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TESI SPERIMENTALE DI DOTTORATO

Risk factors for respiratory function deterioration in patients hospitalised for COVID-19: The ASCL (Age, Sex, CRP, LDH) score

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Summary

1.0 Introduction

The COVID-19 pandemic is a serious threat for global health, with an unprecedent impact over the last 100 years. The last epidemic event of such a dramatic importance, in terms of morbidity and mortality, was probably the "Spanish Flu" in the years 1918-1919, which caused approximately 50-100 million deaths.

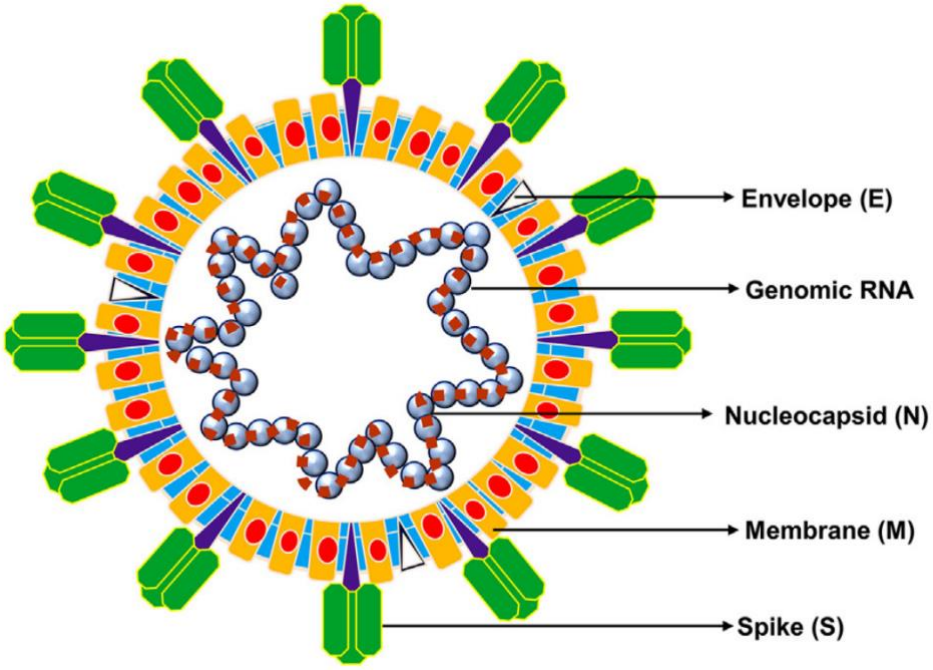
The COVID-19 (which stands for *Coronavirus disease – year 2019*) firstly appeared in the last months of the year 2019 in the Chinese province of Hubei, with its epicentre in the city of Wuhan. Its presence was officially notified by China to the World Health Organization (WHO) in December 2019. The aetiological agent of COVID-19 was recognized in a coronavirus which is strictly related to the virus responsible for the severe acute respiratory syndrome (SARS) and, thus, it was formally named SARS-CoV-2.

Coronaviruses have often been considered like commonplace seasonal respiratory viruses. In the last 18 years however, they have been responsible for severe diseases with high mortality rates: the SARS in the year 2002 and the MERS (*Middle East Respiratory Syndrome*) in the year 2012, with the latter still causing sporadic cases of disease. The disappearance of SARS after two years, as well as the limited geographical spread of MERS, probably caused an underestimation of COVID-19 epidemic at its very beginning. It was indeed considered confined to China and some neighbouring Asiatic countries and reputed extinguished after specific confinement measures (lockdown). Unfortunately, the COVID-19 globally spread with astonishing rapidity until the World Health Organization declared the SARS-CoV-2 infection a pandemic in March 2020. Although the lungs are the main target for SARS-CoV-2 infection, COVID-19 may be also responsible for injury of several organs and can cause disabling *sequelae*. Aside from the medical consequences, the COVID-19 pandemic also caused social and economic implications. In fact, in the year 2020 there was a profound fall of the global Gross Domestic Product (GDP) as a consequence of the pandemic. The GDP fall was even greater than the one documented after the great depression in the year 1929. Despite the availability of effective vaccines against SARS-CoV-2 in 2021, the COVID-19 pandemic still represents a serious hazard after two years from its detection, with significant medical, social, and economic consequences worldwide.

1.1 Aetiology

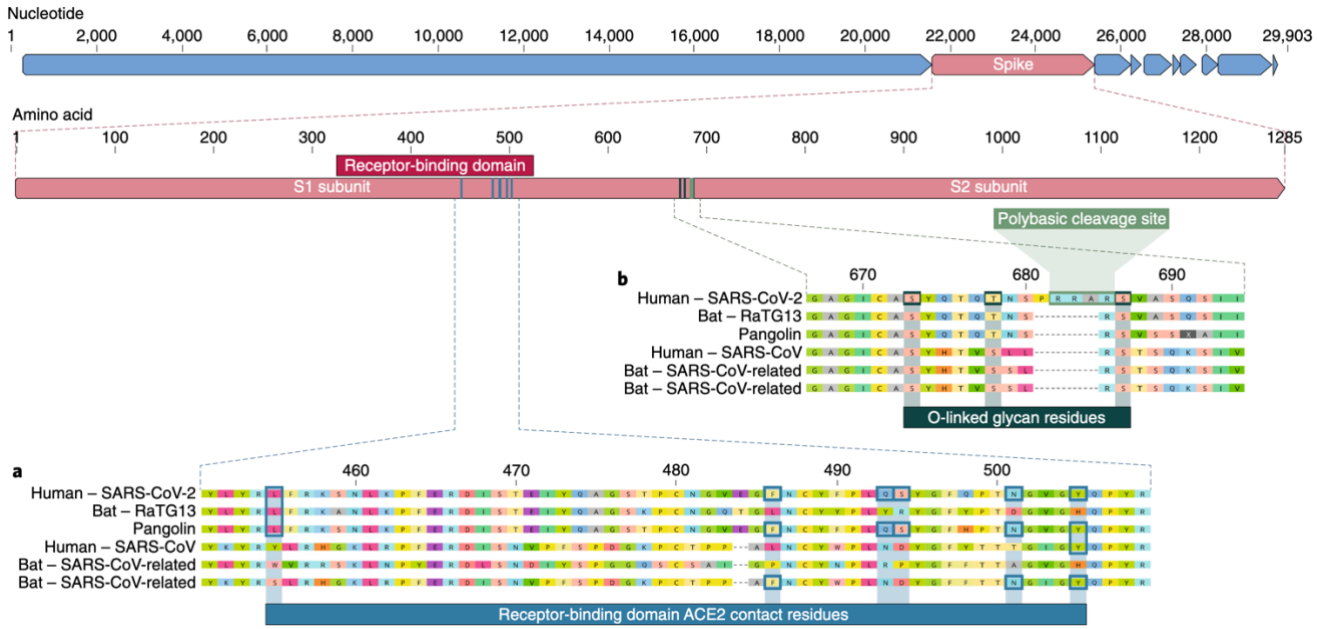
SARS-CoV-2 is a *Betacoronavirus* with single-strand RNA of the *Coronaviridae* family (order *Nidovirales*, sub-order *Corononavirinae*), which comprehend the sub-family *Orthocoronavirinae* (1). The latter includes four different genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus* and *Deltacoronavirus*. Alphacoronaviruses and Betacoronaviruses are capable to infect several mammalians species, while Gammacoronaviruses and Deltacoronavirusus can also infect avian species. The coronavirus virions consist of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N) (Figure 1), six accessory proteins open reading frame (ORFs) (ORF3a, ORF6, ORF7a, ORF7b, ORF8 and ORF10) and 16 non-structural proteins (NSP1-NSP16) (2, 3). The positive-sense, single-stranded RNA genome is encapsidated by N, whereas M and E ensure its incorporation in the viral particle during the assembling process. The spike protein protrudes from the host-derived viral envelope and provide specificity for cellular entry receptors (4).

