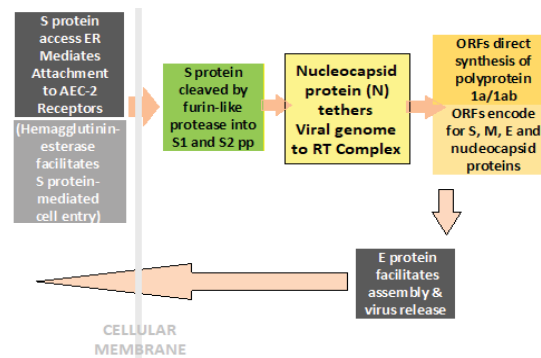


Figure 2: The Simplified Steps in Pathogenesis and Replication Cycle of COVID-19.

respiratory tract and lungs, and gastrointestinal system apart from kidneys and various other organs [11]. Other studies have indicated that the ACE2 is the major cell receptors for 2019-nCoV and the 2019-nCoV may not preferentially use other receptors like aminopeptidase N and dipeptidyl peptidase 4 (DPP4). Further, the 2019nCoV spike (S) protein plays the most important roles in viral attachment, fusion and entry [12]. Considering this, the expression and distribution of the ACE2 in human body may outline the potential infection routes and the organs with high ACE2-expressing tissues are potential sites for 2019-nCoV infection [13]. Further, the ACE2 expression is reduced in SARS-CoV infection and linked with the viral S protein. In mice experiments, the injection of SARS-CoV Spike worsens acute lung failure in vivo, which can be attenuated by blocking the renin-angiotensin pathway [14].

Significantly high ACE2 expression is found in type II alveolar cells (AT2) of lung, upper oesophageal stratified epithelial cells, absorptive enterocytes from ileum and colon, gall bladder and bile ductal cells, myocardial cells, kidney proximal tubule cells and bladder urothelial cells. Apart from this, the ACE2 is also expressed on the oral mucosa and highly enriched in epithelial cells of tongue accounting for a potentially high risk of this route for 2019-nCoV infectious susceptibility [15]. As such, the ACE2 expression is higher in oral mucosa and tongue than buccal and gingival tissues. These findings may underline the prophylactic role of frequent mouth washing and rinsing [16]. The researchers have isolated 2019-nCoV from human airway epithelial cells. Further, the saliva, urine and stool specimens and rectal swabs have demonstrated embedded viruses in the COVID-19 patients [17].

It is worth mentioning that bulk of the ACE2-expressing cells, over 80%, are AT2 (alveolar type 2) cells. In addition, the distribution of ACE2 is more widespread in males than females, which is consistent with the epidemiological finding that men are more prone to COVID-19 than women. The analysis of the ACE2 RNA expression profile indicates that the ACE2 expressing of ACE2 AT2 cells also express many other genes favouring the viral processes. The abundant expression of ACE2 in the AT2 cells may explain the preferential involvement of lungs and severe alveolar damage following the infection. It has also been noticed that the Asian men have a relatively higher ACE2- expressing cell ratio than the Caucasian and African American subjects [18]. But, these reports of the ACE2 expression analysis in lung tissues are controversial, in light of small numbers of subjects. In another study, the ACE2 expression analysis using the RNA-seq and microarray datasets from control lung tissues indicated there were no significant differences between Asian and Caucasian, or male and female [19].

Current and Dormant Therapeutic Possibilities

Clinical Spectrum of COVID-19

The clinical spectrum of COVID-19 varies from asymptomatic or pauci-symptomatic presentation to a moderate to severe states characterized by respiratory failure necessitating mechanical ventilation and ICU support and those manifesting critical clinical conditions like sepsis, septic shock and multiple organ dysfunction syndromes (MODS). The mild disease, i.e., non-pneumonia and mild pneumonia occurs approximately in 81% of cases. A severe disease with dyspnoea and acute respiratory distress syndrome (ARDS) and/or lung infiltrates appearing within 24 to 48 hours occurs in about 14% of cases. Whereas, the critical COVID-19 illness accompanied by respiratory failure, septic shock, and/or multiple organ dysfunction (MOD) or failure (MOF) is likely to occur approximately in 5% of cases [20].

The transition from milder symptoms to acute respiratory distress syndrome (ARDS) is likely due to an unrestrained

cytokine release by the hyperactive immune response. It appears that in patients who are going to have the worst outcomes with COVID-19 infections, the immune system becomes overactive with excessive production of T cells and macrophages, resulting in cytokine storm with release of a large amount of proinflammatory cytokines including interleukins (IL) - 1, 6, 12 and 18. The excessive or uncontrolled levels of cytokines are released then further activate more immune cells, resulting in hyperinflammation and cytokine release syndrome (CRS).

The long-term complications among survivors of infection with SARS-CoV-2 having clinically significant COVID-19 disease are not yet available [21]. A note is to be taken of the clinical evidence suggesting that anosmia and dysgeusia associated with the COVID-19 infection. This may be linked to the rich ACE-2 receptors expressed in nasal mucosa and tongue affected on the viral infection [22]. These subtle symptoms could in many cases be harbingers of a coronavirus diagnosis. Anosmia, in particular, has been seen in patients ultimately testing positive for the coronavirus with no other symptoms [23]. With the recovery from the disease, most patients gradually regain both their sense of taste and smell. The gastrointestinal symptoms in COVID-19 include anorexia, diarrhoea, nausea and vomiting, abdominal colic and, rarely, gastrointestinal bleeding. Diarrhoea is the most common abdominal symptom in children as well as adults in COVID-19 [24].

Specific and Supportive Treatment

There is no vaccine for preexposure prophylaxis or postexposure management. Simultaneously, there is no specific antiviral treatment recommended for COVID-19. The treatment is mostly symptomatic, and oxygen therapy and ventilatory support represents the major treatment options available for patients with severe infection. In the critically ill patients, assisted mechanical ventilation is required for respiratory failure and hemodynamic support is imperative for managing circulatory failure and septic shock [25].

Among other therapeutic modalities, the use of systemic corticosteroids for the treatment of viral pneumonia accompanied by ARDS is not recommended [26]. Further, routine, or inappropriate administration of antibiotics is not recommended. Some studies have cited use of azithromycin along with chloroquine (CQ) or hydroxychloroquine (HCQ) has been tried. So far, although no antiviral treatments have been approved, alpha interferon (e.g., 5 million units by aerosol inhalation twice per day), lopinavir/ritonavir and remdesivir have been suggested to be effective.

Clinical and Experimental Trials

The Anti-Viral Drugs: There have been clinical trials using the combination of the HIV protease inhibitors Lopinavir (acts against the viral 3CL protease and has a modest antiviral activity against COVID-19) and Ritonavir, which increases the bioavailability of the former. The results from the trials were not found to be promising as no benefit has been documented in the primary end point of time to clinical improvement [27]. Though, there was shown slightly smaller number of deaths in the patient group treated with the lopinavir-ritonavir combination. Further, there was no significant effect on viral shedding. In another study in China, Kaletra (lopinavir-ritonavir combination) was not found to be a promising treatment of hospitalized COVID-19 patients with pneumonia in a recent clinical trial in China [28]. There has also been use of chloroquine along with lopinavir-ritonavir combination [29].

A combination of Oseltamivir and Lopinavir and ritonavir, the potent protease inhibitors have been used to treat severe cases of 2019-nCoV. The team of Thai doctors in Rajavithi Hospital, Bangkok who have been caring for COVID-19 patients, used large doses of oseltamivir combined with HIV drugs lopinavir and ritonavir. Though not cured, the patient's condition reportedly improved during a 10 day follow up, with the test result becoming negative within 48 hours following administration of the drug combination [30].

Some studies have reported, Favipiravir (6-fluoro-3-hydroxy-2-pyrazinecarboxamide), a selective RNA polymerase inhibitor, showing useful effects in COVID-19. Eighty patients suffering from COVID-19 participated in the clinical trial of which 35 patients took Favipiravir and 45 formed the control arm [31]. Improvement was noted in lungs on X-rays in about 91 percent patients given the medicine compared to 62% of those without it. In addition, they turned negative for the virus after a median of four days after becoming positive, compared with a median of 11 days for those who were not treated with the drug. It was concluded in the trial that ordinary COVID-19 patients untreated with antiviral previously, Favipiravir can be considered as a preferred treatment because of its higher recovery rate and reduced incidence of pyrexia and cough. There were few associated adverse effects [32].

The anti-viral drug, Remdesivir was found to be beneficial by inhibiting RNA replication in MERS and SARS patients.

The drug acts as nucleotide analogues interfering with the RNA-dependent RNA polymerase. In an in vitro study, Remdesivir blocked coronavirus infection in monkey and human cells. It is held that it may work against COVID-19, as it inhibits the coronavirus that causes MERS in cell culture and improves respiratory symptoms in the animal studies [33]. The drug inhibits viral replication through a premature termination of RNA transcription and has shown in-vitro activity against SARS-CoV-2, and in-vitro as well as in-vivo activity against related beta-Coronaviruses. It appears to be particularly effective in combination with chloroquine [34].

Macrolide Antibiotic - Azithromycin: Azithromycin is a macrolide antibiotic and works by decreasing protein synthesis by bacteria by binding to the 50S subunit of the bacterial ribosome and inhibiting translation of mRNA. A French study has shown promising results for COVID-19 patients with hydroxychloroquine and azithromycin combination. It significantly reduced viral load [35]. The French study was not a controlled clinical trial. A number of researchers have advised that the evidence should be re-examined, and further studies/research are needed. Occasionally, azithromycin can cause cholestatic hepatitis or delirium, whereas its overdose may cause severe heart block and increase the risk of death, especially in patients with heart problems.

Aminoquinolines (4AQs) - CQ and HCQ

Both the drugs, CQ and HCQ, have shown in-vitro activity against SARS-CoV-2, with HCQ having comparatively relatively higher potency. A study in China has documented that CQ treatment of COVID-19 patients had clinical and virologic benefit versus a control group [36]. Based upon in-vitro studies and limited data, CQ or HCQ are being currently recommended for treatment of hospitalized COVID-19 patients in several countries [37]. Though CQ and HCQ, both, have known safety profiles, the main concern is about cardiotoxicity - prolongation of QT interval, risk of hepatic and renal dysfunction with prolonged use and immunosuppression. In general, they are well-tolerated.

CQ has a strong antiviral effect in animal cells, in vitro. Though, the mechanism by which CQ decreases coronavirus infection is not unclear [38]. It appears that CQ raises the pH of endosomes, which prevents fusion and retards the virus from entering the cell. In addition, it may also block enzymes involved in the fusion between the virus and lung cells or thwart the viral replication process. CQ phosphate has demonstrated marked efficacy and acceptable safety in treating COVID-19 associated pneumonia in multicentre clinical trials conducted in China [39]. A combination of CQ with zinc has shown better effectivity. The ionized zinc inhibits the viral RNA polymerase and CQ acts as a zinc ionophore to rapidly transport zinc ions through cell membranes into cytoplasm.

HCQ shares the same mechanism of action as CQ, but its more tolerable safety profile makes it the preferred drug to treat malaria and autoimmune conditions. The optimal dose and duration of HCQ for treatment of COVID-19 are not well established. Various doses used by clinicians for HCQ are - 400mg BID on day one, then once daily for 5 days; 400 mg BID on day one, then 200mg BID for 4 days or 600 mg BID on day one, then 400mg daily on days 2-5 [40].

The immunomodulatory effect of CQ as well as HCQ also may be useful in controlling the cytokine storm that occurs late-phase in critically ill SARS-CoV-2 infected patients [41]. HCQ has a superior in vitro anti-SARS-CoV-2 activity than CQ. In the study, this was demonstrated by the EC50 values for HCQ always being smaller than the EC50 values for CQ, indicating that HCQ has a more potent antiviral activity. Also, it may help mitigating the cytokine storm in critically ill SARS-CoV-2 patients [42].

Conclusion: Faux Espoir, Confusion and Dormant Options

There are no specific FDA-approved drugs for the treatment of patients with COVID-19. At present the clinical management of COVID-19 includes infection prevention and control measures, and symptomatic and supportive care, including supplementary oxygen and mechanical respiratory support when indicated. A number of drugs approved for other indications as well as several investigational drugs are being studied in several clinical trials that are underway across the globe (Figure 3). In addition, several other drugs are being scanned for their likely role in COVID-19 prophylaxis or treatment in the United States and other countries [43].

The several existing antiviral medicines and their combinations are under clinical trials for COVID-19. Favipiravir is an antiviral prodrug and undergoes intracellular phosphor-ribosylation to be converted into an active form, which inhibits the RNA polymerase activity in RNA viruses [44]. Though, favipiravir is considered potentially effective for COVID-19, the sufficient studies for recommending its use in COVID-19 are not available [45]. Remdesivir is another potential

Non-Specific Prophylaxis	Definite prophylaxis	Specific COVID-19 Treatment	Non-specific/Presumptive Therapeutic Options
Social distancing	Specific vaccine Not available	Anti-viral agent(s) Not available	Anti-viral drugs: Remdesivir, Lopinavir-Ritonavir, Oseltamivir, Favipiravir, etc.
Frequent hand washing			CQ and HCQ Disease Modifying Drugs
General hygiene			Convalescent plasma therapy Artificial antibodies - Tocilizumab and sarilumab

Figure 3: Present and Future Prophylactic and Therapeutic Options for COVID-19.

antiviral drug for COVID-19. Also, a prodrug, it is metabolized intracellularly to an adenosine triphosphate analog, which inhibits viral RNA polymerases. Treatment with intravenous remdesivir documented significant improvement for the first COVID-19 case in US [46]. In another study in a cohort of severe COVID-19 patients, the compassionate use of remdesivir was associated with clinical improvement [47]. Ivermectin is a broad-spectrum antiparasitic agent that has shown antiviral activity against a broad range of viruses in vitro. An in vitro study, ivermectin was documented to inhibit the replication of SARS-CoV-2, apparently through inhibiting $IMP\alpha/\beta1$ - mediated nuclear import of viral proteins, which disrupts the immune evading mechanism of virus [48]. But further trials are needed to establish its role in the treatment of COVID-19 [49].

The convalescent plasma (CP) from patients recovered from the COVID-19 infection, contains antibodies against the virus and may be useful in supporting the immune system in severe cases. A recent study reported that the viral loads decreased to becoming undetectable in COVID-19 patients. The CP therapy was well tolerated with improvements in other clinical parameters [50]. The artificial antibodies against the virus, such as tocilizumab (Actemra) and sarilumab (Kevzara), target interleukins like IL-6 for treating the cytokine storms due to hyperactive immune response. In a small unpublished study in China, tocilizumab reduced pyrexia and requirement for supplemental oxygen [51].

The disease modifying and antiarthritic drugs against autoimmune diseases like rheumatoid arthritis work by dampening down the overactive immune system. They could be useful against in COVID-19 associated cytokine release syndrome (CRS). The treatment for less severe CRS is supportive, addressing the symptoms like fever, muscle pain, or fatigue. Moderate CRS requires oxygen therapy, IV fluids and anti-hypertensive agents to deal with the shock. For moderate to severe CRS, the use of immunosuppressive agents like corticosteroids and tocilizumab, an anti-IL6 monoclonal antibody, have been used in some medical centers to treat severe CRS without a proved positive outcome. CQ exhibits an inhibitory effect on SARS-CoV-2 replication cycle. However, the clinical use of CQ may cause severe side effects. The related compound, HCQ exhibits an antiviral effect and likely to attenuate the severe progression of COVID-19 by inhibiting the cytokine storm syndrome (CSS) by suppressing T cell activation, which is frequently fatal [52].

There are vaccine programmes to use messenger RNA to make cells produce anti-viral antibodies leading to immunity. This approach, though, has never been used in a widely available vaccine. There are notable vaccine development programs by biotech Moderna and BioNTech working with Pfizer [53]. Though, there is no vaccine for preexposure prophylaxis or postexposure management. A vaccine for COVID-19 may come in near future.

There are no specific approved drugs for the treatment for the disease. Though, a number of drugs are being scanned and studied in several clinical trials for their likely role in COVID-19 prophylaxis or treatment. As obvious, not all clinical trials will be successful, but having so many efforts in progress, some may succeed and provide a positive solution. Right now, though, confusion and despair prevail. The future seems afflicted with dormant therapeutic options as well as faux Espoir or false hopes. But, having so many efforts in progress, some may succeed and provide a positive solution. Right now, though, confusion and despair prevail.

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Chapter 3: The Agent and Host Factors in COVID-19: Exploring Pathogenesis and Therapeutic Implications

Background

Infectivity and pathogenesis: There is consensus that SARS-CoV-2 originated in horseshoe bats as it shares a high sequence similarity to bat CoVs, ~96% with the bat-CoV-RaTG13 and ~90% with bat-SL-CoVZC45 and bat-SL-CoVZXC21. But, the bat CoVs are not its direct ancestors, instead an intermediate host is involved, which subsequently transferred the virus to humans. The Malayan pangolin harbouring CoVs with a high similarity to SARS-CoV-2, is the most potential intermediate host. There is another consensus, that SARS-CoV-2 has higher infectivity and transmissibility as compared to SARS-CoV, related to a unique peptide (PRRA), four amino acid residue insertion on the S protein between its S1 and S2 subunits and involved in the proteolytic cleavage of the spike protein by cellular furin-like proteases widely expressed in lungs and respiratory tract and various other organs. This furin cleavage site is distinct from SARS-CoV and other CoVs including pangolin CoV which contain only a trypsin or TMPRSS2 cleavage site. This trait, the novel furin cleavage site on the S protein in SRS-CoV-2, has helped the spill-over to humans and potentiated its transmissibility as well as pathogenicity.

The pespective and implications: The sequences of SARS-CoV-2 from various patients from different countries are almost identical, with greater than 99•9% sequence identity, suggesting that SARS-CoV-2 has originated from one source, and has spread relatively rapidly worldwide, within a short period. Through the novel furin cleavage site in its S protein, the SARS-CoV-2 utilizes and affects ACE in multiple ways. Apart from its entry through the ACE2, SARS-CoV-2 subsequently down-regulates ACE2 expression resulting in unopposed angiotensin II accumulation and local RAAS activation, which deranges homeostasis and exacerbates the inflammation and potentiates the tissue injury in lungs and other organs. In this perspective and in light of the studies and available data, it is advisable that an ACE inhibitor or ARB should be continued, rather than withdrawn in favour of another antihypertensive, in known or likely Covid-19 patients with stable condition.

Evolving COVID-19 therapeutics: Presently there are no effective drugs for treatment of the Covid-19. The integrative network-based systems pharmacology methods have been used for rapid identification of repurposable drugs and drug combinations for the potential treatment. While new and repurposed drugs, including antiviral agents are being tested in various clinical trials, some of the promising drugs are simultaneously being recruited off-label for compassionate use or as experimental drugs to treat in desperate situations and otherwise dying patients. There has been a growing interest for the use of chloroquine and hydroxychloroquine as potential treatment in the interim till a specific treatment is available. There have been identified certain proteins in the host immune pathways which can be targeted for blocking viral replication by potential drugs or antibodies. Alternatively, the passive antibody transfer from pooled convalescent patient sera may seem another option. As obvious, not all clinical trials will be successful, but having so many efforts in the direction, some may succeed and provide plausible solutions.

Infectivity and Pathogenesis

The Agent Factors

Origin and Evolution of 2019-nCoV: The Coronaviruses (CoVs) belong to the subfamily Coronavirinae in the family of Coronaviridae of the order Nidovirales. The CoVs are enveloped RNA viruses, having the genome composed of a single-stranded positive-sense RNA (+ssRNA), ~30kb with 5'-cap structure and 3'-poly-A tail. The large CoV genome is related to the special features of the CoV RTC, which contains several RNA processing enzymes and encodes for several structural proteins (sps) and non-structural proteins (nsps) [1]. The sps having a critical role in viral RNA transcription and synthesis, are referred to as replicase-transcriptase proteins, whereas the nsps, also referred to as niche-specific proteins, though nonessential for virus replication but confer selective advantages for survival and tissue invasion [2]. The CoVs are further divided into four lineages, A to D. The lineage B includes the severe acute respiratory syndrome (SARS)-CoV and the novel SARS-CoV-2, whereas the lineage C includes Middle East respiratory syndrome (MERS)-related CoV. The six human CoVs were identified till last year and included HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV and MERS-CoV. The SARS-CoV-2 or 2019-nCoV is the latest and most virulent member of the CoV family that can infect humans [3].

The SARS-CoV-2 shares a high sequence identity to SARS-CoV. The protein sequence analyses have documented that the amino acid similarity of the conserved nsps between SARS-CoV-2 and SARS-CoV is as high as 94.6%, suggesting that they are closely related. The homology between the SARS-CoV-2 genome and CoV bat viruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 is approximately 90 percent, whereas it is approximately 96% with the bat SARS-like CoV (bat-CoV-RaTG13) genome [4]. There is consensus among the scientific community that the SARS-CoV-2 originated in horseshoe bats. But, the bat-SL-CoVZC45 and bat-SL-CoVZXC21 are not direct ancestors of 2019-nCoV. Instead the bat CoV may infected another animal, an intermediate host, which subsequently transmitted the virus to humans (Figure 1). The isolation of a CoV in the Malayan pangolins with high similarity to SARS-CoV-2 links these animals as being the potential intermediate host [5].

The genetic sequence of CoV, discovered in the lung samples of Malayan pangolins has a ~91% similarity to SARS-CoV-2. In addition, the pangolin CoV exhibits 100%, 98.2%, 96.7% and 90.4% amino acid identity with the SARS-CoV-2 E, M, N and S genes, respectively. The ancestor bat CoVs, have a 19 amino acids dissimilarity, whereas the pangolin CoV has only 5 amino acids dissimilarity for the S protein from SARS-CoV-2. In particular, the receptor-binding domain of the S protein of the Pangolin-CoV is virtually identical to that of SARS-CoV-2, with one amino acid difference. The comparison of available genomes appears to suggest that SARS-CoV-2 might have originated from the recombination of a Pangolin-CoV-like virus with a virus similar to bat-CoV-RaTG13, which has a striking 96% similarity to the SARS-CoV-2 virus but lacks the furin cleavage site. Further, the infected pangolins show clinical signs and histopathological features akin to SARS-CoV-2 in experimental models and harbour the antibodies which react with the S protein of SARS-CoV-2 [6]. The genetic sequence analysis of the bat CoVs, pangolin CoV and SARS-CoV-2 appears to link the CoVs, suggesting that viruses from the bats and pangolin may have exchanged the genes at some point before spilling and infecting human-beings [7]. The pangolins are, thus, the most likely intermediate host, though other potential intermediate host(s), cannot be ruled out.

The pangolin CoV lacks a characteristic trait seen in SARS-CoV-2, that has helped it to leap to humans, the adaptation may have been acquired in another, yet unidentified, animal before the Wuhan epidemic spreading around the globe [8]. There has been identified a unique peptide (PRRA) insertion in the human SARS-CoV-2 virus in the S protein between its S1 and S2 subunits, which seems to be involved in the proteolytic cleavage of the spike protein by cellular furin-like proteases, which are widely expressed in a variety of organs including lungs and respiratory tracts, gastrointestinal tract, liver, pancreas and brain [9]. This furin cleavage site is distinct from SARS-CoV and other CoVs which only contain a trypsin or TMPRSS2 cleavage site. With the novel furin cleavage site on the S protein, four amino acid residue insertion at the boundary between the S1 and S2 subunits, the SARS-CoV-2 appears to gain a potentially high infectivity and transmissibility as compared to SARS-CoV, due to the near-ubiquitous distribution of furin-like proteases in various tissues [10]. The sequences of SARS-CoV-2 from various patients from different countries are almost identical, with greater than 99.9% sequence identity. As a typical RNA virus, the average evolutionary rate for CoVs is approximately 10-4 nucleotide substitutions per site per year, with mutations arising during every replication cycle. This finding suggests that SARS-CoV-2 has originated from one source, from Wuhan and has spread relatively rapidly worldwide, within a short period [11].

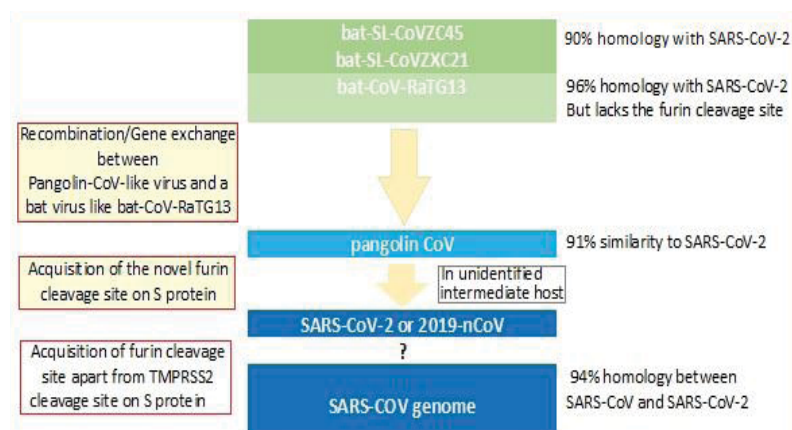


Figure 1: The Origin and Descent of SARS-CoV-2 or 2019-nCoV.

