

The Human Coronaviruses (HCoVs) and the Molecular Mechanisms of SARS-CoV-2 Infection

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Abstract:

In humans, coronaviruses can cause infections of the respiratory system, with damage of varying severity depending on the virus examined: ranging from mild or moderate upper respiratory tract diseases, such as the common cold, to pneumonia, severe acute respiratory syndrome, kidney failure and even death. Human coronaviruses known to date, common throughout the world, are seven. The most common - and least harmful - ones were discovered in the 1960s and cause a common cold. Others, more dangerous, were identified in the early 2000s and cause more severe respiratory tract infections. Among these the SARS-CoV, isolated in 2003 and responsible for the Severe Acute Respiratory Syndrome (the so-called SARS), which appeared in China in November 2002, the Coronavirus 2012 (2012-nCoV) cause of the Middle Eastern Respiratory Syndrome from Coronavirus (MERS), which exploded in June 2012 in Saudi Arabia, and actually SARS-CoV-2. On December 31, 2019, a new Coronavirus strain was reported in Wuhan, China, identified as a new Coronavirus beta strain β -CoV from Group 2B, with a genetic similarity of approximately 70% to SARS-CoV, the virus responsible. of SARS. In the first half of February, the International Committee on Taxonomy of Viruses (ICTV), in charge of the designation and naming of the viruses (i.e., species, genus, family, etc.), thus definitively named the new coronavirus as SARS-CoV-2. This article highlights the main knowledge we have about the biomolecular and pathophysiologic mechanisms of SARS-CoV-2.

Key words: Coronavirus, SARS-CoV-2, Pandemics, Molecular biology, Immunity, Pathology

Introduction

In recent years coronaviruses have been responsible for the two pandemics that of severe acute respiratory syndrome (SARS Covid-1), which began in 2002, is currently at the end of 2019, following a new strain the SARS-CoV-19. [1] The virus was first detected in the Wuhan region of China and caused a pandemic spread to over 114 countries around the world. Coronaviruses are a group of viruses that often cause mild respiratory infections in humans and animals. Most people become infected with coronavirus at least once in their lifetime, having mild to moderate symptoms of the common cold. [2-4] *Coronaviruses* show a notable propensity to transmit themselves to new hosts and this propensity has emerged on several occasions in the evolutionary history of this viral family; suffice it to recall the human coronavirus HCoV-OC43 which has close genetic correlations with the *bovine coronavirus* (BCoV) and which seems to derive from this or the human coronavirus HCoV-229E which after originating from a bat *Alphacoronavirus* has passed back to an animal host generating the swine epidemic diarrhea virus (PEDV) or the most important human coronavirus, SARSCoV and SARS-CoV-2, which seems to have its reservoir in bats. In fact, the sequences of the Wuhan beta coronavirus show similarities to the *Betacoronaviruses* found in bats. Thus, bats are the natural reservoir of numerous *Alphacoronaviruses* and *Betacoronaviruses* [5-10] (Tab1).

Tab1. The seven pathogenic human coronaviruses identified (HCoVs).

Strain	Genus/subgenus	Infect Host
MERS-CoV (Middle East respiratory syndrome coronavirus infection)	<i>Betacoronavirus/Merbecovirus</i>	Humans, Bats, Camels
SARS-CoV (Severe acute respiratory syndrome coronavirus infection)	<i>Betacoronavirus/Sarbecovirus</i>	Humans, mammals
Human coronavirus 2019 HCoV-19 or SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2)	<i>Betacoronavirus/Sarbecovirus</i>	Humans
HCoV-HKU1 (Human coronavirus HKU1)	<i>Betacoronavirus/Embecovirus</i>	Humans, Mice
HCoV-229E (Human coronavirus 229E)	<i>Alphacoronavirus/Duvinacovirus</i>	Humans, Bats
HCoV-NL63 (Human coronavirus NL63)	<i>Alphacoronavirus/Setracovirus</i>	Humans
HCoV-OC43 (Human coronavirus OC43)	<i>Betacoronavirus/Embecovirus</i>	Humans, Cattle

The viral evolution and coevolution

Evolution is the genomic modifications that a population acquires over time; in the case of viruses, it does not only depend on the characteristics of the viral population but also on the physical-biological characteristics of the cell and the structure of the host population. The main success factor of viruses is, despite the small number of genes possessed, the high genetic variability that provides them with the ability to adapt and survive (Tab2).[11]

Tab.2 Main definition for viruses

Properties of viruses
They are not living beings
To exist in nature must be infectious
They must use host cell mechanisms to produce their own components (viral mRNA, proteins, and identical genome copies)
They must encode their own specific proteins for each process they request and not carried out by the cell
The production of new virions occurs by assembly of viral components