Figure 1: A typical structure of coronaviruses exhibiting various structural proteins (i.e., S, M, E) and genomic RNA packed inside the particle by N protein. Taken from *Kirtipal N et al. Infect Genet Evol 2020* (5)



The spike protein of SARS-CoV-2 possess two notable genomic features (6-9): (i) it appears to be optimized for binding the human receptor of angiotensin-converting enzyme 2 (ACE2) and (ii) has a functional polybasic cleavage site at the S1-S2 boundary through the insertion of 12 nucleotides, which additionally led to the predicted acquisition of three O-linked glycans around the site. The receptor-binding domain (RBD) in the spike protein is the most variable part of the coronavirus genome (10, 11). Six RBD amino acids have been shown to be critical for binding the ACE2 receptors and for determining the host range of SARS-CoV-like viruses (6). Five of these six residues differ between SARS-CoV-2 and SARS-CoV (Figure 2a) and this difference in the RBD sequence showed to be optimal for receptor binding (6, 12). The second notable feature of SARS-CoV-2 is a polybasic cleavage site (*RRAR*) at the junction of S1 and S2, the two subunits of the spike (7) (Figure 2b). This allows effective cleavage by furin and other proteases and has a role in determining viral infectivity and host range (13). In addition, a leading proline is also inserted at this site in SARS-CoV-2; thus, the inserted sequence is *PRRA* (Figure 2b). The turn created by the proline is predicted to result in the addition of O-linked glycans, which flank the cleavage site and are unique to SARS-CoV-2. The functional consequences of the polybasic cleavage site in SARS-CoV-2 are unknown, and they will be important to determine its impact on transmissibility and pathogenesis in animal models. The function of the predicted O-linked glycans is also unclear, but they could create a “mucin-like domain” that shields epitopes or key residues on the SARS-CoV-2 spike protein (14). Several viruses indeed utilize mucin-like domains as glycan shields involved immune-evasion (14).

Figure 2: Features of the spike protein in human SARS-CoV-2 and related coronaviruses. *Taken from Anderfsen KG et al. Nat Med. 2020 (15).*



The substitution, deletion, and insertion of aminoacidic sites, which occurred in spike protein and the ORF of SARS-CoV-2, led to many virus variants. These mutations may also alter the virus biological characteristics, including increasing transmissibility and generating immune escape from innate or acquired immune response (16, 17). The SARS-CoV-2 variants were classified as Variants of Concern (VOCs) and Variants of Interest (VOIs) by WHO. At present, WHO described four VOCs, namely, Alpha B.1.1.7 (known as 20I/501Y.V1, VOC 202012/01), Beta B.1.351 (known as 501Y.V2), Gamma P.1 (known as 501Y.V3) and Delta B.1.617.2 (known as 478K.V1) (18) (Table 1). At the end of January 2020, the D614G mutant, which turns aspartic acid (Asp) into glycine (Gly) at site 614 in the amino acid sequence of spike, was first discovered in the UK and quickly became the significant epidemic strain in the world (19, 20). Subsequently during the COVID-19 pandemic, genetic variants of SARS-CoV-2 have been emerging and spreading around the world (21, 22), with the Delta variant being predominant worldwide at the time of November 2021.

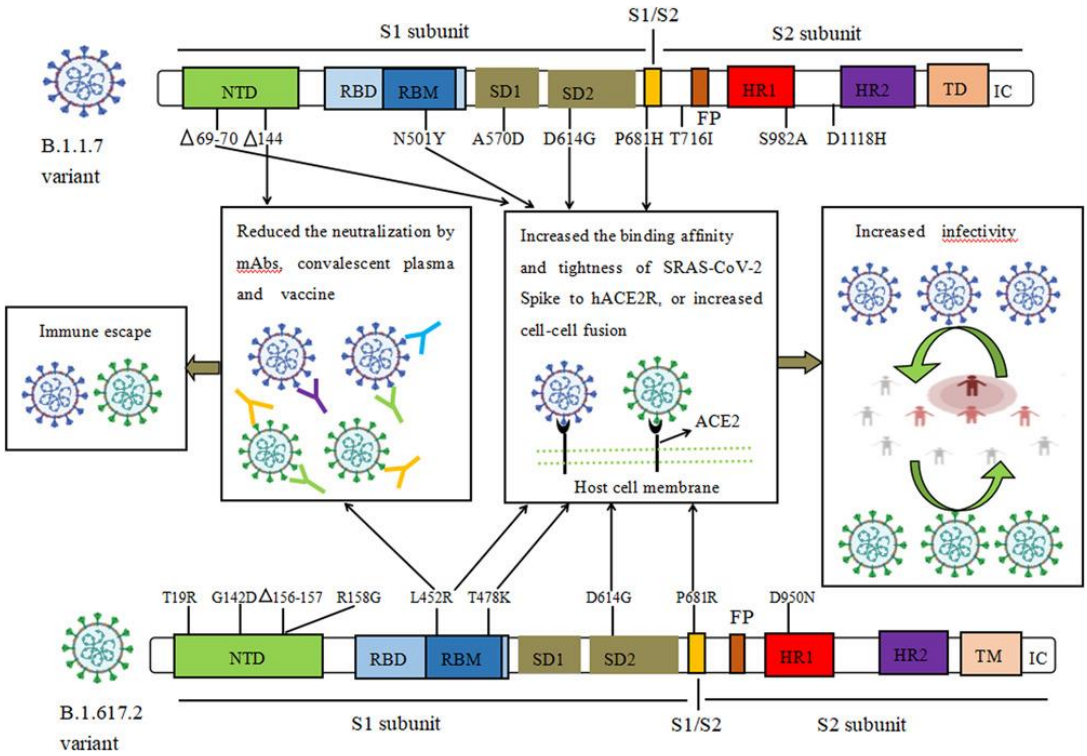
Table 1: Characteristics of SARS-CoV-2 variants of concern. *Taken from Tian D et al J Med Virol 2021 (23)*

WHO label	Alpha	Beta	Gamma	Delta
Pango lineage	B.1.1.7	B.1.351	P.1	B.1.617.2
Amino acid mutations in the spike protein	HV69-70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	D80A, D215G, LLA241-243del, K417N, E484K, N501Y, D614G, A701V	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	T19R, G142D, FR156-157del, R158G, L452R, T478K, D614G, P681R, D950N
Increased the rate of infection than that of wild strain	Increased by 43%–90% in UK. Increased by 59%–74% in Denmark, USA	Increased by 50% in South Africa	Increased by 1.7–2.4-fold in Brazil	Increased by 60% than B.1.1.7 variant in India
Increased the rate of hospitalization and mortality than that of wild strain	Increased by 11% for hospitalization, 1.4% for ICU and 35% for mortality	Increased by 19.3% for hospitalization and 2.3% for ICU	Increased by 20% for hospitalizationand 2.1% for ICU	Increased by 120% for hospitalization,287% for ICU admission and 137% for death

The Alpha variant was first detected in New York in November 2020 (24). It had 10 key amino acid mutations accumulated in the spike protein, with three of them possessing the potential to affect the virus transmissibility (H69-V70del, N501Y and P681H) (25) (Figure 3). Epidemiological studies and dynamic modelling methods suggested that the transmissibility of the Alpha variant in Britain was increased by 43-90% and became the dominant strain in the UK. The B.1.1.7 transmissibility in the US was reported to be 59-74% higher than the wild strain (26). Moreover, the viral loads was higher in B.1.1.7 samples than in non-B1.1.1.7 samples (27). Delta variant was first identified in Guangzhou, Guangdong, China, on May 21, 2021. Except for D614F, B.1.617.2 accumulated eight amino acid mutations in the spike protein, including T19R, G142D, FR156-157del, R158G, L452R, T478K, P681R, D950N (25) (Figure 3). A preprint reported that the time

interval from exposure to the first polymerase chain reaction (PCR) positive was 4 days (IQR: 3.00-5.00) in the Delta epidemic 2021 and 6 days (IQR: 5.00-8.00) during the 2020 epidemic (28). The relative viral loads of cases infected with the Delta variant were 1260 times higher than wild strain when SARS-CoV-2 was first detected. Moreover, 80.65% of samples infected with the Delta variant contained $>6x10^5$ copies/ml in oropharyngeal swabs when the viruses were first detected, compared to 19.05% of samples infected with wild strain containing more than $6x10^5$ copies/ml. Epidemiological investigations showed that typical clinical symptom were observed 2-3 days after infection with the Delta variant. Finally, the basic transmission (basic reproduction number, R0) was 4.04-5.0 times higher than the wild strain (R0: 2.2-3.77) (29).