The CoV Transcription and Expression: The CoVs have complex gene expression and replication cycle, encompassing ribosome frameshifting for genome translation, the synthesis of both genomic and sub-genomic components, and the assembly of progeny virions. Further, the CoV transcription involves the production of multiple sub-genomic mRNAs that contain sequences corresponding to both ends of the genome through a process of discontinuous transcription [12].

The expression of the CoV replicase-transcriptase (RT) protein genes is mediated by the translation of the genomic RNA. Whereas, the sub-genomic messenger RNAs (mRNAs) possess common 5'-leader and 3'-terminal sequences. The genome and sub-genomes of a typical CoV contain at least six open reading frames (ORFs). The first ORFs (ORF1a/b), comprising of about two-thirds of the whole genome length, produce two polypeptides, pp1a and pp1ab, which are processed by virally encoded protease into 16 nsps, 1 to 16. The other ORFs on the rest of one-third of the genome near the 3'-terminus encode 4 main sps - spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. Besides the sps, accessory proteins, such as HE protein, 3a/b protein and 4a/b protein are also encoded. The sps and accessory proteins are translated from the sub-genomic RNAs (sgRNAs) [13].

The 2019-nCoV Structural Components: Spike (S) Protein: The glycosylated S protein is a fusion protein and mediates attachment of the virus to the host cell surface receptors and subsequently facilitate its entry through the cell membrane into the host cell. The S protein has two subunits: the globular S1 subunit is involved in receptor recognition and has a large receptor-binding domain (RBD) structure organized in four distinct domains, whereas the S2 subunit forms the stalk of the spike molecule and contains the key protein segments including the fusion peptide, two heptad-repeat regions (HR1 and HR2) that facilitate virus-cell fusion, and has been conserved across different genera of CoV species [14]. There are various receptors that bind to different CoVs, such as ACE2 for SARS-CoV29 and CD26 for MERS-CoV. There being a structural similarity between the RBDs of SARS-CoV and SARS-CoV-2, SRS-CoV-2 also uses ACE2 as its receptor, despite the presence of amino acid mutations in its S1 RBD [15].

The CoV S glycoprotein has N-terminal region (NTR, domain A) and the C-terminal region of S1 (CTR, domains B, C, and D) which bind to the host ACE2 receptors and function as RBDs [16]. The major difference in S1 subunit of SARS-CoV-2 as compared to SARS-CoV, is the 3 short insertions its N-terminal domain, which confer the sialic acid binding activity and 4 out of 5 key residues changes in the receptor-binding motif [17].

Other Major Structural Proteins: In addition, the CoV genome encodes other major sps, such as, nucleocapsid (N) protein, membrane (M) protein and the envelope (E) protein.

The N protein is the most abundant protein in CoV and binds to the CoV RNA genome, leading to formation of the helical nucleocapsid. It is composed of two domains, an N-terminal domain (NTD) and a C-terminal domain (CTD). The genomic packaging signal binds specifically to the C-terminal RNA binding domain, whereas NTD binds to nsp3, a key component of the replicase complex and the M protein. The N protein is localised to the endoplasmic reticulum (ER)-Golgi region and involved in nascent virion assembly and budding. Its high hydrophilicity has been related to the host cellular response and potent immunity following the viral infection.

The M protein, another major structural protein, helps in defining the shape of viral envelope. It is the central organiser of CoV assembly and interacts with all other major coronaviral sps. Interaction of S with M is necessary for retention of S in the ER-Golgi intermediate compartment (ERGIC)/Golgi complex and its incorporation into new virions. The binding of M to N stabilises the nucleocapsid (N protein-RNA complex) core.

The E protein is abundantly expressed inside the infected cell and localised at the ER, Golgi and ERGIC, and during the replication cycle a small portion of E protein is incorporated into the virion envelope along with a major contribution by M protein [18]. The E protein appears to be involved at multiple stages of the replication cycle, from assembly and induction of membrane curvature to budding and release, and inflammation, apoptosis, and autophagy. The deletion of E from SARS-CoV attenuates the virus.

The hemagglutinin-esterase (HE) protein is attached to the viral surface. It contains a lectin-binding domain that mediates binding to O-acetylated sialic acids. It possesses sialate-O-acetyl esterase receptor-destroying enzyme activity, though which viral attachment to non-permissive cells is prevented. The HE protein appears to facilitate virus entry into target cells after binding to the main entry receptor.

Receptor Tethering, Viral Invasion and Replication: The Viral Tethering and Cell Entry: Following receptor

binding, the SRS-CoV-2 gains access to the host cell cytosol, accomplished by acid-dependent proteolytic cleavage of S protein by a furin like protease. The S protein cleavage occurs at two sites within the S2 portion of the protein, the first cleavage separates the RBD and fusion domains of the S protein and the second cleavage exposes the fusion peptide which inserts into the cell membrane, followed by joining of two heptad repeats, HR1 and HR2, in S2 forming an antiparallel six-helix bundle. The formation of the bundle allows the blending of viral and cellular membranes, resulting in fusion and ultimately release of the viral genome into the cytoplasm.

Replicase Protein Translation and Expression: The next step is the translation of the replicase gene from the viral genomic RNA. Viral RNA synthesis follows the translation and assembly of the viral replicase complexes and generation of genomic and sub-genomic RNAs. The replicase gene encodes two large ORFs, rep1a and rep1b, which express co-terminal polyproteins (pps), pp1a and pp1ab. In order to express the pps, the virus utilizes an RNA pseudoknot that cause ribosomal frameshifting from the rep1a into the rep1b ORF. The pps are subsequently cleaved into the individual nsps by proteases encoded by the virus. Various nsps assemble into the replicase-transcriptase complex (RTC) for RNA replication and transcription of the sub-genomic RNAs. The sub-genomic RNAs serve as mRNAs to generate the structural and accessory proteins (Figure 2).

Functions of CoV non-structural proteins: The nsps contain domains to carry out various genomic as well as supportive functions. The nsps promotes cellular mRNA degradation (nsp1) and blocks host cell innate immune response (nsp1 nsp3), cleave pps (nsp5), bind to prohibitin proteins (nsp2), interact with N protein (nsp3), act as processivity clamps for RNA polymerase (nsp7 and 8) and transmembrane scaffold proteins (nsp4 and 6). In addition, they promote cytokine expression (nsp3), stimulates viral 3'-5' exoribonuclease (nsp10 and 14) and N7 methyltransferase (nsp10 and 14), shield viral mRNA from recognition (nsp16) and bind viral RNA (nsp9). They also modulate the enzymes RNA-dependent RNA polymerase (nsp12), RNA helicase 5'triphosphatase (nsp13) and viral endoribonuclease (nsp15), and act as cofactor for nsp14 and 16 (nsp10).

Virion Assembly and Release: Following replication and sub-genomic RNA synthesis, the viral sps, S, N, M, E and HE are translated and inserted into the endoplasmic reticulum (ER). The sps move along the secretory pathway into the ERGIC. The viral genomes are encapsidated by the N protein bud into membranes of the ERGIC containing viral structural proteins, forming nascent virions or virus like particles (VLPs). The M protein then directs most protein-protein interactions required for assembly of virions and expressed along with E protein to produce virion envelope. The N protein enhances VLPs formation and the S protein is incorporated into virions at this step. The M protein also binds to the nucleocapsid, promoting the completion of virion assembly. Following assembly, VLPs are transported to the cell surface in vesicles and released by exocytosis.

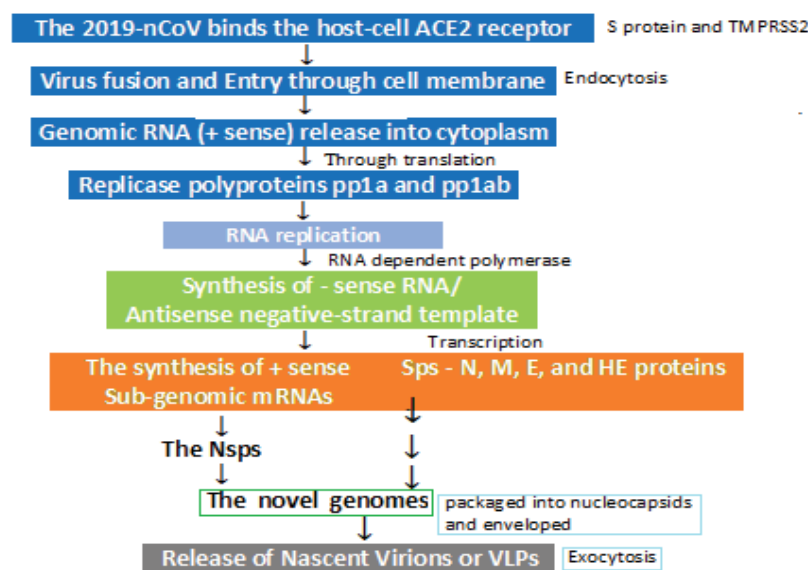


Figure 2: The Outline of the 2019-nCoV Replication Cycle.

The Host Factors

Angiotensin-converting enzyme 2 (ACE2): The ACE2, also known as ACEH (ACE homolog), is an integral membrane protein and a zinc metalloprotease of the ACE family. The human ACE2 protein sequence consists of 805 amino acids, including a N-terminal signal peptide, a single catalytic domain, a C-terminal membrane anchor and a short cytoplasmic tail. ACE2 cleaves angiotensin I and II as a carboxypeptidase. ACE2 is located as an ectoenzyme on the surface of endothelial and other cells. There is an abundant presence of ACE2 in nasal mucosa and nasopharynx, oral mucosa and tongue, airways, and lung alveolar epithelial cells. There is rich ACE2 surface expression on arterial and venous endothelial cells, and arterial smooth muscle cells. The abundant ACE2 expression has been linked with the pathogenesis involving vasculitis, deranged immune function, extensive pulmonary inflammation, and diffuse alveolar damage with hyaline membrane formation, and the severe clinical manifestations [19]. The oral cavity is a potentially high-risk route for SARS-CoV-2 infection and should be a part of future prevention strategy in dental clinical practice as well as daily life [20]. Apart from this, there is a high ACE2 expression in oesophageal upper and stratified epithelial cells, absorptive enterocytes from ileum and colon, cholangiocytes, myocardial cells, renal proximal tubule cells and bladder urothelial cells, skin, lymph nodes and brain. The widespread ACE2 tissue distribution in various organs may explain the multi-organ dysfunction observed COVID-19 in patients.

The ACE2 is an important regulator of the renin-angiotensin system (RAS). Angiotensin I, having no direct biological activity, exists as a precursor to angiotensin II, and converted to the latter through removal of two C-terminal residues by the ACE2 primarily in the lung but also in kidneys, endothelial cells, and brain. Angiotensin II acts on venous and arterial smooth muscle to cause vasoconstriction and increases vasopressin production in the CNS. It also stimulates aldosterone secretion. It appears that apart from gaining its entry through the ACE2, the SARS-CoV-2 subsequently down-regulates the ACE2 expression leading to loss of its protective effects in various organs, which may have significant impact on the pathogenesis of the disease.

There is reduced ACE2 expression with aging in both genders. It is slightly reduced in young-adult and middle-aged groups but reduced significantly in the elderly age group. In animal studies, though there was no gender-related difference of ACE2 expression in adult mice, a higher ACE2 content was noticed in old female than male [21]. The epidemiological studies have documented that different sex and age groups have different susceptibility to SARS-CoV-2 infection, which may be linked to ACE2 expression. There is a skewed relationship with various host factors and the severity and mortality of the disease, with the male sex, elderly population and those with coexisting chronic diseases being the most affected. Further, as per the preliminary epidemiological data, there is an exponential increase in disease severity and mortality in those beyond the sixth decade of life with cardiovascular disease and diabetes [22]. The exaggerated proinflammatory profile is appears to be a salient feature in those suffering from hypertension, heart disease and diabetes. In addition, the elderly, especially those with hypertension and diabetes, have reduced ACE2 expression and upregulation of angiotensin II proinflammatory signaling. The SARS-CoV-2 binding to ACE2 may exaggerates this proinflammatory milieu, predisposing these population groups to greater disease severity and mortality.

S Protein-ACE2-Protease Host Cell Entry: The ACE2, expressed on the surface of various tissues including airway epithelial cells, binds with the S protein. The RBD on the spike, mediates the interaction with the host-cell receptor, ACE2 for both SARS-CoV and SARS-CoV-2. The spike RBD is capable of folding independent of the rest of the spike protein and contains the structural information for host receptor binding. The host protease processing during viral entry is a may be a significant barrier infection, but has been overcome by hCoV including SARS-CoV-2 to the agent's advantage and after binding the receptor, a host protease cleaves the spike, releasing the spike fusion peptide and facilitates the virus entry [23]. The CoV entry into the host cell is a multi-step process. It involves several distinct domains in spike mediating the virus attachment to the cell surface, receptor engagement, protease processing and membrane fusion. After bonding with the host receptor, the host-cell protease cleaves spike and releases the fusion peptide, allowing for host-cell entry. Thus, armed with the SARS-CoV spike, the SARS-CoV-2 is capable of using human ACE2 efficiently, which may account for the human-to-human transmissibility of the virus [24].

The ACE2 is predominantly expressed in surfactant-secreting type II alveolar cells in lungs, bronchiolar epithelium, and endothelium and smooth muscle cells of pulmonary vessels. Further, it is more abundantly expressed in the apical than the basolateral areas of lungs. Furthermore, the ACE2 expression is proportionately correlated to the epithelial differentiation of the alveolar tissue. The undifferentiated cells poorly express ACE2, while well-differentiated cells richly

express ACE2. The studies indicate that infection of human airway epithelia by SARS-CoVs correlates with the state of cell differentiation and ACE2 expression and localization. These findings have implications for understanding disease pathogenesis associated with the SARS-CoV infections [25].

The lungs appear to be the most vulnerable target organs for SARS-CoV and SARS-CoV-2 because of the vast surface area, making the lung highly susceptible to inhaled viruses. About 83 percent of ACE2-expressing cells are alveolar type II cells, which are exposed and can serve as a reservoir for the viral invasion. In addition, the ACE2-expressing alveolar type II cells contain high levels of viral process-related genes, including regulatory genes for viral processes, viral life cycle, viral assembly, and viral genome replication. Further, it has been claimed that ACE2 is not only the entry receptor of the virus but also protects from lung injury and high lethality associated with SARS-CoVs infection could be because of dysregulation of the pulmonary protective mechanisms [26].

Pathogenesis of CoV Infections: Coronaviruses cause a large variety of severe diseases in livestock and other animals such as pigs, cows, chickens, dogs and cats, which has led to significant research on these viruses. For instance, Transmissible Gastroenteritis Virus (TGEV) and Porcine Epidemic Diarrhoea Virus (PEDV) cause severe gastroenteritis in young piglets, leading to significant morbidity and mortality, and economic loss.

The SARS-CoV, a 2b β -coronavirus, was identified as the causative agent of the Severe Acute Respiratory Syndrome (SARS) outbreak that occurred in 2002–2003 in the Guangdong Province of China, leading to approximately 8098 cases and 774 deaths, amounting to a mortality rate of 9%. The rate was much higher in elderly individuals, with mortality rates approaching 50% in individuals over 60 years of age [27]. It appears that the SARS-CoV originated in bats as a large number of Chinese horseshoe bats harbour sequences of SARS-related CoVs and serological evidence for a prior infection with a related CoV. In fact, two novel bat SARS-related CoVs, bat-SL-CoVZC45 and bat-SL-CoVZXC21, were identified as having significant similarity to SARS-CoV. They were also found to use the same receptor, ACE2. Transmission of SARS-CoV was relatively inefficient, as it only spread through direct contact with infected individuals after the onset of illness, and the outbreak was controllable through strict quarantine.

Another novel human CoV, named Middle East Respiratory Syndrome-CoV (MERS-CoV), was found to be the causative agent in a series of highly pathogenic respiratory tract infections in Saudi Arabia and other countries in the Middle East, which emerged in the Middle East in 2012. It was thought that camel-to-human spill over contributed to the outbreak. The outbreak did not accelerate later in 2013, though sporadic cases continued throughout the rest of the year. From 2012 through May 31, 2019, MERS-CoV has infected 2,442 persons and killed 842 worldwide amounting to case fatality rate of ~29 percent [28]. The MERS-CoV utilizes Dipeptidyl peptidase 4 (DPP4) as its receptor in humans and certain species such as bats, camels, rabbits and horses to establish infection. The virus is unable to infect mouse cells due to difference in the structure of DPP4.

Owing to the lack of effective therapeutics or vaccines, the best measures to control hCoVs, remain an efficient public health surveillance system coupled with rapid diagnostic testing, and isolation and quarantine. The past and likely future emergence of pathogenic zoonotic CoVs, the gross social and economic impact of hCoVs infections and the lack of effective antiviral strategies make it obvious that our preparedness to prevent and treat CoV infections is limited [29]. This highlights the importance of advancing our knowledge on the emergence, infectivity, replication, and pathogenesis of the CoVs.

The Host Cell Immune Response: In general, the host cells respond to the virus infection by recruiting an innate antiviral response to limit the spread of the infection and resorting to induce an adaptive immune response to eventually clear the virus. In the case of CoVs and other +RNA viruses, the innate immune system is triggered by recognizing dsRNA and 5'-triphosphate-bearing RNA molecules arising as replication intermediates in the cytosol by the intracellular sensors of the Porcine Epidemic Diarrhoea Virus (PEDV) family, such as retinoic acid-inducible gene 1 (RIG-I) [30] and melanoma differentiation-associated protein 5 (MDA-5) which are expressed in various host cells [31]. The infections are recognized by the cytosolic sensors RIG-1, Mda5 and stimulator of interferon genes (STING) and result in the expression of IFN- β and an inflammasome response. The RLRs, RIG-1, Mda5 and LGP2 recognize the viral ligands. Mda5 is one of the pattern recognition receptors (PRRs) that recognize cytoplasmic viral ligands [32].

For recognition of CoV RNAs, MDA-5 seems the most important cytosolic sensor. The toll-like receptors (TLRs) which are expressed on the cell surface or reside in the endosomes of immune cells can also recognize CoVs nucleic acids or

proteins. Activation of one or more of these sensors generally leads to the activation of the transcription factors IFN-regulatory factor 3 and 7 (IRF3, IRF7) and NF- κ B, which stimulate the expression and secretion of Type-I IFN and pro-inflammatory cytokines. Following this, the JAK-STAT (Janus kinase/signal transducers and activators of transcription) signaling cascade is activated leading to expression of various antiviral interferon-stimulated genes (ISGs) resulting in an antiviral state of the infected cells.

The CoV infection and stages of replication are associated with the endoplasmic reticulum (ER) stress. The ER can sustain a high load of protein content without being overwhelmed. However, when the ER's capacity for folding and processing proteins is exceeded, unfolded or misfolded proteins rapidly accumulate in the lumen and the ER stress response is induced. The ER stress leads to activation of Unfolded Protein Response (UPR) pathways through the induction of protein kinase RNA-like endoplasmic reticulum kinase (PERK). The activated UPR leads to elevated level of phosphorylated Eukaryotic Initiation Factor 2 α (eIF2 α) resulting in the promotion of a pro-adaptive signaling pathway by the inhibition of global protein synthesis and selective translation of Activating Transcription Factor 4 (ATF4). In addition, during conditions of prolonged ER stress, several pro-apoptotic genes are induced, and there is failure of synthesis of anti-apoptotic Bcl-2 proteins. The ER stress-mediated leakage of calcium into the cytoplasm also leads to the activation of apoptosis effectors. These measures eventually trigger apoptosis, which is utilized by the host cells to inhibit viral replication. PKR (serine/threonine protein kinase) is a key player in the innate immune response to RNA virus infection by upregulating antiviral gene expression and enhancing synthesis of interferons (IFNs).

The CoVs Countermeasures: The viruses, on the other hand, have evolved strategies to suppress and overcome the immune responses, which influence the pathogenesis, course of the disease and persistence the virus in the host. The CoVs strategically counteract PKR-mediated signaling to prevent the translational shut-off due to eIF2 α phosphorylation. In case of the MERS-CoV, the ORF4a protein counteracts the PKR-induced formation of stress granules, probably by binding viral dsRNA to shield it from detection by PKR. The S proteins of both SARS-CoV and SARS-CoV-2 also interact with eIF3F, to modulate host translation, including the expression of the pro-inflammatory cytokines, and IL-6 and 8 at a later stage of infection. These interactions play an important regulatory role in CoV pathogenesis, and disease course and prognosis [33].

Apart from modulating eIF2 α phosphorylation, the hCoV manipulates the translation machinery through nsps. The nsp1 protein, an inhibitor at multiple steps of translation, inhibits 48S initiation complex formation and its conversion into the 80S initiation complex, apart from directly binding to the 40S ribosomal subunit to stall translation. Further, the nsp1 and 40S complex induce cleavage of cellular mRNAs to suppress the translation. The viral nsps, especially nsp3, nsp4 and nsp6, appear to drive the formation of replication structures such as double-membrane vesicles (DMVs), tubules, zippered ER and convoluted membranes forming a reticulovesicular network in the cytosol of infected cells. In addition, various RNA-binding proteins in the infected host cells fail to interact and block the CoV 5' UTR, the 3' UTR and poly(A)-tail [34].

In addition, the CoV nsp15 inhibits retinoblastoma protein (pRb), a tumor suppressor protein, resulting in enhanced expression of genes normally repressed by pRb [35]. The overexpression of the SARS-CoV 3a protein leads to G1 arrest and inhibition of cell proliferation. Apart from this, SARS-CoV infection decreases p53 expression related to antiviral effect and able to enhance its replication in the cells lacking p53 depleted cells [36].

The Protein-Protein Interactions (PPIs): The PPIs can be host-host, virus-virus or virus-host PPIs. Through PPIs the viral proteins establish interactions with host proteins. The viral protein domains are basic units for viral-host protein interactions and the mutations at protein interfaces can reduce or increase the binding affinities. During the course of the viral invasion and infection, both the agent and cellular proteins are constantly competing for bonding. The protein-protein interactions (PPIs) in virus-host systems are mediated by domain-domain interactions (DDIs), involving molecular recognition via amino acid residues located at interfaces of interacting domains [37]. Apart from this, endogenous interfaces mediating viral-viral or host-host interactions, are constantly targeted and inhibited by exogenous interfaces mediating the interactions. In case of viral infection, the virus-host PPIs, the interacting proteins are constantly losing and regaining their binding sites in order to evade or optimize interspecific PPIs. The exogenous interfaces lend competition for such molecular reactions and interfere with host-host protein interactions leading to alterations in cellular metabolism [38].

The host factors evolve to retain or restore their recognition capabilities to bind and neutralize viral agent. Whereas,

through virus–host interactions, the viral proteins tend to target more central and highly connected host proteins and maintain and adapt their capabilities via molecular evolutions and, including conservation, HGT, gene duplication and molecular mimicry [39]. Horizontal gene transfer (HGT) is a process of genome recombination by means of which the virus acquires genes from non-parental organisms, optimizes and integrates into its virus–host network. Through gene duplication, the virus acquires duplicated genes in the viral genomes to become more lethal. As the virus evolves at faster mutation rate, it can rapidly acquire new binding partners by mimicking and targeting interfaces of host proteins.

Immune response/Inflammasome activation: There is a complexity of the virus–host interactions that occur within the cell, tissues, or the whole body. The SARS-CoV-infected peripheral blood mononuclear cells lead to the upregulation of expression of various cytokines, including IL-8 and IL-17, and the activation of macrophages and the coagulation pathways. The protein kinases, the key regulators in signal transduction, control a wide variety of cellular processes. They are linked to cellular immune responses, like interleukin (IL) signaling, IL-6 and -8, and influence the CoV infection and CoV-induced inflammation. There occurs downregulation of a large number of mRNAs, including those encoding proteins involved in translation, leading to the host translational shut-off in CoV-infected cells due to a stress response and concomitant mRNA decay. In addition, the heterogeneous nuclear ribonucleoproteins (hnRNPs) influence the maturation of nascent nuclear RNAs into messenger RNAs (mRNAs) and stabilize their cellular transport and control their translation. In general, the difference in the immune and inflammatory responses determines the outcome of the infection and responsible for the differential activation of the Signal transducer and activator of transcription 3 (STAT3) pathway, involved in lung inflammation and cellular repair [40].

The CoV nucleocapsid (N) protein plays a multifunctional role in the virus life cycle, from regulation of replication and transcription to genome packaging and release of VLPs to modulation of host cell defence processes. In addition, various viral proteins interact with host ribosomal and nucleolar proteins, helicases and the hnRNPs [41]. The viruses encode proteins to modulate the cellular factors to modify the immune response pathways to avoid their detection and exploit the extensive network of the host cell's signalling pathways to promote their replication and propagation. The inflammasome activation by CoV E was first reported in porcine reproductive and respiratory syndrome virus (PRRSV), when it documented that PRRSV-encoded small envelope protein E, an ion channel-like protein, triggered the activation of inflammasomes. Blocking ion channel activity with amantadine significantly inhibited activation of the inflammasome [42]. Recently, the transport of Ca^{2+} by SARS-CoV E has been shown to trigger inflammasome activation [43]. Following which there has been shown ER stress and apoptosis to occur, and the inflammatory lung damage in SARS-CoV-infected mice. Inhibiting the CoV E ion channel activity limited the CoV E viroporin induced CoV pathogenicity. Autophagy is a reparative cellular process that recycles excess or damaged cellular material inside the cell ensure its survival. The RNA viruses, including CoVs, appear to exploit autophagy for the purpose of viral replication and propagation.