In general, viral evolution can follow two paths: a) viral population coevolves with the host following a single fate; if the host thrives, the virus thrives too. However, since there are no host alternatives for the virus, a factor may arise that prevents replication of the virus in that host (e.g. antiviral) and therefore the viral population dies out (more common for DNA viruses), b) viral population expands into multiple biological niches by infecting different hosts. If a factor arises that prevents the virus from replicating in one host, the virus replicates in another and the population does not become extinct (more common for RNA viruses such as the *Betacoronavirus* and other).[1]

Selection is the process by which a genotype is fixed or not in the population through the evaluation made by the environment of the phenotype it encoded. For evolutionary purposes, the main characteristics of the virus-receptor interaction are: a) a virus can use different receptors and co-receptors; b) The same receptor can be used by different viruses and other microorganisms; c) the type of receptor used by a virus cannot be predicted on the basis of its phylogenetic position or biological properties; d) One or a few mutations of the viral genome can modify the receptor used, the cell tropism and the pathogenicity. [11-13]

By coevolution we mean a process in dynamic equilibrium (which varies over time) of reciprocal adaptation, by means of genetic modification, between two or more species. In this process, the genetic modifications of one species are the result of the selection made by the other species and vice versa. In the co-evolutionary process, a pathogen and its host are closely related and mutually adapt in opposite directions, just think of some characteristics such as the infectivity of the pathogen and the resistance of the host, or the response of the host to infection and immune evasion by the pathogen. In this context, virulence, i.e. the damage manifested by the host in response to the infection given by the pathogen, is an ambiguous manifestation of the state of coevolution between the two, since it manifests a state of poor fitness of the host that is not always accompanied by an efficient completion of the pathogen replication cycle; it often happens that the adaptation of a virus in a host leads to an attenuation of its virulence. [14] The RNA viruses, such as the Coronaviruses, show a much higher evolutionary speed than DNA viruses, because of the high susceptibility to replication error mediated by RNA-polymerase or reverse transcriptase, by the considerable size of the viral population and by the higher replication speed. These rapid evolutionary modifications allow RNA viruses to quickly generate mutations that can allow them to adapt to a new environment, including a new host species. If RNA viruses show an extreme variability, even the most complex DNA viruses still show a certain ability to generate different variants; the latter do not exhibit high mutation rates like RNA viruses since this would affect their functionality, but are subject to variation of specific sites called “hot

spots". Viruses with genetic material composed of a single small DNA strand which, probably, due to a high replication error rate, can reach an evolutionary speed equal to that of RNA viruses are an exception. [12,13,15]

The genetic variation of viruses

Genetic variation is the process, devoid of specific and predetermined purposes, which underlies the differentiation and therefore the evolution of viruses. The set of viral variants that originate because of different molecular mechanisms allow the virus to adapt to the environment it will act by selecting the form, or viral type, with the winning characteristics. Viruses use the same molecular mechanisms of genetic variation as other life forms: mutation, recombination, and gene reassortment.[2]

Mutation is a copy error of the nucleic acid by RNA- or DNA-polymerase, action of enzymes of the host cell; chemical-physical damage of the nucleic acid and can be divided into three main types: a) transitions: replacement of a purine with a purine or of a pyrimidine with a pyrimidine; b) transversions: replacement of a purine with a pyrimidine or vice versa; c) insertions or deletions (indel): addition or subtraction of one or more nucleotides. The mutation, understood as an event that occurs on a population (set of organisms or genes or traits of them), can be quantitatively expressed through two parameters, the mutation rate, and the mutation frequency. The mutation rate quantifies the number of mutations per single copied nucleotide, regardless of the fate of the error produced, so it is a measure of a biochemical event. The mutation rate of viruses influenced by: a) whether the viral DNA polymerase has proofreading activity; b) whether the virus codes for restorative proteins; c) replicative mechanism of the virus; d) intracellular site of virus replication; e) genome length (on average, the mutation rate in the genome of RNA viruses is 10^{-3} - 10^{-5} nt/site, while that of DNA viruses is at least 300 times lower, 10^{-8} - 10^{-9} nt/site). In an RNA virus with a genome of 10,000 bases there will therefore be on average one mutation for each replicated genome, therefore, in millions of progeny, there will be millions of mutations that create a population of mutants, all very similar but still different. In particular, RNA viruses replicate with a level of fidelity close to the error threshold and in many cases, even if these viruses are potentially very variable, the small size of their genome limits the number of modifications and the positions of the genome in which they can occur without causing functional loss.[11,12,16,17] The quasispecies defined as the viral population within a host composed of a distribution of different but closely related mutants, subject to continuous processes of genetic variation, competition, and selection, which act as a unit of selection. The quasispecies theory is suitable for explaining the cloud of viral variants that created in the host infected with viruses characterized by high mutation rates; RNA viruses are therefore the main viruses to follow this dynamic [15], together with some DNA viruses with small genomes [18].

