COVID-19: An international student's review from Humanitas University

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What is the role of a medical student during the time of a pandemic? What can we learn about the COVID-19? We are three medical students who performed a review of the literature to understand better this infection and to help our fellow colleagues as well. We began with a brief overview of the pandemic development and its epidemiology. We review the up to date information regarding the virology, pathophysiology, immunology, serology, diagnostics, and imaging of COVID-19. We finally reviewed the current pharmacological treatments and hot topics in this area. We found that while there is a vast amount of literature on this topic, there are still essential unanswered questions that need to be addressed.

Introduction

On December 31st, 2019, China reported to the World Health Organization (WHO) a cluster of cases of a pneumonia of unknown etiology in the Wuhan City in the Hubei Province. On January 7, 2020, this was linked to the insurgence of a novel coronavirus, identified via genome sequencing by the CCDC (Chinese Center for Disease Control and Prevention). It is not clear when the outbreak started and this makes it difficult to estimate its rate of spread [1]. Phylogenetic analysis of thirty-eight SARS-CoV-2 genomes suggests that the virus may have been circulating before December 2019 [2,3]. On January 23, 2020, Wuhan City implemented its lock-down, but by this time about five million people had already moved out of the Hubei province due to travels for the Chinese New Year. On January 30, 2020 the WHO declared the outbreak a Public Health Emergency of International Concern (PHEIC), a month later on February 28, 2020 the WHO raised the threat of an epidemic to a "very high level," and finally on March 11, 2020 the WHO declared COVID-19 a pandemic [4,5,6,7].

As this time of lockdown, quarantine, social distancing, smart working, online learning, and disruption of daily activities, medical students particularly find themselves in between a rock and a hard place. We have written this article to inform ourselves of the current pandemic, learning from the doctors on the front lines as well as from professors, with the hope that this can help other medical students learn from this moment in medical history. We hope this will allow us to be better prepared to enter the hospital again when we are allowed to and play our part in this pandemic.

This article gives a brief introduction to the epidemiology of the pandemic, followed by a review of the virology, pathophysiology, clinical presentation, radiology, and pharmacology regarding COVID-19. Our information comes from the most up to date articles in the literature, mostly peer-reviewed, some still under review, as well as from resources and patient cases from our medical school hospital, Humanitas Research Hospital in Milan, Italy. Our information and accuracy have been discussed and reviewed by our professors and doctors working on the front line in Humanitas.

Epidemiology

It is important to note that we are still in the middle of the global epidemic and that many of the numbers and statistics regarding this virus are incomplete, quickly recorded and reported, and are based on varying definitions. Therefore, although we report accurately the data available in the literature, data will need to be "cleaned" at the conclusion of this event, when the true parameters and statistics can be analyzed in a complete way. As of now, data are heterogenous due to differing factors among countries, including (1) surveillance systems, (2) definition of COVID-19 related death, and (3) testing strategies [8]. Additional factors to consider are places that tested pediatric cases, carriers who were asymptomatic but spread the virus, and the difficulty of reporting correct numbers when a majority of the cases were sick or died at home [9].

China officially recorded its first death from COVID-19 on January 11, 2020. Soon after, on the 13th of January the first case of COVID-19 outside of china (Thailand) was recorded. On the 30th of January 2020, the Istituto Superiore di Sanità (ISS) of Italy confirmed the first two imported cases of COVID-19, while on the 21th of February, the first case of Italian origin, without any contact with travelers or connections with people from Wuhan or China in general was reported in Codogno, Lombardy.

SARS-CoV-2 has become a global threat, developing in a pandemic affecting 215 countries and 6 continents. The most up to date numbers from the World Health Organization (WHO) are 4,006,257 infected individuals and a total of 278,892 deaths. The areas with the highest number of cases and also deaths are Europe and the Americas, with both more than 1 million cases [10].

The Incubation period of SARS-CoV-2 has been established to be about five days. However, it has been reported that this timespan can greatly vary, between three and seven days from infection. Also, exceptionally long and short incubation periods have been reported, from a minimum of two days to almost two weeks (max reported is twelve and a half days) [6,7,11].

According to the latest statistics, the mortality of SARS-CoV-2 has been estimated around 7% worldwide, however this parameter has showed great variation among countries; with countries such as Italy with 13.4% and Germany with 3.5 %. Compared to other novel human CoVs this new virus has shown a lower mortality rate, with SARS-CoV having around 10% and MERS-CoV having around 35% [12]. However, given that data have been recorded very rapidly due to the fast onset of the pandemic, and considering the varying data collection measures, it will take time to see the true statistics of this pandemic come to light.

Some sources in the literature hypothesize that the discrepancy in the mortality rates seen in different countries is due to the different mechanics and protocols used for screening and diagnosis, for example Onder G et al. attribute the discrepancy seen between Italy and China to the different definition of COVID-19-related deaths as well as different testing strategies other than to the different demographics (Italy has an overall older population) [13].

The period between symptom onset and death ranged between six and forty-one days, with a median of fourteen days. It is however important to underline that the length of this period is dependent on the status of the patient's immune system and the presence of comorbidities; in particular, this timespan has been shown to be shorter for patients greater than seventy years old compared patients under this age threshold [11,12].

The main control measures applied in the pandemic have been quarantine of infected individuals, different versions of country or state lockdown, travel restrictions, and airport screening for travelers. Combined strategies in which all measures have been implemented has shown to be the most effective in decreasing disease spread. Airport screening has, however, been demonstrated to not be very

effective due to the presence of asymptomatic, pre-symptomatic, and pauci-symptomatic transmission [12].

The European Journal of Allergy and Clinical Immunology has recently published an article regarding the forecast of the possible outcome of coronavirus-associated deaths on the base of R^2 correlations of Italy, Germany, Spain, and New York State after different days of prediction [14]. Based on data obtained by the current situation and current public health interventions, the model predicts two different scenarios for each country by May 31st. The predicted total number of deaths for the country of Italy are a high of about 50,000 and a low of about 29,000 deaths; for Spain a high of about 55,000 and a low of about 28,000 deaths; for Germany a high of about 11,000 and a low of about 6,000 deaths; and for New York State in the US, a high of about 36,000 and a low of about 17,000 deaths. The final outcome could be modified if interventions are implemented on the basis of such predictions.

Virology

Introduction

SARS-CoV-2 is an enveloped, non-segmented, positive single stranded RNA (+ssRNA) virus, classified as a Coronavirus (CoV). Taxonomically, SARS-CoV-2 is classified as a lineage B beta-coronavirus (βCoV) [6,15,16]. Upon electron microscope analysis, SARS-CoV-2 has also shown a typical coronavirus appearance, with a minute dimension and spherical morphology combined with the crown-like appearance provided by the spikes [1,6]. Coronaviruses (CoVs) not only have their morphologic appearance in common but also share a common structural organization at the molecular level. They are made up of four core structural proteins including the **nucleocapsid (N)**, the **membrane (M)**, the **envelope (E)**, and the **spike (S)** proteins. These proteins have important structural as well as functional roles [17]. The S, M, and E proteins are localized in the envelope and are involved in viral entry (S) and envelope formation/viral release (M and E), while protein N is only found at the core of the viral particle, being the protein constituent of the CoV nucleocapsid. Coronaviruses share several characteristics also at the genetic level. They have a peculiarly large genome, with complex mechanisms of gene expression (detailed below) [18].

Coronaviruses generally cause respiratory, enteric, hepatic, and neurological diseases most commonly in mammals and birds. With the introduction of SARS-CoV-2, currently seven CoVs able to infect humans have been discovered [6]. Based on the severity and type of pathology they cause, human CoVs are classified into **common human pathogenic CoVs**, that cause mild and self-limiting upper respiratory diseases, and **novel human pathogenic CoVs**, which cause epidemics with variable clinical severity varying from mild and self-limiting to severe and life-threatening. SARS-CoV-2, together with SARS-CoV and MERS-CoV, is included in the latter group [6,18,19].

Phylogenetics

Genetic analysis of SARS-CoV-2 has shown a high nucleotide identity (and therefore phylogenetic relation) with bat CoVs (89% with SARS-like-CoVZXC21 and 96% with the bat CoV RaTG13). Indeed, when compared with the known genomes of the other novel human CoVs, SARS-CoV-2 is the closest to SARS-like bat viruses in terms of whole genome sequence [16,19,20]. The extreme similarity of the genomes isolated from different patients in Wuhan (0.1% nucleotide difference), summed with the characteristic of RNA viruses to rapidly mutate, suggest that the virus has only recently obtained the capability of human infection [1,11,18].

Despite the general knowledge of CoV phylogenetic evolution and the comparative analyses performed with the genomes of other human and animal CoVs, the origin of SARS-CoV-2 is not yet entirely understood [6,19].

The comparative genomic analyses suggest that the virus evolved from bat CoVs, then became able to infect a currently unidentified intermediate mammalian host from which it entered in contact with humans [6,20,21]. This hypothesis is supported by (1) current knowledge on human CoV viruses (in particular, other novel human Coronaviruses SARS and MERS), (2) the fact that bats are the natural reservoir of *Alphacoronavirus* and *Betacoronavirus*, carrying a highly diverse array of SARS-like-CoVs, and (3) the instance in which the initial transmission of the virus has been reported, a seafood market (the Huanan seafood market in Wuhan City, China) [20,21]. A strong candidate for the role of intermediate host has been given to pangolin. This is because it is sold at Huanan market in Wuhan and because of the genetic similarity of a CoV recently found in this animal species [19,21].

Genetics

SARS-CoV-2 genome is around 29,900 nucleotides long and has a structure similar to that of other coronaviruses, following the shared coronavirus schematic genome organization of **5'-leader-UTR-replicase-S-E-M-N-3'-UTR-polyA-tail**, with accessory genes interspersed within the structural genes at the 3' end [16,19]. Similarly, to other CoVs, two-thirds of the genome consist of the first two open-reading

frames (ORFs) – **OFR1a/b** – which code for **nonstructural proteins (nsps)** responsible for the generation of the **Replication and Transcription Complex (RTC)**. The remaining ORFs code for structural and accessory proteins [16,22].

The major elements involved in CoV genetic regulation are (1) the **5′- and 3′- proximal regions** of coronavirus RNAs (contain key regulatory elements for RNA synthesis which interact with **RNA-binding proteins**), (2) the organization of the genes coding for the structural proteins, ordered **S, E, M and N** from 5' to 3', and (3) the **pseudoknot-induced frameshift between ORF1a and 1b**, resulting in the transcription of the polyprotein 1ab [18,22].