Immunological Basis of Severe COVID-19: In the severely ill SARS-CoV-2 patients, the disease may progress rapidly from viral pneumonia to acute respiratory failure. The neutrophil-to-lymphocyte ratio (NLR) has been identified as an independent risk factor for severe illness in these patients. The patients with age ≥ 50 and NLR ≥ 3.13 denote a severe illness and should have rapid access to intensive care unit and respiratory support [44].

The pyroptosis, a form of inflammatory form of programmed cell death and appears to be the possible mechanism for the increased virulence of SARS-CoV-2 [45]. The SARS-CoV Viroporin 3a triggers the activation of the NLRP3 inflammasome and the secretion of IL-1 β by macrophages, suggesting SARS-CoV induced cell pyroptosis [46]. In addition, the patients infected with SARS-CoV-2 have increased IL-1 β in the serum, an indicator of the pyroptosis [47]. Further, the rising IL-1 β suggests activation of cell pyroptotic activity. The severe SARS-CoV-2 infection is likely to cause cell pyroptosis, especially in lymphocytes, through the activation of NLRP3 inflammasome.

About thirty percent of patients demonstrated neurological manifestations out of a sample of 214 newly diagnosed Covid-19 patients [48]. The central nervous system (CNS) involvement is indicated by headache, dizziness, disturbance of consciousness, acute cerebrovascular disease including epilepsy, and peripheral nervous system symptoms such as decreased taste, decreased smell, and appetite. The SARS-CoV-2 appears to invade the CNS through blood or retrograde neuronal circuits, leading to neurological damage and viral encephalitis.

Evolving COVID-19 Therapeutics

Digital Technologies for Healthcare Systems

The cornerstones of an effective Covid-19 preparedness plan for a health system are mitigating transmission; conserving, supporting, and protecting staff and eliminating nonurgent strains on the healthcare delivery; and good communication [49]. As we understand today, the measures to be applied for effective and efficient control for COVID-19 pandemic need use of quarantine, strict social distancing, and screening at the community level. Similarly, stringent measures and triaging are to be applied for the clinics and hospitals and the care for non-Covid and Covid including the suspected patients is needed to be strictly bifurcated. There are needed clear clinical guidelines in this respect, which are to be updated constantly. An electronic QR code can be that was to be assigned to every patient and the immediate contacts which can be updated every week. In China, the Covid facilities relied much on radiological findings and the CT scan was used as an early investigation [50]. The radiological findings are helpful in screening the severely ill patients from those having moderate clinical features.

In general, the need to look after the physicians and medical professionals involved in the healthcare of the COVID-19 patients is of paramount importance. The innovative indoor and ICU care models improve the healthcare capacity to handle the COVID-19 patients as well as to offer maximum of protection to healthcare professionals. This can be done by utilizing e-consultation and an inpatient tele-consult model to allow subspecialists to reach more patients and to decrease their exposure and unnecessary PPE utilization. The e-ICU program should be developed to enable physicians for video enabled ICU rounds and critical care consultation. There can be a remote patient monitoring program to take care of low and moderate-risk patients and those discharged from the emergency department, sub-intensive units, and indoor wards [51]. Thus, the Covid-19 high infectivity and fatality and has led to changes in healthcare delivery to mobilize hospitals and clinics to cater for large number of patients in need of urgent care, increase in tele and video consults, virtual clinical team meetings, and dissemination of the rapidly-evolving clinical information.

Outlining the Treatment Options

Presently there are no effective drugs for treatment of the Covid-19, which is now pandemic with millions of confirmed cases worldwide. While new and repurposed drugs are being tested in clinical trials, some of the promising drugs are simultaneously being recruited off-label for compassionate use or as experimental drugs to treat in desperate situations and otherwise dying patients. As obvious, not all clinical trials will be successful, but having so many efforts in progress, some may succeed and provide plausible solutions. Right now, though, there prevail confusion and despair. The future seems afflicted with dormant therapeutic options as well as faux Espoir or false hopes.

Understanding the PPIs offers an opportunity to target both viral-host and intraviral interactions to halt the viral replication and propagation. The virus binding and entry are the first steps of the replication cycle that can be targeted with inhibitors. There are inhibitors of endosomal acidification, such as ammonium chloride, chloroquine, and hydroxychloroquine, which appear to block the entry of CoVs including SARS-CoV and SARS-CoV-2. In addition, peptides have been explored which can block recognition and fusion by interfering with the interaction between the HR1 and HR2 domains of the S protein.

Apart from drugs acting as inhibitors directed at viral components, there are evolving therapeutic strategies involving host-directed approaches, based on the pathogenesis and virus-host interactions. The host-directed approach can yield a broad-spectrum therapeutic strategy and may lower the chance of development of antiviral resistance. The interferon (IFN) has been shown to trigger the innate immune response in CoV-infected cells, leading to transcription of various interferon stimulated genes (ISGs) that may play a role in controlling and eradicating the infection [52].

Stalling ACE2-mediated COVID-19 Entry

1. Spike protein-based vaccine: Development of a spike-1 subunit protein-based vaccine relies on the fact that ACE2 is the SARS-CoV-2 receptor [53]. The subunit vaccines for SARS-CoV may work by eliciting an immune response against the S-spike protein to prevent its docking with the host ACE2 receptor. In this context, the Novavax Inc, headquartered in Maryland, has developed immunogenic virus-like nanoparticles based on recombinant expression of the S-protein [54]. The Clover Biopharmaceuticals is developing a subunit vaccine consisted of a trimerized SARS-CoV-2 S-protein using their patented Trimer-Tag technology [55]. Another subunit vaccine being developed and tested is comprised of the

receptor-binding domain (RBD) of the SARS-CoV S-protein [56]. Initial findings have documented that the SARS-CoV and SARS-CoV-2 RBDs exhibit more than 80% amino acid similarity and bind to the same ACE2 receptor offer an opportunity to develop either protein as a subunit vaccine.

2. Inhibition of TMPRSS2 activity: The S protein of CoVs facilitates viral entry into target cells through attachment with ACE2 receptors. The priming of S protein by protease, namely, transmembrane protease serine 2 (TMPRSS2) is a prerequisite for S protein cleavage at the S1/S2, which frees S2 subunit essential for entry and translocation of the SARS-CoV-2 genome through interaction with the ACE2 receptor (Figure 3).

The protease, TMPRSS2 is a potential target for therapeutic intervention and the serine protease inhibitor, camostat mesylate, has been shown to block TMPRSS2 activity and to prevent SARS-CoV-2 entry into the host cells [57]. In the animal study, Camostat protected 6 out of ten mice from lethal infection with SARS-CoV. The results, thus, indicate that camostat or similar serine protease inhibitors may be a potential treatment option for SARS-CoV and SARS-CoV-2 infections [58]. The drug, camostat mesylate is already approved in Japan for the treatment of a number of non-infectious conditions.

3. Convalescent SARS Sera: The convalescent SARS patients exhibit a neutralizing antibody response directed against the S protein, which can be detectable as long as 24 months or more following the infection [56]. It has been found that the antibodies found in the recovering SARS patients sera cross-neutralized SARS-2-S-driven entry as well, as apparent from being able to hamper the SARS-CoV-2 S protein-driven entry into Vero-E6 cells, in vitro [59]. The finding is important as it reveals an important commonality between SARS-CoV-2 and SARS-CoV infection and a potential therapeutic intervention.

4. Modifying ACE2 Expression: The interaction between ACE2 and SARS-CoV is a therapeutic target. Theoretically, the anti-ACE-2 antibodies can block SARS-CoV-2 binding to the receptor, disrupt the interaction, prevent the virion entry and the alveolar cell damage. The treatment with anti-ACE-2 antibodies, thus, disrupts the interaction between virus and receptor. The same results can be achieved through enhancing the ACE2 expression by treatment with soluble ACE2.

It has been demonstrated in mice experiments that SARS-CoV downregulates ACE2 protein by binding its spike protein, contributing to severe lung injury [60]. It appears that, ACE2 competitively binds with SARS-CoV-2 not only to neutralize the virus but also rescue cellular ACE2 activity which negatively regulates the renin-angiotensin system (RAS) to protect the lung from injury [61]. Apart from this, angiotensin-(1-7) is generated by degradation of Ang II by ACE2 has vasoprotective functions. The enhanced ACE activity and decreased ACE2 availability may, thus, contribute to oxidative stress and subsequent lung injury. ACE2 deficiency may be critical in a variety of co-existing disease states, including hypertension, diabetes, renal impairment, and cardiovascular disease, and in elderly with physiological reduced ACE2 availability.

Functionally, there are two forms of ACE2. The membrane-bound ACE2 contains a transmembrane domain and an extracellular domain, which is used by SARS-CoV-2 as the receptor. The other, soluble form of ACE2 lacks the membrane anchor and circulates in small amounts in the blood. In this context, treatment with a soluble form of ACE2 may slow the

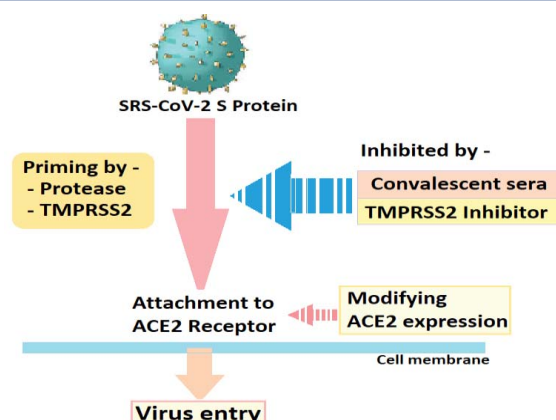


Figure 3: Therapeutic options to stall ACE2 mediated SRS-CoV-2 entry.

viral entry into host cells, as the soluble ACE2 acts as a competitive interceptor of SARS-CoV by preventing its binding to the membrane-bound ACE2, limits cell entry and spread, and protects the lungs from injury. The recombinant human ACE2 (rhACE2; APN01, GSK2586881) has been found to be safe in healthy volunteers and in a small cohort of patients with ARDS [62].

The Stapled Peptides

Therapies using small-molecule drugs, allows the drugs to cross cell membranes but may lack selectivity. Further, the small-molecule drugs may not be able to disrupt PPIs efficiently. The larger molecules and protein-based therapies, including the engineered antibodies, on the other hand, are potent and more selective, but have restricted ability to reach the target tissues and cross the cell membranes. The stapled peptides are large enough to be specific and potent for viral infections to inhibit PPIs and can be modified to enhance their membrane permeability to reach the target cells [63]. The stapled peptides have the potential to act at multiple levels in virus replication cycle. Drug-development-wise, the development of stapled peptides can be expressed and has a short market time.

Blocking CD147 inhibits CoV replication

The CD147 (basigin), transmembrane glycoprotein belonging to the immunoglobulin superfamily, is important factor in the host defence and regulates the expression of extracellular matrix metalloproteinases (EMMPs). It is expressed in various tissues and cell types, including leukocytes, platelets, epithelial cells and endothelial cells and fibroblasts. CD147 has been shown to play a prominent role in the induction of pro-inflammatory and pro-thrombotic events in various disease models [64]. The CD147-SP interaction enhances viral invasion for host cells and blocking CD147 on the host cells has shown an inhibitory effect on SARS-CoV-2 [65]. It has been documented that Meplazumab, a humanized anti-CD147 antibody, can competitively inhibit the binding of S protein and CD147, and prevent the viruses from invading host cells.

Developing/Repurposing Antiviral Drugs

The antiviral agents are being tested in clinical trials. Remdesivir, a broad-spectrum antiviral and nucleotide prodrug, inhibits replication of hCoVs in tissue cultures. The anti-retroviral and other investigational antiviral drugs are being tested for efficacy against COVID-19. Beta-D-N4-hydroxycytidine (NHC), plitidepsin and favipiravir are other anti-viral drugs in clinical trials. There have been efforts for using oligonucleotides against SRS-CoV-2 RNA genome or repurposing currently available various antiviral medications. The integrative network-based systems pharmacology methods have been used for rapid identification of repurposable drugs and drug combinations for the potential treatment of 2019-nCoV/SARS-CoV-2 [66].

In the cell culture infection models, a cyclophilin inhibitor, cyclosporin A (CsA), inhibited the replication of CoVs. CsA binds to cellular cyclophilins to inhibit calcineurin, a calcium-calmodulin-activated serine/threonine-specific phosphatase, and CoV replication [67]. The CK2 inhibitors Emodin and Rhein, anthraquinone compounds and chrysin, a flavonoid compound, target autophagy via different upstream pathways including the AKT/mTOR-axis and transcription of autophagy-related proteins. Whereas, emodin has been shown to block the S protein and ACE2 interaction in a dose-dependent manner.

The translation inhibitors enable the ongoing translation of messenger ribonucleoproteins (mRNPs) that encode antiviral factors such as interferon-stimulated genes (ISGs) despite the arrest of bulk translation [68]. Whereas, the viral transcription inhibitors, like sirolimus, temsirolimus and everolimus (mTOR inhibitors) block the mTOR, a serine/threonine protein kinase that regulates protein synthesis, autophagy, and transcription. The toll-like receptor 3 (TLR-3) agonist, rintatolimod is being tested as a potential treatment for COVID-19 in Japan.

Immuno-modulators and Other Drugs

The interleukin-6 inhibitors, such as tocilizumab (Genentech), may ameliorate severe damage to lung tissue caused by cytokine storm in patients with severe COVID-19. Tradipitant (Vanda Pharmaceuticals), a neurokinin-1 (NK-1) receptor antagonist, may have efficacy for inflammatory lung injury associated with severe SARS-CoV—19 infection. Corticosteroids are not generally recommended for treatment of COVID-19 or any viral pneumonia [69].

Conclusion: Perspectives And Implications

CQ and HCQ in treating COVID-19

There has been a growing interest in the use of chloroquine (CQ) and hydroxychloroquine (HCQ) as potential

treatments in the interim till a specific treatment is available, based on several in vitro and few in vivo studies reporting antiviral activity of CQ and HCQ against SARS-CoV-2 [70]. It has been claimed that HCQ is more potent than CQ against SARS-CoV-2, in vitro [71]. There are some in vivo studies data at present, though limited [72]. A number of potential mechanisms of action of CQ/HCQ against SARS-CoV-2 have been postulated. CQ may reduce glycosylation of ACE2, thereby preventing SARS-CoV-2 from effectively binding to host cells. Furthermore, CQ might block the production of pro-inflammatory cytokines (such as IL-6), thereby blocking the pathway that may lead to acute respiratory distress syndrome (ARDS). CQ is also believed to raise the pH level of the endosome, which may interfere with virus entry and/or exit from host cells.

The prophylactic use of HCQ is not backed-up by research data and trials are underway relating to preexposure and post exposure prophylaxis [73]. Encouraging clinical results were reported from China in February 2020, revealing that the treatment of over 100 patients with chloroquine phosphate in China had resulted in significant improvements of pneumonia and lung imaging, with reductions in the duration of illness [74]. At present, considering all the data, there is insufficient evidence to endorse that CQ/HCQ are safe and effective treatments for Covid-19 [75].

In addition, HCQ has a potential QT-prolonging effect by blocking critical potassium channels in cardiac conduction system, with the possibility of arrhythmias and sudden cardiac death. The COVID-19 patients with a baseline QTc value greater than or equal to 500 milliseconds and those that experience an acute QTc reaction with a QTc greater than or equal to 60 milliseconds from baseline after starting treatment with one or more QTc-prolonging drugs are at greatest risk for drug-induced arrhythmias.

Interplay between COVID-19 and the RAAS

The perspective that ACE inhibitors, like ramipril and ARBs, like losartan may increase ACE2 expression, have raised concerns about their safety in patients with COVID-19. The interaction between the SARS-CoVs and ACE2 has been proposed as a potential factor in their infectivity by some researchers, and there are concerns about the use of these drugs may alter the ACE2 expression and attended by its fallouts [76]. Currently, there are insufficient data available to translate it into clinical practice, though studies are underway. On the other hand, it is held that an abrupt withdrawal of these drugs in high-risk patients, having heart failure, coronary heart disease and hypertension may result in clinical instability and adverse outcomes. Further, despite substantial structural homology between ACE and ACE2, their enzyme active sites are distinct and the ACE inhibitors and ARBs in clinical use do not directly affect the ACE2 activity. Thus, it is advisable that an ACE inhibitor or ARB should be continued in known or likely COVID-19 patients with stable condition [77].

The SARS-CoV-2 utilizes and affects ACE in multiple ways. Apart from its entry through the ACE2, SARS-CoV-2 subsequently down-regulate ACE2 expression leading to loss of its protective effects in various organs leading to uncontrolled angiotensin II activity, which may potentiate organ injury in COVID-19. The continued viral infection and replication contribute to reduced membrane ACE2 expression as the ACE2 sites are taken up and damaged by subsequent viral invasions. The reduced ACE2 activity in the lungs results in unopposed angiotensin II accumulation and local RAAS activation, which leads to inflammation and neutrophil infiltration. In the experimental mouse models, exposure to SARS-CoV-1 spike protein induced acute lung injury, which was prevented by RAAS blockade. Similarly, in the COVID-19 patients having elevated levels of plasma angiotensin II, which correlated with viral load and degree of lung injury and the restoration of ACE2 through the administration of recombinant ACE2 appeared to reverse the devastating lung-injury process [76]. SARS-CoV-2 binding to ACE2 creates an acutely exaggerated proinflammatory background and it is advised that clinicians continue treating patients with ACE inhibitors and ARB [78].

Convalescent Plasma and COVID-19 Antibodies

Considering the potential options to treat COVID-19, there have been identified certain proteins in the host immune pathways which can be targeted for blocking viral replication by potential drugs or antibodies [79]. These include antiviral pathways in the innate immune response, such as the stress granule protein, G3BP1, which is an antiviral protein that induces the innate immune antiviral response.

The passive antibody transfer from pooled convalescent patient sera is another obvious therapeutic option in severe SRS-CoV-2 infection. The conceptual framework for using it as treatment for COVID-19 has been outlined in two recent review papers [80,81]. To date, two small case series have been published about its use and effectiveness by Chinese researchers [82,83].

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Chapter 4: Adverse Outcomes for Elderly in COVID-19: Annihilation of the Longevity Dream

Background

The Sars-Cov-2 Infection in Older Adults: The COVID-19 pandemic is impacting the elderly people in drastic ways. As a novel disease, it often presents with higher morbidity and adverse outcomes including mortality in the elderly. As a special age group, they face a significantly high risk for contracting the infection and developing severe illness due to physiological changes with aging and potential underlying health conditions.

Clinical Spectrum and Course of COVID-19: The clinical spectrum of COVID-19 varies from asymptomatic or paucisymptomatic presentation to a moderate to severe state characterized by respiratory failure necessitating mechanical ventilation and ICU support. Further, those developing critical clinical condition manifest complications like sepsis, septic shock, and multiple organ dysfunction syndromes. The elderly people are more likely to develop a severe and critical illness as compared to the younger age groups.

The Host Factors for COVID-19 in Elderly: In general, the elderly men display a higher mortality than women, especially if there are one or more underlying chronic health conditions. The age-related decline in physiological functions, attributable to reduced organ reserve and gradual functional decline in various organs in the elderly, is the key factor. In addition, the aging associated alterations in the immune response, the immunosenescence accompanied by subclinical pro-inflammatory state and inflamm-aging, expose the elderly to higher infectivity and a severe form of illness. There is significantly reduced ACE2 expression with aging in the elderly age group in both genders, which is further reduced in the elderly with hypertension, heart disease and diabetes. The SARS-CoV-2 binding to ACE2 may exaggerates the proinflammatory milieu, predisposing this age group with the reduced ACE2 expression, to greater disease severity and mortality. There are certain socio-economic reasons, as well. The elderly people are prone to experience a drop in the quality of life and neglected healthcare, which compromise their health in general and when exposed to COVID-19.

Conclusion: Annihilation of Longevity Dream: The aging is a complex process and affects virtually all organs of the body. A vast body of knowledge can now explain the changes that take place with aging at molecular and cellular level. The possibility of aging slowly and living longer, and lengthy-healthy life is alluring. As the life expectancy has increased with the improved lifestyle, progress in healthcare and technology have made possible to slow aging and achieve significant longevity. But, the high morbidity and mortality in the elderly with COVID-19 shows us that aging weakens the physiological systems and compromises function of various organs especially in those with the underlying conditions like diabetes and heart disease. The older adults or elderly age group, in light of its vulnerability to COVID-19 associated infectivity, disease severity, unfavourable prognosis and worsened mortality, now face the loss of the longevity dream.

The COVID-19 Infection in Elderly

The Clinical Disease in Elderly

The COVID-19 is a novel disease with variable clinical presentations. For the most of those infected with SARS-CoV-2, the disease may pass on as a non-event with few mild symptoms and recovery without any significant illness. Those with significant but moderate disease, the hospital admission with supportive treatment without need for assisted ventilation may suffice. In those with severe disease, the SARS-CoV-2 infection damages the alveolar tissue and triggers an overreaction of the immune system leading to hyperinflammation and cytokine storm. The variable spectrum of disease severity attended by variable course of the disease in an individual patient may make the therapeutic decisions difficult and requires a close and intense monitoring. There are, though, certain clear risk factors, including the age and underlying chronic health conditions, which foretell adverse outcomes for the COVID-19 in older adults or elderly age group [1].

As per the United Nations projections, by 2050, there will be more than twice as many people over 65 as there are children under 5, and the number of people 65 years of age or older globally will surpass the number of people 15 to 24 years of age [2]. The global aging trends are likely to create widespread public health challenges, dramatically increasing the burden of noncommunicable diseases as well as exposing the vulnerability to infectious diseases like COVID-19. Protecting the elderly population will become a major issue in maintaining global health.

Steps in Progression of COVID-19

Once inside the airways, the S protein on the SARS-CoV-2 viral surface recognize and stick to the ACE2 receptors, followed by the virus infecting the ACE2-bearing cells lining the upper as well as the lower respiratory tracts and with the dying cells sloughing down and filling the airways the virus is carried deeper into the lungs as the thin layer of surfactant coating the airways becomes even thinner and the brush border less efficient to defend from the invading virus. The virus is able to transmit while still confined to the upper airways, before invading the lower respiratory tract and lungs and causing symptoms. In addition, the virus is able to infect ACE2-bearing cells in other organs, including the blood vessels, gastrointestinal tract, and kidneys.

The SARS-CoV-2 orf1ab, ORF10 and ORF3a proteins attack the 1-beta chain of hemoglobin leading to dissociation of iron to form porphyrin and the viral structural and non-structural proteins appear to bind to the porphyrin leading to formation of denatured hemoglobin and loss of the capacity to carry oxygen and carbon dioxide, which exacerbates respiratory distress [3]. Further, in the elderly and those with comorbid conditions, the pro-inflammatory states may be responsible for insufficient erythropoiesis. The red blood cells (RBCs) are dynamic reservoirs of cytokines and their structural and functional alterations cause decreased deformability leading to their lysis with release of the intracellular contents including various inflammatory cytokines in severe illness. The increased turnover of erythrocytes leads to increased red cell distribution width (RDW) in COVID-19 patients, which can be regarded as an index of enhanced RBC fragility and higher vulnerability to adverse outcomes [4].

With the viral infestation, the activated immune system leads to inflammation and pyrexia, and in extreme cases may damage the host tissues and organs. The blood vessels become inflamed and leaky, leading to pulmonary edema. The immune hyperactivation is accompanied by the excessive cytokine release, during which, the immune system apart from damaging the host tissues, leads to increased susceptibility to infectious bacteria. The storms can also affect other organs besides the lungs, especially in those suffering chronic diseases.

The Clinical Spectrum of COVID-19

The clinical spectrum of COVID-19 (Figure 1) varies from asymptomatic or pauci-symptomatic presentation (Stage I) to moderate to severe states (Stage II a and IIb) characterized by respiratory failure necessitating mechanical ventilation and ICU support and those manifesting critical clinical condition (Stage III) with complications like sepsis, septic shock, and multiple organ dysfunction syndromes (MODS). The mild to moderate disease occurs in approximately 81% of cases. The severe disease with dyspnoea and acute respiratory distress syndrome (ARDS) and lung infiltrates appearing within 48 hours occurs in 14% of cases. Whereas, the critical COVID-19 illness accompanied by respiratory failure, septic shock, and multiple organ dysfunction (MOD) or failure (MOF) has been seen in approximately 5% of cases [5].