Recombination is a widespread mechanism of genetic variation that occurs for both RNA and DNA viruses and often with the participation of the replicative complex of the virus [12]. To have recombination, the same host cell must be coinfecting by at least two different viral genomes; the persistence of a viral genome in the cell (persistent infection) favors the establishment of a coinfection for the subsequent penetration of another virus and therefore increases the probability of a recombination event. A recombination event is suspected when the phylogenetic analysis of the same viral strain shows discordant positions for the different genes; in these cases, confirmation must be done by statistical evaluation to differentiate a true recombination from possible convergent mutations which arose independently.[12]

The gene reassortment is the exchange of genome fragments between two or more viruses (RNA or DNA) with a segmented genome that infect the same host cell. It is an evolutionary phenomenon that allows for major changes and is greatly exploited by these viruses, just think of the frequent generation of new variants that occurs in this way for the flu virus. These different molecular mechanisms of genetic variation make it possible to distinguish two types of viral evolution which can be differentiated precisely by the molecular mechanisms involved, the times with which they occur and the effects they have on the virus. The “genetic drift”, is the progressive and scaling modification over time, by small successive steps, of the viral genome; it is the genetic diversity resulting from single mutations (due to duplication errors) that occur each time the genome replicates and from the subsequent selection made by the environment. The “genetic shift”, is the massive and single-stage modification of the viral genome as a consequence of recombination or reassortment; it is the genetic diversity resulting from a relatively rare event that occurs only in certain circumstances [11].

The cross-species transmission

When we refer to emerging viruses, we mean the causative agent of a new or never previously encountered viral infection in the population such as SARS-CoV-2. The emergence of the virus leads to an increase in the incidence of the disease caused by it in a new host, or in the traditional host following events that alter the epidemiology; in the latter case, it would be more correct to speak of re-emergence. Viruses undergo a process of random evolution resulting from the biological interactions they establish with the host and, in conjunction with certain events or factors, this evolution can lead to the emergence of a disease. The emergence of new viral diseases can therefore affect the passage of a pathogen from one animal to another, or the passage of a pathogen from an animal to man. The emergence of new zoonoses represents the main threat to public health and are events that have repeated themselves with some frequency in the last decades, suffice it to recall the past SARS virus, the influenza, Nipah, Hendra, Ebola, and today the pandemic SARS-CoV-2 viruses. [19-21]

To have the emergence of a disease, two elements are required: a) the introduction of the pathogen into the population and b) its consequent spread and maintenance in the population itself. The factors that can lead to the emergence of infectious diseases are different and genomic adaptability plays an important role [22]. For this reason, the more a virus can mutate and adapt, the greater its ability to pass on to a new host and establish a productive infection there. The other factors are mostly environmental, ecological and sociological influences that affect the likelihood of potential pathogens to come into contact with new hosts; in fact, many of these factors alter the spread of pathogens and their hosts or vectors. The emergence of a non-pre-adapted virus in a new host follows four phases (Fig.1) [12,20,23]

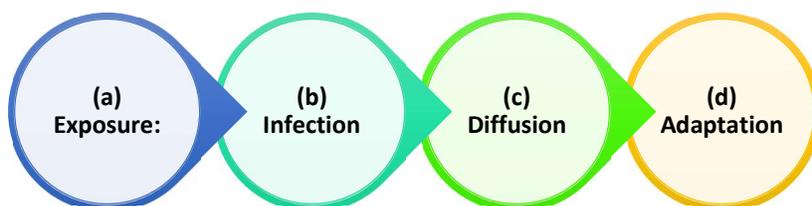


Fig 1 The emergence of a non-pre-adapted virus in a new host follows four consecutive phases: a) **Exposure** (contact between the donor host and the recipient, which can be influenced by geographical, ecological and behavioral factors); b) **Infection** (the passage of the virus from the donor host to the recipient, which can be influenced by the ability of the virus to overcome the species barriers and by the compatibility with the new host: binding to the cell receptor, ability to complete the replication cycle, evasion the immune response, etc.) c) **Diffusion** (transmission of the virus between subjects belonging to the new population, which can be influenced by the ability of the virus to complete its replication cycle in the new host and by the contact between the subjects that make up the new population); d) **Adaptation** (evolution of the virus in the new host so as to remain in equilibrium within the population, which can be influenced by the genetic variability of the virus) [23]