Virology

The **receptor-entry process** involves the spike glycoprotein present on the viral envelope, **Protein S**, which is a type 1 transmembrane glycoprotein which associates in homotrimers. The S protein is formed by two subdomains: **S1**, involved in receptor binding and therefore major determinant of host range and tissue tropism, and **S2**, mainly involved in triggering the fusion of the viral envelope with the host cellular membrane [6,18,19,20]. The cellular protein used as receptor for SARS-CoV-2 entry is the **ACE2 enzyme**, the same receptor used by SARS-CoV, which is an extracellular membrane enzyme acting as a negative regulator in Renin Angiotensin System (RAS). ACE2 is expressed in various tissues including lung type II pneumocytes, nasal goblet cells, heart pericytes, kidney and testes, upper stratified epithelial cells of the esophagus and absorptive enterocytes of ileum and colon. Distribution of the virus receptor explains the multi organ involvement, the variety of symptoms that are possibly associated with the disease and the various possible transmission routes [11,19,23,24]. (*Figure 1*).

The **receptor binding domain (RBD)** of SARS-CoV-2 and SARS-like-CoVs consists of approximately 193 amino acids. In particular, SARS-CoV has a well-defined RBD, which comprises 14 binding residues that directly interact with ACE2. Of these amino acids, 8 have been conserved in the RBD of SARS-CoV-2 S protein which presents a discrete similarity with the SARS-CoV RBD. These variations in key amino acids have been shown to attribute a higher affinity for human ACE2, potentially explaining the higher infectivity of SARS-CoV-2 compared to SARS-CoV [1,7,16,21]. Interaction between the viral spike RBD and its cellular target take place through van der Waals interactions. This generates a relatively weak interaction among the two, making it possible for the virus to infect multiple. Recent studies showed that the SARS-CoV-2 RBD, besides human ACE2, is able to interact with the ACE2 of Chinese Horseshoe bat, civet, and pig [15,19].

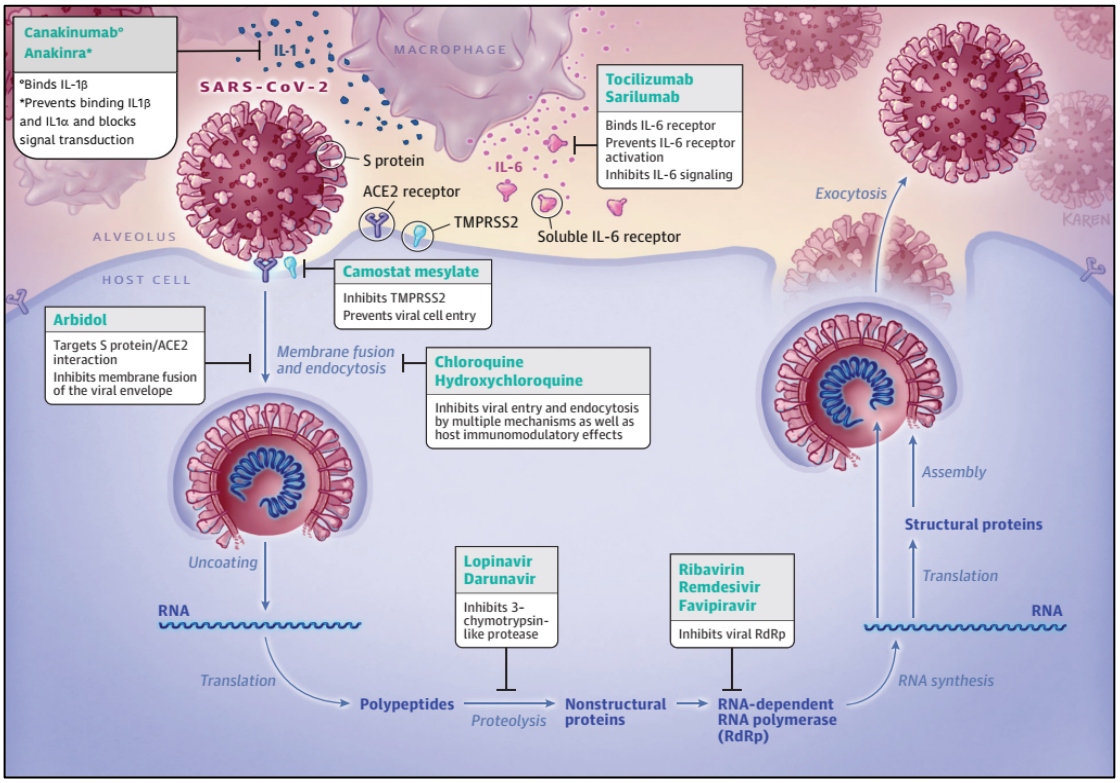

Figure 1 - A schematic review of the host immune response induced by SARS-CoV-2 and possible drug targets, adapted and modified from Sanders et al., *Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review* [25]

Beside receptor binding, CoV S-glycoproteins also require extracellular, membrane-associated, or lysosomal **proteases** that cleave the S protein at two sites called the **S1/S2 site** and the **S2' site**. This process is known as **S protein priming**. This is dependent on the extracellular protease **TMPRSS2** and, due to the presence of a poly basic motif (PRRARSV) at the S1/S2 site, supposedly on the intracellular protease **furin** (which cleaves the S1/S2 site during its biosynthesis, similarly to the one of MERS) [1,15,20,21].

Intracellular life cycle

After receptor binding at the membrane, the virus enters the cell via the **endosomal pathway**, through a process of **receptor-mediated endocytosis**, and then the fusion between the viral envelope and the endosomal membrane takes place, allowing the **nucleocapsid** to be released in the cytoplasm. The viral genome is then uncoated, and the transcription and replication steps take place [15,18]. Initially there is the transcription and translation of the first polycistronic ORFs (ORF1a and ORF1b), generating **polyproteins 1a (pp1a)** and **1ab (pp1ab)**, which are then proteolytically cleaved by viral proteases into **16 non-structural proteins (nsps)** responsible for the assembly of the **replication and transcription complex (RTC)**. The RTC is localized in double membrane vesicles and has a variety of enzymatic activities including RNA-dependent RNA polymerase, helicase, RNA cap-modifying methyltransferase and an exoribonuclease [6,16,18,22]. It is responsible for replication and transcription of the viral genome, which produces (1) **sub-genomic mRNAs (sg mRNAs),** which are translated into structural and accessory proteins, and (2) **new genomic RNAs**.

Among the proteins translated from the sg mRNAs, the most relevant are the structural proteins N, M, E and S (the latter is described above in entry). The **N protein** interacts with the viral RNA forming the **nucleocapsid** and is required for viral RNA packaging during viral assembly. The **M protein** is the main structural component of the **envelope**, it exists in higher quantities than any of the other proteins in the viral particle and gives shape to the envelope. Together with the E protein, the M protein is critical for

directing virus assembly. The **E protein** is required for assembly and release of the newly formed viral particles form the host cell [1,6]. Translation of structural viral proteins is ultimately followed by their assembly, together with viral RNAs, into **virions** which are transported via vesicles to the plasma membrane and released out of the cell [15].

Many **nsps** have multiple functions in the **synthesis or processing of viral RNA**, and in addition some are also involved in **virus-host interactions.** Among the possible host-virus interactions are the interference with antiviral defenses and host gene expression. Therefore, also these interactions are decisive for infection/pathogenesis [18].

Transmission

As described above, it is most probable that this disease has an initial zoonotic origin. As with other respiratory pathogens and in particular similar CoVs, person-to-person transmission occurs mainly through **droplet transmission**, by coughing and sneezing, from infected individuals [7]. However, transmission related to close contact with the infected individual (**fomite transmission**) and respiratory transmission through **aerosol transmission** have also been demonstrated as viable routes for viral spread, the latter in particular in case of prolonged exposure to the aerosol in closed spaces [6,11,19]. Despite no cases of **fecal-oral transmission** being confirmed up to this point, the confirmed shedding of viral particles in urine and feces, combined with the already acknowledged high environmental resistance of SARS-CoV-2, makes the occurrence of a fecal-oral transmission possible [23,26,27,28]. Despite symptomatic patients being the main transmitters, transmission also from pre-symptomatic, asymptomatic, and pauci-symptomatic individuals has been confirmed [2,29,30]. Not enough data are present to either confirm or disprove the possibility of intrauterine transmission or transmission during labor [11,31,32]. Despite its overall high environmental resistance, SARS-CoV-2, like other CoVs, is sensitive to ultraviolet rays, heat and lipid solvents [6].

Immune Response

The immune response is vital for the control and resolution of CoV infections, however requiring precise regulation since it can also lead to immunopathogenesis, associated with out of control immune responses [16]. The innate immune response is initiated by recognition of viral RNAs, acting as **pathogen-associated molecular patterns (PAMPs)**, by the **pattern recognition receptors (PRRs)**. Toll-like receptor (TLR) 3, TLR7, TLR8, and TLR9 sense viral RNA and DNA in the endosome compartment [16]. The innate immune system is also triggered by the dsRNA and 5′-triphosphate-bearing RNA molecules that arise as replication intermediates in the cytosol. These molecules, foreign to the cell, can be recognized by the intracellular sensors of the **RIG-I-like receptor (RLR) family** which are expressed in almost all cells [18]. This array of PRRs recruits various adaptors, including TIR-domain-containing adaptor proteins to trigger down-stream cascades molecules that lead to the activation of the transcription factor **nuclear factor-κB (NF-κB)**, of **interferon regulatory factor 3 (IRF3)** and to the production of **type I Interferons (IFN-α /β)** together with a series of **pro-inflammatory cytokines** [16,18]. Hence, virus-cell interactions lead to the production of diverse immune mediators. Plasma levels of cytokines and chemokines were increased in COVID-19 patients, including **IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, granulocyte-colony-stimulating factor (G-CSF), macrophage CSF (M-CSF), IP-10, MCP-1/CCL2, MIP-1α/CCL3, hepatocyte growth factor (HGF), IFN-γ and TNF-α** [33].

The increase of pro-inflammatory cytokines with a dysregulated immune response develops into a cytokine storm where the innate immune response is out of control and the adaptive immune response is impeded. The major players in this are the macrophages, which produce cytokines in an unregulated manner [34]. Patients showing this immunopathology have increased neutrophil counts and **lymphopenia**. Both Zheng M et al. and Qin C et al. indicate that severe patients show a high neutrophil count and a low lymphocyte count. The **neutrophil-to-lymphocyte ratio (NLR**), therefore, is increased, indicating a dysregulation of the immune system and possibly worse prognosis [35]. It is hypothesized that one of the major effects COVID-19 has on the immune system is the damage to **lymphocytes**, especially **T lymphocytes**. Lab results include **lymphopenia** and **elevated levels of infection-related biomarkers** [35].