The transition from milder symptoms to severe disease and ARDS has been related to an uncontrolled cytokine release by the hyperactive immune response. In patients who are going to have the worst outcomes with COVID-19 infections, the immune system becomes overactive with excessive stimulation of T cells and macrophages, resulting in cytokine storm with release of a large amount of proinflammatory cytokines including interleukins (IL) - 1, 6, 12 and 18. The excessive or uncontrolled levels of cytokines released, further activate more immune cells, resulting in hyperinflammation and cytokine release syndrome (CRS).

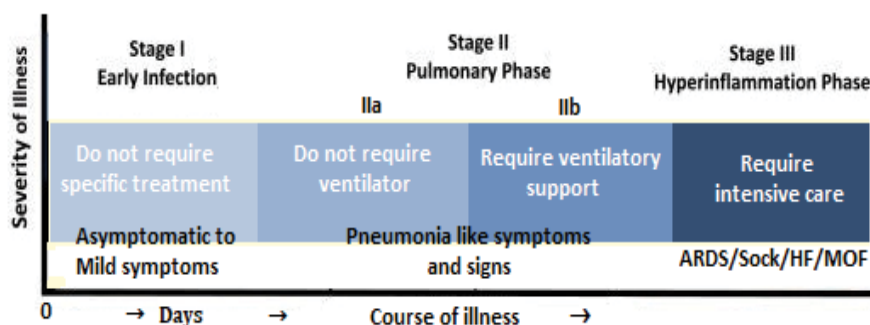


Figure 1: The spectrum and clinical stages of COVID-19.

The Morbidity and mortality in Elderly

The COVID-19 pandemic is impacting the elderly people in drastic ways. As a special age group, they face a significantly high risk for contracting the infection and developing severe illness due to physiological alterations with aging and potential underlying health conditions. As per WHO, over 95% of the deaths occurred are in those aged 60 years or older. More than 50% of all fatalities involved people aged 80 years or older. Further, 8 out of 10 deaths due to COVID-19 occur in individuals with a comorbidity, such as cardiovascular disease, hypertension, and diabetes and other underlying diseases [6]. As per the report posted on 31 March 2020 by the CDC, based on 7162 COVID-19 patients about whom the CDC had complete information, 37.6% had one or more underlying health condition. Further, out of Covid-19 ICU patients, 32% had diabetes, 29% heart disease, 21% chronic lung disease, and 9% had compromised immune systems [7].

As compared to COVID-19, the earlier human Coronavirus disease outbreaks, the Severe Acute Respiratory Syndrome (SARS), which occurred in 2002–2003, registered a mortality rate of 9%. The rate was much higher in elderly individuals, with the fatality rates approaching 50% in individuals over 60 years of age [8]. Another novel human CoV, named Middle East Respiratory Syndrome-CoV (MERS-CoV), was found to be the causative agent in a series of highly pathogenic respiratory tract infections in Saudi Arabia and other countries in the Middle East, which emerged in the Middle East in 2012. Like, the SARS, the MERS outbreak was also associated with a higher mortality in the elderly age group [9].

The Host Factors in Older Adults

The COVID-19 is a novel disease, but the available studies point to a genetic factor making certain persons or a genetic stock more susceptible. In general, the men display a higher mortality than women, which could be due to one or more underlying health risk factors, such as smoking and drinking. In addition, women appear to have a stronger immune system than men. There are individual differences in immune response, which may lead one person to have severe disease [10]. The physiological changes associated with aging, decreased immune function and multimorbidity lead the older adults to high infectivity, disease severity and adverse outcome from COVID-19. It is becoming clear that the health status, in general, plays a crucial role in prognosis from COVID-19. People aging healthily are at less risk, compared to those suffering with poor health and compromised physical fitness.

There are underlying reasons for the elderly people's greater susceptibility to COVID-19. The pre-existing chronic illnesses mark declining immunity and biological aging, which is more important than the chronological age. In addition, other factors like individual genetic make-up, the amount of virus load, the other microbes in the body including gut microbiota may play a role in acquirement of the infection and its progression.

The Aging Process and COVID-19

The age is an important epidemiological factor and the elderly people are at risk of severe infections possibly because the inefficient initial anti-viral immune response. The presence of the chronic diseases marks declining immunity and biological aging, which is more important than the chronological age. Due to various factors, the clinical severity and outcomes including the mortality from COVID-19 in elderly patients is higher than that in young and middle-aged patients [11]. The elderly patients are more susceptible to severe illness and more likely to need admission to ICU than the younger patients [12]. Further, among the admitted patients, the incidence of ARDS, and MOD and MOF was higher in the elderly age group than that in the young and middle-aged groups. Furthermore, the response to drugs including Lopinavir and Ritonavir, supportive care, oxygen therapy and mechanical ventilation was statistically less effective in the elderly than those young and middle-aged.

In terms of laboratory tests, the proportion of elderly patients with an increased number of total leucocyte count and neutrophils in was significantly higher than the young and middle-aged groups, suggesting that elderly SARS-CoV-2 infected patients are more likely to suffer from a bacterial infection. In addition, the decrease in lymphocytes is delayed in the elderly patients, compared to the young and middle-aged patients. This may be due to delayed recovery in the elderly due to asthenia including muscle atrophy and age-related changes in the lungs compromising the physiological functions of the respiratory system and reduced airway clearance, reduced lung reserve, and reduced defence barrier function [13]. The C-reactive protein levels in elderly patients are significantly higher than that in the young and middle-aged groups. In context of imaging, the incidence of multilobe lesions in elderly patients is significantly higher than in young and middle-aged patients [14].

Finally, the lethality of COVID-19 infection in the elderly is significant and the age could be held as an independent risk factor for severity of disease and associated mortality. As reported by Verity et al, the age-related case fatality for COVID-19, age-group-wise was - for less than 30 years ~ 0.06%, for those 30-39 years 0.14%, 40-49 years 0.29% and for 50-59 years 1.2%. It rose significantly for elderly to 3.9% in the 60-69 age group, 8.6% to those 70-79, and 13.4%. For those 80 and over [15]. In the US, the CDC has reported that during 12 February to 16 March 2020, the adults older than 65, were 31% of total cases, 45% of hospitalizations, 53% of ICU admissions, and 80% of all deaths associated with Covid-19, whereas those aged ≥ 85 years showed the highest percentage of severe outcomes [16].

As much as 63% the deaths due to COVID-19, have been reported among the people who were over 60 years, while 60 and above age group formed 19% of all cases, as reported by Ministry of Health, Government of India. Further, 86% deaths were associated with comorbidity related to diabetes, chronic kidney disease, hypertension, and heart disease. Compared to this, 47% were infected with the virus in age group below 40 years with death rate 7 % and 34% infected between 40 to 60 years age group with 30% deaths [17].

The Biological aging and its Fallouts: The organ reserve is the ability of an organ to endure recurring stressful conditions and restore the homeostasis and normal physiological functions following such conditions [18]. A part of the age-related decline in physiological functions is attributable to reduction in organ reserve as present in various body systems and the gradual functional decline in specific tissues or organs (e.g., immune-, musculoskeletal-, nervous systems) is a key characteristic of aging. Although organs vary in the rate of functional decline with age, the gradual linear decline of reserve capacity with age shows values ranging from 0.5% to 1.4% per year [19]. The decline appears to accelerate by the fifth decade of age, which may explain, in part, the age-related increase in vulnerability to disease and disability (Figure 2).

On the cellular level, several metabolic pathways exhibit excess metabolic capacities, such as, bioenergetics pathways and antioxidants system. The excess metabolic capacities can be viewed as an adaptability mechanism that substantiates organ reserve and contributes to the cellular defence systems. With aging, the metabolic excess capacities or organ reserves are impaired or exhausted, the ability to cope with stress is reduced leading to cell senescence, transformation, or apoptosis [20]. The decrease in organ mass, tissue anatomy and physiological function with age have been demonstrated in the human heart, brain, liver, kidney, salivary glands, stomach, and muscle tissue. The major storage reservoir of protein in the body is skeletal muscle, which tend to be lost with age manifesting as decrease in muscle mass and strength. By 6th decade of life, voluntary muscle contractile strength is decreased by over 20% in both men and women. By the time men and women are in their 7th or 8th decade of life, on average they have lost about 20% to 40% of the contractile strength of voluntary muscles, and over 50% by the 9th decade. The rate, and severity of decline varies between individuals and even between specific organs within individuals and may not always correlate with age. Further, the clinical trials have shown that declines in organ mass and function are reversible in some tissues, such as muscle and brain.

Apart from, the effacement of the excess metabolic capacities, aging at the molecular level is characterized by the progressive accumulation of molecular damage through environmental and metabolically generated free radicals, spontaneous errors in biochemical reactions and nutritional components. The damage to the maintenance and repair

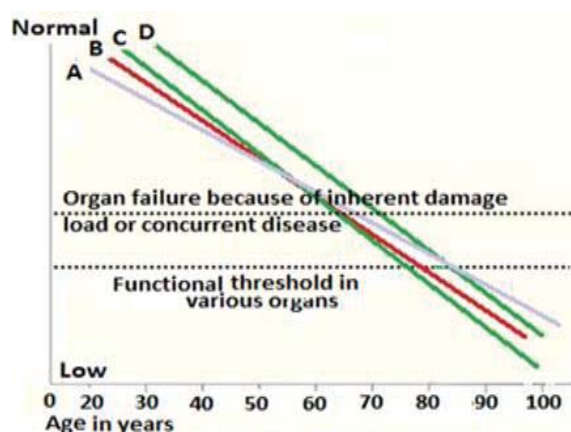


Figure 2: Aging and Functional Threshold and Reserve of Various organs.

pathways comprising homeo-dynamic mechanisms leads to age-related failure of homeo-dynamics, increased molecular heterogeneity, reduced stress tolerance, altered cellular functioning, senescence, and apoptosis [21]. These factors compromise the bioenergetics reserve capacity and accelerate the process of aging and increase the risk of age-related disorders.

With age, the functional reserve and redundancy of various organs wear out. Most organ functions decline by about one percent per year, the decline starting at about age forty or earlier, continues throughout life often without perceptible loss of function. Further, the age of functional decline is variable for different organs in different individuals. Nevertheless, the internal organs including brain, heart, lungs, and kidneys show a slow and gradual decline. The older adults often have multiple age-related functional impairments at level of various organs due to loss of organ reserve and redundancy.

Concepts of Genome Stability and Aging: Pathophysiologically, a number of diverse stimuli induce senescence. These factors appear to converge on certain pathways that influence cell cycle regulation, DNA repair and apoptosis, and the process of cellular senescence. At the cellular level, these pathways are regulated by the tumor suppressor proteins p53 and pRb. The p53 is a crucial mediator of the cellular response to damaged DNA and dysfunctional telomeres, and in turn activates the cyclin-dependent inhibitor p21. It is considered that senescence occurs via the p53 pathway in response to DNA damage and telomere attrition, whereas the p16/pRb pathway mediates senescence caused by oncogenic stimuli, chromatin disruption, and other cellular stresses [22]. In addition, the chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in animal studies as well as in the human endothelial cells (Figure 3).

The Bio-Homeo-Dynamic space: The living systems owe their survival and health to a series of complex biochemical pathways of maintenance and repair. Simultaneously, the defence systems create the homeo-dynamic space characterized by stress tolerance, molecular damage control and continuous remodeling. Further, the biological systems maintain the biological rhythms, sense, and respond to intra- and extra-cellular stressors through innate and adaptive immune responses, scavenge and remove of reactive oxygen and other free radical species and keep thermal regulation and neuro-endocrine balance [23]. The aging and the age-related diseases can be held as the consequences of a progressive shrinkage of the homeo-dynamic space, due to the failure of maintenance and repair. Aging is a complex process due to the interaction of many lifelong influences which include heredity, environment, culture, diet, exercise and leisure, past illnesses, and various other factors.

The aging organs slowly lose function, which may not be noticeable because the organs are rarely used to their fullest ability and have a reserve ability to function beyond the usual needs. Some body systems begin aging as early as age 30, whereas other aging processes are not common until much later in life. Further, age affects each individual differently from others. The cell membrane changes affect diffusion of oxygen and nutrients and removal carbon dioxide and other wastes. With aging, the connective tissue becomes stiffer making the organs, blood vessels and airways more rigid.

Senescence, Debility and Age-related Diseases: The major changes in organ reserve occur in the heart, lungs, and kidneys, usually appearing slowly and over a long period. The stressors producing an extra workload include illness, significant life changes and sudden increased physical demands on the body as a result of change in activity level. The recovery from illnesses is seldom complete, leaving some residual disability. The loss of reserve also makes it harder to restore homeostasis in the body with robustness.

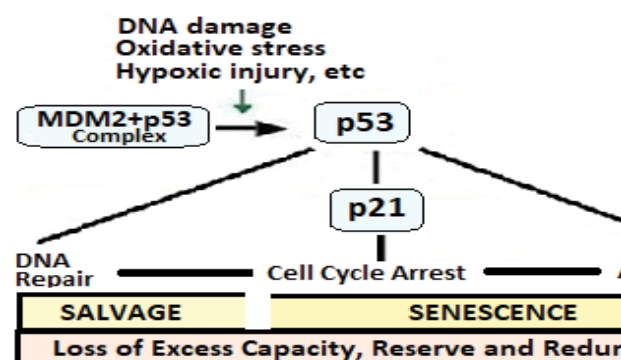


Figure 3: The Concept of Genome Stability and Aging.

The process of aging of organs is universal, progressive, and vastly irreversible. Some individuals may age without much deterioration in body organs and systems, whereas others may be ridden with the extreme age-associated changes. Further, the aging process may affect some body systems more severely whereas others are spared from a serious disability. Thus, aging is associated with a progressive but varying decline in numerous physiological functions and significantly affects the body organs and various systems including the lungs, heart, and vasculature, kidneys, and brain.

The Immune Response in COVID-19

The high risk to COVID-19 in the elderly, is due, in part, to the immunosenescence which encompasses the changes in the immune system with age. The immune system has two sets of defences against viruses and other pathogens. The first response is, in form of naïve leukocytes that attack invading microbes within minutes to hours. The second line of response is, in form of precisely targeted antibodies and T cells that surge later over several days.

The immunosenescence and inflamm-aging: With advancing age, the body has fewer naïve T cells, that have not yet been programmed to defend against a specific microbe. Whereas, the body does retain the memory T cells. Another age-related change is the natural killer cells do not work efficiently, responding too late and too little (Figure 4). Similarly, the B cells in the elderly do not produce enough antibodies. In general, there occurs a progressive age-related decline of innate and adaptive immune responses [24]. In addition, the age-associated chronic diseases such as heart disease, metabolic including diabetes, autoimmune diseases, and malignancy, along with the effects of their treatments on general health, substantially affect responses to infectious diseases, both bacterial as well as viral [25].

Cells in the elderly: The immunosenescence is accompanied by subclinical accumulation of pro-inflammatory factors and inflamm-aging [26]. The immunosenescence and inflamm-aging, acting in concert, mutually influence each other and lead to a decreased adaptive immune response, which reinforces the stimulation of the innate immune response. Thus, the innate immune response is conserved and the immune cells are constantly maintained in an alert state due to chronic low-grade inflammation, which is a physiological response to the life-long antigenic stress but without the essential counter-regulation by anti-inflammatory molecules as seen with aging.

The adaptive immune system is composed of the cellular and the humoral immune response. The cellular response includes CD4+ (helper) and CD8+ (memory) T cell populations. With aging, there is an increase in the number of CD8+ T memory cells and, later on, that of B cells due to a continuous chronic antigenic stimulation. The chronic antigenic stimulation, thus, leads to the phenomenon of inflamm-aging and immune exhaustion, characterized by the emergence of inhibitory receptors, such as PD-1 and CTLA-4. The CD4+ T cell population also undergoes similar changes to CD8+ T cells and there is an increase in Treg population as well as the pro-inflammatory Th17 subpopulation also increases with aging. Finally, the B cell compartment is also altered with aging. The clonal expansion, cytokine production, and specific antibody production are compromised leading to increased incidence of infections, chronic diseases, and cancer in the elderly [27]. The changes affect the innate immune system, which overtakes the altered adaptive immune system.

The cumulative changes in critical B- and T-cell subpopulations, from natural killer cells to the activated T cells and

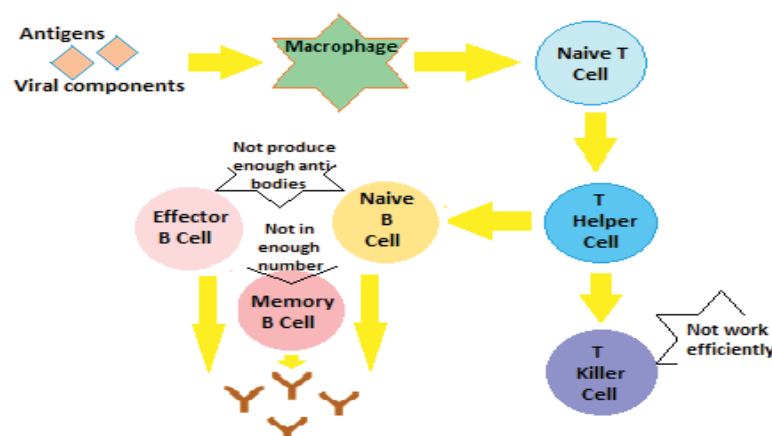


Figure 4: Immunosenescence: Dysfunctional T Cells and B.

B cells, and innate immune system lead to increased likelihood of infection and bringing about a proinflammatory state. With aging, there are defects in monocytes/macrophages function and expression of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α , and anti-inflammatory cytokine IL-10 by these cells after stimulation with lipopolysaccharide (LPS) and IFN- γ . Aging seems to affect the immune system both quantitatively as well as qualitatively, leading to deregulated response to infection, manifesting as exaggerated inflammation and excessive tissue damage.

The Host Cell Immune Response: In general, the host cells respond to the virus infection by recruiting an innate antiviral response to limit the spread of the infection and resorting to induce an adaptive immune response to eventually clear the virus. In the case of SARS-CoV-2, the innate immune system is triggered by recognizing dsRNA and 5'-triphosphate-bearing RNA molecules arising as replication intermediates in the cytosol by the intracellular sensors of the RIG-I-like receptor (RLR) family, such as retinoic acid-inducible gene 1 (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5) which are expressed in various host cells [28,29]. The toll-like receptors (TLRs) which are expressed on the cell surface or reside in the endosomes of immune cells can also recognize CoVs nucleic acids or proteins. This is followed by the expression of IFN- β , secretion of Type-I IFN and pro-inflammatory cytokines, and an inflammasome response resulting in an antiviral state of the infected cells. In addition, the viral infection and replication are associated with the endoplasmic reticulum (ER) stress. The ER can sustain a high load of protein content without being overwhelmed. However, when the ER's capacity for folding and processing proteins is exceeded, unfolded or misfolded proteins rapidly accumulate in the lumen and the ER stress response is induced. The ER stress leads to activation of Unfolded Protein Response (UPR) pathways eventually triggering apoptosis, which is utilized by the host cells to inhibit viral replication.

Immunological Basis of Severe COVID-19: There is a complexity of the virus-host interactions that occur within the cell, tissues, or the whole body. The SARS-CoV-infected peripheral blood mononuclear cells lead to the upregulation of expression of various cytokines, including IL-8 and IL-17, and the activation of macrophages and the coagulation pathways. The protein kinases, the key regulators in signal transduction, control a wide variety of cellular processes. They are linked to cellular immune responses, like interleukin (IL) signaling, IL-6 and -8, and influence the CoV infection and CoV-induced inflammation. In general, the difference in the immune and inflammatory responses determines the outcome of the infection and responsible for the differential activation of the Signal transducer and activator of transcription 3 (STAT3) pathway, involved in lung inflammation and cellular repair [30].

The pyroptosis is an inflammatory form of programmed cell death and appears to be the possible mechanism for the increased virulence of SARS-CoV-2. The SARS-CoV Viroporin 3a triggers the activation of the NLRP3 inflammasome and the secretion of IL-1 β by macrophages, leading to SARS-CoV-2 induced cell pyroptosis. The patients have increased IL-1 β in the serum, an indicator of the pyroptosis. The severe SARS-CoV-2 infection is likely to cause cell pyroptosis, especially in lymphocytes, through the activation of NLRP3 inflammasome. The response is an overdrive, in form of a fusillade of inflammatory molecules called cytokines, which attack the virus infested lungs, leading to a hyperinflammatory state, which is a harbinger of ARDS, a common cause of COVID-19 deaths.

The cytokine fusillade, however, varies by gender, with the older men having more cytokine-producing cells and thus faring worse than older women [31]. In the severely ill SARS-CoV-2 patients, the disease may progress rapidly from viral pneumonia to acute respiratory failure. The neutrophil-to-lymphocyte ratio (NLR) has been identified as an independent risk factor for severe illness in these patients. The elderly patients with $NLR \geq 3.13$ are more likely to develop severe pneumonia and ARDS and should have rapid access to intensive care unit and respiratory support [32]. A number of elderly people dying from COVID-19, die because of complications related to the coexisting chronic diseases without developing pneumonia or ARDS. Further, the immunosenescence leaves older people who have survived COVID-19, without robust immunity, if they be exposed to the virus again. The associated chronic conditions like CVD, lung disease, diabetes, or kidney disease also weaken the body's immunity. The elderly people having a weaker immune memory than the young people, are more vulnerable to the disease.

The ACE2 Homeostasis and COVID-19

Angiotensin-converting enzyme 2 (ACE2) The ACE2, or ACEH (ACE homolog), is an integral membrane protein and a zinc metalloprotease of the ACE family. The human ACE2 protein sequence consists of 805 amino acids, including a N-terminal signal peptide, a single catalytic domain, a C-terminal membrane anchor and a short cytoplasmic tail. ACE2 cleaves angiotensin I and II as a carboxypeptidase. Functionally, there are two forms of ACE2. The membrane-bound

ACE2 contains a transmembrane domain and an extracellular domain, which is used by SARS-CoV-2 as the receptor. The other, soluble form of ACE2 lacks the membrane anchor and circulates in small amounts in the blood.

There is rich ACE2 surface expression in nasal mucosa and nasopharynx, oral mucosa and tongue, respiratory tracts, and lung alveolar epithelial cells. In addition, there is an abundant presence of ACE2 on arterial and venous endothelial cells, and arterial smooth muscle cells and oesophageal upper and stratified epithelial cells, absorptive enterocytes from ileum and colon, cholangiocytes, myocardial cells, renal proximal tubule cells, urinary bladder and urothelial cells, and skin, lymph nodes and brain. The abundant ACE2 expression has been linked with the pathogenesis involving vasculitis, deranged immune function, extensive pulmonary inflammation, and diffuse alveolar damage with hyaline membrane formation, and the severe clinical manifestations and the multi-organ dysfunction observed COVID-19 in patients [33]. It appears that apart from gaining its entry through the ACE2, the SARS-CoV-2 subsequently down-regulates the ACE2 expression leading to loss of its protective effects in various organs including lungs and the vasculature, which may have significant impact on the pathogenesis of the disease.

Interaction between ACE2 and COVID-19: The epidemiological studies have documented that different sex and age groups have different susceptibility to SARS-CoV-2 infection, which may be linked to ACE2 expression. There is significantly reduced ACE2 expression with aging in the elderly age group in both genders. In animal studies, a higher ACE2 content was noticed in old female than male, which may explain comparatively worse prognosis of COVID-19 in elderly men³⁴. In addition, the elderly, especially those with hypertension and diabetes, have reduced ACE2 expression and upregulation of angiotensin II proinflammatory signaling. The SARS-CoV-2 binding to ACE2 may exaggerates this proinflammatory milieu, predisposing the elderly people to greater disease severity and mortality. The ACE2 is predominantly expressed in surfactant-secreting type II alveolar cells in lungs, bronchiolar epithelium, and endothelium and smooth muscle cells of pulmonary vessels. Further, it is more abundantly expressed in the apical than the basolateral areas of lungs. Furthermore, the ACE2 expression is proportionately correlated to the epithelial differentiation of the alveolar tissue. The undifferentiated cells poorly express ACE2, while well-differentiated cells richly express ACE2. The studies indicate that infection of human airway epithelia by SARS-CoVs correlates with the state of cell differentiation and ACE2 expression and localization [35].

The lungs appear to be the most vulnerable target organs in COVID-19, because of the vast surface area making the lung highly susceptible to inhaled viruses. About 83 percent of ACE2-expressing cells are alveolar type II cells, which are exposed and can serve as a reservoir for the viral invasion. The ACE2 is not only the entry receptor of the virus but also protects from lung injury and the angiotensin-(1-7) generated by degradation of Ang II by ACE2, has vasoprotective functions. In addition, it appears that ACE2 competitively binds with SARS-CoV-2 not only to neutralize the virus but also rescue cellular ACE2 activity which negatively regulates the renin-angiotensin system (RAS) to protect the lung from injury [36]. The high lethality associated with SARS-CoV-2 infection could be because of dysregulation of the pulmonary protective mechanisms [37]. Whereas as demonstrated in mice experiments, the SARS-CoV downregulates ACE2 protein by binding its spike protein, contributing to severe lung injury [38]. The decreased ACE2 availability may contribute to oxidative stress and subsequent lung injury.