In the case of viruses, emergence with pandemic effects, is more frequently associated with RNA viruses, such as the *Betacoronaviruses*, that manifest more than other viruses, probably thanks to their greater genomic flexibility, mutation, recombination and reassortment phenomena that allow them to overcome species barriers, adapt to hosts different and give rise to new variants will have isolated events and the primary host will always be needed to have the infection of the new one, while, in the second case, the pathogen will stabilize in the new population without needing the primary host and an event will develop epidemic that may become endemic.[23]

In general, the interactions between virus and host can therefore be divided into four types: a) stable, keeps the virus in the environment (the host and viral populations are in equilibrium and are maintained in the environment), behavior characteristic of the virus in the primary host or reservoir; b) evolving, passage of the virus from the traditional population to a new one, belonging to the same host species or to different species, with progressive adaptation of the pathogen; c) dead-end or dead-end, passage of the virus from the traditional host to a new one in which the virus is unable to transmit effectively and therefore does not adapt, it may also be due to the high lethality of the virus which causing death too quickly the new host does not allow the transmission; d) resistant: the infected host completely blocks the replication cycle of the virus (non-receptive host). To infect a new host, a virus must be able to overcome the species barriers and carry out its replication cycle in its cells; this process can be limited at various levels, including receptor binding, cell entry, replication, gene expression and the spread of an infecting progeny. [23,24]

The various factors that influence the ability of the virus to transmit from one host species to another, such as the ability to bind to the cell receptor of the new host but not only, are generally influenced by the phylogenetic constraints existing between the host species in question; in the sense that the more the original host and the new host are phylogenetically related, the higher the probability that host barriers will be successfully overcome (it is easier for a pathogen to be transmitted from one mammal to another mammal than from a plant or insect to a mammal). 40,45

The main factor influencing the contact between donor and recipient host is man, as population dynamics, commercial and non-commercial transport, action on the environment and social behaviors, modify the dynamics and interactions, among others. Some animal species creating potentially dangerous promiscuity situations. Once the virus has acquired the ability to infect the new host, further evolutionary adaptations may be necessary to have the pathogen spread; in fact there are cases of pathogens that replicate and give disease in a new host but are not able to transmit from this to other subjects of the same or different species [12].

The main obstacle to the emergence of a virus, following a jump in species, is therefore represented by the ability to obtain an efficient transmission of the virus itself between different individuals, from the infected to the healthy, belonging to the new host species. A tool for assessing the possibility of emergence of a pathogen is the basic reproductive number (R_0), which refers to the number of new infected generated by an already infected individual in a completely sensitive population [23,25]. This parameter depends on the number of infected-healthy contacts, the probability of transmission and the contagiousness of the pathogen. R_0 is a function of the balance that is created between the pathogen and the host and, therefore, represents a measure of the degree of adaptation that the pathogen shows in giving an effective infection in each host. For each pathogen, in a specific host of infection and at a precise moment, its R_0 can be calculated, which will have high values depending on the adaptation of the virus in it (if R_0 shows values of 1 the disease will spread in the population and the pathogen will stabilize in it, furthermore, if the R_0 value is close to 1, the infection will have an endemic character, while if the value is greater than 1, the infection will have an epidemic character). For SARS-Covid-19, R_0 reveals that for each infection directly generates 2-4 infections. But days of infectivity latency are variable from 3 or 4, and if use $R=4$ the number of cases will quadruple. [23,25,26]

Ultimately, the probability that the adaptation of the virus to the new host takes place successfully and that the pathogen therefore remains in it, depends on four factors [21]: a) the number of primary infections, determined by the amount of virus that is transmitted to the new host, by the number of infected individuals and by the repetition of multiple transmissions between the primary and new host; b) the higher the value of R_0 in the new host at the beginning of the transmission, the greater the probability; c) the number of mutations or genetic changes necessary for the virus to adapt to complete its replicative cycle in the new host; d) the specific characteristics of the pathogen, i.e. the genetic variability of the virus, the probability that the necessary mutations will occur and how much R_0 will change with each mutation [26]