Essential players of the immune system during a viral infection are the **cytotoxic lymphocytes**, including cytotoxic T lymphocytes and natural killer cells. A reduced presence or function of these cells is inversely correlated with progression of the infection [36]. In patients with COVID-19 we see a decreased total numbers of both NK and CD8+ T cells and Zheng M et al. hypothesize that the SARS-CoV-2 infection could possibly be breaking down the antiviral immune system response at the initial stages. Intracellular cytokine staining showed that there is evidence of functional exhaustion of cytotoxic lymphocytes and T cell apoptosis in the early stage of COVID-19 infection, which has been correlated to disease progression [36].

In the immune system functions, CD4+ and CD8+ T cells regulate the immune response by playing a regulatory role on the innate immune system response to a viral infection. Qin C et al. showed that there was a high naïve CD4+ T cell to CD4+ memory cell ratio, indicating weakened immune system response [35]. Giamarellos-Bourboulis EJ. et al. also showed that Th17 function is downregulated in COVID-19 infection. This supports the hypothesis that the innate immune system is out of control, while the adaptive immune system is not present in a functioning or regulatory capacity.

The **adaptive immunity response** of COVID-19 patient has been observed through antibody testing. It begins with an antigen presentation to B and T lymphocytes in the lymph nodes. Once humoral immunity is activated, there is a massive production of antibodies against the infective agent, and detection of these antibodies can be useful in diagnosis and evaluation of disease progression [37,38]. This typically occurs about one week after the initial infection [33]. In a viral infection, IgM are the antibodies of the first line of defense preceding the long-term production of IgG antibodies [37,39]. IgG

antibodies are important for the immune memory and long term responses of the body to the virus, as they provide a more robust immunity [37]. In serology testing, IgM antibodies are detected in the early infections, whereas IgG are detected further along the course of infection, with concentration increasing gradually, while IgM levels are dropping. Testing these antibodies can give insight into the time course of the viral infection in a particular patient, additionally, the presence of these antibodies could potentially be used as an added tool in the diagnostic tests for COVID-19 [39]. Is has been shown that after some time, from two weeks to 35 days of testing positive, patients' IgM antibodies decrease, while IgG rise and then reached a plateau [33,37,39,40,41]. This is typically seen one month post-infection [40]. Studies argue that IgG antibodies will be more informative for vaccine development than IgM in this situation. It may be argued that a delayed antibody response in a patient indicates improper immune activation, which would orient the prognosis [33].

Despite antibodies being usually regarded and correlated to the efficacy of the immune system in protecting against the invading pathogen, **high antibody titers** and **rapid seroconversion** has been correlated with disease severity in patients with SARS [42]. Indeed, it is the quality and the quantity of the antibody response to dictate its functional outcome. Antibody mediated clearance of SARS-CoV has been associated with the direct activity of neutralizing antibodies (blocking viral entry, fusion or regress) or by their interaction with other immune components (such as complement, phagocytes and NK cells) [42]. On the other hand, in rare cases, neutralizing antibodies are able to promote pathology, resulting in a phenomenon known as **antibody-dependent enhancement** (ADE; phenomenon documented also for other viruses, such as dengue). The response initiates via the engagement of Fc receptors on various immune cells (including monocytes, macrophages, and B cells), where pre-existing SARS-CoV-specific antibodies promote the entry of virus-antibody immune complexes in FcR-expressing cells. The entry of the virus in these cells is non-productive (no viral replication), resulting in the immune cell activation by the (1) engagement of viral PAMPs via PRRs, such as the TLRs previously discussed, and (2) the activation of FcR signaling machinery. This results in high production of **inflammatory cytokines** (IL-6 and **TNFα** by macrophages), increased production of **chemokines** (**CCL2** and **CCL3**) and decreased levels of **anti-inflammatory cytokines** (**IL-10** and **TGFβ**). Overall, this promotes inflammation and tissue injury [42].

Multiple factors are involved in determining whether an Ig neutralizes the virus or is a trigger for **ADE** and **acute inflammation**: including epitope specificity (varying both among different target elements, es. N and S targeting Igs, or among epitopes of the same element), concentration, affinity and isotope [42]. ADE is induced when stoichiometry is below the threshold for neutralization, therefore higher affinity antibodies are less likely to cause this phenomenon since they reach it at lower concentration. IgMs are considered more pro-inflammatory compared to IgGs due to the higher efficacy in complement activation. Recent studies on COVID-19 have associated anti-N IgM and IgG (at all times during infection) with a worse disease outcome [42]. Furthermore, higher titers of anti-N and anti-S IgM and IgG have been correlated to worse clinical outcome and older age.

Recommendations include a combination test for both diagnostics as well as prior infection detection, possibly for asymptomatic infections [33,37,38,39,43]. However it is to be noted that a positive COVID-19 RT-PCR test with a negative serology test does not rule out infection, due to the fact that it takes a few days and up to a week for these antibodies to show their presence in the blood [41]. The tests currently being proposed in the literature show high consistency and precision [37]. Testing is important also to obtain information regarding disease recovery, as these viral antibodies remain in the body after the infection has past [37]. Zhang et al. showed that both IgG and IgM can be detected after 5 days on infection onset [37]. Important to note, a study tested that plasma antibodies for SARS-CoV-2 have no cross-reactivity with other coronaviruses, except for SARS-CoV. The authors argue that it is unlikely that these patients were previously infected with SARS-CoV, and if they were, the IgM reactivity would not have lasted that long [43].

Pathophysiology

The COVID-19 patient population is divided into two main groups – mild-moderate and severe-critical. These groups are not evenly divided, as 80% of patients are mild-moderate, 13.8% are severe, and 6.1% are critical [44,45]. Risk factors for the severe-critical group of patients are over 60 years old, hypertension, diabetes mellitus, cardiovascular disease, chronic respiratory disease and immunocompromised. The difference between these groups lies in the pathophysiology, that consists in a viral pneumonia that can further develop into ARDS, a cytokine storm, sepsis, multi-organ failure, and death.

The ACE2 enzyme that is the viral receptor for entry into the host cells is present on type II pneumocytes, vascular endothelium, and many other cell types [46]. The infection begins in host Type II pneumocytes, enters the blood stream, and spreads to the rest of the body. We will first describe the ACE2 enzyme physiological function and then move to the pathophysiology of SARS-CoV-2 infection in the lungs and the rest of the human body.

The ACE2 enzyme is a primarily membrane-bound, interferon-inducible gene and plays a physiological role, along with the angiotensin converting enzyme (ACE), in the **Renin Angiotensin System (RAS)** [47,48]**.** The ACE enzyme converts ANGI to ANGII, which is a peptide that induces vasoconstriction, hypertension, decreased renal blood flow, and increases the activity of the sympathetic nervous system [49]. ACE2 converts (1) ANGI to ANG1-9, inhibiting the formation of ANGII from ANGI, and (2) ANGII to ANG1-7, thus deactivating ANGII [50]. We see therefore that the ACE2 enzyme opposes the action of the ACE enzyme. The molecule ANG1-7 is a immunoregulatory, vasodilatory peptide that acts via a GPCR system, the Mas receptors, and has a protective role in vascular, heart, and kidney cells [51]. This protection includes anti-arrhythmic effects, anti-inflammatory effects, vasodilation, stimulation of renal Na+ secretion, and modulation of the sympathetic nervous system [49]. Additionally, cleavage of ANGI and ANGII enhances the effects of both ACE-I and ARB drugs. These two enzymes have opposing functions within the same axis, and in normal physiology are maintained in balance.

Many studies hypothesize that the link between the SARS-CoV-2 virus and the dysregulation of RAS is strong evidence for why this infection becomes severe [47,50,52,53]. Interestingly enough, ACE2 also plays a protective role during acute, severe lung injury and conversely, ANGII promotes lung disease pathogenesis, such as edema and decreased lung function [47,50]. In COVID-19, ACE2 molecules are essentially “inactivated” due to viral binding and endocytosis [47]. This leads to an unbalance of the RAS axis, with increased presence of ANGII and decreased activity of ACE2, thus promoting pathogenesis and inactivating a defense mechanism. Additionally, the destruction of the Type II pneumocytes by the virus inactivates a second defense mechanism, as Type II pneumocytes proliferate during injury resolution to both differentiate into Type I pneumocytes as well as remain Type II pneumocytes to produce surfactant [54].

The entry of SARS-CoV-2 virus in type II pneumocytes via the ACE2 enzyme leads to the development of an interstitial viral pneumonia [55]. The syndrome of a **pneumonia** is the effect of an inflammatory response of the host rather than an intrinsic action of the pathogen itself. The presence of viral RNAs in the host cells elicits an inflammatory response leading to fever, infiltration of lymphocytes into the interstitial space, production, and release of pro-inflammatory cytokines (such as IL-1, IL-8, and TNF-alpha) and alveolar capillary leakage. This uncontrolled alveolar damage develops **acute respiratory distress syndrome (ARDS)**, which is characterized by severe acute dyspnea, hypoxemia, and diffuse pulmonary infiltrates and can lead to respiratory failure [54,56]. Alveolar capillary leakage develops into alveolar filling, decreased lung compliance, and increased pulmonary shunting. The pulmonary edema can become severe enough to cause alveolar damage and hyaline membrane formation, as seen in autopsy reports of COVID-19 patients [52]. Given that the alveolar space and the pulmonary capillaries are anatomically very close, this alveolar damage also induces pulmonary vascular injury via

microthrombi and fibro-cellular proliferation [54]. This pathophysiology and increased dead space in the lungs manifests as dyspnea, hypoxemia, possible hemoptysis, and peripheral increase in neutrophil counts.

Genetic susceptibility and inflammatory cytokines are associated with development of ARDS and with a worse COVID-19 outcome [52]. The innate immune response needs to be precisely regulated to eliminate the virus, otherwise it can result in pathology. An overactivation of the inflammatory response in ARDS pathophysiology can lead to a **cytokine storm,** which is essentially a hyperinflammation leading to diffuse damage to the body's own tissue [45]. This storm is seen in severe forms of COVID-19 and is a strong determinant of prognosis [16,45,52]. As explained above, the dysregulated immune system leads to neutrophilia and lymphocytopenia in severe patients.

Closely intertwined to this immune dysregulation found in the diffuse alveolar and interstitial inflammation is the dysregulation of the clotting system. Multiple clinical reports indicate the presence of **disseminated intravascular coagulation (DIC)** and **immune-thrombotic events** in the COVID-19 patient population [42,57]. The three principle effects seen with DIC are fibrinolysis, ischemic tissue damages, and consumption of clotting factors and platelets [54]. In COVID-19 we see this evidenced by increased D-dimer and fibrin degradation products (FDP), ischemic tissue damages, and **thrombocytopenia**. A case study in France also indicated the presence of possible **thrombocytopenic purpura**, and other studies indicate the findings of hypercoagulability in COVID-19 patients [57,58]. A Chinese study found that a D-dimer level >1.0 ug/L upon admission was correlated with an 18-fold increase in chance of death [8].