The RAAS Activity and COVID-19 Prognosis: The perspective that ACE inhibitors, like ramipril and ARBs, like losartan may increase ACE2 expression, have raised concerns about their safety in patients with COVID-19. The interaction between the SARS-CoVs and ACE2 has been proposed as a potential factor in their infectivity by some researchers, and there are concerns about the use of these drugs may alter the ACE2 expression and attended by its fallouts [39]. Currently, there are insufficient data available to translate it into clinical practice. On the other hand, it is held that an abrupt withdrawal of these drugs in high-risk patients, having heart failure, coronary heart disease and hypertension may result in clinical instability and adverse outcomes. Further, despite substantial structural homology between ACE and ACE2, their enzyme active sites are distinct and the ACE inhibitors and ARBs in clinical use do not directly affect the ACE2 activity. Thus, it is advisable that an ACE inhibitor or ARB should be continued in known or likely COVID-19 patients with stable condition [40].

The SARS-CoV-2 utilizes and affects ACE in multiple ways. Apart from its entry through the ACE2, SARS-CoV-2 subsequently down-regulate ACE2 expression. Further, with the continued viral infection and replication contribute to reduced membrane ACE2 expression as the ACE2 sites are taken up and damaged by subsequent viral invasions. In addition, the SARS-CoV-2 binding to ACE2 exaggerates proinflammatory background in the elderly patients. The reduced

ACE2 activity in the lungs results in unopposed angiotensin II accumulation and local RAAS activation, contributing further to inflammation and neutrophil infiltration and associated with high morbidity and mortality in the elderly patients with COVID-19.

General health and Socioeconomic factors

There are certain socio-economic reasons, as well. In many societies, elderly people are prone to live in poverty, experience a drop in the quality of life and neglected healthcare, which compromises their health in general [41]. Further, prioritizing younger patients in case of limited resources such as availability of ventilators and ICU beds, may exclude the elderly people, who already carry a poor prognosis, thus, still worsening their chances of survival [42]. Many of the elderly are able to receive only compassionate care, which means they may lose provision of the scarce resources and overburdened intensive care in favour of the younger COVID-19 patients having a better chance at survival [43].

Conclusion: Annihilation of the Longevity Dream

The concepts in aging and longevity

The aging is a complex process and affects virtually all organs of the body. A vast body of knowledge can now explain the changes that take place with aging at molecular and cellular level. There do exist possibilities of being able to reverse the aging process [44]. The possibility of a lengthy-healthy life is alluring. As the life expectancy at birth rises and there is taking place an improvement in average and maximum lifespan, the possibility of living life more than never seems logical. The science gives visions; the technology makes the visions possible. The future technology appears to offer us visions that rival the dreams of myth and legend. As per the Arthur C. Clarke's Third Law - 'any sufficiently advanced technology is indistinguishable from magic' [45]. One of these magical dreams, is that of exponential life extension.

The longevity research has always looked forward to retard aging of immune system, reverse stem cell aging and to keep alive or preserved through good health practices, nutritional supplements, caloric restriction (CR) and organ transplantation, and finally cryopreserved the human body after death till the advanced molecular repair technologies are available. The realization of dream of the state of non-aging to achieve immortality or eternal life was a far-fetched dream which dwelt on the possibility is that technology will be able to repair the damage done to our tissues with age and thus granting us longevity with good quality of life.

Retarding aging and the longevity dream

But the aging is universal in the kingdom of living. We find people aging; we ourselves age and grow older. There has evolved a whole novel understanding of the biology of aging. As per rational thinking, it is unlikely that something like a pill or potion, can reverse the changes and dysfunction associated with aging. At the same time, the improved lifestyle, progress in healthcare and technology has made possible to slow aging, achieve significant longevity [44]. There are established strategies aiming to reduce the oxidative stress which exacerbates aging process and leads to debility to human body in general and CVS in particular. The life-style changes in form of healthy diet, regular physical exercise, other interventions like smoking cessation and intervention in sleep disorders are established practical ways to prevent metabolic diseases, CVDs, and neuro-degenerative disorders (Figure 5).

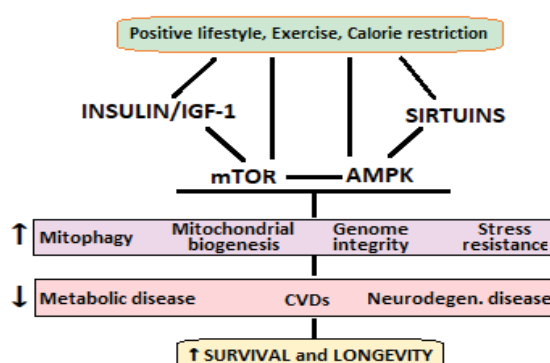


Figure 5: The synergistic pathways for disease protection and longevity.

Annihilation and loss of the longevity dream

The high morbidity and mortality in the elderly with COVID-19 shows us that aging weakens the physiological systems and body organs. The irrational hopes from the unproven concepts of longevity research move us away from terra-ferma and are detrimental to rational scientific behavior. The elderly apart from physiological alterations in the immunity and various organs, frequently suffer with the underlying conditions like diabetes and heart disease, which increase the infectivity risk as well as pose them to unfavourable prognosis from COVID-19. Further, the lifetime exposure oxidants, various endocrine disruptors and air pollution exacerbate the aging related physiological deficits in the lungs, heart and vasculature and other organs and make them vulnerable to SARS-CoV-19 infection. The older adults or elderly age group, in light of their vulnerability to COVID-19 associated infectivity, disease severity and worsened mortality, now face the loss of the longevity dream.

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Chapter 5: Identifying Patterns in COVID-19: Morbidity, Recovery, and the Aftermath

Background

The Infectivity and Pathogenesis: SARS-CoV-2, the causative agent of COVID-19, involves Angiotensin-converting enzyme 2 (ACE2) receptors on type II alveolar type 2 (AT2) cells in lungs. Apart from, the upper and lower respiratory tracts, the disease affects the gastrointestinal system prominently, as evidenced by the significant GI symptoms, early in the course of the disease. In addition, the virus infects ACE2-bearing cells in other organs including the heart and blood vessels, brain, and kidneys.

Clinical Features and Morbidity: The clinical spectrum of COVID-19 varies from asymptomatic or pauci-symptomatic presentation to moderate to severe states characterized by respiratory failure necessitating mechanical ventilation and ICU support and those manifesting critical clinical condition with complications like sepsis, septic shock, and multiple organ dysfunction failure. The CT chest is an important tool for early identification of COVID-19 pneumonia as well as for prognostic purposes.

The Recovery and Residual Damage: The recovery and other outcomes vary depending on age and other aspects including sex, comorbidities, and genetic factors. The outlook for older adults, who account for a disproportionate share of critical disease, is unfavorable, and most of those who survive are unlikely to return to their previous level of functioning. The disease affects their long-term health and quality of life as well as brings in propensity for truncated post-disease survival.

COVID-19 Aftermath and Follow Up: The patients discharged from hospital following severe COVID-19, continue to suffer with lingering impact of the disease as well as that of the emergency treatments that saved their life. The post-infection reduced exercise tolerance and other subtle factors, like post viral fatigue syndrome, post-traumatic stress disorder, impaired concentration, delirium, and disturbed sleep-wake cycle often underly the functional impairment. In fact, there is need of step-down care and later a multidisciplinary support involving regular clinical assessment, respiratory review, physiotherapy, nutritional advice, and psychiatric support.

Conclusion: The life after COVID-19: After recovery from the disease, the virus SARS-CoV-2, may persist for uncertain period. In addition, the chance of reinfection cannot be ruled out. The vitamin D supplementation may be helpful. In general, the quality of life (QOL) in ICU survivors improves but remains lower than general population levels, but most of the patients adapt well to their level of self-sufficiency and QOL. Also, the debility due to co-morbidities may further compromise the activity of daily living and QOL issues. The Age and severity of illness appear to be the major predictors of post-discharge physical functioning.

The Infectivity and Pathogenesis

The Agent and Infectivity

The causative agent of COVID-19, SARS-CoV-2 involves Angiotensin-converting enzyme 2 (ACE2) receptors on the host tissues to invade and infect. The analysis of the ACE2-RNA expression profile indicates that the ACE2-virus receptor expression is mainly concentrated in type II alveolar cells (AT2) in lungs. Further, it is more widespread in males than females, and the Asian men have a higher ACE2- expressing cell ratio than white and African American subjects [1]. In addition, the virus is able to infect ACE2-bearing cells in other organs, including the gastrointestinal tract, heart and blood vessels, and kidneys. The ACE2-expressing AT2, in addition, also express various other genes that positively regulate viral entry, replication and transmission. Once inside the airways, the SARS-CoV-2 through its S protein on viral surface, is able to recognize and stick to the ACE2 receptors, followed by the virus infecting the ACE2-bearing cells lining the upper as well as the lower respiratory tracts. With the dying cells sloughing down and filling the airways, the virus is carried deeper into the lungs as the thin layer of surfactant coating the airways becomes even thinner and the brush border less efficient to defend from the invading virus. The virus is able to transmit while still confined to the upper airways, before invading the lower respiratory tract and lungs and causing symptoms [2]. The virus infects the gastrointestinal (GI) system prominently, at least in the early phase, as evidenced by the significant GI symptoms [3]. It is able to infect and multiply in the intestinal epithelial cells [4]. It appears that further intestinal invasion is effectively stalled by the fast dividing intestinal epithelial cells with help of the gut microbiota [5].

Immune Response and Pathogenesis:

With the viral infection, the first response of the human body is to destroy the virus and prevent its replication. The virus invasion and intracellular replication leading to the disease manifestation, however, depends on various host factors. With the weakened immunity with aging, due to co-morbidities like cardiometabolic diseases and other factors which alter the immunity and compromise the defence system, the body is unable to stall the virus, aggravating the disease process. The infection activates the immune system leading to inflammation and pyrexia, and in severe cases, may damage the host tissues and organs. The blood vessels get inflamed and leaky, leading to pulmonary edema in lungs. In the critical disease, the SARS-CoV-2 infection extensively damages the alveolar tissue and triggers an overreaction of the immune system accompanied by the excessive cytokine release, which apart from damaging the host tissues, leads to increased susceptibility to infectious bacteria, septic shock and multi-organ dysfunctions (Figure 1).

Other Factors Related to Pathogenesis:

Due to the interaction of multiple factors, which may include gender-associated and genetic linked susceptibility, the COVID-19 is a disease with variable clinical presentations, requiring a close clinical follow up, meticulous monitoring and ensuring availability of various therapeutic options including mechanical ventilatory support [6]. Such a precarious clinical scenario from virtually asymptomatic to mild to moderate, and severe to critical presentation of the disease with a high infectivity and fast changing prognosis, may make the therapeutic decisions in individual cases challenging with a high error factor on part of the care providers. The propensity to get infected themselves, adds further to the difficulties faced by the medical and paramedical workers.

Clinical Features and Morbidity

The Clinical Spectrum of COVID-19

The clinical spectrum of COVID-19 varies from asymptomatic or pauci-symptomatic presentation (Stage I) to moderate to severe states (Stage II a and IIb) characterized by respiratory failure necessitating mechanical ventilation and ICU support and those manifesting critical clinical condition (Stage III) with complications like sepsis, septic shock, and multiple organ dysfunctions (Figure 2).

The mild to moderate disease occurs in approximately in 81% of cases. The severe disease with dyspnoea and acute respiratory distress syndrome (ARDS) and lung infiltrates appearing within 48 hours occurs in 14% of cases. Whereas, the critical COVID-19 illness accompanied by respiratory failure, septic shock, and multiple organ dysfunction (MOD) or failure (MOF) has been documented in approximately 5% of cases [7].

The transition from milder symptoms to severe disease and ARDS has been related to an uncontrolled cytokine release by the hyperactive immune response. In patients, going to have the worst outcomes with COVID-19 infections, the immune system becomes overactive with excessive stimulation of T cells and macrophages, resulting in cytokine storm with release of a large amount of proinflammatory cytokines including interleukins (IL) - 1, 6, 12 and 18. The excessive or uncontrolled levels of cytokines released, further activate more immune cells, resulting in hyperinflammation and the cytokine release syndrome (CRS).

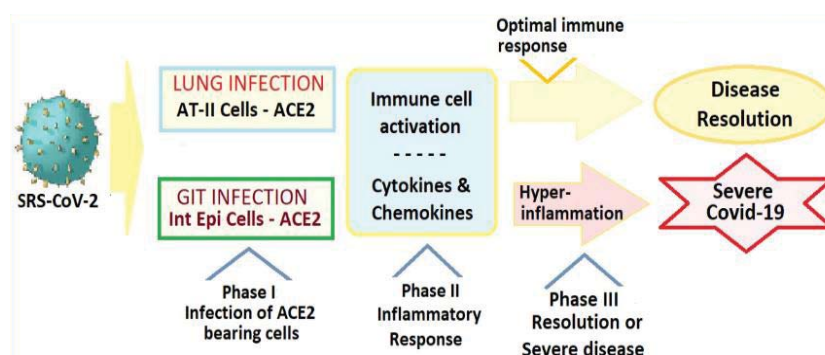


Figure 1: The host immune response to SARS-CoV-2 and COVID-19.

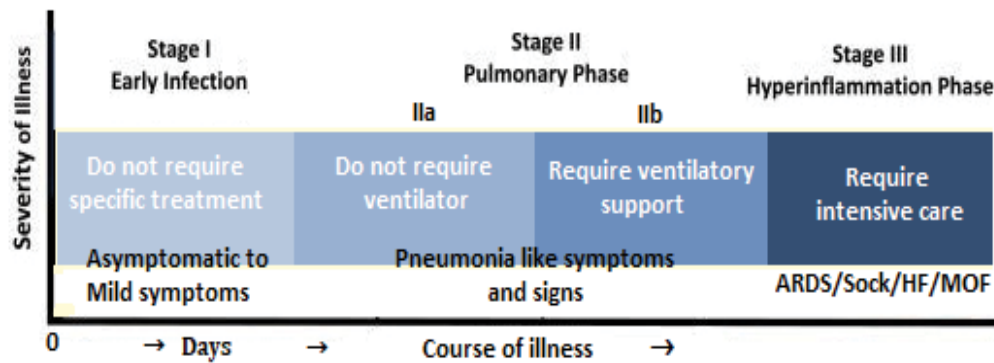


Figure 2: The spectrum and clinical stages of COVID-19.

The Clinical Features of COVID-19: The most common sign is pyrexia, which is present in nearly 9 in 10 of those infected [8]. About 7 in 10 have a dry cough, of which nearly a third have cough with mucus. Tiredness and feeling of lethargy are common. Less commonly, there is sore throat, headache, muscle-aches and arthralgias. There may be nausea, vomiting or diarrhoea. Anorexia may be present, as are ageusia and anosmia. Some may complain dyspnoea, shortness of breath and a tight band wrapped around the chest. A skin rash - viral exanthema, may occur especially in younger patients, as may be spots of swelling and redness. A specific finding called COVID toes, occasionally present especially in children, is superficial small clots in blood vessels close to the skin in toes and fingers. The clinical course of the disease, in general, depends on the host's immune response. It is to be noted that the patients need a drug to boost the immune system early on in the disease, and later one to tamp it down if the disease progresses and cytokine begin to rise. The drugs that target the immune system to lower the risk of cytokine storm, may also tamp down the immune response, making it hard to clear the virus in the long run.

Lab and Radiological Investigations

The patients may present with lymphopenia and thrombocytopenia. There are raised CRP and elevated liver enzymes. Raised lactate dehydrogenase is a bad prognostic sign as it tends increase in cytokine storm [9]. Abnormal coagulation parameters, such as elevated D-dimers and fibrinogen concentrations, and prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT) are associated with unfavourable prognosis [10]. The radiological investigations like x-ray and CT imaging of chest are of particular significance relating to the diagnosis, defining the extent of pulmonary involvement and disease prognosis [11].

The CT Imaging in COVID-19 Patients: The CT chest is an important tool for detecting pneumonia in asymptomatic or with mildly symptomatic patients with COVID-19 patients who are covert transmitters and can deteriorate clinically in a short period [12]. The typical imaging findings are ground glass opacities (GGO) with peripheral distribution, unilateral or bilateral, involving one or more lobes particularly the lower lobes, often combined with subpleural curvilinear line and fine reticulation [13]. The CT abnormalities in COVID-19 pneumonia more frequently exhibit a peripheral predominance, with less frequent pleural effusion and lymphadenopathy.

On follow up, the maximum lung involvement peaks at approximately 10 days from the onset of initial symptoms. There are 4 stages on the CT chest - Stage 1 (0-4 days): ground glass opacities; Stage-2 (5-8d days): increased crazy-paving pattern; Stage-3 (9-13days): consolidation; and Stage-4 (≥ 14 days): gradual resolution of consolidation [14]. In a series of 919 patients, the CT findings in the intermediate stage of the disease were characterized by an increase in the number and size of GGOs, a progressive transformation of GGO into multifocal consolidation, with septal thickening and development of a crazy-paving pattern [15]. The presence of centrilobular nodules, mucoid impactions and unilateral segmental or lobar consolidations suggest a bacterial origin of pneumonia, or superinfection.

The Complications during COVID-19 Illness

Often the infection caused by SARS-CoV-2 is an asymptomatic or mild disease and recover by themselves. But, approximately 1 to 5% of all COVID-19 patients are moderately severe, severe, or critically ill, and may suffer from various disease related complications (Figure 3).

Complication	Inflamm. response	Structural abnormalities	Clinical outcome
The abnormal clotting or thrombosis	Lymphopenia Thrombocytopenia ↑ IL-6, CRP ↑ D-dimer	Venous thrombosis Intravascular coagulopathy Myocardial injury Cerebral infarction	DVT DIC Myocardial infarction Stroke
Acute cardiovascular syndrome (ACovCS)	↑ Troponin ↓ ST or ↑ ST Arrhythmias	Wall motion abnormalities De novo systolic dysfunction Myocarditis or cardiomyopathy	Heart failure Pericardial effusion Ac coronary syndrome
Ac respiratory distress syndrome (ARDS)	Alveolar damage Influx of inflam cells Protein exudation	Pulmonary edema Pneumonia, Pleural effusion Secondary bacterial infection	Acute dyspnoea Severe hypoxia Respiratory failure
The Cytokine storm syndrome (CSS)	Macrophage activation ↑↑ Cytokine release ↑ Thrombin ↓ Anticoag	Activation of coagulation paths Widespread Microthrombi ↑ Vascular permeability	DIC Shock Multi-organ failure

Figure 3: Complications related to severe COVID-19 infection.

These patients need hospitalization for advanced treatment, apart from symptomatic and supportive, with antiviral drugs, antibiotics and other drugs and assisted respiratory support in a special medical facility or an intensive care unit – ICU [16]. The survival odds and the potential for long-term complications, influence the decisions in ICU care, which are difficult and grey areas clinicians as well as the family members.

The abnormal clotting or thrombosis: In the severe or lethal form of COVID-19, there occur widely scattered thrombi in multiple organs early in course of the disease. The abnormal clotting plays a major role in morbidity and mortality and occurs at various sites involving small veins like in COVID toes and the large vessels in the legs leading to deep vein thrombosis (DVT), in the lungs causing to pulmonary embolism (PE) and in cerebral arteries resulting in a stroke. The etiopathology of thrombosis in COVID-19 is not fully explained, but it appears that the disease may predispose to the venous and arterial thromboembolic events due to excessive inflammation, hypoxia, immobilisation and diffuse intravascular coagulation (DIC). Added to this, is hypotension and shock, ventilator use, and multiple drug treatments themselves affecting the various organs adversely including the lungs, heart, brain, liver, and kidneys. Using medicines to prevent clotting may end up causing severe bleeding, further adding to the insult.

There has been reported a remarkably high incidence of thrombotic complications manifesting as acute PE, DVT or ischemic stroke, apart from acute coronary syndrome, myocardial infarction, and systemic arterial embolism. In a Dutch study, 31% of patients hospitalized with COVID-19 suffered with thrombosis clots while on antiplatelets like aspirin or clopidogrel [17]. Further, in a very recent study, the US investigators led by Oxley T, et al, have reported five cases of large vessel stroke over a 2-week period in COVID-19 patients under age 50 years, representing a sevenfold increase from what can be normally expected. The study links COVID-19 infection to large vessel stroke in young adults [18].

Acute COVID-19 cardiovascular syndrome (ACovCS): The ACovCS describes the impact of the disease on cardiovascular system. The ACovCS is a myocarditis-like syndrome associated with acutely reduced left ventricular ejection fraction in the absence of obstructive coronary artery disease. The acute myocardial injury, the fulminant myocarditis, evidenced as elevated troponin, occurs in 20-30% of hospitalized COVID-19 patients [19]. The ACovCS involves demand ischemia, microvascular ischemic injury, myocarditis, acute coronary syndrome, and myocardial infarction (MI). The details of the mechanisms of ACovCS are not clear. The endomyocardial biopsy of a patient with ACovCS and cardiogenic shock showed low-grade myocardial inflammation, with localization of SARS-CoV-2 within macrophages but not cardiomyocytes. Given the exposure risks involved in these patients, the acute myocardial injury is advised to be managed conservatively, with rest and abstinence from aerobic activity for a period of 3-6 months until normalization of troponin or resolution of myocardial inflammation on magnetic resonance imaging [20].

Respiratory Distress and Ventilatory support: There is a high mortality rate in ventilated COVID-19 patients. In a large study, involving 3,883 COVID-19 patients, the mortality was as high as two thirds among COVID-19 patients who required ventilation. As per the United Kingdom's Intensive Care study, 66.3% patients who required mechanical

ventilation died, compared with 19.4% of the patients who required basic respiratory support. The new ICNARC (Intensive Care National Audit & Research Centre) findings are consistent with other previous reports from smaller case series. For example, a single-center study involving 52 patients treated in Wuhan, China, showed that 37 (71%) required mechanical ventilation and 32 (61.5%) died within 28 days of ICU admission [21]. Another study from Seattle, also found that a higher proportion of COVID-19 patients, over 50%, requiring mechanical ventilation died compared to about 35% patients without coronavirus who had viral pneumonia and who needed ventilation [22].

The COVID-19 pneumonia is a specific disease with dissociation between the severity of hypoxemia and the maintenance of relatively good respiratory mechanics. The lungs of COVID-19 patients tend to be more elastic and compliant than those of other ARDS patients so it is possible to allow patients to take slightly bigger breaths with a bit more tidal volume in each breath and allow them to be less sedated, more awake, and more in sync with the ventilator. They may be able to be extubated earlier. In this light, the mechanical ventilation protocols and strategies need revision and modification. It has been advocated that some COVID-19 patients might need gentler positive end-expiratory pressure because they present with an atypical form of ARDS [23]. The respiratory distress in COVID-19 is of two types, Type 1: Near normal pulmonary compliance with isolated viral pneumonia, non-ARDS patients, and Type 2: Decreased pulmonary compliance and present with ARDS.

In the Type 1, the lung's gas volume is high, the recruitability is minimal, and the hypoxemia is likely due to the loss of hypoxic pulmonary vasoconstriction and impaired regulation of pulmonary blood flow. High PEEP and prone positioning do not improve oxygenation through recruitment of collapsed areas, but redistribute pulmonary perfusion, improving the ventilation/perfusion (VA/Q) relationship. In the Type 1 patients, PEEP levels should be kept lower with higher tidal volume, the respiratory rate should not exceed 20 breaths/min, closely monitored, but too much interventions should be avoided, in general. Whereas, the Type 2 is present in 20% – 30% of the COVID-19 patients admitted to the ICU, manifesting severe hypoxemia is associated with compliance values < 40 ml/cmH₂O, indicating severe ARDS [24]. In the Type 2 patients, the standard treatment for severe ARDS should be applied with lower tidal volume, prone positioning, and relatively high PEEP.

Regarding alternate methods of advanced respiratory support, like Extracorporeal membrane oxygenation (ECMO), which are special interventions for when standard therapy is not successful. The ECMO therapy is not realistic and an impractical alternative [25]. Further, it is expensive and has associated complications and a high mortality rate in patients on ECMO.

The Cytokine Storm Syndrome (CSS): The Pathophysiology of CSS: As the SARS-CoV-2 virus attacks the ACE2 bearing alveolar type 2 (AT2) cells, the macrophages are recruited and increase cytokine production and attract additional immune cells to the affected area such as T-helper cells CD4 and CD8 which work to combat the infection. Pattern recognition receptors (PRRs) of the immune cells recognize the virus and signal release of the pro-inflammatory cytokines such as interferon gamma (IFN- γ), tumor necrosis factors (TNFs), interleukins (ILs), and chemokines. IFN- γ activates macrophages which produce IL-6, TNF- α , and IL-10. Once the virus is cleared, the immune pathways go quiescent. In event of defective immune response to Covid-19 infection, however, this process does not shut down and leads to overproduction of proinflammatory cytokines, TNF, IL-6, and IL-1 β causing cytokine storm. The CSS is associated with an increased vascular hyperpermeability along with activation of coagulation pathways, which predispose to the development of micro-thrombosis, disseminated intravascular coagulation (DIC), and ultimately multi-organ dysfunction [26].