The characteristics of 2019-nCoV structure

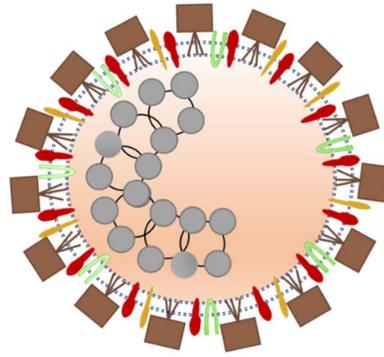
The *Coronavirus* (CoV), order *Nidovirales* and family *Coronaviridae*, are a group of viruses equipped with single-stranded, linear not segmented genome, positive polarity RNA (ssRNA+) and provided with envelope, which have a helical symmetry core and a characteristic crown morphology. Until a few years ago, all the viruses belonging to the family *Coronaviridae* were subdivided in only two genera: *Coronavirus* and *Torovirus*, and all the viruses classified in the genus *Coronavirus* were subdivided in their turn in only three antigenic groups (group I, group II and group III). The recent discovery of a wide variety of new coronaviruses in different host species has prompted the Coronavirus Study Group of the International Committee for Taxonomy of Viruses (ICTV) to propose the reclassification of the *Coronaviridae* family into two subfamilies: *Coronavirinae* and *Torovirinae*. In particular, the subfamily *Coronavirinae* now includes three

genera: *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus*. [11,27] The SARS-Cov-2 has 29 proteins that can be of three types: structural, non-structural and accessory (Tab.3).

Tab 3 The three types of SARS-CoV-2 proteins.

SARS-CoV-2 proteins		
Structural	Non structural	Accessory
Glycoprotein S or Spike (S or ORF2), Nucleocapsid (N or ORF9a), Membrane (M or ORF5), Envelope (E or ORF4)	NSP1, NSP2, NSP 3, NSP 4, NSP 5, NSP 6, NSP 7, NSP 8, NSP 9, NSP 10, NSP 11, NSP 12, NSP 13, NSP 14, NSP 15, NSP 16	ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, ORF10

SARS-Cov-2 shows projections on its surface (about 20 nm long) formed by the glycoprotein S (spike) that join in groups of three that thus make up a trimer that together with the others form the structures that resemble a crown that surrounds the virion. The main differences of this new Coronavirus compared to SARS virus appear to be localized precisely in this spike protein and that glycoprotein S is the one that determines the specificity of the virus for epithelial cells of the respiratory tract, that is to be able to bind the ACE2 receptor (angiotensin converting enzyme 2), expressed by the cells of the capillaries of the lungs and studies have shown that SARS-Cov-2 has a higher affinity for ACE2 than SARS-CoV. Instead the membrane protein M crosses the lining (envelope) interacting within the virion with the RNA-protein and the protein E helps glycoprotein S to attach to the membrane of the target cell. The Dimer-hemagglutinin esterase (HE) is a coating protein, smaller than glycoprotein S, and plays an important role during the release phase of the virus within the host cell. The Envelope it is the coating of the virus, consists of a membrane that the virus "inherits" from the host cell after infecting it. Coronaviruses are Enveloped viruses that carry a genome of positive (+) polarity RNA as their genome. RNA consists of a single strand of positive RNA of large size (27 to 32 kb in different viruses) and are not known larger RNA viruses. RNA originates seven viral proteins and is associated with protein N (nucleoprotein), which increases their stability (Fig.2) [28,29,30]



■ Spike Glycoprotein S ■ Membrane protein M (ORF5) ■ Protein E (ORF4) ■ Hemagglutinin-esterase (HE) □ Envelop ■ Nucleocapsid N (ORF9a) ■ RNA genom

Fig 2 Coronavirus SARS-Cov-2 structure: **Glycoprotein S** (spike) is cleaved into two glycosylated subunits, S1 (binds to the host's receptor, ACE2) and S2 (aid viral and host membrane fusion). **Membrane Protein M** Aid to the assembly and budding of viral particles to ER-Golgi-intermediate compartment and interact with ORF9a for RNA packaging into virion. **Protein membrane E** type III (single pass and forms a homopentameric ion channel and is a viroporin), interacts with ORF5 and ORF9a, which aids in viral assembly, budding and pathogenesis. **Dimero hemagglutinin-esterase (HE)** plays an important role during the release phase of the virus into the host cell. **Genome** consists of a single strand of large size positive polarity RNA + (26 to 32 kb in different viruses). **Nucleocapsid N (ORF9a)** plays its role in genome protection, viral RNA replication, virion assembly and immune evasion (including IFN-I suppression). It binds to viral genomic RNA forming a helical ribonucleocapsid and interacts with M and NSP3 proteins. **Envelope** is the coating of the virus, consisting of a membrane that the virus "inherits" from the host cell after infecting it.