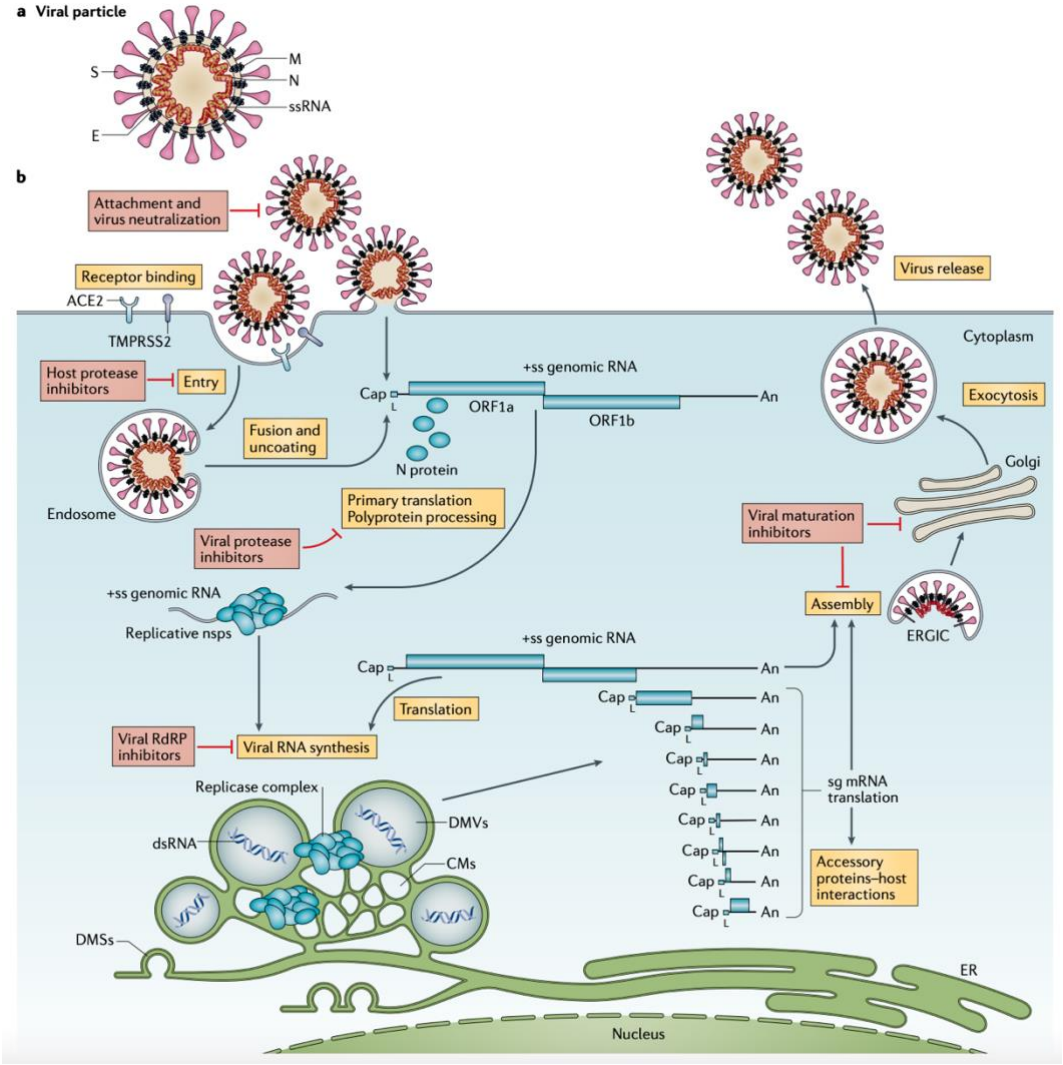
Figure 3: The biological characteristics of key amino acid mutations of spike protein in B.1.1.7 and B.1.617.2 variant. Taken from Tian D et al J Med Virol 2021 (23)



Since the first reports of COVID-19 pneumonia in Wuhan, Hubei province, China, there has been considerable discussion on the origin of the causative virus, SARS-CoV-2. As many early cases of CODIV-19 were linked to the Huanan market in Wuhan (10, 11), it is possible that an animal source was present at this location. Given the similarity of SARS-CoV-2 to bat SARS-CoV-like coronaviruses (10), it is likely that bats served as reservoir hosts for its progenitor. Although the bat's coronavirus RaTG13 sampled from a *Rhinolophus affinis* bat is 96% identical overall to SARS-CoV-2 (11), its spike diverged in the RBD, suggesting that it may not bind efficiently to human ACE2 (6). Malayan pangolins (*Manis javanica*) illegally imported into Guangdong province contained coronaviruses similar to SARS-CoV-2 (30). Although the RaTG13 bat virus remains the closest to SARS-CoV-2 across the genome, some pangolin coronaviruses exhibited strong similarity to SARS-CoV-2 in the RBD, including all six key RBD residues (30). This clearly shows that the SARS-CoV-2 spike protein optimized for binding to human-like ACE2 is the result of natural selection. It is possible that a progenitor of SARS-CoV-2 jumped into humans, acquiring the genomic features described above through adaptation during undetected human-to-human transmission. Once acquired, these adaptations likely enabled the pandemic to take off and produce a sufficiently large cluster of cases to trigger the surveillance system that detected it. The replication cycle of SARS-CoV-2 is shown in Figure 4 (4). The initial steps of coronaviruses infection involve the specific binding of the coronavirus S protein to the cellular entry receptors, which have been identified for several coronaviruses and include human aminopeptidase N, ACE2 and dipeptidyl peptidase 4 (DDP4). Specifically, SARS-CoV-2 binds the ACE2 receptor. The expression and tissue distribution of entry receptors consequently influence viral tropism and pathogenicity. Coronavirus particles bind to cellular attachment factors and specific S interactions with the cellular receptors, together with host factors, promote viral uptake and fusion at the cellular or endosomal membrane. Following entry, the release and uncoating of the incoming genomic RNA subject it to the immediate translation of two large ORFs (ORF1a and ORF1b) that occupy two-thirds of the capped and polyadenylated genome. ORF1a and ORF1b encode 15-16 NSPs, of which 15 compose the viral replication and transcription complex (RTC) that

includes, amongst others, RNA-processing and RNA-modifying enzymes and an RNA proofreading function necessary for maintaining the integrity of the >30 kb coronavirus genome. Translated structural proteins translocate into endoplasm reticulum (ER) membranes and transit through the ER-to-Golgi intermediate compartment (ERGIC), where interaction with N-encapsidated, newly produced genomic RNA results in budding into the lumen of secretory vesicular compartments. Finally, virions are secreted from the infected cell by exocytosis.

Figure 4: The coronavirus virion and life cycle. Taken from *V'kovski P et al. Nat Rev Microbiol 2021 (4)*



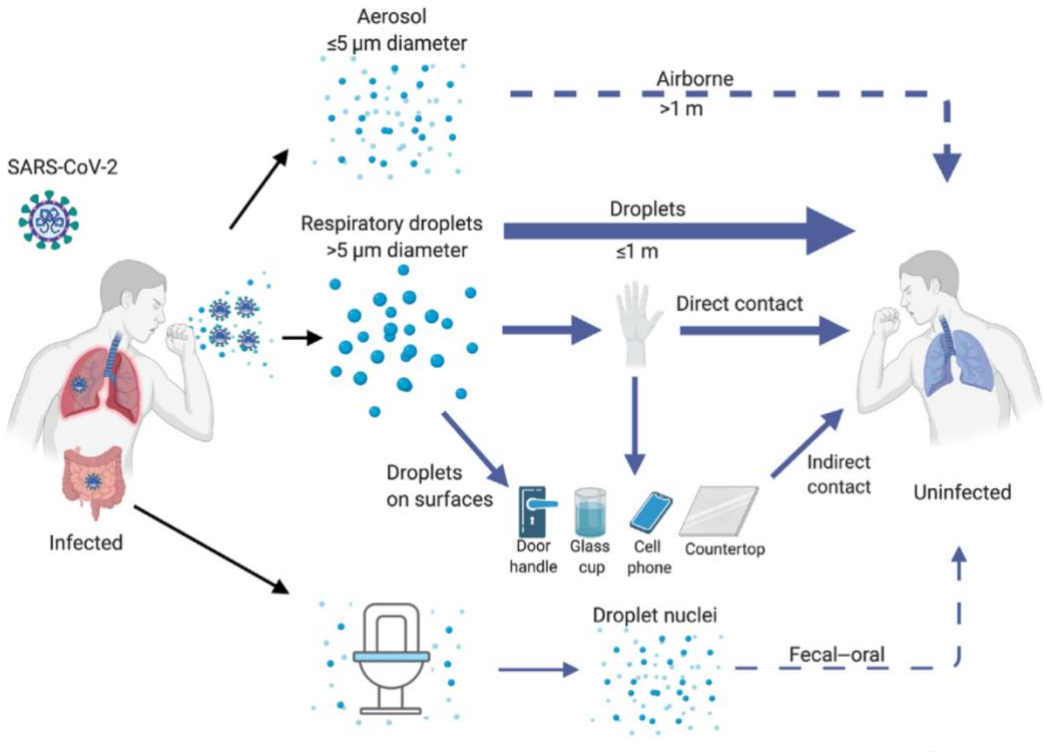
1.2 Epidemiology and risk factors

Human coronaviruses are transmitted primarily through respiratory droplets, but aerosol, direct contact with contaminated surfaces, and faecal-oral transmission were also reported during the SARS epidemic (Figure 5) (31-33). Early reports of patients with cough, lung ground glass opacities, and symptom progression to severe pneumonia, suggested communicability of SARS-CoV-2 via the respiratory route (10, 34, 35). Direct transmission by respiratory droplets is reinforced by productive SARS-CoV-2 replication in both the upper respiratory tract and lower respiratory tract, and the increasing number of reports indicating human-to-human spread among close contacts exhibiting active coughing (36-38). So far, the basic reproduction number (R_0) is about 2.2, based on early case tracking during the beginning of the pandemic, with a doubling time of 5 days (37, 39). Furthermore, there is evidence for non-symptomatic/pre-symptomatic spread of SARS-CoV-2, which is in contrast to the transmission dynamics of SARS-CoV (40). This finding underscores the ability of SARS-CoV-2 to colonize and replicate in the throat during early infection (41-43). Based on these apparent disparities in virus transmission, one study modelled the transmission dynamics of SARS-CoV-2 in pre-symptomatic individuals and indicated that the pre-symptomatic R_0 has approached the threshold for sustaining an outbreak on its own ($R_0 > 1$). By contrast, the corresponding estimates for SARS-CoV were approximately zero (39). Similarly, asymptomatic spread of SARS-CoV-2 has been documented throughout the course of the pandemic (38, 42, 44-47). Understanding the relative importance of cryptic transmission to the current COVID-19 pandemic is essential for public health authorities to make the most comprehensive and effective disease control measures, which include mask-wearing, contact tracing, and physical isolation.