DIC occurs in the small peripheral blood vessels, and downstream from here there is the possibility of a **thrombotic microangiopathy (TMA)**, which is the presence of thrombosis in the capillaries and arterioles [54]. Along with the DIC, these microthrombi lead to ischemic tissue injury which is a life-threatening complication. TMA, which ultimately results in organ dysfunction, can occur in various clinical scenarios, including **pathogenic complement activation.** It is thought that, in SARS-CoV-2 infected patients, the damage of vascular endothelial cells may be caused by TMA [42].

Murine studies investigating the role of complement in novel human CoV infections suggest that a significant part of SARS-mediated damage is immune mediated [42]. This finding was supported by experiments in C3 knockout mice, which presented less respiratory dysfunction and reduced cytokine levels despite equal viral loads compared to wild-type infected mice. In a murine model of MERS-CoV infection, elevated levels of C5a and C5b-9 were observed in lung tissue and treatment via blocking the anaphylatoxin C5a resulted in alleviated pulmonary and extrapulmonary damage, a decreased cytokine response, and decreased viral replication [42].

Clinical data on the role of the Complement System in the pathogenesis of SARS-CoV-2-related ARDS is scarce, but certain inferences have been made on its role. The published clinical summaries of severe COVID-19 patients paint a clinical situation that is consistent with excessive complement activation. This includes elevated LDH, D-dimer, and Bilirubin; decreased platelets; mild anemia; renal and cardiac injury; and diffuse TMA [59]. In addition, lung biopsies from severe COVID-19 patients indicated excessive complement activation, which was confirmed with increased C5a levels in the serological tests of these patients [59]. Finally, the clinical condition of COVID-19 patients was seen to rapidly improve upon treatment with anti-C5a antibodies. This involved increased oxygenation of the lungs and decreased systemic inflammation [59].

In particular, it was also noticed that COVID-19 cardiac dysfunction seems to mimic cardiac dysfunction in **atypical hemolytic uremic syndrome-thrombotic microangiopathy (aHUS-TMA)** (condition that is treatable with complement inhibition, that ultimately leads to reversal of cardiac and renal

dysfunction). Predisposition to TMA in COVID-19 might result from genetic predisposition to pathogenic complement activation [42].

Complement inhibition might be a promising treatment for COVID-19, by reducing the innate immune-mediated repercussions of the severe CoV-associated pathology, possibly in combination with antiviral therapy [42]. Further studies need to investigate the effects of targeting specific complement pathways, as well as the time window in which this treatment would be most beneficial. A particularly relevant advantage of complement inhibition, in particular regarding C3 blockade, is that blocking this central component of the innate immune cascade would result not only in the decreased action of C3 itself but also in the decreased generation of downstream mediators such as C5a and IL-6 [59].

DIC, as a secondary condition, can be caused both by pathologic activation and widespread **injury of the endothelium**. We know that **TNFα** is a mediator of endothelial injury, that **deposition of antibody-antigen complexes** can also injury the endothelium (this leads to activation of the classical complement pathway), and it is currently an open question in the literature whether SARS-CoV-2 directly attacks the endothelium via the presence of the ACE2 enzyme on these cells [42,51,59]. It is hypothesized that the increased D-dimer and fibrin degradation products are a result of increased pulmonary vascular bed thrombosis and fibrinolysis, which can lead to downstream pulmonary hypertension-induced ventricular stress, seen in elevated cardiac enzymes (BNP, CK, Troponin I).

The increased inflammation and pro-coagulation factors activated in the cytokine storm already puts the body at risk for cardiovascular events such as myocardial infarction, congestive heart failure, arrythmias, sepsis, and clotting [54]. The development of ARDS in a patient is followed by **sepsis** and **multiple organ failure**. The common features of sepsis involve the signs of infection, organ dysfunction, tachypnea, hypotension, and hepatic, renal, or hematologic dysfunction [54]. At this point also, the virus has reached the circulation and is infecting extra-pulmonary organs. The presence of the virus in the blood, and separately also in anal swabs, is a strong indicator of disease progression and worsened prognosis [60]. It is unclear at this time whether the organ damage is due to a systemic dysregulated immune and coagulation response with widespread endothelial damage, a viral infection of extra-pulmonary organs, or a combination of both. Given the severe decompensation seen in some patients, DIC and microvascular complications leading to both pulmonary and extra-pulmonary ischemic injury are valid possible mechanisms of pathogenesis. Multiple studies have indicated the presence of the virus at extra-pulmonary sites, which is also an indication of disease severity [60,61]. The extra-pulmonary organs primarily involved in a severe COVID-19 infection are the heart, the GI tract, the liver, the kidneys, the CNS, the olfactory tract, and the integumentary system.

Cardiac involvement of COVID-19 makes up a conservative percentage of the total cases, however patients with this presentation have a worsened prognosis. It is not currently clear if the myocardial injury is due to a viral myocarditis or due to the cytokine storm that could cause a secondary injury to the myocardium via respiratory dysfunction and hypoxemia [62,63]. A recent meta-analysis of COVID-19 patients with severe disease showed increased cardiac troponin I in the majority of cases [64]. A case study from Italy described a COVID-19 patient, with no prior cardiovascular complications, who developed severe left ventricle dysfunction and acute myopericarditis [65].

Recent studies also show that the presence of the virus in the **GI tract** has a longer duration than its presence in the respiratory tract, as patients are remaining positive for SARS-CoV-2 presence in stool samples after their respiratory samples have changed from positive to negative [66]. This, along with other studies, supports the hypothesis that COVID-19 also has a fecal-oral mode of transmission. A small portion of patients also present with GI symptoms such as nausea, vomiting, and diarrhea. The ACE2 enzyme is present on both the smooth muscle cells of the GI tract and the enterocytes of the small intestine. This indicates that viral infection and replication can also occur along the GI tract.

Liver damage has been found in patients with severe COVID-19 infection. The ACE2 enzyme was shown to be expressed on bile duct epithelial cells and a very low level in hepatocytes [67]. Additionally, COVID-19 pathological findings indicate "microvascular steatosis and mild lobular activity" [52]. Laboratory analysis of severe patients showed elevated liver enzymes and bilirubin [68]. At this time, it is unclear whether liver pathology is due to direct viral damage, an immune-related injury, or a toxic effect of the drugs administered during hospital stay [67].

Although it has been demonstrated that ACE2 enzyme expression is present in the kidney parenchyma, and that severe cases of COVID-19 are more likely to have elevated blood urea nitrogen and creatinine levels, there are few studies in the literature evidencing the presence of kidney disease in COVID-19 patients [68,69]. One study from China found that the presence of **acute kidney injury (AKI)** on admission or during hospital stay was associated with mortality [70]. Another study showed that patients with higher levels of plasma troponin T were more likely to develop acute kidney injury. The most probably hypothesis is that AKI develops secondary to the sepsis, hypotension, DIC, possible viral infection of these cells, and in-hospital treatment of these patients [47,55]. AKI is seen in a small population of COVID-19 patients and its pathophysiology with respect to the virus is still under discussion [70].

A study of **neurological symptoms** in COVID-19 patients showed that these symptoms more commonly manifest in severe COVID-19 conditions compared to non-severe cases. The most common CNS symptoms are dizziness and headache, while the most common PNS symptoms are hypogeusia and hyposmia. Authors hypothesized that SARS-CoV-2, a respiratory virus, could possibly enter the CNS via retrograde neuronal routes, or a hematological route [71]. A separate study hypothesized that the mode of entry into the CNS could be similar to that of SARS-CoV, where brainstem involvement has been demonstrated. Studies show that other coronaviruses have the ability to infect the medullary cardiorespiratory center from the mechanoreceptors and chemoreceptors in the lung and lower respiratory airways [72]. The target areas in the brainstem are the **nucleus of the solitary tract**, which receives sensory information from respiratory tract, and the **nucleus ambiguous**, which, along with the nucleus of the solitary tract, provides efferent innervation of airway smooth muscle, glands, and blood vessels. One hypothesis is that death could be due to the dysfunction of this cardio-respiratory center in the brainstem [72].

In addition to these hypothesis, a third one proposes entrance from the **olfactory tract** via the **cribriform plate and oral mucosa** [72,73]. There is a high expression of ACE2 enzyme on the mucosa of the oral cavity and SARS-CoV showed cerebral involvement through the cribriform plate of the ethmoid bone. This involvement would lead to altered sense of smell and taste in patients, which was reported by a recent study in Milan, Italy in which patients reported olfactory and taste alteration symptoms. Interestingly enough, these symptoms appeared to be at the initial states of the disease, as their onset was reported by patients prior to hospitalization or in the initial days of hospital stay. Italian doctors have proposed that this could be an early sign of infection, contrasting other extra-pulmonary symptoms that indicate severity of disease progression.

A final presentation of COVID-19 patients included the **integumentary system**, showed dermatological manifestations, which may be an additional aid for the achievement of rapid diagnosis. COVID-19 cutaneous manifestations have an extremely various clinical presentations, including (ordered from the most common to the rarest) maculopapular exanthem (morbilliform, in 36.1% of cases), papulovesicular rash (34.7%), urticaria (9.7%), painful acral red purple papules (15.3%), livedo reticularis lesions (2.8%) and petechiae (1,4%) [74].

Lesions were found to be localized in the majority of cases on the trunk (66.7%), however, 19.4% of patients experienced cutaneous manifestations in the hands and feet [74]. It was also reported that skin lesion development may occur before the onset of respiratory symptoms or COVID-19 diagnosis (described in 12.5% of the patients). In the majority of the studies analyzed by Sachdeva M et al.,

lesions spontaneously healed in all patients within 10 days. Furthermore, the majority of the studies reported no correlation between COVID-19 severity and skin lesions [74].

From the pathophysiological point of view, cutaneous lesions are thought to be caused by different mechanisms. Most of the lesions are considered to result from the hematogenous spread of the virus through the cutaneous vascular system [75]. On the other hand, it is possible that activation of the immune system and creation of immune complexes at the cutaneous level may lead to CD4 + T helper lymphocytes activation, resulting in the production of pro-inflammatory cytokines (IL-1, IFN-γ, and TNF-α) and to the recruitment of eosinophils, CD8+ cytotoxic T cells, B cells and natural killer (NK) cells leading a lymphocytic thrombophilic arteritis [75]. Finally, sepsis or severe viral infections could activate the cytokine cascade inducing a DIC phenomenon.