A new variant of the spike protein D614G that increase infectivity and transmissibility has been immersed. Recently it is reported after an analysis of the number of variants in the open reading in frame 1ab of SARS-CoV-2 genomes, by final cleaved protein of who showing deletions in non-structural proteins NSP1, NSP4, NSP6, NSP8, RdRp, NSP14 (Exon N), endoRNase, OMT. One of the most important determinants of the pathogenicity of SARS-CoV-2 is the NSP1 protein and this is quite new, as coronaviruses undergo a moderate rate of mutations, due to NSP4 with proofreading activity. It promotes viral gene expression and immune evasion in part by interfering with interferon-mediated signaling. It probably blocks host translation by interacting with the 40S ribosomal subunit, thus leading to degradation of host mRNA. The data clearly identify the new SARS-CoV-2 viral strain present in individuals from different areas of the world such as Europe, North and South America (Fig.3) [31-33]



Fig 3 The Genomic epidemiology of novel coronavirus (Global subsampling, 43 of 4672 genomes sampled between Feb 2020 and Aug 2020). The non-structural protein mutation new SARS-CoV-2 viral strain Country presence, 22-23/08/2020 (source https://nextstrain.org/ncov/global?f_recency=1-2%20days%20ago&l=unrooted).

Bio-pathogenesis from SARS-Cov-2

Coronaviruses infect numerous animal and humans hosts and are characterized by a remarkable frequency of recombination, which, together with the high rate of mutation, encourage their adaptation to new hosts and ecological niches.[11] In most patients the SARS-CoV-2 virus is attacked by the immune system and patients exhibit a wide range of variability in disease severity ranging from asymptomatic to extremely critical. The transmission occurs mainly through secretions and fecal excretions. The upper and the lower airway tract system in the first involved in SARS-Cov2 infection and other such as gastrointestinal, cardiovascular, kidney, integumentary system, central nervous system (CNS) through synaptic connections, peripheral nervous system (PNS) and psychiatric/mental disorders.[34,35,36] Respiratory infective Flüge drops infect the cells of epithelia/endothelia in the upper respiratory tract and progresses to lower regions of the lung and essentially causing respiratory damage of varying severity, pulmonary macrophages (containing angiotensin-converting enzyme 2), neurons, microglia and other. The life cycle of SARS-Cov-2 when it infects a person follows five steps: attachment, penetration, biosynthesis, maturation, and release. Once the viruses bind to the receptors, it enters the host's cells. [34,37-42] As we mention, protein modeling experiments on virus protein S have suggested that SARS-Cov-2 has affinity with enzyme 2 receptors of human cell angiotensin conversion (ACE2) to use it as an entry "port" into the cell and each SARS-CoV-2 virion is approximately 50-200 nanometers in diameter.[28,37]

The virus escapes the control of the immune system and decreases the number of lymphocytes so that they do not finish the virus and monocytes/macrophages are not regulated and start pumping "cytokine storms"(neutrophil-to-lymphocyte ratio (NLR) is the leading indicator of

hypercytokinaemia). The virus direct pro-inflammatory effects response can cause acute damage to lung tissue, and subsequently the hyper-inflammation lead to the acute respiratory distress syndrome. The development of SARS-CoV-2 infection towards the lower airways depends on the damage caused to the bronchioles which disrupts the protective coatings of the surfactant released by type II pneumocytes. This leads to alveolar alteration and desquamation of the endothelial cells and therefore impaired gas exchange and therefore hypoxemia which will lead to the Acute respiratory distress syndrome (ARDS). But ARDS itself will in turn cause an overactive immune defect (by localizing neutrophils and increasing cytokines), which will lead to an increase in free radicals, cell debris and proteases and edema resulting from the increase in proteins in the interstitial space and the consequent vasoconstriction through platelet activation that further alter alveolar gas exchange with severe hypoxemia and tissue hypoxia which will lead to Multi Organ Failure (MOF).[37,43,44] The mechanism of thrombocytopenia in patients then has a multifactorial origin. Furthermore, the combination of viral infection and mechanical ventilation can give additional endothelial damage which will also lead to platelet thrombosis in the lung, thus causing excessive consumption of platelets and a decrease or capillary morphological alternation can lead to altered platelet defragmentation. But another factor would be that coronaviruses can infect the bone marrow resulting in the activation of an autoimmune response against blood cells.[37,40,45,46] Lung 's histological examination of patients who died from ARDS shows diffuse alveolar damage with desquamated pneumocytes II and lymphocytic infiltrates (CD4 and CD8 lymphocytes) located mainly in the interstitial spaces, around the larger bronchioles and in many cases there are foci of hemorrhage and small vessels, appear to contain platelets and small thrombi. The CD4 and CD8 cells (killer lymphocytes) collaborate with B lymphocytes responsible of the production of antibodies. The CD8 destroy the infected cells by cytolysis and necrotizing cytokines instead the CD4, release interferons and interleukins which eliminates the pathogens (beneficial effect) but on the other hand may had also harmful effects (immunopathology). [47-49]