Aerosol transmission (spread > 1m) was implicated in the Amoy Gardens outbreak during the SARS epidemic, but the inconsistency of these findings in other settings suggested that SARS-CoV was an opportunistic airborne infection (31, 48). Similarly, no infectious SARS-CoV-2 virions have been isolated in the air of COVID-19 hospital wards, although viral RNA was detectable (49). Generation of experimental aerosols carrying SARS-CoV-2 have offered the plausibility of airborne transmission, but the aerodynamic characteristics of SARS-CoV-2 during

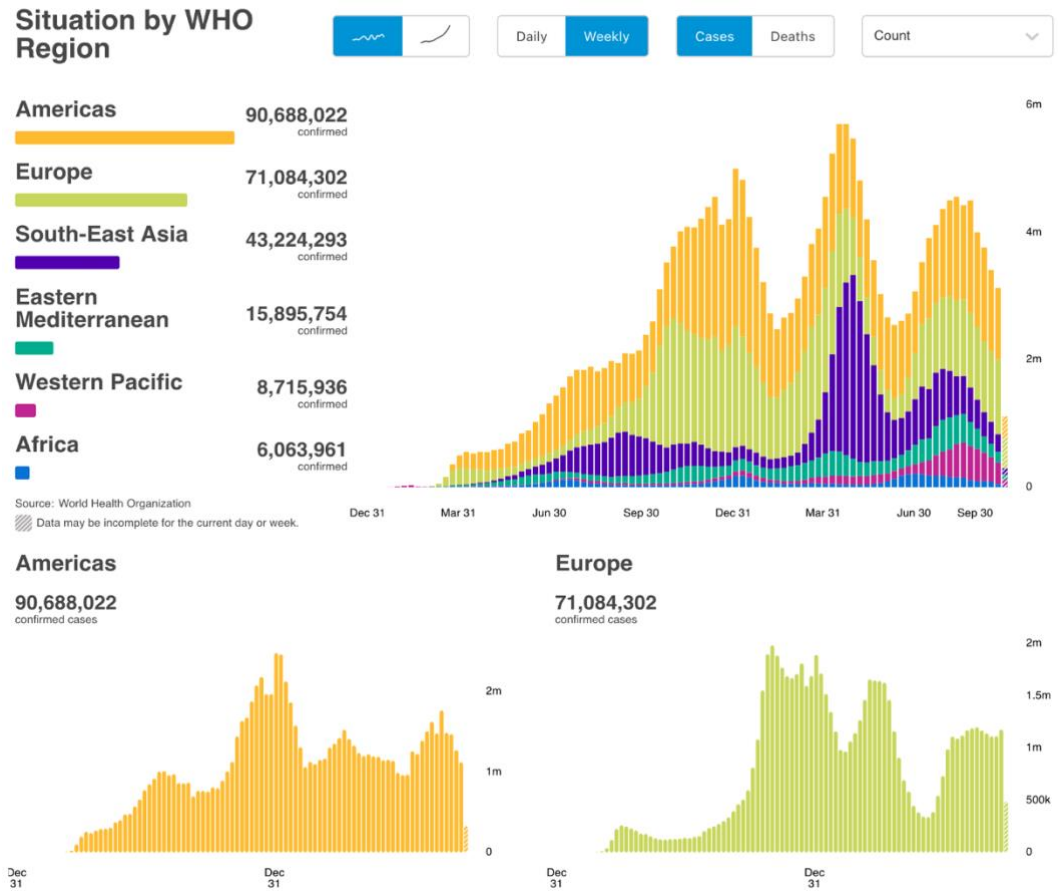
a natural course of infection is still an area of intense inquiry (50). Nonetheless, deposition of virus-laden aerosols might contaminate objects (e.g. fomites) and contribute to human transmission events (49, 51). Finally, faecal-oral transmission has also been considered as a potential route of human spread, but remains an enigma despite evidence of RNA-laden aerosols being found nearby toilet bowls, along with detectable SARS-CoV-2 RNA in rectal swabs during the precursor epidemic of COVID-19 in China (49, 52, 53).

Figure 5: Proposed SARS-CoV-2 transmission routes. Taken from *Harrison AG et al. Trends Immunol 2020 (54)*



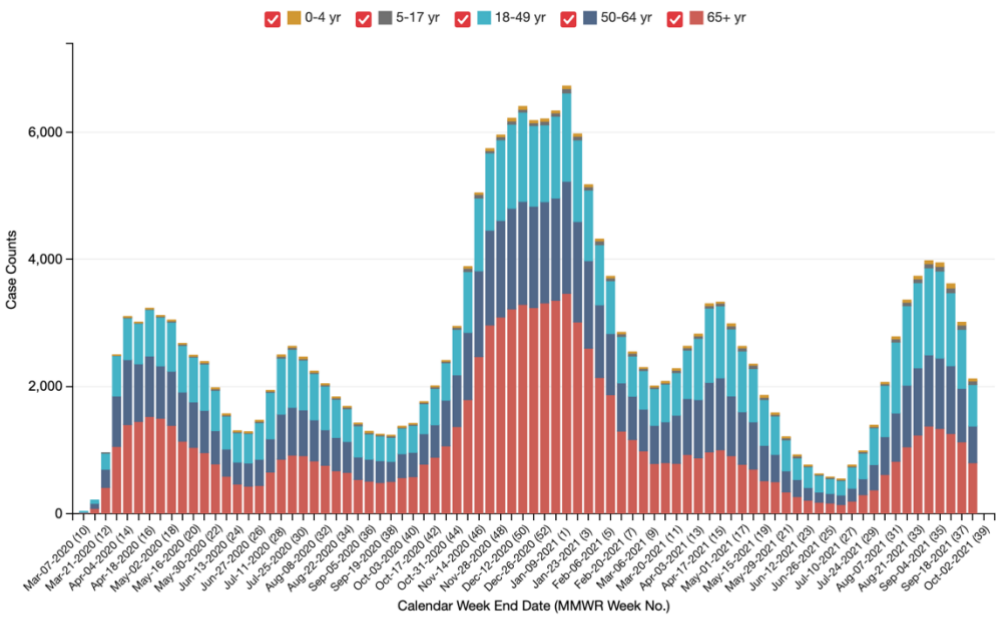
With regards to the global diffusion of SARS-CoV-2, on the 6th of October 2021, there were 235,673,032 confirmed cases of COVID-19, including 4,814,651 deaths reported to the WHO (55). Most COVID-19 cases were reported from the Americas and Europe (Figure 6).

Figure 6: Situation of global COVID-19 cases by WHO. Taken from *WHO Coronavirus (COVID-19) Dashboard (55)*



According to data provided by the Centre for Disease Control and Prevention (CDC), most patients hospitalized with COVID-19 in the USA in the 2020-2021 period (and up to the end of September 2021) were aged ≥ 18 years (98%), with those aged >65 years being the most representative patients affected by SARS-CoV-2 infection (42%) (Figure 7). Most of the adult patients had at least one co-morbidity (91.4%). In particular, 57,1% of adult patients hospitalized for COVID-19 in the USA had hypertension, while 50.3% and 42% were obese or had a metabolic disease, respectively (Figure 8).