Laboratory testing that is done on COVID-19 patients includes total Leukocyte counts, Neutrophil counts, Lymphocyte counts, Platelet Counts, Hemoglobin level, Albumin level, PT and PTT Time, D-dimer, Pro-calcitonin, C-reactive protein (CRP), IL-6, Lactate Dehydrogenase (LDH), ALT and AST, Total Bilirubin, Creatine Kinase (CK), and Creatinine [68]. For severe disease, the most common laboratory findings are leukocytosis, neutrophilia, lymphocytopenia, thrombocytopenia, decreased hemoglobin levels, hypoalbuminemia, prolonged bleeding time, positive D-dimer, elevated CRP, increased IL-6 levels, increased LDH, increased ALT and AST, increased total bilirubin, increased CK, and increased creatinine. A positive/increased level of pro-calcitonin is seen in a small population of severe patients, indicating a co-infection.

Diagnostics and Imaging

Triage

Different protocols and guidelines have been adopted by different countries for a first patient access to the hospital. The main plan regards, as suggested by WHO, the modification of the rules for a common access of an ill patient into the hospital (*Figure 2*). All patients are still accepted by emergency rooms, which have been organized table into specific areas for suspected and non-suspected cases in order to limit the possible infection of patients/staff. The protocols require a pre-triage area in which patients are asked to specify their main complaints in order to possibly evaluate and distinguish between a suspected case of coronavirus from a non-suspected one [76]. An example from the protocols, adopted by Humanitas Research Hospital *(Figure 3*) and several emergency departments of the area outside Milan, indicated that the primary indications for a **suspected positive COVID-19 patient** include cough, fever (>37.3C), dyspnea, SpO2 <94% or recent contact with a possibly COVID-19 case.

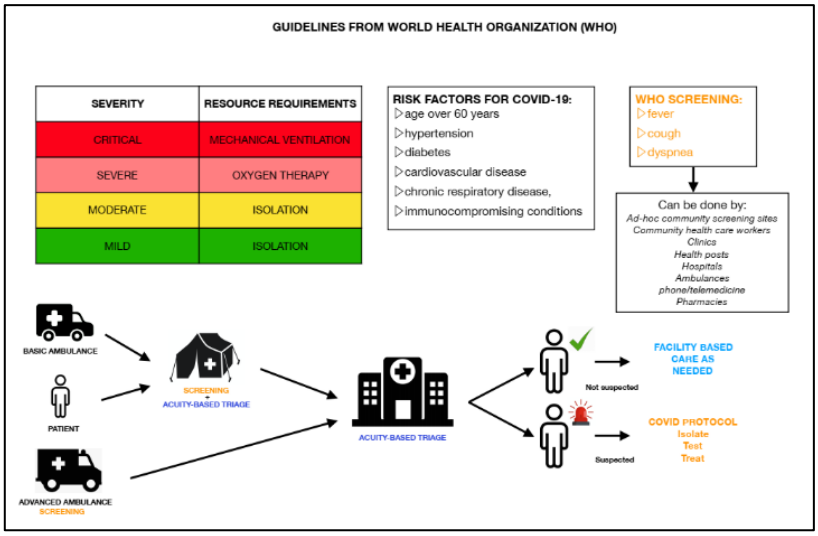

Figure 2 - Case management of COVID-19 in health facility and community [76]

Every patient that presents one of those specific symptoms or that has respiratory symptoms must wear a surgical mask and gloves (after having disinfected his/her hands with an alcoholic solution) [77]. Temperature is best measured with an infra-red device, whereas the oxygen saturation can be assessed by means of a portable pulse oximeter and performing a simple test, known as “walking test”. This test involves the patient fast walking forty steps and then having the oxygen saturation measured. This measurement is compared to baseline and is used to assess whether there is an underlying respiratory disorder. Once the suspected cases have been isolated, it is then possible to assess the severity of the patient. Such assessment is done following some criteria like blood oxygen level and imaging [78].

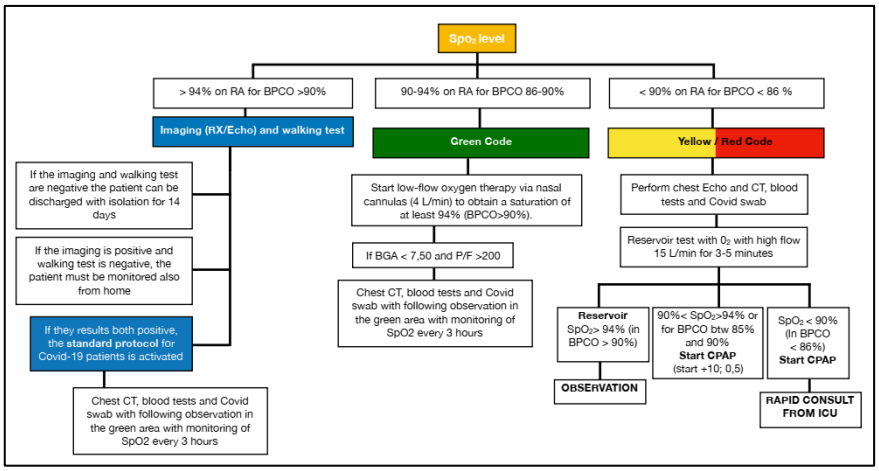

Figure 3 - Protocols adopted by Istituto Clinico Humanitas

Legend: RA = room air, BGA = Blood gas analysis, P/F = PaO2/FiO2 Ratio, ICU = intensive care unit

Physical examination

The WHO report described the typical signs and symptoms seen in over 55,000 positive laboratory confirmed cases. These results are summarized in the table below (*Table 1*), with the major symptoms including fever, dry cough, fatigue, and sputum production and additional recent findings of anosmia,

dysgeusia and possible dermatological manifestations maculopapular exanthem, papulovesicular rash, urticaria, painful acral red purple papules, livedo reticularis lesions and petechiae [74]. The general description of the clinical course is mild respiratory symptoms and fever, that appear on average 5-6 days following infection. Incubation period is described as 5-6 days on average, with a range of 1-14 days [6].

This said, positive Covid-19 patients can have diverse clinical presentations based on symptoms and varying degrees of severity. The CDC has therefore categorized COVID-19 into three stages:

- **Stage 1 Mild to moderate** - Mild symptoms presentation including mild fever, dry cough, nasal congestion, sore throat, general malaise, and myalgia up to mild pneumonia [78]
- **Stage 2 Severe** – Increased severity of symptoms including high fever, severe dyspnea, severe respiratory
- distress, hypoxia (SpO2 < 90% on RA), and tachypnea (> 30 breaths/min). A diagnosis for severe pneumonia can be made clinically or with imaging to assess further complications [78]
- **Stage 3 Critical** – Respiratory failure, with varying levels of ARDS. This is the main indication for transfer/admission of patients to the Intensive Care Unit, as well as a predictor of poor prognosis and mortality. Also shock and multiorgan system disfunction are part of this category of patients [78]

Signs and Symptoms	%
Fever	87.9
Dry Cough	67.7
Fatigue	38.1
Sputum production	33.4
Shortness of breath	18.6
Sore throat	13.9
Headache	13.6
Myalgia or Arthralgia	14.8
Chills	11.4
Nausea and Vomiting	5.0
Nasal congestion	4.8
Diarrhea	3.7
Hemoptysis	0.9
Conjunctival Congestion	0.8

Table 1 - Signs and Symptoms and their percentage [44]

Patients with mild symptoms may not present positive signs of the disease, sometimes patients may be also totally asymptomatic, but with a positive swab test. Patients in severe conditions develop shortness of breath, moist rales in the lungs, weakened breath sounds, dullness in percussion and increased or decreased tactile speech tremor. The outcome of a complete physical examination depends on the additional comorbidities of the patients and on the organs involved [44]. An additional tool to define the disease progression was highlighted by Tan et al. in an article published by Nature, where they sustained the fact that lymphopenia could be considered a predictor of prognosis in COVID-19 patients, and in particular, patients with a lymphocyte level of less than 5% are normally critically ill with high mortality rate and a need of ICU admission [79,80].

Diagnostics

The current standard method for the diagnosis of COVID-19 is a real time reverse transcription polymerase chain reaction (RT-PCR) test. This is based on the collection of specimens from mainly of the upper respiratory tract (naso-oropharyngeal samples), lower respiratory tract (expectorated sputum; endotracheal aspirate; bronchoalveolar lavage), and less frequently, fecal samples. Broncho-alveolar lavage (BAL), following Doctor Simpson's report, is considered the most sensitive method, and can be performed specifically in mechanically ventilated patients. Given that BAL is an aerosol-generating procedure, health care workers are more exposed to the virus particles and at a greater risk of contracting the virus. Considering the swab methods, sputum and nasopharyngeal swabs are equivalent in sensitivity while a throat swab is less sensitive [81].

Studies from China and published in The Lancet detail an extended duration of viral shedding in the feces, indicating a possible fecal-oral transmission, which was already seen in SARS-CoV and MERS-CoV viruses. An RT-PCR of stool samples is therefore indicated following the clearance of RNA presents in a patient's respiratory sample. There are no suggestions for this test to become a common diagnostic tool, or for its use as a replacement diagnostic. Although, GI involvement has been reported and potential fecal-oral transmission could increase the risk of infection in public places like dormitories, hotels, public toilets, and cruise ships [26].

One or more negative tests do not rule out the possibility of a SARS-CoV2 infection. In the case of conflicting test results, WHO Guidelines state that a swab test must be re-done. A number of factors could lead to a negative result in an infected individual, including poor quality of the specimen, poor handling and shipping, too early/late testing, or technical reasons due to DNA mutations or PCR inhibition. In the specific case of a swab test of the upper respiratory tract being negative, but the patient presenting signs and symptoms of COVID-19, the best solution is to re-do the swab test but with a sample from the lower respiratory tract [82].

Imaging

RT-PCR is currently the required laboratory test to confirm the diagnosis of COVID-19, even though early reports of test performance showed variable sensitivities, ranging from 37% to 71% [83,84]. Since during a pandemic the primary task is the identification and isolation of positive patients in order to contain the dissemination of the infection and to protect healthcare personnel, imaging can be used as an additional tool to better identify positive patients.

Differently to the position of the major Radiological Scientific Societies in Europe, UK and US, a consensus paper from the Fleishner Society advises the use of imaging in patients with COVID-19 and worsening respiratory status and in a resource-constrained environment for medical triage of patients with moderate-to-severe symptoms and high-pretest probability of disease [83,85,86,87,88,89]. The choice of the imaging modality, either chest X-ray or computed tomography (CT), is left to the judgment of clinical teams, according to local expertise and resources [83].