Normally, thrombin promotes clot formation by activating platelets and by converting fibrinogen to fibrin. The thrombin generation is regulated by negative feedback loops and physiological anticoagulants, such as antithrombin III, tissue factor pathway inhibitor, and the protein C system. During hyper-inflammation, the feedback loops can be impaired leading to unabated production of thrombin and reduced anticoagulant concentrations due to reduced production and increased consumption. The defective procoagulant–anticoagulant balance leads to the activation of coagulation pathways [27]. On the other hand, the excess thrombin further augments inflammation via proteinase-activated receptors (PARs), especially PAR-1. In addition, the defective immune response to COVID-19 infection predisposes to secondary bacterial infection in setting of severe COVID-19 pneumonia [28].

The patients with CSS present with, apart from a surge in IL-6, IL-1, TNF- α and IFN- γ , elevated C-reactive protein, serum lactate dehydrogenase, D-dimer, and ferritin levels. The CSS in COVID-19 pneumonia in setting of hospitalised

patients is associated with unfavourable prognosis. Various therapeutic modalities have been tried. The prophylactic use low molecular weight heparin (LMWH) may be recommended for prevention of venous thromboembolism, especially in those with significantly raised d-dimer concentrations. The LMWH has anti-inflammatory properties, which may be beneficial in COVID-19 [29]. The IL-inhibitors such as, tocilizumab (IL-1 inhibitor) may be tried, which has a large therapeutic window and a short half-life. The concentrated globulin prepared from pooled human plasma may be useful. The convalescent plasma collected from recovered COVID-19 patients is being studied as a potential treatment. The immunosuppression with corticosteroids, is controversial but may be useful in treating hyperinflammation.

The Recovery and Residual Damage

The Fatality and Other Severity Outcomes

The CFR (case fatality rate) from COVID-19 increases with age, the overall death rate being 1.4% in those with no comorbid conditions, rises sharply to 4% for those in their 60s, 8.6% for people in their 70s and 13.4% for those age 80 and older. The CFR is higher among males compared to females (4.7% vs. 2.8%). By occupation, patients who reported being retirees had the highest CFR at 8.9%. The patients with comorbid conditions had much higher rates: 13.2% for those with cardiovascular disease, 9.2% for diabetes, 8.4% for hypertension, 8.0% for chronic respiratory disease, and 7.6% for cancer. The data on the progression of disease indicate that the time period from onset to the development of severe disease, including hypoxia, is 1 week. Among patients who have died, the time from symptom onset to outcome ranges from 2-8 weeks [30].

The recovery and other outcomes vary depending on age and other factors [31]. The outlook for older adults, who account for a disproportionate share of critical disease, is not favourable, and most of those who survive are unlikely to return to their previous level of functioning [32]. The disease affects their long-term health prospects and quality of life (QOL) as well as brings in propensity of truncated post-disease survival.

Recovery Estimates for COVID-19

About 8 in 10 people who get COVID-19, are asymptomatic or suffer with mild illness. The early estimates predict that the overall COVID-19 recovery rate is between 97% and 99.75%. It may take 2 weeks for to recover from illness in mild cases. For those with severe or critical cases, recovery can take up to 6 weeks. For Severe Illness need to stay in the hospital may last 2 weeks or more [33]. In general, the recovery depends on how a person's immune system responds to COVID-19. Reinfection may occur, after recovery. One early study on monkeys found that they did not get infected a second time.

According to the preliminary data from a recent UK study, involving 775 patients with COVID-19 admitted to critical care; 79 died, 86 survived and were discharged to another location, and 609 were still being treated in critical care, with uncertain prognosis [34]. Another, small study of 24 critically ill COVID-19 patients treated in Seattle hospitals, documented that over 50% died within 18 days. Of those who survived, three remained on ventilators in intensive care units, four left the ICU but stayed in the hospital, and five were discharged home. Many COVID-19 patients who need a ventilator never recover [35]. The survival rates may vary across studies and countries. A report from London's Intensive Care National Audit & Research Centre found that 67% of reported COVID-19 patients from England, Wales, and Northern Ireland receiving advanced respiratory support died [36]. Those least likely to recover seem to be frail older patients with other pre-existing illnesses such as chronic lung disease or heart disease. The ARDS mortality is usually between 30% and 40%. For older people, who tend to have more infections, mortality rates are as high as up to 60% or more.

The Effect of ARDS on lungs

The COVID-19 directly impacts the lungs and damages the alveoli. There occurs damage to the wall and the lining of the alveolus and capillaries. The debris from the damage, which is plasma protein accumulates on the alveolus wall and thickens the lining and impairs the oxygen transfer to the red blood cells. As the alveoli are damaged, there occurs influx of inflamed cells and protein leak, leading to pneumonia, further impairing the oxygen intake and exchange by the lungs. The immediate assault on the body by the disease, is extensive. It targets the lungs, and through hypoxia and widespread inflammation damages the heart, kidneys, brain, and other organs. Even after coming off the ventilator, the patient often needs assistance in form of oxygen therapy, through a mask or a continuous positive airway pressure (Cpap) ventilator. The COVID-19 survivors, who have spent time on a ventilator often suffer with muscle wastage and long-term disability and may need retraining to breathe. The breathing exercises are to be advised to the recovering COVID-19 patients [37].

Although it is too early to say about lasting disabilities in COVID-19 survivors, the clues come from studies of severe pneumonia and ARDS. Though, most of the pneumonia and ARDS patients eventually recover their lung function, the conditions may lead to scarring resulting in long-term breathing problems. In addition, in the recovered patients, the prolonged inflammation seems to increase the risk of future illnesses, including coronary heart disease, stroke, and kidney disease. The people hospitalized for pneumonia have a risk of heart disease about four times as high as that of age-matched controls in the year after the discharge, and about 1.5 times as high in each of the next 9 years.

The Disabilities and Rehab Measures

The older adults are at greatest risk of both severe disease and long-term impairment. The best practices for geriatric care are difficult to be followed in setting of the highly infectious disease in the hospital and ICU. During ICU care, the aim is to keep patients as lucid and mobile as possible, which can reduce the muscular weakness and allow to wean off early from the ventilator and has a positive effect on their long-term odds. The disease is highly infectious, and the rehabilitation measures can be challenging tasks.

Further, the patients who spend long time in an ICU, regardless of their illness, are prone to a set of physical, cognitive, and mental health difficulties known as post-intensive care syndrome. An associated factor for hospitalized patients is risk of delirium, a state of confused thinking that can lead to long-term cognitive impairments such as memory deficits. The COVID-19, like severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), can directly damage the brain or the associated wide-spread inflammation can compromise the cerebral blood flow to cause neuronal deficit.

In addition, the commonly prescribe sedative drugs to suppress violent coughing and help patients tolerate the distress associated with endotracheal tube or benzodiazepines to allay anxiety can increase the risk and intensity of delirium. To overcome this and improve patient care and outcomes, many ICUs, now adopt a frequent interruption of narcotics and sedatives plus a decrease of ventilator pressure to test whether patients can wake up, breathe, and tolerate the ventilator without drugs. This practice, of course, requires close monitoring and risk of exposure to the COVID-19 infection. Early reports from the ICUs care, suggested that the COVID-19 patients should be put on ventilators early in the course of the disease. But there are the downsides of early ventilation [38]. It may be possible to differentiate the subtypes and stage of respiratory failure in COVID-19 patients, who are more likely to need to ventilatory support.

Post-Recovery Complications

There are immediate as well as the delayed post-recovery complications. Those patients who have suffered with high fevers or severe diarrheal illness can remain mildly dehydrated and suffer with electrolytes disturbance, and various fallouts. They may not tolerate nutrition as well and get suboptimal nutrition and suffer with weight loss and emaciation. Frailty is common, associated with ongoing fatigue and lethargy. The long-term complications include myocarditis and cardiomyopathy, which can exacerbate their propensity to coronary artery disease. There occurs an increased risk for future illnesses, including MI, stroke, and kidney disease. With other pre-existing illnesses like diabetes, there is more risk for complications.

In addition, the underlying conditions like chronic obstructive pulmonary disease (COPD) and other chronic lung diseases are exacerbated. Some of these patients may have significant scarring of the lungs and suffer with reduced lung function. Those who remain for a long period on a ventilator are prone to respiratory and extra-respiratory muscle atrophy and weakness. Their immune system is less robust, and they are prone to infections. In addition, many of them may suffer with post-traumatic stress disorder (PTSD), impaired concentration, delirium and disturbed sleep-wake cycle afterwards. A study of people hospitalized for SARS found that more than one-third had moderate to severe symptoms of depression and anxiety one-year later [39].

COVID-19 Aftermath and Follow Up

The Post-Discharge Period

The patients hospitalized with severe COVID-19, after discharge suffer with lingering impact of the disease as well as that of the emergency treatments that allowed them to survive it. The post-infection reduced exercise tolerance suggests that there may be more subtle factors, like post viral fatigue, underlying the functional impairment. In fact, there is need

of step-down care while in the hospital before discharge to home or the community care with primary care support [40]. Later, the additional inputs are likely to be multidisciplinary for regular clinical assessment, recomposing respiratory review, physiotherapy, nutritional advice, and psychiatric support as the discharged patients regain their health.

The Post-Discharge Follow Up

About 50% of patients admitted to hospital will require no further input on discharge. 45% will need some form of low level medical or social input for recovery, and a predicted 5% of patients will require more focused, ongoing intense rehabilitation (Figure 4). In general, a combination of physical interventions such physiotherapy and graded exercise programmes, good nutrition and clinically validated mental health support interventions such as cognitive-behavioral therapy (CBT) focusing on changing the automatic negative thoughts that can contribute to and worsen emotional difficulties, depression, and anxiety, and possibly antidepressants may provide benefits. A designed care approach for severe COVID-19 discharged patients may include monitoring them using computers and smartphones and treating them remotely in hopes of preventing readmission to the hospital, and planning visits at home on case to case basis.

A two year follow up study involving 106 patients aged 80 years or over, has documented that 40 (37.7%) died in the ICU and 66 (62.2%) patients were discharged alive from the ICU. Out of this, 25 patients died before the one-year evaluation. Of the 33 survivors at one year, seven refused the evaluation. Of the 26 remaining patients, three had dementia and the self-sufficiency could be assessed by the relatives. QOL was assessed in the rest, who were found to have significantly higher scores for psychological health, social relationships, environment, fear of death and dying, expectations about past, present, and future activities, and intimacy (friendship and love). Of the 23 patients, 18 agreed for another ICU admission should the need arise [41].

Conclusion: The Life after COVID-19

The Recovery and SARS-CoV-2 virus

During the recovery period, the viral particles can be found not only in the nasal passages, throat, and respiratory tract, but also in tears, stool, the kidneys, liver, pancreas, and heart. They have been found in the cerebrospinal fluid (CSF) in a patient with meningitis. the virus could persist in the body for up to two weeks or more after symptoms had vanished. The virus has been shown to survive in a Chinese patient's respiratory tract for 37 days, well above the average of 24 days for those with critical disease status [42]. There are further reports from China that 14% of recovering patients were retested positive [43]. It is possible that the virus may persist as a latent infection, like chickenpox, lying dormant in the body, re-emerging periodically as shingles, or become a chronic infection, like hepatitis B, living within the body for a sustained period of time, causing long-term damage.

Recovery from COVID-19 and Vitamin D

Vitamin D levels are often low in the aging population, which is also the most vulnerable group of population for COVID-19. Vitamin D has been shown to protect against acute respiratory infections [44]. A team of researchers have found the link between low levels of vitamin D and COVID-19 mortality rates across Europe. They found that levels of

Physiotherapy and Graded exercise programme
Adequate good nutrition Mineral and vitamin supplementation
Mental health support and Cognitive-behavioral therapy
Drug therapy for symptoms and co-morbidities
Evaluation and documentation
Physical examination and follow up
Monitoring health through electronic devices
Data evaluation and documentation

Figure 4: The COVID-19: Post-discharge follow up, Evaluation and interventions.

vitamin D among citizens of 20 countries in Europe was strongly associated with the mortality caused by COVID-19 and advised vitamin D supplementation to protect against COVID-19 infection [45].

Post-Discharge Period: Things that Matter

The human lifespan has increased across the globe as a result of economic progress, technological advances, and improved healthcare. Simultaneously, there has been a steady increase in the proportion of elderly individuals in different societies. As the natural fallout of a growing number of elderly and very elderly patients are being admitted to the ICU. Compared with the general population, ICU survivors report lower QOL prior to ICU admission. After hospital discharge, QOL in ICU survivors improves but remains lower than general population levels. Age and severity of illness are major predictors of physical functioning [46].

The critical care seeks to ensure survival as well as restore to the pre-admission level of function and QOL. Few data are available on QOL in elderly and very elderly ICU survivors compared to the general population. The study by Tabah, et al. inferred that in the highly selective cohort of the elderly patients one year after ICU discharge, the patients were satisfied with their level of self-sufficiency and QOL [41]. Further, the QOL, physical health, sensory abilities, self-sufficiency, and social participation had slightly lower ratings than other domains like social relationships, environment, and death related issues.

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Chapter 6: The New Revelations: Little-known Facts about COVID-19 and their Implications

Introduction

The Subsidiary Factors: The SARS-CoV-2 attacks the respiratory tract and other organs to cause highly contagious disease, COVID-19. The serious illness and fatality from COVID-19 seem concentrated among older adults and those having an underlying chronic illness or debility, such as diabetes, hypertension, cardiovascular disease, or respiratory disease. The compromised lung function in setting of lung diseases, such as chronic obstructive pulmonary disease (COPD), with a smoking history, can add to the risk for serious complications and worsen the prognosis.

Smoking and Substance use Disorders: The smokers are likely to be more vulnerable to COVID-19 as the act of smoking involves frequent contact of fingers with lips, in addition to the increased the possibility of transmission of virus from sharing of cigarettes and vaping devices. The smokers may already have lung disease or reduced lung capacity which increases the risk of serious illness and complications. Further, smoking and vaping, both tobacco as well as other substances of drug abuse, are associated with the adverse progression and unfavourable outcomes in COVID-19 patients.

The Immunity and Immune Deficiency: The immune system and immune response against an infection function on a continuum. Presently, our understanding the novel coronavirus, SARS-CoV-2, is limited. Although those exposed to the agent and developing from asymptomatic to severe disease, seem to produce antibodies and the recovered COVID-19 patients have shown antibodies, there is lack of long-term data. Further, it is not known how long the SARS-CoV-2 antibodies persist or whether they protect against reinfection or not, making the concept of development herd immunity in a community uncertain.

Aging and Comorbidity linked Frailty: Frailty is a state of increased vulnerability to physical or psychological stressors and compromised capacity to maintain homeostasis. The COVID-19 infection in setting of different stages of frailty in older adults is associated with severe disease, increased hospitalisation and ICU admission, and reduced recovery and adverse survival outcomes.

The COVID-19 associated Infections: In the COVID-19 patients, there occurs damage to epithelial lining of the respiratory tract and inflammation in lungs, leading to COVID-19 pneumonia. These patients are at risk of developing secondary infections by various pathogens including *M. pneumoniae*, which may be responsible for the increased severity of and fatal progression. The co-infection with *Mycoplasma* species in COVID-19 may lead to hemodynamic dysfunction, autoimmune activation and cytokine storm, and other complications.

Conclusion: The new Revelations: The virus being novel and highly infectious, can spread exponentially, in the absence of adequate public health measures, which would be disastrous. Further, the concentration of COVID-19 hospitalized cases in older adults has led to the hypothesis that there is a widespread transmission in younger individuals, who remain asymptomatic or suffer with mild and often undetected illness. But, in absence of herd immunity and efficient vaccination programs, the feasible option is to effectively suppress recurrent outbreaks through public health measures aided by technology and gadgets through mobile software applications including apps to help in contact tracing and exposure notification.

COVID-19 and the Subsidiary Factors

The SARS-CoV-2 attacks respiratory tract and other organs to cause highly contagious disease, COVID-19. The serious illness and fatality from COVID-19 seem concentrated among older adults and those having an underlying chronic illness or debility, such as diabetes, hypertension, cardiovascular disease, or respiratory disease [1]. The compromised lung function in setting of lung diseases, such as chronic obstructive pulmonary disease (COPD), with a smoking history, can add to the risk for serious complications and worsens the prognosis [2].

In a case series of total 72 314, the confirmed cases comprised 44 672, based on data from the Chinese Center for Disease Control and Prevention (China CDC), the overall case-fatality rate (CFR) was 2.3% and 6.3% for those with chronic respiratory disease. Further, the cases were classified as mild (non-pneumonia and mild pneumonia), severe (with dyspnoea, respiratory rate ≥ 30 /min, blood oxygen saturation $\leq 93\%$, partial pressure of arterial oxygen to fraction

of inspired oxygen ratio <300 , and/or lung infiltrates $>50\%$ within 24 to 48 hours), and critical (with respiratory failure, septic shock, and/or multiple organ dysfunctions), comprising of 81%, 14% and 5% respectively. While the CFR was 49.0% among critical cases, the overall CFR was elevated among those with pre-existing comorbid conditions - for cardiovascular disease, 10.5%; diabetes, 7.3%; chronic respiratory disease (6.3%), hypertension, 6.0%; and cancer, 5.6% [3]. Further analysis of the emerging data highlights the preponderance in men as compared to women for COVID-19 infection and its severity. While the gender distribution was almost equal in the survivors of COVID-19, the percentage of male were higher in the deceased group than in the survived group (70.3 vs. 50.0). Thus, compared to women, men appear to be more prone to have higher severity and fatality for COVID-19 independent of age and other factors [4].

The Extended Factors for COVID-19

Smoking and Substance use Disorders

The smokers are likely to be more vulnerable to COVID-19 as the act of smoking means that fingers are in frequent contact with lips, increasing the possibility of transmission of virus from hand to mouth. Sharing of cigarettes and other smoking products such as water pipes involving the sharing of mouth pieces and hoses, could facilitate the transmission of COVID-19 in communal and social settings. In addition, the smokers may already have lung disease or reduced lung capacity which increases the risk of serious illness. Further, smoking and vaping, both tobacco as well as other substances of drug abuse, are associated with the adverse progression and unfavourable outcomes in COVID-19 patients (Figure 1).

COVID-19 transmission and disease severity: The symptoms due to the neuro-invasive nature of the SARS-CoV-2, have been explored. There is likelihood that the COVID-19 related neurological manifestations can be exacerbated by smoking, due to the functional interactions and co-expression of human ACE2 receptor (hACE2) and nicotinic receptors in neuronal cells, leading to augmented expression of the hACE2 [5]. The asymptomatic COVID-19 patients often complain about anosmia (loss of smell) and ageusia (loss of taste) due to the involvement of the neurons of the olfactory mucosa and olfactory bulb, located in the forebrain. Further, the underlying structures in the brain are prone to the infection with SARS-CoV-2, culminating in a rare encephalopathy called Acute Necrotizing Encephalopathy (ANE), leading to brain dysfunction with seizures and mental disorientation. The infection may also damage the medulla oblongata, which regulates vital functions like respiration and cardiovascular activity.

The Tobacco smoking: Tobacco smoking, using cigarette, cigar, or country-made cigarette, is associated with adverse prognosis in a number of diseases including respiratory diseases. It is also detrimental to the immune system and its responsiveness to infections, making smokers more vulnerable to infectious diseases bacterial as well as viral including influenza, SARS, MERS, and COVID-19 [6]. As the COVID-19 afflicts mainly the lungs, there is a serious threat to those who smoke [7]. The smoking is more prevalent in men than women in various population groups. In China, 52.9% of men and 2.4% of women are reported to smoke [8]. It could be a relevant point to explain the disparity contributing to the COVID-19 related higher morbidity and mortality observed in men compared to women [4]. Further, active smoking has been correlated to the disease severity and resultant fatality [9].

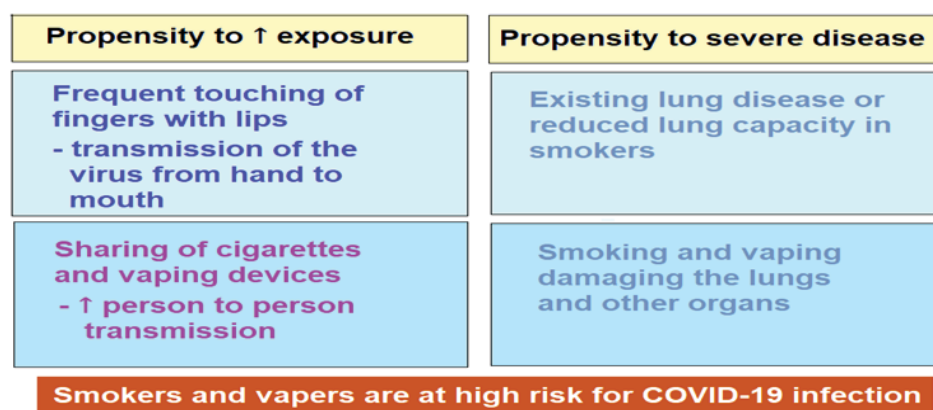


Figure 1: Smoking and vaping associated increased risk for COVID-19 transmission and disease severity.

In general, the smokers are more likely than non-smokers to contract COVID-19 and have more severe clinical course and higher mortality. Zhou, et al. studied the epidemiological characteristics of 191 individuals infected with COVID-19, among which there were 54 deaths, while 137 survived. Among those that died, 9% were current smokers compared to 4% among those that survived [2]. Similarly, in another study involving 140 patients with COVID-19, the results showed that among severely hit patients ($n = 58$), 3.4% were current smokers and 6.9% were former smokers, in contrast to non-severe patients ($n = 82$) among which 0% were current smokers and 3.7% were former smokers [10]. In another small study of COVID-19 among 41 patients, none of those admitted to an ICU ($n = 13$) was a current smoker, in contrast to three patients from the non-ICU group were current smokers [11]. In another study involving 78 patients with COVID-19, the adverse outcome group had a significantly higher proportion of patients with a history of smoking (27.3%) than the group that showed improvement or stabilization (3.0%). In the multivariate logistic regression analysis, the history of smoking was a risk factor of disease progression [12]. In a larger involving study involving 1099 patients with COVID-19 from multiple regions of mainland China, 173 patients had severe symptoms, out of which 16.9% were current smokers and 5.2% were former smokers, in contrast to remaining 926 patients with non-severe symptoms where 11.8% were current smokers and 1.3% were former smokers. Further, in the patients that either needed mechanical ventilation, admission to an ICU or died, 25.5% were current and 7.6% were former smokers, as compared to those without adverse outcomes, 11.8% being current and 1.6% former smokers [1].

Substance Use Disorders (SUDs): The persons with opioids and methamphetamine use may be vulnerable to severe form of COVID-19 because of the drugs affecting the respiratory physiology and pulmonary health. The opioids acting on the brainstem slow breathing and may also cause hypoxemia, which can cause damage to the brain if persists for longer periods and occurs frequently. In setting of chronic respiratory disease, there is increased opioid overdose mortality risk and the diminished lung capacity from COVID-19 infection can similarly endanger the life. The methamphetamine use constricts the blood vessels to contribute to pulmonary vascular damage and pulmonary hypertension [13]. The methamphetamine abuse can lead to adverse outcome in those with COVID-19 infection.

Additionally, individuals with SUDs are likely to experience decreased access to health care, general and social neglect, homelessness, and greater likelihood of incarceration exposing them to close contact with others with higher risk for COVID-19 [14]. The compromised health, in general, due to smoking or vaping with opioid, methamphetamine, cannabis, and other substance use disorders also lead to an increased risk of COVID-19 and its complications. The forced quarantine and other public health measures may disrupt their access to syringe services, medications, and other support needed by people with opioid abuse. Further, there is risk of discrimination against them with a rise in COVID-19 cases and added burden on the healthcare system.

The E-cigarettes and Vaping: The e-cigarettes heat nicotine (extracted from tobacco), flavourings and other chemicals to create an aerosol to inhale. The electronic cigarettes (e-cigarettes, vape pens, and other vaping devices) are claimed to be a way to ease the transition from traditional cigarettes to not smoking at all. Though vaping may be less harmful than smoking, it has also been linked to lung disease and other disorders. There is emerging evidence that exposure to aerosols from e-cigarettes harms the alveolar cells and diminishes their ability to respond to an infection, both viral as well as bacterial. In a NIH-supported study, for instance, influenza virus-infected mice exposed to the aerosols had enhanced tissue damage [15]. In Jan 2020, the US Center for Disease Control (CDC) confirmed 60 deaths in patients with e-cigarette or vaping product use associated lung injury (EVALI) [16].

In EVALI, patients often report a gradual start of symptoms, including breathing difficulty, shortness of breath, and/or chest pain before hospitalization. There can be mild to moderate gastrointestinal illness including vomiting and diarrhoea, or other symptoms such as fevers or fatigue [17]. The vaping products containing tetrahydrocannabinol (THC) and other cannabinoids, along with diluents and other additives like pesticides, heavy metals, and other toxins, have been associated with the illness. As per the FDA Preliminary Lab Analysis 73% of cases were linked to products containing THC. Of these, 81% of products were having vitamin E acetate, 32% aliphatic esters and 9% polyethylene glycol as diluents. In addition, vitamin E acetate is also used as thickening agent in THC vaping products.