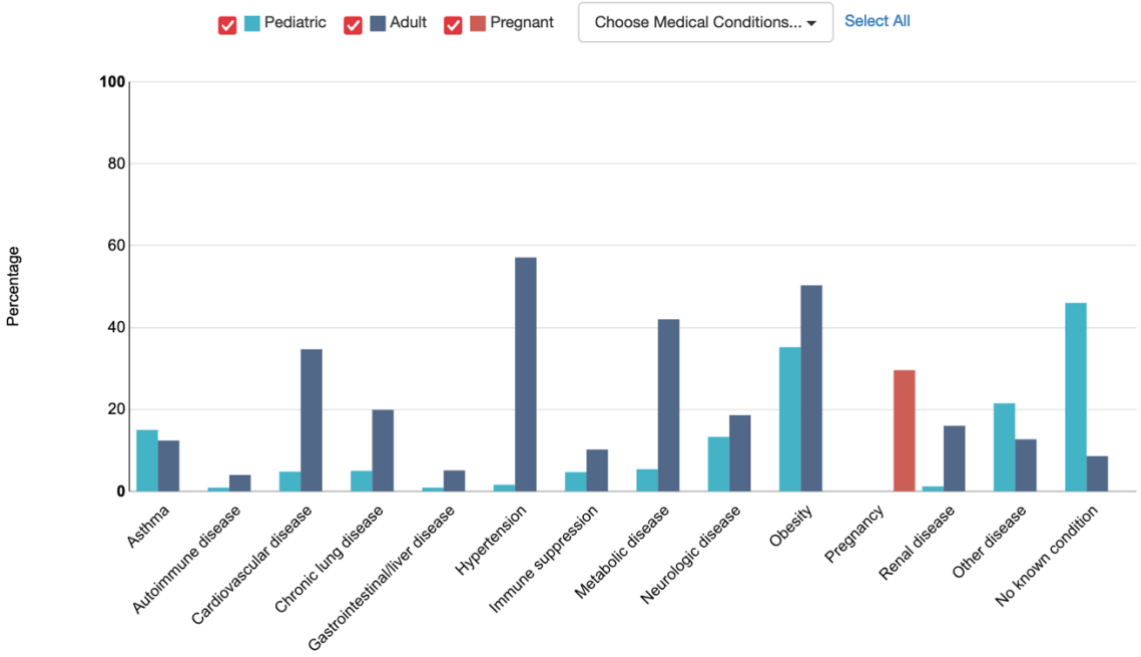
Figure 7: *COVID-19-associated hospitalizations by age in the USA. Taken from* CDC COVID-NET (56)



Cumulative case count by age group

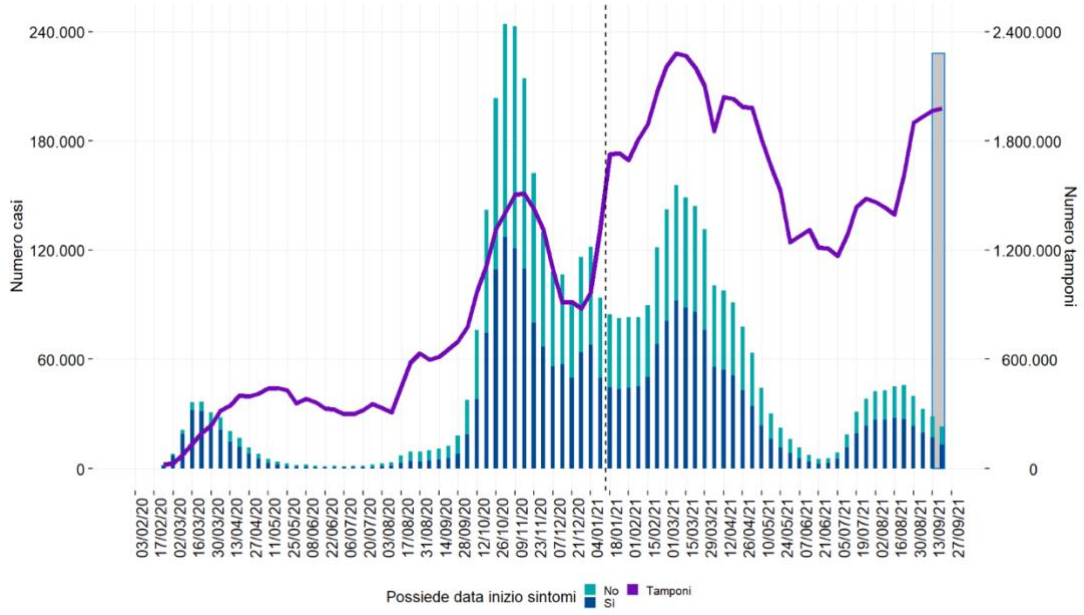
	0-4 yr	5-17 yr	18-49 yr	50-64 yr	65+ yr	Total
2020-21	1603	2625	61806	61545	92960	220539

Figure 8: Prevalence of comorbidities in patients hospitalized for COVID-19 the USA. *Taken from CDC COVID-NET* (56)



In Italy, on the 29th of September 2021 there were a total of 4,669,279 confirmed cases of COVID-19 and 130,259 deaths, with a fatality rate of 2.8% (57). Italy, as per most of industrialized countries, has experienced 4 different peaks in COVID-19 diagnosis (The so-called COVID-19 "waves") since the beginning of the pandemic, with the "second wave" in the period September 2020 – December 2020 being the most severe in terms of new confirmed cases and deaths (Figure 9). According to data provided by Italian authorities (57) on the 1st of October 2021, the weekly Italian incidence of COVID-19 were decreasing from 48 cases on 100,000 inhabitants to 39 cases on 100,000 inhabitants. The transmissibility index (based on data from hospitalized patients) were also decreasing from 0.86 (95% confidential intervals [95CI]: 0.82 to 0.90) to 0.80 (95CI: 0.77 to 0.84). The median age of patients with SARS-CoV-2 (39 years) had been stationary in the previous 14 days.

Figure 9: Weekly cases of COVID-19 in Italy and number of performed swabs. *Taken from ISS Epidemia COVID-19 aggioramento nazionale (57)*



For what concern the risk factors for COVID-19, male sex, older age and hypertension were early associated with severity of the disease (Figure 10). Age showed to be undeniably related to both frequency and severity of SARS-CoV-2 infection. While the prevalence of COVID-19 among different age groups was

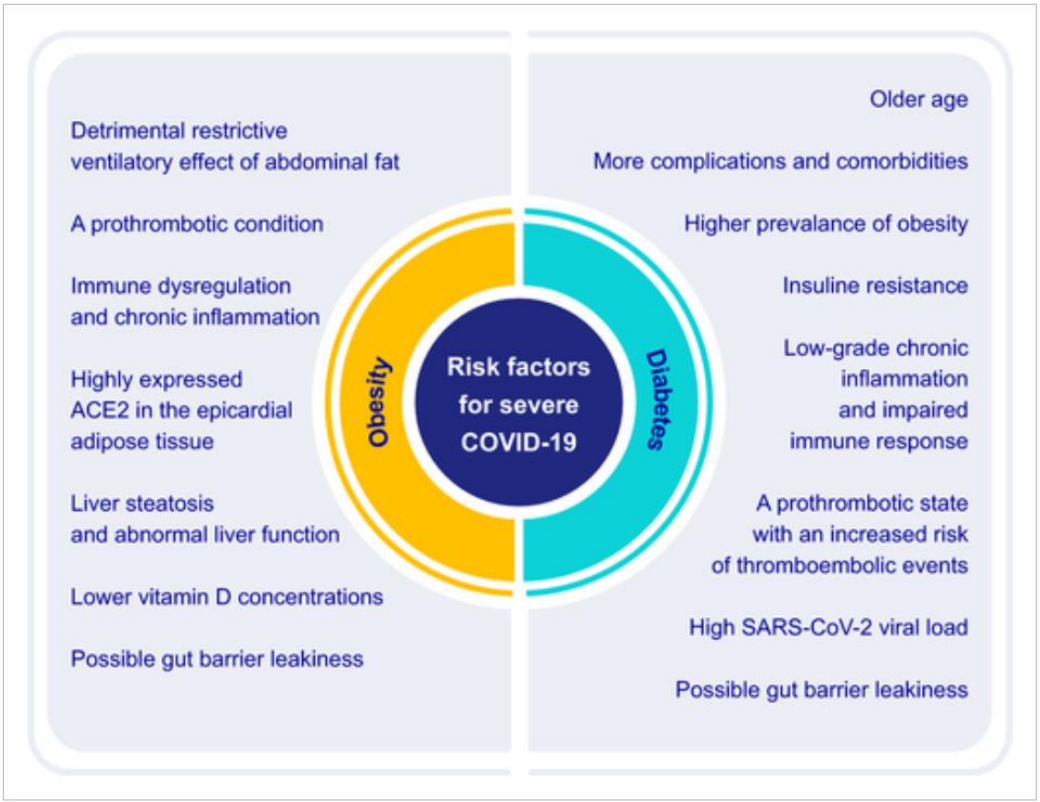
already discussed (Figure 7) (56), it is important to stress that older age was significantly associated with severe disease and mortality related to SARS-CoV-2 in almost all case series, particularly among patients aged > 60 years (58-62). In fact, median age of patients requiring intensive care was higher than those not admitted to intensive care unit (ICU) (66 vs 51 years). According to data from 79,394 confirmed cases in China (63), patients aged below 30 and above 59 were 0.6 (0.3 to 1.1) and 5.1 (4.2 to 6.1) times more likely to die after developing symptoms of SARS-CoV-2 infection, respectively. Together with the age, male sex also represents an indisputable risk factor for severe COVID-19 (64). In a US study, 83.8% of patients who received invasive mechanical ventilation were male, while significantly lower age was observed among patients who had been weaned successfully from mechanical ventilation. Arterial hypertension was more frequently observed in severe COVID-19 patients compared to non-severe patients (65, 66). In particular, a higher prevalence of hypertension was reported among COVID-19 patients requiring ICU compared with those not admitted to ICU (58.3% vs 21.6%, $p<0.001$) (67). However, hypertension is more frequent in the elderly, and this may represent a confounding factor. In another study, it was found that hypertension was an independent risk factor for COVID-19 (OR: 2.01, $p<0.05$). According to the CDC, individuals with hypertension might be at increased risk for severe illness from COVID-19 (68). In a retrospective study on 803 COVID-19 patients with hypertension, high average systolic blood pressure and high systolic/diastolic blood pressure variability during hospitalization were independently associated with in-hospital mortality, ICU admission and hearth failure, suggesting that lower and stable blood pressure is predictive of a better prognosis (69).