Computed Tomography (CT)

Common CT findings

Diffuse alveolar damage and inflammatory exudation are some of the pathological mechanisms involved in COVID-19 patients. Consequently, the most common corresponding CT abnormalities are **ground glass opacities (GGO)** (*Figure 4*) and **consolidation** (*Figure 6*). Pulmonary damages are frequently bilateral and multilobar, with a predominant distribution in the peripheral and posterior areas of the lungs [88,89,90,91,92,93,94]. Less common features include reticular and/or interlobular septal thickening, crazy paving pattern and bronchiectasis. On contrast, pleural and pericardial effusions and lymphadenopathy are rare findings, described only in a small proportion of patients (*Table 2*) [89,92,94].

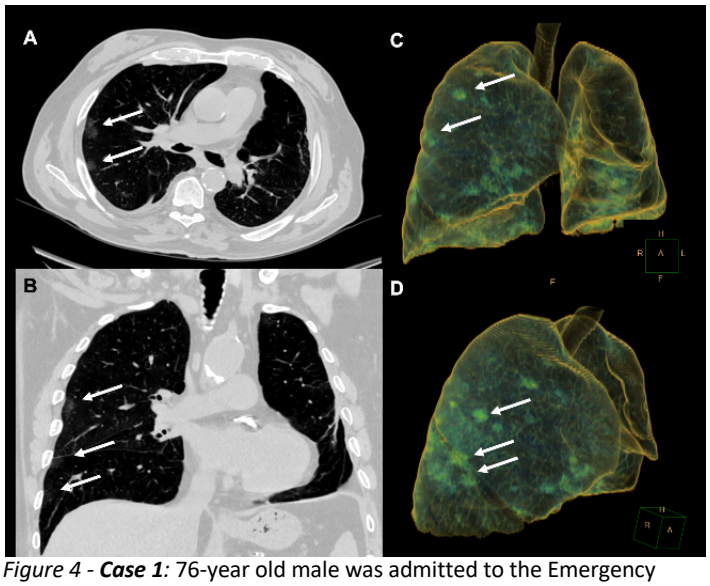

Figure 4 - ***Case 1****:* 76-year old male was admitted to the Emergency Department due to fever and dyspnea for few days. RT-PCR diagnosed COVID-19 infection. *Axial (A) and coronal (B) non-contrast-CT images and corresponding volume rendered three-dimensional CT of the lungs (C, D) showed few peripheral ground glass opacities (arrows) within the lower and middle lobes of the right lung.*

Vascular changes deserve a special mention. Although this aspect has been less investigated than parenchymal abnormalities, Caruso et al. reported sub-segmental vascular enlargement in 89% of confirmed COVID-19 patients [94]. In addition, Bai et al. demonstrated that vascular thickening was more commonly identified in COVID-19 patients than in patients with other types of viral pneumonia (59% vs. 22%, respectively) [95]. Despite the fact that the underlying mechanism of vascular pathology is still unknown, this finding has been related to the effect of pro-inflammatory factors released locally during the infection [96].

While GGOs have been described in the absence of consolidation, pure consolidative lesions have been reported only in a very limited proportion of patients, indicating that they represent a more advanced stage of the disease [89,92]. GGOs are indeed more frequently reported than consolidation in asymptomatic patients and in the early stage of the disease with a time interval between the onset of symptoms and chest CT scan < 4 days [93]. Differences in the incidence of imaging features were not found between hospitalized and non-hospitalized patients [94].

Recent studies have identified the presence of acute pulmonary embolism as a common complication of COVID-19 infection, determining direct admission of patients to the critical care unit [97]. Therefore, D-dimer test and CT pulmonary angiography may be considered as additional diagnostic tools in these patients [98,99].

CT findings according to disease stage

Several groups analyzed the most common CT findings according to different phases of the disease time-course (*Figure 5)*. All studies reported an initial phase of progressive increase in the number and severity of lung lesions followed, first, by a short plateau period and, subsequently, by a gradual resolution of lung changes [92,100,101]. In particular, Pan et al. identified four temporal stages of the disease [100]:

(1) *Early stage* (0-4 days after beginning of symptoms): GGO is the most representative feature with mainly sub-pleural distribution in the lower lobes, unilaterally or bilaterally.

(2) *Progressive stage* (5-8 days): GGO, crazy-paving pattern and consolidation coexist with bilateral and multilobar distribution.

(3) *Peak stage* (9-13 days): consolidation is the prevalent finding but GGO, crazy-paving pattern and residual parenchymal bands are still present.

(4) *Absorption stage* (≥14 days): consolidations are gradually reabsorbed and GGOs are now present as a sign of the healing process.

Further long-term longitudinal studies are warranted to evaluate potential pulmonary sequelae and their relationship with residual respiratory function.

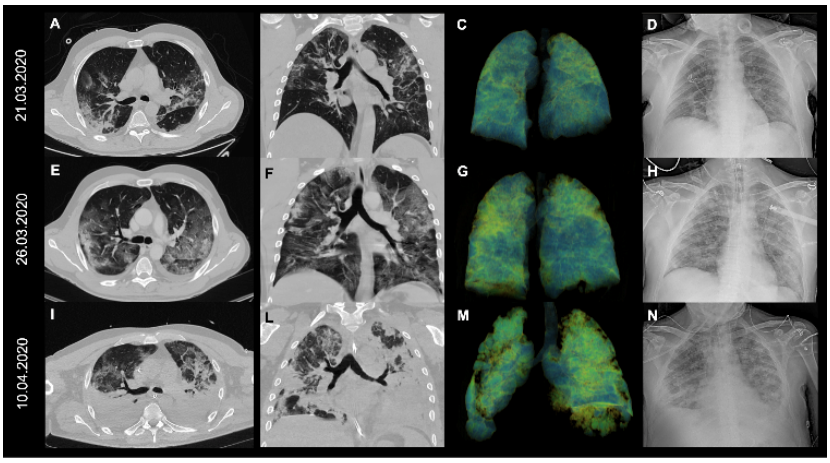

*Figure 5 - **Case 2**:* 55-year old man presented to the Emergency department with fever for 10 days. Peripheral capillary oxygen saturation was 88%. RT-PCR diagnosed COVID-19 infection.
Axial (A, E, I), coronal (B, F, L) and volume rendered three-dimensional (C, G, M) non-contrast CT images (C). At hospital admission CT (A, B, C) showed bilateral ground glass opacities associated to interlobular septal thickening (crazy-paving pattern) and partial consolidation with a predominant peripheral distribution.
CT (E, F, G) performed during the 5th day of hospitalization demonstrated enlarged region of ground glass opacities with partial consolidation. After two weeks
CT (I, L, M) showed significantly increase of the consolidative areas mainly located in the posterior regions of the lungs. Chest X-rays (D, H, N) demonstrated progressive increase of the number and the size of the patchy opacities within both lungs. In addition, pleural effusion was detected on the chest X-ray performed on April 10th (N).

The knowledge of disease progression is of paramount importance not only to understand the natural history of the disease but also to predict potential complications or negative patient outcomes [92]. In this perspective, a more objective and reproducible method than visual assessment is required to assess the percentage of lung parenchyma involved by the disease. So far, several semi-quantitative CT scores have been developed calculating the number of lung segments with abnormalities and the rate of involvement of each segment. Pan et al. demonstrated that lower pulmonary lobes had higher CT score compared to upper and middle lobes [100]. Similarly, Yang et al.

confirmed the predominant involvement of the lower lobes [102]. An optimal CT score threshold of 19.5 (maximal total score: 40) has been reported to identify patients with severe clinical symptoms compared to mild cases, with a sensitivity of 83% and a specificity of 94%. Inter-reader agreement for CT score calculation was excellent [102]. A further step toward the development of a fully quantitative parameter as surrogate of disease burden was obtained by the group of Huang et al. [103]. Despite the small sample size, they demonstrated that lung opacification percentage calculated by deep learning algorithm differed significantly among patients at different clinical stages of the disease. Furthermore, visual and software-based quantification of well-aerated lung parenchyma on chest CT at admission were identified as independent predictors of ICU admission and death in an Italian population of 236 COVID-19 patients [104].

Diagnostic accuracy

Several publications investigated the accuracy of CT in the diagnosis of COVID-19 having RT-PCR as reference standard (*Table 3*). While reported sensitivities are consistently high, ranging between 72% and 97%, data on specificity vary greatly, from 24% to 94% [90,94,95]. These data have been confirmed by a recent meta-analysis, which showed a pooled sensitivity and specificity of 94% and 37%, respectively [105]. The low specificity may be first explained by the intrinsic limitations of the test assumed as reference standard. Although RT-PCR is still required to confirm the diagnosis of COVID-19, its rate of false negative results, especially in the early phase of the disease, is not negligible [90,100,106]. In particular, Ai et al. highlighted that RT-PCR turned from negative to positive in 10/15 (67%) patients, of those 14/15 (93%) had initial positive findings on CT.

On the other hand, CT findings commonly described in COVID-19 patients (e.g. GGO and consolidation) are neither unique nor specific [107,108]. Since they are signs of acute lung injury, they are frequently seen in other infections such as H1N1 influenza virus, SARS, H5N1 or H7N9 pneumonia, and non-infectious diseases [91,107,108]. Therefore, positive CT findings should be always interpreted taking into account the prevalence of the disease and the patient's pretest probability [105]. More specifically, the typical imaging pattern for COVID-19 should be interpreted with caution outside the pandemic time.

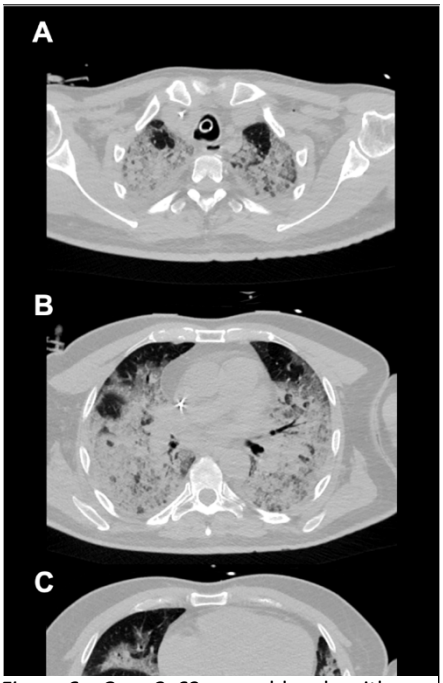

*Figure 6 – **Case 3**:* 63-year old male with confirmed diagnosis of COVID-19 was intubated due to respiratory insufficiency. *Axial non-contrast-CT images (A, C, C) showed extensive consolidations involving both lungs.*

Chest X-ray

Wong et al [101] described the principal radiographic features of COVID-19 pneumonia showing that common chest X-ray features mirror those described on CT imaging. In particular, consolidation has been reported as the most common finding, followed by ground glass opacity and pulmonary nodules. The distribution of the damage has been mainly described in peripheral and lower zones and most of the abnormalities presented bilateral involvement. Signs of pleural effusion were rare. Nevertheless, the sensitivity of chest X-ray in the detection of lung abnormalities is only 25% when compared to CT [109]. The main factors influencing the detectability of lung lesion on chest X-ray are lesion density and volume [109].