Among youth, e-cigarettes are more popular than any traditional tobacco product. In the US, the e-cigarette use among high school students and teenagers has increased during recent years [18]. There are reasons for popularity of e-cigarettes. First, many teenagers believe that vaping is less harmful than smoking. Second, e-cigarettes have a lower per-use cost than traditional cigarettes. Third, vape cartridges are often formulated with flavourings such as apple pie

and watermelon that appeal to young users. And, finally, both youths and adults find the lack of smoke appealing. In addition, with no smell, e-cigarettes reduce the stigma of smoking. The recommendations are that e-cigarette, or vaping, products should never be used by youths, young adults, or women who are pregnant. Adults who do not currently use tobacco products should not start using e-cigarette, or vaping, products. The best way to avoid potentially harmful effects is to not use THC-containing e-cigarette, or vaping, products [19].

Immunity and Immunodeficiency States

Immune Response Continuum and COVID-19 Antibodies: The immune system and immune response against an infection function on a continuum. Thus, there can be null to mild to moderate to exaggerated immune response. With some pathogens, such as varicella-zoster virus, the infection confers long-lasting resistance [20]. Whereas, the individuals infected with HIV often have large amounts of antibodies that do not prevent or clear the virus [21]. Presently, at the early stage of understanding the novel coronavirus, nCoV-2019 or SARS-CoV-2, the place of COVID-19 infection and disease on the immunity spectrum is not known [22]. Although those exposed to the agent and developing from asymptomatic to severe disease, seem to produce antibodies and the recovered COVID-19 patients have antibodies for at least two weeks or more (there is lack of long-term data). Further, it is not known how long the SARS-CoV-2 antibodies persist or whether they protect against reinfection or not.

The concept that presence of antibodies to the SARS-CoV-2 virus could provide protection from infection, forms the basis of herd immunity as a potential end point to the COVID-19 pandemic [23]. The concept of herd immunity is important for governments and policy planners also, as the immunity status in a population group is the key to getting with social and economic activities as well as to provide a clearer picture of the COVID-19 pandemic [24]. In the real world, unlike the diagnostic tests to confirm the presence the virus, the antibody tests help to determine the virus spread in a particular population group [25].

Another issue is the period to which immunity against SARS-CoV-2 may last. The best estimate for the period to which the immunity to COVID-19 may persist, comes from the closely related coronaviruses (CoVs). The immunity to seasonal CoVs causing common colds, starts to decline in few weeks after infection and there is vulnerability to reinfection within a year. But, relating to the SARS-CoV, causing SARS, which shares a considerable amount of its genetic homology with SARS-CoV-2, the antibody tests show that the SARS-CoV immunity peaks at ~4 months and offers protection for about 2-3 years. Further, in those with the antibody response, immunity might wane, but is detectable beyond 1 year after hospitalization [26]. This possibility that the duration of immunity may be 1 year or more, means the possibility of another wave of COVID-19 cases in 3 or 4 years. The specific T-lymphocyte immunity against Middle East respiratory syndrome CoV, however, are detectable for up to 4 years. considerably longer than antibody responses [27].

The uncertainty about COVID-19 protective immunity could be addressed by monitoring the frequency of reinfection with SARS-CoV-2. There are reports of reinfection from China and South Korea that some patients who seemed to have cleared SARS-CoV-2 infection, nevertheless harboured virus persistently. In this context, it is imperative to collect seroprevalence data. Further, even if the antibodies develop in COVID-19 infection and remain for some period, it is not yet certain that they can prevent re-infection [28]. The neutralizing antibodies against SARS-CoV-2 are needed to prevent re-infection by binding to the virus or its components to stall it from invading the host cells [29]. In contrast, the non-neutralizing antibodies though recognize parts of the pathogen, but do not bind effectively and do not prevent it from invading host cells [30]. This is explained by the fact that the target site in SARS-CoV-2, is most likely the receptor-binding domain, the S protein, which being a glycoprotein, does not act as an efficient stimulus for immune response.

The research on real-life immunity to SARS-CoV-2 being in preliminary stages, uncertainties remain. In fact, one study found no correlation between viral load and antibody presence, whereas other studies appear to suggest that some non-neutralizing CoV antibodies, in fact may trigger a harmful immune response upon reinfection or cross infection with other CoVs. In fact, both hyperimmune globulin and vaccine development face the common hurdle risk of antibody-mediated disease enhancement [31].

COVID-19 Patients with Immune Deficiency and HIV: In the COVID-19 infection, the first response is to destroy the virus and limit its replication. But, the first response mechanism is impaired in those having immune-deficient states including cancer or immune deficiency disease like HIV infection. In addition, apart from those on steroid drugs, certain endocrinopathies such as Cushing's syndrome, are also characterised by deficient immune response.

The COVID-19 infection has the potential to lead to severe disease and adverse outcomes for people living with HIV (PLWH) [32]. Besides the risk of COVID-19 disease itself, the indirect effects of socio-economic handicaps in PLWH including housing and shelter-in issues, rampant unemployment, and deprived health and concurrent diseases may interact synergistically to worsen the prognosis. Limited access and adherence to antiretroviral therapy, apart from neglected healthcare are possible hazards for PLWH with COVID-19 infection [33].

The PLWH belong to socio-economically deprived backgrounds and are exposed increased risk for SARS-CoV-2 infection. Recent studies also suggest that COVID-19 is more common among African American and Latinx populations, the groups with higher prevalence of HIV in the US. Additionally, comorbid diseases, like diabetes and cardiovascular disease, that are common among PLWH, may lead to worse COVID-19 outcomes. The COVID-19 pandemic has the potential to greatly disrupt HIV care continuum among PLWH [34]. In addition, the unintended prioritizing patients may affect the HIV care continuum for PLWH [35].

Aging and Comorbidity linked Frailty

The Frailty in Elderly and Associated Factors: Frailty is defined as a state of increased vulnerability to physical or psychological stressors because of a decreased physiological reserve and redundancy in multiple organ systems limiting the capacity to maintain homeostasis [36]. It occurs when multiple physiological systems decline. The presence of frailty in elderly is associated with poor clinical outcomes, including increased hospitalisation and reduced survival [37]. The frailty has a biological component, and results from cumulative cellular damage with aging. There is evidence that both malnutrition and sarcopenia may have similar causal pathways. The chronic inflammatory state is a causal factor for frailty, promoting protein degradation directly or indirectly by altering metabolic processes.

The figure 2 outlines a simplified clinical frailty measurement technique based on general parameters like the level of day-to-day activities and dependency for personal care in a person [38]. The clinical assessment of frailty in hospitalised older adults is of prognostic value [39].

The COVID-19 infection in setting of different states of frailty in older adults, is related to adverse outcomes [40]. As such, the frailty is a known complication affecting more than 20% of those with type 2 diabetes (T2D). It is highly likely that advanced age, the presence of frailty and diabetes will individually and collectively impose additional risks to COVID-19 related complications [41]. The diabetes in elderly and associated frailty has been dubbed as triple jeopardy for COVID-19 related vulnerability [42]. Factors such as age-related impairment in immune function, low-grade chronic inflammatory states, and the increased health hazard due to co-existing comorbidities such as cardiovascular disease, hypertension and diabetes may increase the risk of adverse clinical outcomes [43].

The Impact of the Comorbid Conditions: Diabetes is a high-risk factor for severe disease, hospitalisation, and increased CFR for the COVID-19 infection. It leads to an impaired immune-response to infection both in relation to cytokine profile and to changes in immune-responses including T-cell and macrophage activation. The poor glycaemic control impairs several aspects of the immune response to viral infection and poses risk for potential bacterial secondary infection in the lungs. It is likely that many of the patients with diabetes in China were in poor metabolic control when infected by COVID-19. In a series, diabetes was a comorbidity in 22% of 32 non-survivors in a study of 52 intensive care patients [44]. In a large study comprising of 1099 patients with confirmed COVID-19 infection, indicated in 173 with

1. Very fit – Active, energetic	2. Well – Fit, no active disease	3. Managing well – No physical problems
4. Vulnerable – Independent, but ↓ ADL	5. Mildly frail – Compromised ADL	6. Moderately frail – Need help with ADL
7. Severely frail – Dependent for personal care	8. Very severely frail – Completely dependent for personal care, ↓ recovery from mild illness	9. Terminally ill – Approaching end of life, Life expectancy may be < 6 months

Figure 2: The simplified clinical assessment of frailty in older adults based on activity level (ADL = Activities of daily living) and the help required for personal care (observed dependency).

severe disease there existed hypertension in 23•7%, diabetes mellitus in 16•2%, coronary heart diseases in 5•8%, and cerebrovascular disease in 2•3% [1]. In another study of 140 patients who were admitted to a hospital with COVID-19, 30% had hypertension and 12% had diabetes [45].

The increased morbidity and mortality are particularly seen in older adults and those presenting with co-morbidities such as overt diabetes, obesity, and hypertension [46]. In fact, the number of comorbidities is a predictor of mortality in COVID-19. Many patients with type 2 diabetes are obese and obesity, especially metabolic active abdominal obesity, is also a risk factor for severe COVID-19 infection. The abnormal secretion of adipokines and cytokines like TNF- α and interferon characterising a chronic low-grade inflammation in abdominal obesity may induce an impaired immune-response. In addition, patients with severe abdominal obesity also have mechanical respiratory problems, with reduced ventilation of the basal lung regions increasing the risk of pneumonia as well as reduced oxygen saturation of blood [47]. Further, late diabetic complications such as diabetic kidney disease and ischaemic heart disease may complicate the health for people with diabetes, making them frailer and increasing the severity of COVID-19. In addition, COVID-19 infection can cause acute cardiac injury and heart failure, leading to circulatory instability [48].

A recent meta-analysis from China involving 46,248 patients showed that the most prevalent co-morbidity in people infected by COVID-19 was hypertension followed by diabetes, cardiovascular disorders, and chronic respiratory disease. The latter being surprisingly less than the components of metabolic syndrome. Patients with severe disease were 2.36 times more likely to have hypertension, 2.46 times likely to have respiratory disease and 3.42 times likely to have underlying cardiovascular disease, as compared to those with mild disease not needing hospitalization [43]. In a cohort of 131 patients with COVID-19 infection admitted to a hospital in Wuhan, hypertension was the most common associated comorbidity, 30%; followed by diabetes, 19% and coronary artery disease, 8% [49]. The patients complicated with myocardial injury as evidenced by raised cardiac enzymes, and N-terminal pro-B-type natriuretic peptide, had mortality rate as high as 48.1% [50].

COVID-19 Pneumonia and other Infections

In the COVID-19 patients, there occurs damage to epithelial lining of the respiratory tract, causing inflammation, which in turn stimulates the nerves in the airways to cause cough. On further progression, the alveolar tissue damage responds by outpouring out fluid and inflammatory cells leading to pneumonia and compromising the gaseous exchange. The COVID-19 associated pneumonia shows up as distinctive hazy patches on the outer edges of the lungs, on CT imaging. There is no established treatment for COVID-19 pneumonia. These patients are at risk of developing secondary infections and should be treated with anti-viral medication and antibiotics, apart from supportive treatment [51].

Most patients with COVID-19 recover from the infection, but in a significant fraction the disease progression leads to a fatal outcome. In this context, a co-infection, or the activation of a latent bacterial infection along with pre-existing health conditions in COVID-19 disease may worsen the prognosis. *Mycoplasma*, especially *M. pneumoniae* has been identified co-infecting and may be responsible for the severity of signs and symptoms in certain COVID-19 patients with progressive disease. Moreover, the presence of pathogenic *Mycoplasma* species or other pathogenic bacteria in COVID-19 pneumonia may show propensity to autoimmune activation, hemodynamic dysfunction, cytokine storm, and other complications [52].

Mycoplasma are smallest lifeforms (150-250 nm) and free-living organisms which lack a cell wall and have a unique deformable cell membrane composed of sterols. Unlike viruses, they are able to grow in cell-free media and contain both RNA and DNA. *Mycoplasma pneumoniae* are atypical bacteria that commonly causes mild infections of the respiratory system, the *Mycoplasma pneumoniae* pneumonia (MPP) is often called 'walking pneumonia' but have propensity to cause a severe disease [53]. The overall mortality of MP infection is low, but significantly higher mortality has been reported among the elderly requiring respiratory support and ICU admission [54]. The *mycoplasma pneumoniae* infection is often found as co-infection with influenza and has been reported in patients with SARS virus infections, and recently, in COVID-19 [55]. In a study with 138 COVID-19 patients, 26.5% of COVID-19 patients were found to have co-infection with *Mycoplasma* [56]. Thus, *Mycoplasma* should be considered as a possible co-infection in a case of severe COVID-19 pneumonia.

The pathogenic mycoplasmas, such as *M. pneumoniae*, are known to cause immunological manifestations like inflammatory reactions and immune suppression. The mycoplasmal lipoproteins can induce inflammatory responses through Toll-like (TLR) and other receptors, and release of pro-inflammatory cytokines. In addition, *Mycoplasmas* can

stimulate the generation of reactive oxygen species (ROS) that damage host alveolar cell membranes, mitochondria, and other structures. The SARS-CoV-2 virus affects the host innate immune response systems that utilize pattern recognition and TLR receptors involved in initial responses. Later, when the adaptive immune responses are initiated, involving T cell lineages and B cell production of antibodies, SARS-CoV-2 may initiate immune suppression by inducing apoptosis of T cells. The host cells, in turn, turn on production of cytokines, chemokines, and interferon-stimulated gene (ISG) responses to counter the infection. In setting of COVID-19 pneumonia, the MPP is associated with severe disease and high fatality [57].

Conclusion: The new Revelations

The Update about Herd Immunity

A study led by the Pasteur Institute has reported that about 2.8 million people, 4.4% of the French population, have been infected by the SARS-CoV-2, which appears to be too low to achieve the herd immunity. The herd immunity refers to a situation when enough people in a population harbour immunity against the infection, here COVID-19, to be able to effectively stop the disease from spreading (Figure 3).

Assuming a basic reproductive number of $R_0 = 3.0$, it would require around 65% of the population to be immune for the epidemic to be controlled by immunity alone. Thus, the study has found the French population immunity, insufficient to avoid a second wave of outbreaks if the control measures are lifted at the end of the lockdown [58]. But the study also shows the massive impact of the lockdown in curtailing the transmission. A Spanish study published on 12 May 2020, also documented similar findings that about 5% of the country's population had contracted the disease and that there was no herd immunity in Spain. Depending on these findings, it has been suggested that, without a vaccine, herd immunity on its own will be insufficient to avoid a second wave of the infection if the efficient control measures are not maintained [59].

Surveillance and Control for Outbreaks: On the exposure to COVID-19 infection, many people will either develop no symptoms or mild symptoms not detected through healthcare-based surveillance. Whereas, the concentration of hospitalized cases in older adults has led to hypothesis that there may be a widespread silent transmission in younger individuals [60]. In the hospitalized patients, two patterns have been identified - those dying soon after hospital admission (15% of fatal cases, mean time to death of 0.67 days) and others who die after a longer period (85% of fatal cases, mean time to death of 13.2 days). The proportion of fatal cases in the first pattern, remains approximately constant across age-groups, largely depending on the underlying comorbidities [43].

The virus being novel and highly infectious, can spread exponentially, in the absence of adequate public health measures depending on demography and population density. Letting the virus spread naturally would be disastrous, its infection fatality rate being 0.5–1% as the available data. In addition, the uncontrolled second outbreaks can be deadlier. Further, the large sero-surveys indicate that the outbreaks may be unavoidable as, on average, approximately 95% of the population remains susceptible to the virus. The best feasible option at present, is to effectively suppress recurrent outbreaks. To detect the outbreaks and suppress them, the governments ought to implement wide range of public health measures aided by technology [61].

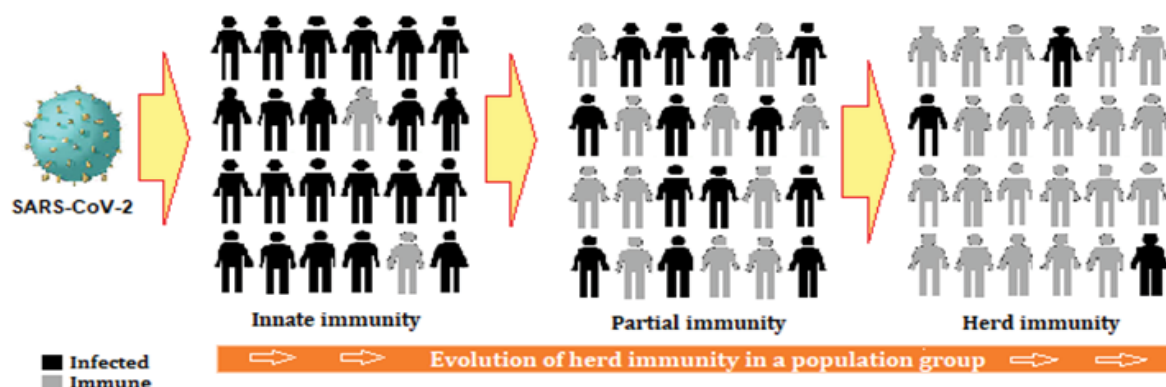


Figure 3: The possible evolution of herd immunity for COVID-19 in a population group.

Help from Technology and COVID-19 Apps: The technology and gadgets through Apps, along with public participation, can help in proximity tracing and exposure notification for COVID-19. The COVID-19 apps are mobile software applications that use digital tracking to aid to identify persons having been in contact with an infected individual [62]. These apps are built on Application Programming Interface (API) platforms, which can be linked to other computer programs, mobile applications, and web services to make use of the data and services available. They usually employ Bluetooth beaconing and the user's daily random exposure key, which alert the frequently changing broadcasted proximity identifiers. The data should be decrypted only when a user tests positive and has given consent, to preserve the privacy of contacts at home and stored only for as long as necessary, typically for 14 days. The apps should run in the background. Moreover, beyond testing and proximity-tracing, the apps should allow people to self-report symptoms to aid public health systems to find blind spots in the disease transmission.

The Aarogya Setu is an updated version of an earlier app, Corona Kavach, newly designed, developed and hosted by National Informatics Centre and launched during April 2020 by the Government of India [63]. The app is a tracking app which uses the smartphone's GPS and Bluetooth features to track the COVID-19 infection and available for Android and iOS mobile operating systems. It has four sections: Your Status to tell the risk of getting COVID-19 for the user, Self-assess to let the user know the risk of being infected, COVID-19 Updates on local and national COVID-19 cases, and the E-pass.

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Chapter 7: Fear, Reaction and Rational Behaviour to COVID-19 in Public, Health Professionals and Policy Planners

Background

The COVID-19 Iceberg: Being highly infectious disease, COVID-19 can spread exponentially but most of those infected remain asymptomatic or develop mild symptoms, thus may remain undetected. In fact, the majority of COVID-19 iceberg is made up of the dormant undetected cases forming the submerged part of the iceberg. The undetected cases of COVID-19 infection can be quantified through serological tests for presence of antibodies against the SARS-CoV-2 in a given population which form the basis of sero-surveys to provide crucial data.

Age Groups and Perceived COVID-19 Threat: The millennials or Gen Y (women more than men) appear to be more fearful compared to other age-groups for contracting the infection. Whereas, the older generations (Gen X and older, Gen Alpha), are the most vulnerable but less afraid of the virus and the disease than millennials. Across the Generations, those belonging to the Gen Z are found to have least fear and low susceptibility.

Fear and other Reactions to COVID-19: Though case-counts and fatalities are been relatively higher in Europe and the US, as compared to most Asian countries, fear to the viral infection by residents of Asian countries including India was on higher side. Most of the respondents to global survey have been found to be fearful of contracting the infection. Apart from this, fears about the availability of essentials and access to various amenities afflicts the susceptible populations. The key concerns by the healthcare professionals involve prevention from exposure to the infection, the adequate protection, and their personal and social support.

Conclusion: Protecting Communities: The aphorism, prevention is better than the cure, fits the COVID-19, as presently there are no specific treatment modalities available. The fear may be the key to prevent the disease by using barriers like face masks and to practice social distancing. The lockdown measures enforced by various governments in countries around world work on basis of curtailing infection by distancing, isolation, quarantine measures. With the swift of growth of critically ill COVID-19 patients, it requires utilizing all clinicians within a medical center to significantly improve the critical care capacity. As the elective surgery and alike activities are cancelled or postponed, the hospitals become focused facilities for COVID-19 with all the medical and paramedical staff working for the cause.

COVID-19 Iceberg: Identifying Dormant Cases

The COVID-19 is highly infectious disease, but most of those infected are asymptomatic or develop mild symptoms, and thus remain undetected clinically. In fact, the total infected cases represent an iceberg with the apparent cases being those who are confirmed positive cases and the dormant undetected cases forming the major submerged part of the iceberg (Figure 1).

These undetected cases of COVID-19 infection can only be quantified through universal test for presence of antibodies against the SARS-CoV-2 in a given population [1].

The serological tests to detect presence of antibodies in the blood can only establish a prior infection. The antibodies tests for the presence of SARS-CoV-2 antibodies indicating a previous infection are invasive tests through blood samples as compared to the non-invasive molecular tests, the swab tests, to determine the presence of the virus in a person's airways to identify active infection. The swab tests do not identify if a person has been infected earlier with SARS-CoV-2 and recovered.

The serological tests analyse blood samples for two types of antibodies, anti-SARS-CoV-2 S protein IgG and IgM, using an ELISA (enzyme-linked immunosorbent assay) technique [2]. These tests are called sero-surveys and involve collection and analysis of mass blood samples to provide crucial data including the herd immunity for epidemiological models and help to outline the extent to which the nCoV-19 has spread undetected in the communities and populations. The data collected can also help to measure the impact of the enforcement measures like lockdown and public health efforts and form the basis and guide for planning the future prophylactic as well as treatment-oriented steps. A recent study led by the Pasteur Institute has reported that about 2.8 million people, 4.4% of the French population, have been infected by the SARS-CoV-2 [3]. Another project, a Spanish study published on 12 May 2020, also documented similar findings, stating that about 5% of the country's population has contracted the disease [4].

Fear and other Reactions to COVID-19

The spread of COVID-19 pandemic is a major public health concern threatening people's general and mental health and safety of life all over the globe. There are a number of studies for assessment and validation of the anxiety symptoms related to COVID-19 [5,6]. It has been documented that though the number of cases and fatalities have been relatively higher in the Europe and the US compared to most Asian countries, the fear of the viral infection appears to be higher in residents of Asian countries including India, except Singapore, than various European nations and the US. In India, 62% of respondents to the survey stated that they were fearful of contracting the disease. The analysis is based on a large-scale global survey conducted by market research firm YouGov in association with the Institute of Global Health Innovation (IGHI) at Imperial College London [7]. The survey was initially started with eight Asia-Pacific countries in early in February 2020 and was extended to cover 26 countries by mid-March. The survey was run for over five weeks or more, planned to gather information about COVID-19, and aimed to offer an understanding about the people's reactions to the pandemic spread of the virus and the measures taken by governments to contain it. The agency, YouGov has recently shared the data about views and behaviours of people around the world [8].

Another study involving general Indian population, found that the anxiety levels were high, in general. More than 80 % felt preoccupied with the thoughts of COVID-19 and 72% found the need to use masks and sanitizers, whereas 12.5% had sleep difficulties, 37.8% suffered from fear about acquiring the infection and 36.4% felt distress related social media [9] A small study involved 263 participants, 106 males and 157 females with the mean age of 37.7 ± 14.0 years, and 74.9% a high level of education. The mean IES (Impact of Event Scale) score to measure current subjective distress in the participants was 13.6 ± 7.7 and it was concluded that the COVID-19 was associated with mild stressful impact in the sample, with the pandemic still ongoing [10].

Reaction to COVID-19 among Healthcare Professionals

Assessment of the change in behavior and attitudes focusing on the healthcare providers' concern about getting infected and related concerns in light of the COVID-19 pandemic was assessed using a structured questionnaire sent through an electronic medium like email or a chat-app like WeChat, WhatsApp, etc. It was found that the fear of getting COVID may outweigh other concerns as the hospitals were often regarded as COVID-19 infection reservoirs by general public as well as healthcare professionals. There was a large concern about the aerosolized spread of the virus through being exposed to coughing respiratory patients and aerosol-generating procedures, associated with the apprehension that they may not be able to maintain a safe social distance appropriately. Almost everyone expressed concern about screening and testing and measures taken once a patient screened positive to keep the remaining patients and themselves safe.

A study was done to examine the abnormality in mental health and explore their resilience and social support issues in Chinese health care workers dealing with the COVID-19 pandemic. Out of a total of 1521 health care workers, 147 had a prior emergency care experience while 1374 did not have. The results from the Symptom Check-List-90 (SCL-90), Chinese version of Connor-Davidson resilience scale (CD-RISC) and Social Support Rating Scale (SSRS) showed that those without emergency care experience had inferior performance in mental health, resilience, and social support than others [11].