The angiotensin system is involved in viral infection. This activity linked to the blood coagulation cascade. By blocking the ACE2 receptor, severe vasoconstriction occurs, which can lead to the accumulation of fluid in the lungs. There are clotting factors outside the blood vessels (e.g. von Willebrand factor acts on factor VIII) that when the damage starts to occur as it also happens with the surrounding vessels, these enter the blood and this acts on the clotting factors which can cause phenomena of thrombosis. These form in the lungs and cause damage, acute respiratory distress syndrome, stroke, heart and other. ACE2 is one of the main enzymes in the renin-angiotensin system (RAS) which regulates blood pressure, fluids, electrolyte balance and systemic vascular resistance. [34,37,45] In the lungs, activation of local RAS can influence the pathogenesis of lung damage through multiple mechanisms, such as increased vascular permeability and alterations in alveolar epithelial cells. Activation of pulmonary RAS involves renin, the initial enzyme of the RAS activation cascade; renin cleaves angiotensinogen generating angiotensin I (Ang I, a decapeptide hormone, inactive). ACE converts Ang I to angiotensin II (Ang II, an octapeptide hormone), which is very active, which exerts vasoactive effects by binding to its receptors, type I (AT1) and type II (AT2). There is multiple evidence on the importance of AT2 and ACE2 in the regulation of inflammation, both through activation of the AT2 receptor via Angiotensin2, and through activation of the MAS receptor, via Ang 1-7 produced by ACE2, with a reduction of inflammatory interleukins such as Interleukin 6, Il-5, TNF-alpha, NF-kB and others. In addition, some studies show that lung ACE2 deficiency correlates with a worse prognosis since ACE2 and AT2 have a protective action on the

lung; ultimately ACE2 is not only the receptor for the virus but also the protector of lung damage.[35,43,47] Some evidence shows that SARS-CoV2 is able to deregulate the balance of the protective system of the lung formed by the balance between ACE/AngII/AT1 and ACE2/AT2/Ang 1-7 and MAS receptor. Is a possible explanation from the ACE2 receptor in the severity of the infection induced by SARS-CoV2? Several studies have shown that the host's cells defend themselves from attack by decreasing the presence of ACE2. Finally, the scholars analyzed the presence of ACE2 also to try to clarify the animal origin of the Sars-CoV-2 coronavirus, initially connected to the bat but later also attributed to the pangolin. The analysis carried out showed a greater similarity between the ACE2 protein of human cells and those of pangolins: a result that supports the hypothesis that the small mammal could have been the original host of the Sars-CoV-2 virus or an intermediate host between bat and man (Fig 4). [35,43,50]