Figure 10: Mechanisms of age, sex, and hypertension on the severity of COVID-19. *Taken from Gao Y et al Allergy 2020* (70)

Aside from hypertension, diabetes and obesity were also associated with an increased risk of severe COVID-19 (Figure 11). Diabetes is indeed a common comorbidity in COVID-19 patients and was suggested to be a risk factor of severe and fatal COVID-19 cases. In fact, a meta-analysis showed that COVID-19 patients with diabetes had a higher risk of severe disease or death (RR: 2.96; 95%CI: 2.31-3.79) (71), as well as higher rates of ICU admission (72). Another meta-analysis demonstrated that the odds ratios (ORs) of diabetes for ICU admission and mortality were 2.79 (95%CI: 1.85 to 4.22) and 3.21 (95%CI: 1.82 to 5.64), respectively (73). Another study showed that patients with hyperglycaemia at admission had a higher risk of composite outcomes (ICU admission, mechanical ventilation and death), with OR 5.47 (95%CI: 1.56 to 19.82) (74).

With regard to obesity, in a large cohort study, obese patients with COVID-19 were at increased risk of hospitalization (adjusted relative risk [aRR]: 2.20) and severity (aRR: 2.30) compared with non-obese patients; this was notable in the population younger than 50 years (aRR: 13.80) (75). In another study, a higher severity and a longer hospital stay in obese COVID-19 patients were reported, and it was positively correlated with the body mass index (BMI) (aOR: 3.00 for obesity, aOR 1.13 for BMI) (76). Obese patients with BMI $\geq$ 35 kg/m^2 had an increased risk of admission to the ICU (OR: 3.6) in COVID-19 patients aged < 60 years (77). Moreover, BMI above 40 kg/m^2 was found to be an independent risk factor associated with mortality and this effect was more pronounced in patients younger than 50 years (aOR: 5.1) (78).

Figure 11: Mechanisms of diabetes and obesity on the severity of COVID-19. *Taken from Gao Y et al Allergy 2020* (70)



Patients with pulmonary comorbidities are also at high risk of severe COVID-19. For instance, chronic obstructive pulmonary disease (COPD) did not show to be a predisposing factor for SARS-CoV-2 infection, but it was associated with an elevated risk of hospitalization (aOR: 1.36), ICU admission (aOR 1.20) and invasive mechanical ventilation (aOR: 1.49) (79). Patients with pre-existing interstitial lung disease (ILD) are also more susceptible to progressing to a severe or critical form of COVID-19 due to a restrictive ventilatory dysfunction and limited pulmonary reserve. In addition, SARS-CoV-2 may trigger an exacerbation of underlying ILD and result in poor outcome (80). In fact, COVID-19 patients with pre-existing ILD had a poorer prognosis with fatality rate ranging from 30% and 60% and ORs from 3.2 to 5,5 (81-83). Other comorbidities associated with severe COVID-19 and high fatality rates are chronic liver disease (84) and chronic kidney disease (85). Patients with cancers and haematologic malignancies are vulnerable to SARS-CoV-2 infection due to compromised immunity (86) and might be at risk of severe forms of COVID-19 probably depending on tumour type, duration and specific anti-tumoral treatment (87-89).

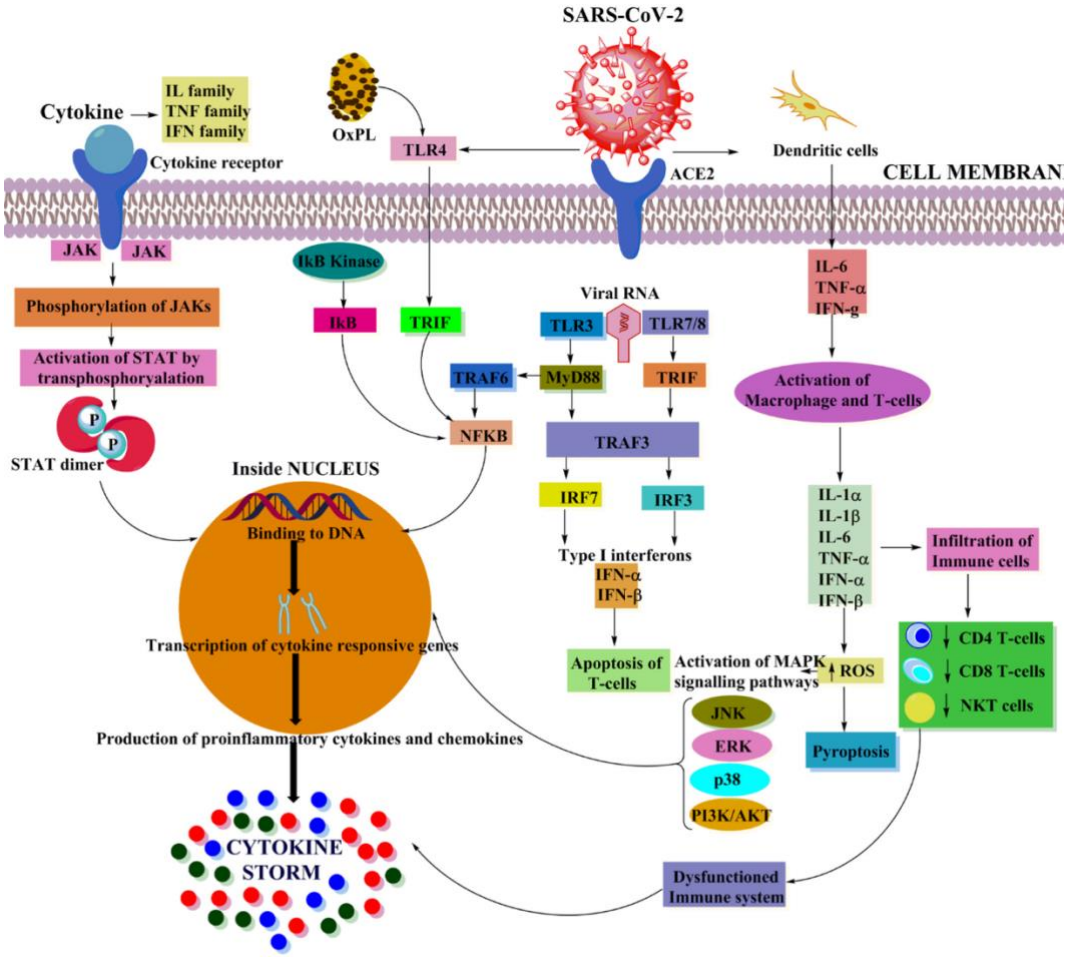
Finally, pregnancy was recognized as risk factor for SARS-CoV-2 infection and severe COVID-19 due to the physiological changes in the immune and respiratory system in pregnant women (90). In particular, placental immaturity and the early ACE2 expression can make the first trimester the most susceptible period for SARS-CoV-2 infection (91). A report by the CDC showed that the prevalence of COVID-19 in pregnant women was 9.0% (92). Moreover, pregnant women with COVID-19 showed higher ICU admission rate than non-pregnant COVID-19 women (1.5% vs 0.9%) and 0.5% of pregnant women required mechanical ventilation compared with 0.3% of non-pregnant women (92).