In addition, the anxiety and stress may also be caused by organizational factors and fear about resource shortages

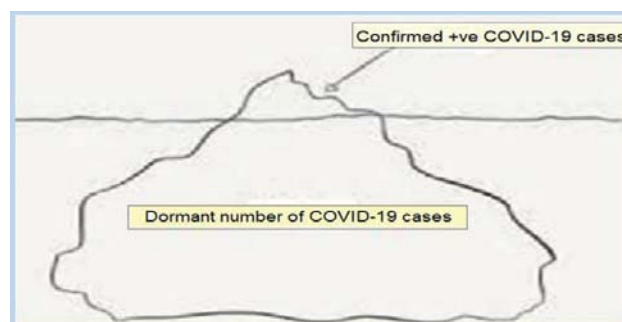


Figure 1: The Iceberg phenomenon for COVID-19 infection.

such as personal protective equipment (PPE) and protective masks are common, and concerns about not being able to provide competent care if deployed to a new area, about rapidly changing information and lack of communication, lack of specific drugs, the shortage of ventilators and intensive care unit beds necessary to take care for the surge of critically ill patients, and significant change in their own daily social and family life [12]. Some fraction of fear and anxiety is common but a severe degree of the symptoms were found in 2.2 % to 14.5 % of all participants and the severity of symptoms was influenced by age and gender, and their role in healthcare and specialization and proximity to COVID-19 patients [13].

The COVID-19 Situation and Lockdown Measures

An analysis from a recent survey indicates that more than half of urban Indians think that the COVID-19 situation in the country is getting worse. Whereas, the remaining half believes that the situation will resolve in India in the next three months or a little longer. About 32% respondents think that it may take significantly longer, to wear off by the end of August to end of October 2020. A lower number of respondents anticipated to improve it both locally and globally, respectively 7% and 10%, till the end of the year [14].

In most countries, fear levels were stated to rise in response to the mounting case count and governmental efforts to contain the pandemic through lockdown measures, over the last few weeks. As schools and offices are shut down and the government has imposed mobility restrictions to complete lockdown, the fears in public appears to have accentuated. The increase in fear levels was relatively high in countries such as France, Australia, and the UK, where the proportion reporting high levels of fear nearly doubled after lockdown measures were introduced. The greatest shift in fear levels was in Sweden, where the number of people fearful of contracting COVID-19 infection rose from 7% to 46% during last few weeks.

The lockdown measures may have been authoritarian in some countries like China, whereas most of the countries across the world have promulgated softer versions reacting to public opinion and with idea of limiting the damage to economic activities. But, in general, the lockdown measures have generated fears about the availability of essential goods and access to various amenities. Following the lockdown, the people across the world, became worried about the availability of essentials items like food, medicines, and other supplies. Many, about 37%, feared about falling sick, whereas about 25% feared their likely handicapped access to health services. A significant number, about 20% expressed fear about losing their jobs and other vocation related issues. The survey also found that while approving of the government's lockdown strategy, a significant number of citizens among various nations including Indians were fearful of the impact of lockdown on availability and quality of the essential services [8].

Age Groups and the Perceived Threat

Generations or the age-groups are often considered by their periodic span along the timeline, though there is no agreed upon formula to define the length of the span. In general, those born between 1981 to 2000 can be called Millennials (witnessed the dawn of the Millennium) or Generation Y. The Generation X is born between 1961 to 1980, whereas the Baby Boomers, Generation O, are those born between 1941 to 1960. Those born between 1921 to 1940 form the Silent or Generation Alpha. Whereas, Generation Z (or Gen Z for short, or Zoomers) is the demographic cohort succeeding Millennials or Gen Y. Most members of Generation Z are born in the mid-to-late 1990s and the early 2010s, and most of the Gen Z have used digital technology since relatively young age and are comfortable with the Internet and social media, and are the successors of Gen Y.

Through the collected data, in the survey by YouGov and the Institute of Global Health Innovation, it has been inferred that among the populations groups within India and elsewhere, it is millennials or Gen Y who appear to be most fearful to COVID-19 infection compared to other age-groups. Among the millennials in India, women were more scared of the infection than men [14]. Across Generations, those belonging to the Gen Z generation (post-millennial adults) were less scared than millennials. Even the older generations (Gen X and older, Gen Alpha), who are more vulnerable to COVID-19 than millennials, were less afraid of the virus and the disease than millennials (Figure 2).

The Experience and Lessons in Covid Care

The Experience and Lessons from China

As we see in retrospective, the measures applied by the government and health care dispensed out have turned out to effectively and efficiently controlled the COVID-19 pandemic in China. At the community level in the affected areas, everybody was kept under quarantine, and strict social distancing and screening were enforced [15].

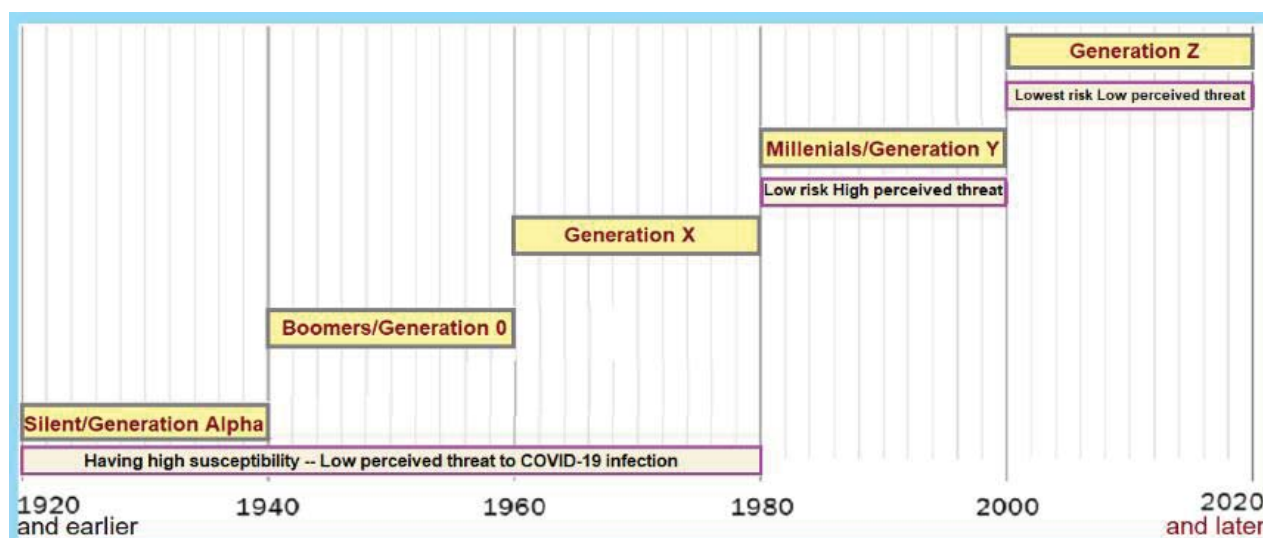


Figure 2: Age-groups and Generations Alpha, O, X, Y and Z and Risk and Perceived threat to COVID-19.

Similarly, stringent measures and triaging was applied for the hospitals and the care for non-COVID and COVID including the suspected patients was strictly bifurcated. The physicians who were not designated to treat COVID patients, did not have contact with such patients. There were designated and well-announced medical facilities in the cities for COVID patients including the suspected ones, and the non-COVID medical units worked routinely and intensively screened their patients epidemiologically and symptomatically before being entered to the facility. In case, suspected, they were immediately sent to COVID-designated facilities.

Clinically, the doctors working in COVID units were well informed, and the communication from the government down to the physicians and to potential patients and to society was effective. There were clear clinical guidelines, which were updated constantly. There was a massive response from the physicians. The COVID facilities relied much on radiological findings and the CT scan was an early investigation even before a serological test was being done. Every person was assigned an electronic QR code that was to be updated every week.

During the initial phase of the COVID-19 outbreak in China, more than half of the 1210 respondents from 194 cities in China from general population rated the psychological impact as moderate-to-severe, and about one-third reported moderate-to-severe anxiety, with 53.8% of respondents rating the psychological impact of the outbreak as moderate or severe, 16.5% had moderate to severe depressive symptoms, 28.8% reported moderate to severe anxiety symptoms, and 8.1% suffered with moderate to severe stress levels [16].

In a limited survey of Chinese physicians involving 450 responders, the issue of fear of infection and reaction to the pandemic was noted. About 20% of the responders stated that they needed psychological support during that time. While about 50% of all the responders stated that they were afraid that they could be infected by their patients, they still continued working. On inquiring about considering leaving the medical profession or switching into being a medical professional in non-clinical settings, about 11% to 15% of physicians stated that they had those thoughts but did not make any steps toward it. On asking about the use of digital medicine and telemedicine, 45%, said that they see telemedicine as a good alternative in the future.

In general, the care of physicians and medical professionals during this outbreak has been high and strong. There have been measures to assure their health and protection. The physicians were well prepared and briefed about the disease. The panic and the mass hysteria that is being observed all around the world was not present in China and this has helped health care professionals to proceed systematically and consistently to succeed in terms of focusing on patient care. The lessons learnt from Chinese handling of the COVID-19 pandemic were importance of maximum of protection to healthcare professionals, more stress on radiological including CT scan than serological testing and recording epidemiological and clinical data to create a big data to be useful in future [17].

The Experience in European Countries

COVID-19 Pandemic in Italy: Italy reported its first cases of COVID-19 on 31 January 2020. The Italian government reacted by declaring the state of emergency, quarantined the cluster areas, and lockdown. Italy being the first country in Europe to be hit by COVID-19, the lockdown of business and various services, anxiety and fear disoriented people [18]. The health professionals in Italy faced the threat of infection and encountered difficulties in protecting themselves. The unprepared Italian health system, having a modest number of ICU beds and few sub-intensive care beds about 8.4 per 1,00,000 population, was handicapped by the surge of COVID-19 patients and as the hospitals were overcrowded, the infection rate in medical personnel increased amounting to nearly 9,000 medical professionals infected as on March 30, leading to further loss of capacity for healthcare system.

Seen in retrospect the number of cases and deaths in Italy cannot be explained simply because of the epidemic starting in the country earlier than other countries in Europe [19]. The specific age structure of the Italian population is an important factor, which the most elderly population in Europe. According to the study by G Onder, et al, for the Italian people infected with COVID-19 and died, the median age of people has been 80 years, whereas the average age was 67 years for patients requiring critical care support. Further, because presence of concomitant diseases such as heart disease and history of chronic smoking, the elderly Italian population was vulnerable to the COVID-19 associated morbidity and mortality. About 99% people who died had at least one comorbidity, and 48.6% had 3 or more diseases that contributed to their death.

As of 30 May 2020, Italy has 43,691 active cases, with 232,664 confirmed cases and 33,340 deaths. Due to the limited number of tests performed, the real number of infected people in Italy is estimated to be much higher. The healthcare workers have been affected by COVID infection, with a high number of the infected healthcare workers being women because of their predominance among nurses. This resulted in death in a considerable number of medical professionals, more than 145 doctors in Italy had died with COVID-19 by 22 April [20]. Healthcare personnel are also subject to high levels of stress and the risk of professional burn-out is considered high, particularly among nurses and doctors alike especially in more affected areas.

COVID-19 Pandemic in Spain: The ongoing COVID-19 pandemic was first reported to have spread to Spain, simultaneously with Italy, on 31 January 2020. By 13 March, COVID-19 cases were confirmed in all 50 provinces of the country [21]. Despite the lockdown imposed on 14 March 2020, by late March, the Community of Madrid has recorded the most cases and deaths in the country. Medical professionals and those who live in retirement homes have experienced especially high infection rates [22]. As of 31 May 2020, there have been 239,429 confirmed cases and 27,127 deaths, but the actual number of cases is considered to be much higher, as many people with only mild or no symptoms have been tested [23].

Recently, on 13 May, the Spanish Government nation-wide seroprevalence study have shown that the percentage of population which could have been infected during the pandemic is approximately 5%, amounting to about 2 million people [24]. The number of persons testing sero-positive is about ten times higher than the number of confirmed cases. Similarly, the number of deaths, recorded as confirmed deaths due to COVID-19, may be an underestimate due to lack of testing and reporting.

COVID-19 Pandemic in France: The COVID-19 pandemic in France was confirmed to have reached on 24 January 2020, earlier than any other European country [25]. Following the arrival of pandemic, France went onto lockdown on 16 March 2020, which was extended twice and ended on 11 May 2020. As of 30 May, France has reported over 151,496 confirmed cases, 28,771 deaths, and 68,268 recoveries. A study led by the Pasteur Institute has reported that about 2.8 million people, 4.4% of the French population, appear to have been infected by the SARS-CoV-2, by 11 May 2020 when the lockdown measures were eased [26]. The study underlines the massive impact of the lockdown had on SARS-CoV-2 transmission in France and suggested that efficient control measures to limit transmission risk will have to be maintained beyond the 11 May 2020 to avoid a second outbreak of COVID-19 epidemic.

The US Experience in New York

The first COVID-19 case was confirmed in New York City on 1 March 2020. On March 3, the first person-to-person transmission in New York was reported. The COVID-19 cases have multiplied since then exponentially [27]. On March 20,

the New York was closed down non-essential businesses, but the public transportation system remained open. By April, loss of jobs soured mainly affecting low income jobs in the retail, transportation, and restaurant sectors. Some of the most affected communities included densely populated neighbourhoods in New York with high immigrant populations. This was accompanied by xenophobia and racism against Asians. By early May, over 5,200 Latinos in the city had died of COVID-19, the largest ethnic group with fatalities from the disease [28].

Innovative ICU Care Models for COVID-19: With the swift growth of critically ill COVID-19 patients, it was required to utilize all clinicians within medical centers in New York to improve the critical care capacity. Simultaneously, the cancellation of elective cases and the consolidation of outpatient practices created a large pool of clinicians available for redeployment. With the innovative models, various hospitals became focused facilities caring for critically ill COVID-19 patients [29].

Utilizing the innovative ICU care model, the NewYork-Presbyterian, in association with Weill Cornell Medicine and Medical School of Columbia University structured a supervised pyramid-staffing model to serve 550 incremental ICU beds. The surge ICU beds were created in medical and surgical units, and in procedural areas such as the cardiac catheterization lab and in the operating rooms. There evolved concept of the emergency department ICU. The multidisciplinary team consisted of anaesthesiologists, pulmonologists, general surgeons, and cardiologists, to assist in critical care and develop the physician staffing complement for a COVID-19 ICU. The ICU frontline role staffed by residents, advanced practice providers, and certified critical care nurse.

This was supported by utilizing e-consultation and an inpatient tele-consult model to allow subspecialists to reach more patients and to decrease staff exposure and PPE utilization. There was developed an e-ICU program which enabled physicians for video enabled ICU rounds and critical care consultation. Simultaneously, a remote patient monitoring program took care of low and moderate-risk patients discharged from the emergency department, sub-intensive units, and indoor wards.

Rational Behaviour for COVID-19 Prophylaxis

As regards the fear to contracting the virus, respondents in Asian countries have reported taking more precautions (such as avoiding public places and wearing face masks) compared to Western peers. On most parameters, Indians ranked close to the median. The survey also noted that in case of personal hygiene (washing hands, using sanitizers, etc.), people from some countries were relatively less cautious about hygiene compared to others from more developed countries. The residents from most of the countries, rated their governments' actions to contain the pandemic favourably. As per the survey, more than 80 percent respondents in Vietnam, India, and Denmark approved their government's response to handling COVID-19.

There are various surveys in offing and underway. The COVID-19 Symptoms & Social Distancing Web Survey by Harvard, aims to gather information on the prevalence of COVID-19 symptoms and social distancing behavior [30]. Another survey, the Knowledge, Attitude and Practice Survey on COVID-19 is a platform to receive feedback from the public on measures to improve efforts in the fight against COVID-19 and encourage behaviour change [31]. There is also an International Survey on CoronavirusCOVID19-survey.org to measure Worldwide COVID-19 Attitudes and Beliefs [32].

Anxiety and Concerns among Healthcare Professions

The healthcare professionals suffer with various concerns, which are different from the public at large [33]. The responses from 8 listening sessions with groups of physicians, nurses, advanced practice clinicians, residents, and fellows have disclosed the sources of anxiety and concerns among healthcare workers. Hear me, protect me, prepare me, support me and care for me was the message from health care professionals dealing with the COVID-19 pandemic delivered to their organizations, according to an article published in JAMA [34].

In general, the healthcare professionals are worried about availability of appropriate personal protective equipment, risk of exposure to COVID-19 at work and taking the infection home to their family, uncertainty that their organization will support for their personal and family needs if they develop infection, and issues about strenuous duties and long working hours and being able to provide competent medical care and access to up-to-date information and communication [35].

On the part of the policy planners, along with maintaining critical supplies, there should be maintenance of an

adequate healthcare workforce to maximizes the ability of each healthcare worker to handle increased number patients and overall workload. The simple and genuine expressions of gratitude for the commitment of health care professionals, should be properly supplemented by concrete support and facilities.

Evolving Care-Practices for COVID-19

To combat the COVID-19 pandemic, the healthcare planners need a clear, systematic approach to quickly evaluate critical needs and identify areas of weakness. In addition, the healthcare setups need to proactively deploy a robust preparedness strategy. By acting early and rationally the healthcare systems may avoid being crippled by a sudden surge of the patients leading to crisis.

The cornerstones of an effective COVID-19 preparedness plan for a health system are mitigating transmission; conserving, supporting, and protecting staff and eliminating nonurgent strains on the healthcare delivery; and good communication [36].

The hospitals and clinics are likely hubs for the spread of COVID-19. The strategies should be taken to minimize unnecessary exposure and transmission of COVID-19 by limiting those entering the health care facility, reducing the staff to essential minimum, and screen and isolate people entering the facility. All nonurgent procedures should be cancelled or rescheduled. The mild and stable COVID-19 patients should be sent home for self-quarantine. The moderate COVID-19 cases should be evaluated for respiratory symptoms, distress, and hypoxia.

Separate spaces required to separate services like screening, diagnostic and other testing, and for healthcare providers. The healthcare providers need to feel optimally protected. Finally, there should be clear communication about disease, clinical state of the patient and risks involved, and the services available.

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Postscript: La Confusion - Caring for COVID-19 patients and the raging, engulfing and debilitating pandemic

These are turbulent times with the novel phenomenon COVID-19, engulfing, imprisoning, and debilitating the humanity all over the Globe. The disease being highly infectious and having a high case fatality rate, various possible modalities are being explored and tried. There are innumerable studies and research are taking place on the agent factor, the pathogenesis, clinical features, and prognostic outcomes in COVID-19 patients. There are taking place animal studies and preclinical studies especially as regards the immunity and immune response profiles to COVID-19 and related to the vaccines being developed. The data from these studies are being developed on daily basis and half-concocted opinions and views are being floated, with the aim to prevent the infection from acquiring and to find a treatment modality to save lives of COVID-19 patients.

The research related to COVID-19 apart, the daily profuse updates bring a lot of confusion to the frontline and other healthcare professionals. Seems like, there is an elephant in the room! The research, fact-finding studies, and concepts and recommendations based on them, if not contradictory at least amount to the loss of focus. La confusion prevails! Meanwhile, the COVID-19 pandemic rages, involving newer communities and countries, defying a plausible solution.

COVID-19 Epidemiology: New Insights

Analysis of contact survey data in Wuhan, where the COVID-19 pandemic originated, has documented that the lockdown measures reduced the number of daily contacts per participant sevenfold at the epicentre of the outbreak. In addition, the study estimated that the closure of schools reduced peak incidence by 40% – 60% [1].

There another update shows that a Covid positive person may not be necessarily infectious. According to experiments in monkey cells, the RNA-positive samples collected more than eight days after a person's symptoms began did not infect the cells, suggesting that people who test positive for viral RNA are not necessarily infectious [2] Thus, the hospital patients testing positive for Covid RNA weeks after their illness might not have to be strictly isolated.

The Disease Impact: New Findings

It appears that nose is the most probable starting point for COVID-19 infection. The virus infects various cells in the respiratory tracts, the cells of nasal cavity being the most infected and the least infected are deeper areas in the lungs. Thus, there is a gradient of infectivity that decreases from the upper to the lower respiratory tracts [3]. As such COVID-19 affects most abundantly the lungs, it also impacts the kidneys, liver, heart, brain, and blood [4].

As per the recently published findings from the autopsies of 12 COVID-19 subjects, 7 had venous thromboembolism and 4 had pulmonary embolism. The post-mortem CT revealed reticular infiltration of the lungs with bilateral, dense consolidation, while 8 subjects showed diffuse alveolar damage. In addition, SARS-CoV-2 RNA was detected in the lungs at high concentrations, with 5 out of 12 patients had high viral RNA titres in the liver, kidney, and heart [5]. The histology in the patients who died of acute respiratory distress syndrome (ARDS) from COVID-19. Show the diffuse alveolar damage with perivascular T-cell infiltration in the lungs. In addition, the lungs show disrupted cell membranes and presence of intracellular virus, and widespread thrombosis with microangiopathy in the pulmonary vasculature [6]. Further, the degree of angiogenesis has been related to the duration of hospital admission [7].

Myocardial injury is a common finding in hospitalized COVID-19 patients hospitalized with COVID-19. The patients with cardiovascular disease are more likely to have myocardial injury than patients without CVD. Troponin concentration may be normal but those with elevated troponin levels have been documented to have a higher risk of mortality [8].

The long-term psychological impact of the Covid-19 pandemic on front line health care workers has yet to be fully understood. However, health systems lack a practical model for providing mental health support to front-line staff engaged with the pandemic [9].

The latest Developments

A study by Max Crispin et al has analysed glycosylation of the SARS-CoV-2 spike protein and concluded that the protein is not densely glycosylated, which is good news for immunization strategies targeting the SARS-CoV-2 spike protein. Vaccine development is focused on the principal target of the humoral immune response, the spike (S) glycoprotein,

which mediates cell entry and membrane fusion [10]. Wang, et al. used a humanized monoclonal antibody, termed 47D11, having neutralizing activity against SARS-CoV-2, thus establishing the existence of alternative mechanism of SARS-CoV-2 neutralization apart from anti-spike antibodies [11].

Another study by Daniel Wrapp, et al. using single-domain antibodies, called nanobodies, cloned from a llama immunized with SARS-CoV-1 spike protein also has neutralizing activity against SARS-CoV-2 [12]. The nanobodies have higher stability and ease of production and can be delivered via nebulization.

Controversial use of HCQS for COVID-19

The endorsement from the Indian Council of Medical Research (ICMR) for preventative use of hydroxychloroquine (HCQS): recently claimed to be based on observational and case control studies in India showing no major side effects of the drug. The ICMR has expanding its advisory for the prophylactic use of HCQS as a preventative measure, has extended its recommendation the frontline workers to prevent Covid infections [13]. Various scientists have criticised the government for issuing advice when it is uncertain that the drug can actually prevent infection.

There are though studies claiming that HCQS did not prevent illness or confirmed infection after high-risk or moderate-risk exposure to COVID-19 within 4 days after exposure [14,15]. Another study found that the evidence for the benefits of using HCQS or chloroquine (CQ) to treat COVID-19 is very weak and conflicting [16]. Very recently, the chief investigators of the RECOVERY Trial, on review of the unblinded data, found no beneficial effect of HCQS in hospitalised COVID-19 patients in relation to mortality or duration of hospital stay [17]. The WHO has now resumed testing HCQS for COVID-19 treatment, despite having suspended the clinical trials over safety concerns earlier.

Recommendations for Antivirals

Favipiravir is an antiviral agent that inhibits the RNA-dependent RNA polymerase of RNA viruses. The drug undergoes an intracellular phosphor-ribosylation to convert into an active form, inhibits the RNA polymerase activity [18]. The favipiravir is considered potentially effective for COVID-19, though confirmed studies related to COVID-19 are not available [19]. Remdesivir is another potential antiviral drug for COVID-19. It is a prodrug which is metabolized intracellularly to an adenosine triphosphate analog, which inhibits viral RNA polymerases. Treatment with intravenous remdesivir showed significant improvement for the first COVID-19 case in US [20]. In a cohort of severe Covid-19 patients, the compassionate use of remdesivir was associated with clinical improvement [21].

Ivermectin is a broad-spectrum antiparasitic agent that has shown antiviral activity against a broad range of viruses in vitro. In an in vitro study, ivermectin was found to be an inhibitor of the SARS-CoV-2, likely through inhibiting $IMP\alpha/\beta$ 1- mediated nuclear import of viral proteins which disrupts the immune evasion mechanism of virus [22]. But further trials are needed to determine its role in the management of COVID-19 [23].

The Genetic and Blood group links

The researchers have shown a link between COVID-19 and blood groups. It appears that people with blood type A+ have an increased risk of lung failure compared with those with other blood types, whereas those with type O blood were protected to some extent [24]. The study has associated it with a variant on chromosome 3, that interacts with the molecular receptor the virus uses to enter human cells. In addition, the researchers have identified two human gene variants that could make people more susceptible to lung failure associated with COVID-19 [25].

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*La Confusión, the lyrics and song by Juhn.

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