Ultrasonography

Lung ultrasound (US) is a surface imaging technique. Therefore, it is well suited for the detection and evaluation of the typical COVID-19 abnormalities, which present mainly a peripheral distribution [110]. Of note, use of lung US may prevent nosocomial spread of the virus limiting the number of health care

workers and medical devices exposed to suspected or confirmed cases of COVID-19 [110,111]. The typical lung abnormalities detected by US in COVID-19 patients are as follows:
(1) Irregular, thickened pleural line with discontinuities.
(2) Multiple, focal, or diffuse B-lines representative of thickened sub-pleural interlobular septa.
(3) Sub-pleural consolidations with possible discrete and localized pleural effusion.
(4) Alveolar consolidation with static and dynamic air bronchograms associated with severe and progressive disease [112].
Experience is required to generate high-quality and reproducible images [110].

Positron emission tomography (PET/CT)
Although not routinely employed in the diagnostic work-up of COVID-19 patients, 18F-FDG PET/CT may allow for a better understanding of the pathological mechanisms involved in COVID-19 pneumonia. Currently, only few, isolated cases of COVID-19 pneumonia documented by 18F-FDG PET/CT have been described reporting peripheral GGOs and consolidative opacities in more than two pulmonary lobes [113,114]. The peculiarity of these lesions is the high FDG uptake reflecting a significant inflammatory reaction, which leads to the activation of several inflammatory cells. Among these, activated neutrophils are strongly dependent on anaerobic glycolysis resulting into a higher consumption of glucose and, therefore, in high FDG uptake [114]. In addition, increase in nodal FDG uptake has been detected without significant nodal enlargement [113,114].

Role of imaging in extra-pulmonary findings

Heart
Cardiac involvement has been detected in many patients infected by SARS-CoV 2 as a result of myocarditis, microvascular dysfunction and systemic inflammatory response [64,65,115,116]. Since cardiac involvement has been associated with an increased risk of mortality, a longitudinal monitoring of cardiac biomarkers is strongly suggested from the admission and during hospital stay to identify this subgroup of patients [64,117]. On the other hand, cardiac imaging is advised only if it is likely to change patient management, following all the hospital's protection guidelines in order to limit at minimum the possible transmission [118].

Central nervous system
A single case report of acute necrotizing encephalopathy (ANE) associated to COVID-19 disease has been recently published [119]. Imaging features described are as follows: (1) symmetric, bilateral hypoattenuating lesions within the thalamus on non-contrast CT (2) bilateral hemorrhagic rim enhancing lesions within the thalamus, medial temporal lobes and sub-insular regions [119].

Tables

Table 2 - Prevalence of CT lung abnormalities in COVID-19 patients

Author	N°of pts	Days	Bilat. involve	Periph Distrib	Post Distrib	Multi-lobar involvement	GGO	Cons	Pleural effusion	Pericardial effusion	Lymph.
Chung M et al [88]	21	Adm	76%	33%	-	71%	57%	29%	0%	-	0%
Pan F et al [100]	21	0-4	42%	54%	-	42%	75%	42%	-	-	-
		5-8	77%	59%	-	77%	82%	47%	-	-	-
		9-13	86%	62%	-	86%	71%	91%	-	-	-
		> 14	80%	70%	-	80%	65%	75%	-	-	-
Bernheim A et al [92]	36	0-2	28%	22%	-	28%	44%	17%	0%	-	0%
	33	3-5	76%	64%	-	78%	88%	55%	0%	-	0%
	25	6-12	88%	72%	-	92%	88%	60%	0%	-	0%
Caruso et al [94]	58	Adm	91%	89%	93%	93%	100%	72%	3%	5%	58%
Ming-Yen et al [91]	21	3	-	86%	-	-	86%	62%	0%	0%	0%

Legend
Cons, consolidation; GGO, Ground glass opacity; Lymph, Lymphadenopathy

Table 3 - Prevalence of CT lung abnormalities in COVID-19 patients

Study	Patients enrolled	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Ai, T et al [90]	1014	580	105	308	21	97 (96-98)	25 (22-30)	65 (62-68)	68 (65-70)
Caruso, D et al [94]	158	60	54	42	2	97 (88-99)	56 (45-66)	59 (53-64)	96 (87-99)
Bai, HX et al [95]*	424	158	192	13	61	72 (66-78)	94 (89-97)	92 (87-96)	76 (70-81)
Bai, HX et al [95]**	424	157	181	24	62	72 (65-78)	88 (83-92)	87 (81-91)	74 (69-80)
Bai, HX et al [95] ***	424	206	49	156	13	94 (90-97)	24 (18-30)	57 (52-62)	79 (67-88)

Legend
TP, true positive; TN, true negative; FP, false positive; FN, false negative; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.
* Reviewer 1; ** Reviewer 2; *** Reviewer 3

Pharmacology

Standard clinical treatment

Beyond symptomatic and empirical treatment, there are no currently approved drugs that directly target the SARS-CoV-2 virus in patients [55,77]. However, some present protocols give indications on how to stabilize the patient and avoid further complications. It is of extreme importance to rapidly recognize patients with possible severe hypoxemic respiratory failure due to ineffective standard oxygen therapy and then provide the appropriate advanced ventilatory support.

The WHO has approved several protocols for the primary treatment of COVID-19 positive patients based on the severity of symptoms, which include:

- **Mild symptoms** - symptomatic treatment, such as antipyretics
- **Severe symptoms** with possible **respiratory distress, hypoxemia,** or **shock** - oxygen therapy to achieve at least a $Sp0_2$ > 94% with continued monitoring of patient
- **Sepsis** or **severe acute respiratory infection** - empiric antimicrobials to treat all likely pathogens on the basis of the microbiology results and clinical judgment [77].

Antimicrobials with potential activity against Covid-19

Several studies are underway looking at potential antiviral direct targets to SARS-CoV-2, including some medication previously used in other infections. These medications include **chloroquine**, **hydroxychloroquine**, **lopinavir/ritonavir**, and **Remdesivir**. These drugs, their actions, and potential side effects are summarized in Table 4 (*Figure 1*).

Chloroquine is an antimalarial drug whose mechanism of action is that it binds to and inhibits DNA and RNA polymerase and inhibits prostaglandin effects, among other effects. Wang 2020 hypothesized that this drug may change the pH at the cell membrane surface and inhibit viral fusion, and/or it could inhibit the glycosylation of viral proteins [120]. **Hydroxychloroquine** is a second antimalarial drug, more potent than chloroquine, that has immunosuppressive, anti-autophagy, and antimalarial effects. Its direct mechanism of action is not fully clear, but it interferes with antigen presentation and cytokine production and raises intralysosomal pH [121].

Lopinavir/Ritonavir is a drug combination used to treat HIV. They are both anti-retroviral drugs that are protease inhibitors, which prevent viral replication. This action is hypothesized to be a potential mechanism of action in SARS-CoV-2 replication. **Remdesivir** is a broad-spectrum antiviral whose mechanism of action is an adenosine analogue which causes premature termination of nascent viral RNA chains of virus. This drug was effective in treating SARS and MERS [122]. It interferes with the activity of RNA-Dependent RNA polymerase, which is used by coronaviruses to replicate [123,124].

Name of the drug	Mechanism of the drug	Side effects
Chloroquine phosphate	Block of viral entry into the cells by inhibition of glycosylation of host receptors, proteolytic processing, and endosomal acidification + immunomodulation	QT prolongation, hypoglycemia, neuropsychiatric disorders, and retinopathy
Hydroxychloroquine sulphate	Same mechanism of action of Chloroquine, but is more potent	QT prolongation, hypoglycemia, neuropsychiatric disorders, and retinopathy
Lopinavir/Ritonavir	Agent for treating HIV, it inhibits the 3-chymotrypsin-like protease	GI distress such as nausea and diarrhea + hepatotoxicity
Ribavirin	It is inhibiting viral RNA-dependent RNA polymerase	Hematologic and liver toxicity
Remdesivir	Interferes with the viral RNA-dependent RNA polymerase and evades proofreading by viral exoribonuclease, causing a decrease in viral RNA production	Nausea, vomiting and possible liver toxicity

Table 4 - Antimicrobials with potential activity against SARS-CoV-2 [25]

Supportive and adjunctive therapy for the management of the patient

Beyond potential antimicrobial treatments to target SARS-CoV-2, there are additional therapies in use/under study that target the immune system of the patient. These include **IL-6 receptor inhibitors**, **anti-IL-1 and IL-1 receptor antagonist, convalescent plasma transplant,** and **corticosteroids** [125]. The primary target in these supportive therapies is the inhibition of the excessive inflammation that comes from an uncontrolled immune response and the reduction of damages is induced by inflammatory cytokines such as **IL-1, IL-6, TNF, and others.** Monoclonal antibodies like **anti IL-1/6 and receptors antagonists** have been proved effective in the reduction of the inflammatory response [55] (*Table 5*).

Name of the drug	Mechanism of the drug	Side effects
Monoclonal Antibodies (Tocilizumab and Sarilumab)	Dampen the "cytokine storm" caused by IL-6 thanks to their activities as IL-6 receptor antagonists and improve clinical outcomes	Headache, HTN, hematologic and liver toxicity, infections, GI perforations and hypersensitivity reactions
Anti-IL-1 (Canakinumab) and IL-1 receptor antagonist (Anakinra)	The first binding to IL-1β and the second one preventing the binding of IL-1β and IL-1α and blocking signal transduction	GI symptoms, headache Skin reactions and possible cold symptoms
Convalescent Plasma	Via passive antibody therapy, a means of providing immediate immunity to susceptible persons. In SARS-CoV-2 possible sources of antibodies are only human convalescent sera from individuals who have recovered from COVID-19.	
Corticosteroids	Decrease host inflammatory responses in the lungs, in order to prevent possible acute lung injury and ARDS	Possible delayed viral clearance with increased risk of secondary infections

Table 5 - Supportive and adjunctive therapy for patients infected by SARS-CoV-2 [25,55]

Immunosuppressant drugs

Cytokine targeting therapy has been at the center of attention of several researches. Similarly, to H1N1 influenza, SARS-CoV-2 seems to trigger a cytokine storm in a subcategory of patients, therefore specific trials have been done to target the inflammatory cytokines involved in the cytokine storm syndrome (CSS) [126]. A possible explanation for this CSS could be a macrophage activation syndrome (MAS)-complicating pneumonia based on the elevated levels of CRP, hyperferritinaemia and a specific cytokine profile based on increased IL-1β, IL-2, IL-6, IL-17, IL-8, TNF and CCL2 [51]. Immunosuppressant drugs have been considered to be potentially beneficial in patients with an overactivation of inflammatory mediators and therefore an exaggerated inflammatory reaction [55].