1.3 Pathogenesis, immunity, and vaccines

The pathogenesis of SARS-CoV-2 infection takes place through virus entry into the host, binding with the host cell receptors and viral replication. The virus enters human cells binding to the ACE2 receptor, which is expressed in the lungs, kidney, heart, liver, testes, and intestine (93). The spike protein of SARS-CoV-2 binds to the ACE2 receptor with the help of the cellular protease transmembrane protease

serine 2 (TMPRSS2) and other protein clathrin by endocytosis (94, 95). Subsequently, SARS-CoV-2 RNA is released into the nucleus of infected cells and viral replication starts. At this point, the dendritic cells, macrophages, and respiratory epithelial cells secrete cytokines and chemokines to produce immune response to clear out the pathogen from the body. The inflammatory patterns activated at this stage include, among the others, the interleukin-6 (IL-6)/janus kinase (JAK)/STAT signalling pathway (96, 97), the interferon (IFN) cell signalling pathway (98), the tumor necrosis factor (TNF)α-nuclear factor kappa (NF-κB) pathway (99) and the T-cell receptor (TCR) pathway (97, 100). The result of the triggered inflammation is the presence of low levels of the antiviral IFN and high levels of proinflammatory cytokines (IL-1ß, IL-2R, IL-6, IL-7, IL-8, IL-17 and TNFα) and chemokines (CCL-2, CCL-3, CCL-5, CCL-7, CXCL-10). These secretions from pro-inflammatory cells lead to an uncontrolled inflammatory response (the "cytokine storm") that plays a key role in the pathogenesis of COVID-19 and worsens the infection (101) (Figure 12). The cytokine storm is responsible for severe COVID-19 complications (e.g., Acute Respiratory Distress Syndrom [ARDS] and multiorgan damage that leads to death). Clinical and laboratory alterations, such as lymphopenia, ground-glass infiltrates, hyperferritinaemia, elevated lactate dehydrogenase (LDH), C-reactive protein (CRP) and IL-6 are also related to cytokine storm (102, 103). Studies suggested that various complications associated with COVID-19 are probable related to the immune dysfunction and the effect of cytokine storm: cardiovascular complications, neurological, thrombotic, haematologic etc. (104-106).

Figure 12: Inflammatory signalling cascade activated in COVID-19. *Taken from Choundhary et al Microb Pathog 2021 (107)*



In order to fully understand the pathogenetic characteristics of COVID-19 and the role of vaccines effective against the infection, it is pivotal to discuss in the details the immunity response subsequent to SARS-CoV-2 exposure. Humoral immune responses to SARS-CoV-2 are mediated by antibodies that are directed to viral surface glycoproteins, mainly the spike glycoprotein and the nucleocapsid protein. Such antibodies neutralise viral infection of human cells and tissue expression of ACE2 (108-110). Functional neutralising antibodies specific to SARS-CoV-2 that are produced following infection, vaccination, or both (anti-spike glycoprotein and anti-RBD) are considered important for viral neutralisation and viral clearance. For these reasons, antibody titres might be good biomarkers for the protective efficacy of antibodies and successful humoral immune response after SARS-CoV-2

exposure. Indeed, a strong correlation between neutralising antibody response against the spike glycoprotein, the nucleocapsid protein, and RBD proteins was detected in patients with PCR-confirmed COVID-19 (111). Most patients with COVID-19 or those who are convalescent have virus-specific IgM, IgA and IgG responses in the day after infection, suggesting that antibodies mediate protective immunity to SARS-CoV-2 (112, 113). The antibody kinetics of antibody response against SARS-CoV-2 are the same to those for SARS-CoV, which are characterized by robust seroconversion (IgM and IgG) after 7-14 days from the onset of symptoms and persisting of antibody concentrations for weeks to months after infection and viral clearance (114). A longitudinal study assessing the kinetics of spike glycoprotein-specific antibodies in patients with COVID-19 found that IgA antibodies are produced early in the first week of the disease and peak in concentration at 20-22 days, while IgM antibodies reach high titres at 10-12 days and subsequently dropped 18 days after the onset of symptoms (113). On the other side, a seroprevalence study on IgG responses to spike glycoprotein in 40 patients with COVID-19 after symptoms onset showed that IgG titres increase during the first 3 weeks and begin to decrease by 8 weeks (115). The antibody response seemed to be more pronounced in patients with severe disease compared with those with mild COVID-19; the former indeed showed higher IgG titres and a slower decay pattern of IgG in the weeks following the infection (116-118). Nevertheless, a study of adaptive immunity to SARS-CoV-2 showed that the concentration of neutralising antibody was not correlated with COVID-19 severity, suggesting that cellular immune response is also important for the clearance of SARS-CoV-2 infection (119). Initial reports on cellular immunity to SARS-CoV-2 showed that the proportion of CD38+, HLA-DR+ T-cells (both CD4+ and CD8+) increases during the first 7-10 days of COVID-19 symptoms and begins to return to baseline around day 20 (120-122). In some reports, the increase in the proportion of SARS-CoV-2 specific T-cells seemed to correlate with disease severity (123, 124). The CD4+ T-cell response mainly consists of T-helper-1 (Th1) cells, characterised by high concentrations of IFNγ secretion and a propensity for the structural spike glycoprotein and the nucleocapsid protein, although non-structural protein were also targeted (123). On the other hand, CD8+ T-cell response consists of IFNγ and

TNFα, also reflecting a response towards Th1 cells and showing a preference for structural proteins over non-structural proteins (123).

Through an unprecedent research and development process, in early 2021, just one year after the COVID-19 pandemic started, there were several vaccines commercially available against SARS-CoV-2 infection. First approved vaccines were those from Pfizer-BioNTech, Moderna and AstraZeneca, and other pharmaceutical companies have continued their research efforts in the developments of new vaccines. In fact, clinical trials are being conducted worldwide on over 80 vaccines, half of which have reached the final phase of experimentation, and at least 180 experimental products are currently in preclinical phase of trials and animal testing has started (125). Regardless of the technology used in their development, all vaccines approved or still under study were developed to stimulate an immune response targeting the blockage of SARS-COV-2 spike protein which has a key role in the viral entry into human cells. As the end of May 2021, the European Medicines Agency (EMA) had approved four COVID-19 vaccines: two of them are mRNA vaccines and two are viral vector vaccines (Table 2), while the Food and Drug Administration (FDA) had approved three vaccines: two are the same mRNA-based vaccines approved by EMA, while one is a viral vector vaccine.

Table 2: Characteristics of the four COVID-19 vaccines approved by regulatory authorities and available in Europe as of the end of May 2021

Vaccine	Type	Efficacy	Administration	Side Effects	Cold chain
Pfizer-BioNTech COMIRNATY	mRNA	95%	2nd dose 21-28 day	Fever, local reaction, allergy	-70° (2-8°C x 5d)
Moderna-mRNA 1273 (NIAID)	mRNA	92%	2nd dose 28d and up to 42d	Fever, local reaction, allergy	-20°C (6 months)
Oxford-AstraZeneca AZD1222	ChAdOx1	70-80%	2nd dose 8-12 weeks	Fever, local reaction, allergy	2-8°C
Johnson & Johnson Ad26.COV2.S	Ad26	85%	Single dose	Fatigue, headache, myalgia, fever	2-8°C (two months) -20°C (two years

Pfizer/BioNTech (BNT162) and Moderna (mRNA-1273) vaccines were approved by EMA in December 2020 and January 2021, respectively, and by FDA, which issued emergency use authorizations in December 2021 and extended them in May 2021 to include adolescents between 12 and 15 years of age (126). They are both mRNA vaccines which contain the instruction for the synthesis of the spike protein in the cells of the vaccinated subject. The Pfizer/BioNTech vaccine phase trial involved 43,998 subjects of age between 12 and 85 years (127). This vaccine was 95% effective in preventing COVID-19 (95%CI: 90.3 to 97.6), with 170 infections reported at 4 weeks from the second administration. Most of the adverse events reported were mild-to-moderate pain at the injection site, fatigue, and headache. The incidence of serious adverse events was low and was similar in the vaccine and placebo groups. The efficacy of BNT162 vaccine against the Alpha variant of SARS-CoV-2 was reported to be lower compared against its efficacy against the wild strain (72.1% 95CI: 66.4 to 76.8) (128). Similarly, the efficacy against the Delta variant of SARS-CoV-2 was reduced, with reported efficacy of 88.0% (95CI: 85.3 to 90.1) after two doses (129). The Moderna phase 3 trial on the mRNA-1273

vaccine involved 30,000 participants (130). This vaccine showed an efficacy of 94% (95%CI: 90 to 97%), with 196 infections reported at 2 weeks from the second administration. However, in another study the mRNA-1273 showed an efficacy of 86% (95CI: 61 to 95%) among patients aged more than 55 years, compared to 95% (95%CI: 67 to 100%) of the Pfizer/BioNTech BNT162 vaccine (131). The efficacy of mRNA-1273 vaccine seems to be not reduced among patients with B.1.1.7 and B.1.617.2 SARS-CoV-2 variants (132, 133). Fatigue, muscle and joint pain, headache and injection site erythema were reported after mRNA-1273 vaccine administration (130). Serious adverse events were rare, and the incidence was similar in the treatment arm and in placebo group.