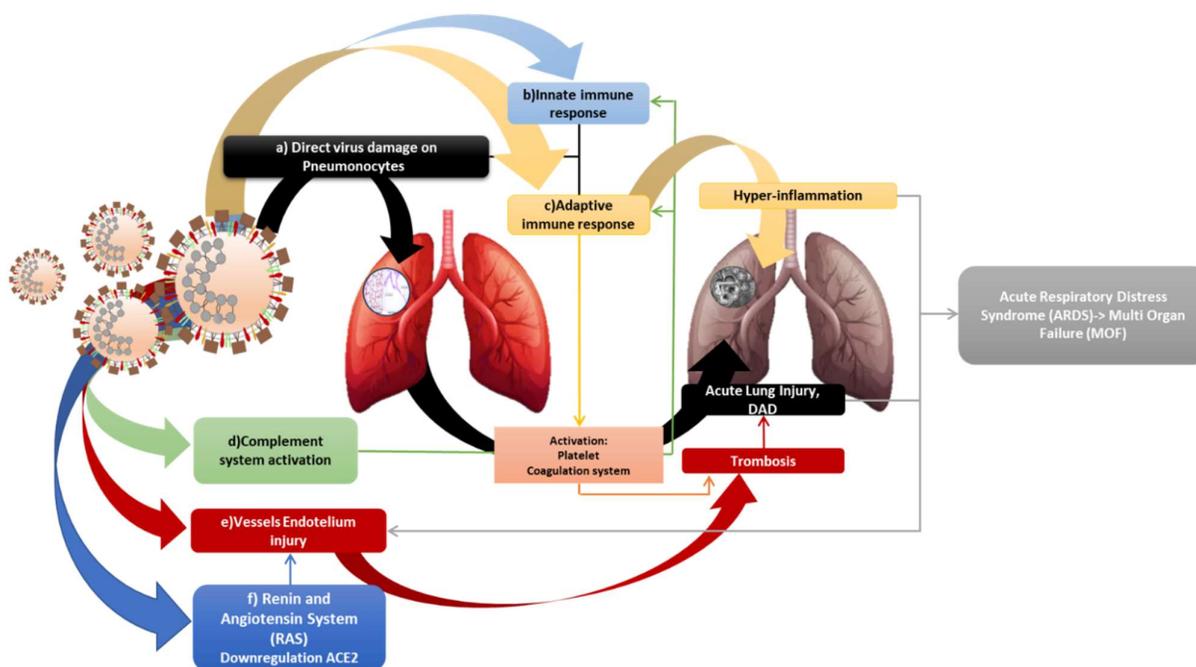


Fig.4 The virus causes direct and indirect effects that lead to severe lung damage. Direct actions: a) induces damage directly to pneumonocytes I,II with cytopathic consequences through pro-inflammatory status (cytokines/chemokines), b) recruitment activation of the innate immune response (macrophages/PMN, proinflammatory status (cytokines/chemokines tempest), c) recruitment activation or the adaptive immune response (antiviral CD8, Antiviral B cell, antibodies), d) complement system (generation of C3a, C5a, membrane complex), e) endothelial activation and injury leading to endothelial dysfunction and vascular leakage) and f) RAS: down regulation of ACE2 which increases ACE/AT1 with pro-inflammatory, pro-oxidative effects, vasoconstriction and pulmonary endothelial damage. Activation of the adaptive immune system causes lung tissue hyper-inflammation, activation of coagulation system (which in turn reactivates complement), activation of platelets (which in turn activates coagulation system), down regulation of inactivity and apoptosis. The activation of the system coagulation and the dysregulation of the ACE2 also lead to endothelial damage thus causing an increase in the possibility of formation of clots and microthrombi/thrombi (such as pulmonary embolism, deep vein thrombosis, large vessel stroke, arterial and venous thromboembolism), increase the risk of lung damage with edema, diffused alveolar destruction (DAD), severe hypoxemia and can evolve into Acute respiratory distress syndrome (ARDS) and afterwards Multi Organ Failure (MOF).

The Immune responses and immunity

The virus can be detected by PCR in whole blood and in stool samples (real-time reverse transcriptase-polymerase chain reaction: RT-PCR) and the serological blood test looks for antibodies created by the immune system.[51,52] Some aspects regarding the progression of SARS-CoV-2 still remain uncertain. One of these is the role of cross-immunity with other *Coronaviruses* (at least 4 coronaviruses that cause flu in humans) and following the SARS-CoV epidemic and studies led to the belief that cross-immunity between the common cold virus and SARS-CoV-2 is very likely directed against the antigens common to all coronaviruses and not the specific antigens of SARS-Cov-2. [53,54] According to what has emerged so far, all patients who have contracted Sars-Cov-2 and are cured have produced antibodies, but there are conflicting theories on their duration (from a few months to the end of the year). If at first it was thought that antibody immunity for Sars-Cov-2 could last at least a year (as ascertained for the SARS-CoV and MERS-CoV coronaviruses), but there may be a drop in plasma antibody levels 2-3 months later healing in both patients who were asymptomatic and in those with symptoms. If so, even those who have already contracted the virus once could become vulnerable after a short time and if the infected quickly lose antibodies, they could be negative on serological tests. However, immunity conferred by antibodies is not the only possible one. In several studies was also highlighted the immunity mediated by specific T lymphocytes, neutralizing antibodies, and other molecules produced by B lymphocytes capable of neutralizing the virus. In addition, some studies reported the possible enhancement of the immune response by probiotic administration. [46,55-58] The presence of antibodies, of any type, is not a guarantee of absolute immunity and the persistence of the antibodies produced remains uncertain. [53,59,60]

Conclusion

The Sars-Cov-2 pandemic emergency has shown the world that only an international scientific effort can stop a threat of this magnitude also because in some cases it has shown the whole world the weakness of local health systems. The research effort of the scientific community continues to understand how the new virus works and thus to have an early diagnosis, better management, and adequate therapy. The virus is now well-researched in a short time but the victims around the world continue to be present. It causes no or mild symptoms such as flu like, taste and smell disorders and others but also serious diseases such as the diffuse alveolar damage (DAD), acute respiratory distress syndrome (ARDS), multi organ failure (MOF), thromboembolic phenomena that make it necessary to study the virus pathophysiology continuously. However, it seems that the virus is "changing" and probably this will make it less pathogenic because it shows the deletion in non-structural proteins (such as NSP1), the most important determinants of the pathogenicity of SARS-Cov-2. On the other hand, cross-immunity and immunity are questioned at a certain time that a patient can develop and maintain over time. This is important for the reinfection in a short time of the patients themselves healed and highlights the search for an effective vaccine over time.

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