ANTI IL-6

Tocilizumab

A clinical trial was carried out in China with a specific drug called **Tocilizumab**, which is a humanized monoclonal antibody and specifically an IL-6 receptor blocker (*Figure 7)*; this drug has previously been approved for use in the case of cytokine release syndrome and as an anti-rheumatic [55,126]. IL-6 is a cytokine specific for the activation of the inflammatory response and is implicated in a number of different pathological processes. In blocking its receptors, the drug can inhibit the JAK-STAT signaling pathway that is responsible for processes such as the immune response, cell division, cell death, and tumor formation. Janus kinase (JAK) inhibition might affect both inflammation and viral entry in COVID-19 patients. In every patient with a severe COVID-19 infection it is important to check for inflammatory markers such as ferritin, platelet counts or erythrocyte sedimentation rate to better identify the groups of patients for whom immunosuppression could be most dangerous [127].

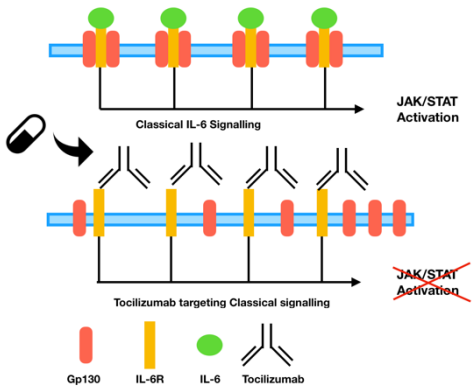

Figure 7 – Scheme of the action of Tocilizumab

ANTI IL-1

Interleukin-1 (IL-1) is a specific and highly active pro-inflammatory cytokine central in the inflammatory response, that drives the IL-6 signaling pathway, contributing to damage tissues. IL-1 interacts with type 1 IL-1 receptor (IL-1R1) and the adaptor protein IL-1RAcP triggering signal transduction and NF-kB activation. To limit IL-1-dependent hyperinflammatory responses, two main drugs are used: Anakinra (IL-1Ra) and Canakinumab (anti IL-1β) (*Figure 8*) [128,129].

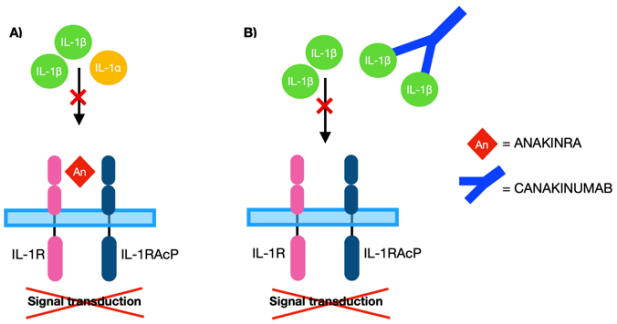

Figure 8 - Scheme of the mechanism of action of Anakinra and Canakinumab

Anakinra is a recombinant IL-1 receptor antagonist and was approved in the past for the treatment of rheumatoid arthritis and inflammatory diseases. The efficacy of this drug is based on the block of IL-1α or IL-1β activity and downregulation of signal transduction [130].

Canakinumab is a human monoclonal antibody specifically binding and inhibiting IL-1β, reducing inflammation ameliorating the condition of the patient [131].

Convalescent Plasma

A study by Doctor Shen from Shenzhen reported the benefits of **convalescent plasma transfusion** in the treatment of critically ill patients with SARS-CoV2 infection. In particular, the parameters for the selection of specific patients were (1) respiratory failure and mechanical ventilation, (2) shock and elevated lactate levels despite fluid resuscitation, and (3) multiorgan failure requiring admission to ICU. While the parameters for possible donors were (1) testing positive for coronavirus and (2) asymptomatic after the infection for at least 10-14 days. Several tests were done on donors including swabs for SARS-CoV2, respiratory viruses, HIV, HBV, HCV, and syphilis. After a treatment with various antiviral agents and steroids, in between the 10 and 22 days after admission, convalescent plasma was administered to five patients. Following transfusion, inflammatory biomarkers like CRP, procalcitonin and IL-6 decreased, and CT also demonstrated an improvement of the pulmonary lesions and gradual resolution. Body temperature was reduced, pO2/FiO2 (P/F) improved and the correlation between high viral loads and disease severity and progression was demonstrated. This study was based on significant evidence from prior tests on patients with MERS, SARS, and influenza A (H1N1) and the only limitation was the small case series and the uncertainty that the improvement was due to only the infused plasma. Undoubtedly, the P/F improvement is a good indicator/predictor [132].
The FDA, on March 26 2020, approved, on the basis of past effective treatments for polio, measles, and mumps respiratory infections, the convalescent plasma containing antibodies against the SARS-CoV2 as a possible effective treatment [133].

Are NSAIDs dangerous, can they worsen the infection?

The European Medicine Agency has analyzed the fact that Ibuprofen and other NSAIDs could worsen the illness caused by SARS-CoV 2 based on the fact that the National Agency for the Safety of Medicines and Health Products of France has suggested that the infections from chicken pox and other bacterial ones could be exasperated by the use of anti-inflammatory drug and so there is a current doubt as to whether it can also be valid for coronavirus or not [134]. In fact, in the warning messages of the same non-steroidal anti-inflammatory drugs stat that they can mask the symptoms of a worsening infection, but the European Medicines Agency decided, in accordance with all the national guidelines, that physicians can continue to prescribe anti-inflammatory drugs with all the already existing recommendations and so use them at the lowest effective dose for the shortest period possible.
An additional article was published by BMJ that clarified the position on NSAID use, in particular referring to past observational studies [135]. Prior to the outbreak of SARS-CoV-2, these studies showed

that the long-term use of NSAIDs such as Ibuprofen, Naproxen and Diclofenac lead to a higher rate of cardiovascular damage, such as myocardial infarction, heart failure, and stroke. Even a short-term use of NSAIDs could be associated with an increased cardiovascular risk. Recent studies have also shown that NSAID use increases the risk of complications and the dissemination of infection following respiratory tract infections, PNA, or pleural effusions. The explanation could be the fact that these drugs inhibit cyclooxygenase, inhibit the synthesis of resolvins, delaying a possible tissue resolution [136].

Across the board all studies indicate possible complications of respiratory tract infections due to NSAIDs use, but, on the other hand, the data obtained are not considered strong enough suspend their use because in some trials it appeared that intermittent or occasional use could help patients infected by SARS-CoV2, for example to relieve night symptoms and let the patient sleep in order to help the immune system.

To summarize, all patients with possible chronic disorders should continue their therapy, like in the case of aspirin use (that has an anti-inflammatory effect only at dosages like 1-4g per day). Accordingly, there is strong evidence of a link between NSAIDs administration and possible respiratory or cardiovascular adverse effects, but until now they are limited to the common treatments and there is no evidence or link to the worsening of COVID-19 patients due to NSAIDs administration.

Discussion of ACE-I and Anti-HTN Medicine

The Council of Hypertension of the European Society of Cardiology has recently published a specific statement on the importance of the continuation of the usual treatments for hypertension in COVID 19 patients, because there is no clinical or scientific evidence that suggests treatment with its ACE-I or ARBs should be discontinued due to infection from Covid-19 [137]. The main doubts of the scientific community came out from the specific interaction of the virus with the ACE2 receptors inside the lungs, specified in the article "*Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection?"* published by the Lancet Journal, which focused mainly on the clinical aspect [138]. From their point of view, being ACE2 (expressed in epithelial cells of lungs, kidneys, blood vessels and intestine) a specific target for SARS-CoV and SARS-CoV2, the expression of ACE2 receptors is increased in hypertensive patients and diabetic patients treated with ACE-I or ARBs. The authors hypothesized that an upregulation and overexpression of ACE2 could lead to an increased viral entry into the cells, putting the patient at a higher risk for severe or fatal COVID-19 infection [139]. Actually, patients that take ACE-I or ARBs generally do have higher risk for severe COVID-19, not because of the drugs but because of the cardiovascular diseases they are being treated for; theoretically, it might be good to monitor them for ACE2 modulating medications and Ca channel blockers could be a possible alternative in order to avoid such risks, but, practically, there are no such evidences for COVID-19 patients and then treatments must be continued. On the other hand, ACE2 receptors are important also as reducers of inflammation and have been suggested as a possible new therapy against inflammatory lung diseases.

Conclusion

Having considered the existing literature on the topic COVID-19 published mainly between December 2019 and April 2020, some possible considerations can be made. In particular, we concluded that SARS-CoV-2 is a highly infective and environmentally resistant virus that in particular was showed to be more infective than SARS-CoV despite the lower mortality ascertained to date. Both the exact phylogenetic origin and the array of possible animal hosts are not completely understood yet even though various studies are being performed.

The exact mechanism of entry of the virus involving the ACE2 enzyme and host proteases such as TMPRSS2 and furin, has been properly defined and the same is valid for the generalities of the intracellular life cycle and viral genetics. Despite this, a knowledge gap still exists regarding the function of various viral proteins, genetic regulatory mechanism, and interactions with host factors. Having recognized that the virus is not yet fully known and that infected patients are not said to have developed adaptive immunity; it is really dangerous to consider herd immunity as a possible choice. For most patients, this disease manifests and resolves as a respiratory illness. Severe cases, on the other hand, involve a cytokine storm and extra-pulmonary illness including multi-organ failure and sepsis.

Triage is very important in this time of pandemic to correctly identify and isolate positive COVID-19 patients, in order to limit the spread of the infection. After accurate assessment of patient symptoms, RT-PCR is required to confirm the diagnosis. Imaging is an additional diagnostic tool, which helps clinicians not only in the diagnosis but also in the follow-up and in the prediction of potential complications related to COVID-19 infection.

Open questions

Is fecal-oral route really an option for viral transmission? Is intrauterine and vaginal transmission also possible? Why are children less affected than adults, is there a possible explanation for this? Is it a real possibility that a new pandemic event is going to develop next autumn? Is this kind of virus going to become a new seasonal type of respiratory virus?

Limitations of the study

Being medical students, we do not have the clinical experience in order to give a clear idea of the impact of the virus and our ability to research and discriminate among literature was limited by the continuously growing number of published papers on the topic.

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