Astrazeneca/Vaxzevria's vaccine (ChAdOx1) is a viral vector-based vaccine. It uses a modified version of chimpanzee's adenovirus which is not able to replicate but can provide the instructions for the spike protein synthesis. Once the protein is produced, it can stimulate a specific immune response of both cellular and humoral nature. The phase 3 trial on ChAdOx1 vaccine involved 32,451 subjects with an efficacy of 69.7% (95CI: 60.7 to 76.6) in preventing COVID-19 after a vaccination schedule of two doses (second dose after 28 days) (134). However, most patients in the United Kingdom received the booster dose more than 12 weeks from the first administration, and the vaccine efficacy was higher in this population (81.3%) compared with those who received the second administration earlier (135). The efficacy of ChAdOx1 vaccine against the Alpha and Delta variants of SARS-CoV-2 showed to be 74.5% (95CI: 68.4 to 79.4) and 67% (95CI: 61.3 to 71.8), respectively. Adverse events after ChAdOx1 were similar to those related to mRNA-vaccines, but local and systemic reaction were less frequent in older age groups (136). Doubts on safety arouse due to two cases of transverse myelitis described after vaccination. Both cases were later reported to be unlikely related to the vaccine. However, the use of this vaccine was later associated with some deaths in different European countries that were attributed to thromboembolic events. These were attributed to a response similar to the one occurring in heparin-induced thrombocytopenia and prompted some EU countries to suspend the administration of the vaccine. On 18th March 2021 the EMA affirmed that the vaccine was not associated with an increased risk of thromboembolic events nor that specific

batches were related to those events. WHO confirmed the declaration provided by EMA and therefore recommended the continuation of vaccination campaign (137). However, in April 2021, as further episodes of thromboembolic events were described, the EMA's safety committee (PRAC) advised that very rare cases of thromboembolism associated to thrombocytopenia should be included as possible side effects of ChAdOx1 vaccine (138). Finally, the other viral vector-based approved vaccine is the Johnson&Johnson's Ad26COV2.S vaccine (139). This vaccine uses type 26 human adenovirus administered intramuscularly and it requires a single administration. It was tested in over 43,000 subjects of different age groups, including 34% of patients aged >60 years and it was especially evaluated in patients with comorbidities (e.g., obesity, diabetes, cancers, HIV) obtaining results reaching 100% of efficacy in preventing hospitalization or death, and 85% against severe forms of COVID-19 (140). There are no robust data on the efficacy of Ad26COV2.S vaccine against SARS-CoV-2 VOCs. Adverse reactions after this vaccine comprehend headache, fatigue, myalgia and nausea (141).

1.4 Clinical Manifestations

The majority of patients with COVID-19 present common symptoms that include fever, shortness of breath, cough (either with or without sputum), sore throat, nasal congestion, dizziness, chills, muscle ache, arthralgia, weakness, fatigue or myalgia, chest tightness and dyspnoea (142-144). Although fever is not the only initial clinical manifestation of SARS-CoV-2 infection, it is considered to be critical (35, 145). Fever, cough, and fatigue are the three most prevalent symptoms in COVID-19 patients (146, 147). Other less characteristic symptoms include headache, diarrhoea, abdominal pain, vomiting, chest pain, rhinorrhoea or pharyngalgia (148-150). Approximately 90% of the patients present more than one symptom (151, 152). An approximate proportion of severe versus common cases of COVID-19 is estimated to 1:4 (153) and it is suggested that an early onset of shortness of breath constitutes a poor prognostic factor for patients. Among 81 fatal cases of COVID-19 patients from Wuhan, the most common cause of death was a respiratory failure (46.9%), followed by septic shock (19.7%), multiple organ failure (16.0%) and cardiac arrest (8.6%). Rarer causes of death were acute coronary syndrome,

malignant arrhythmia, or disseminated intravascular coagulation (154). Clinical characteristics might differ between critically ill and non-critically ill patients (155). Regarding non-respiratory findings, isolated sudden-onset anosmia, with or without taste dysfunction, is reported to be the fourth most common symptom of SARS-CoV-2 infection (156, 157). In fact, patients who present sudden olfactory and/or gustatory dysfunction should be suspected of SARS-CoV-2 infection (158, 159). The pathogenesis of olfactory and gustatory dysfunctions in patients with COVID-19 remains undiscovered. After the recovery from the infection, some of the olfactory dysfunction might persist and gustatory dysfunctions might be resolve, and vice versa. Cases of complete losses of olfactory functions have also been reported (160). Nevertheless, the mean duration of smell and taste disorders due to SARS-CoV-2 was estimated to be 7.5 days (161).

Several studies showed that SARS-CoV-2 actively infects and replicates within the gastrointestinal tract, inducing digestive symptoms primarily via the overexpression of viral receptor ACE2 found in the gastrointestinal epithelial cells (52). The most common digestive symptoms in patients with COVID-19 include nausea and/or vomiting, diarrhoea, anorexia and loss of appetite (162-164). Less common symptoms include abdominal pain, abdominal distension, tenesmus, dysgeusia, gastrointestinal bleeding, or haematochezia (165-167). COVID-19 patients can also present gastrointestinal disorders before the occurrence of respiratory symptoms (168). Apart from the aforementioned gastrointestinal disorders, SARS-CoV-2 infection can also cause liver impairments of a wide spectrum of severity (169, 170). COVID-19 patients may indeed present with increased levels of ALT and AST. Furthermore, serum bilirubin and GGT might also be elevated during the course of the disease (171, 172). Elevated concentrations of ALT and AST might be observed both in severe and non-severe cases of COVID-19 (173), but it was reported that liver injury due to SARS-CoV-2 infection occurs more prevalently in severe cases rather than mild cases of COVID-19.

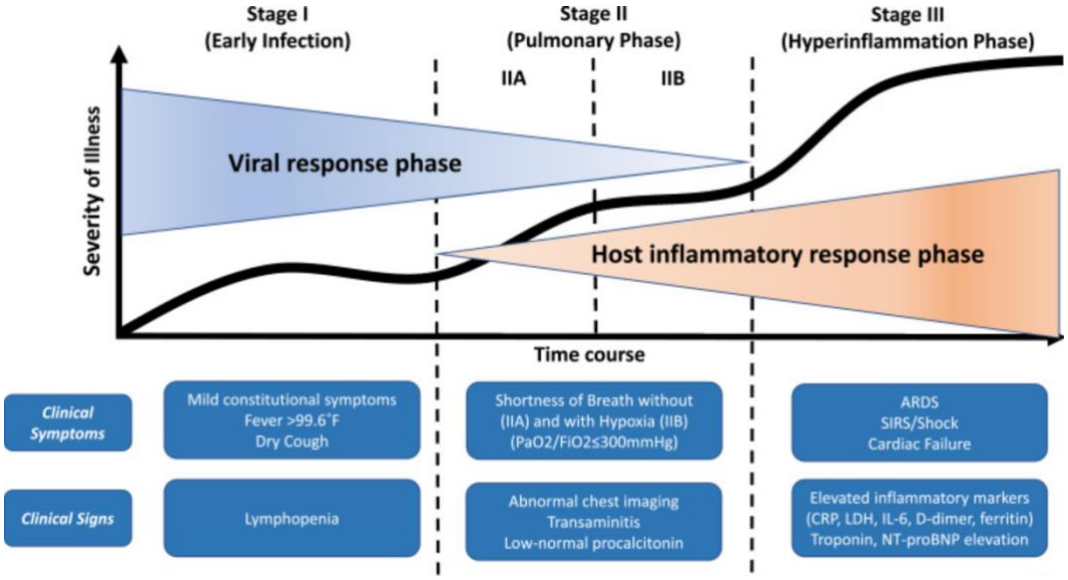
Cardiovascular diseases can significantly worsen the clinical outcome of COVID-19 patients; however, SARS-CoV-2 infection may also induce cardiac and vascular complications *de novo* (174). The most prevalent cardiovascular complication of COVID-19 is acute myocardial injury, with a prevalence of 8-12% (175, 176).

Other most prevalent complications include either brady- or tachyarrhythmias, with an estimated incidence of 16.7%, acute pericarditis, left ventricular dysfunctions, heart failure, cardiogenic shock, blood pressure abnormalities, or myocarditis (36, 177, 178). The mortality rate of patients with cardiac injury due to SARS-CoV-2 infection is much higher compared to those without cardiovascular complications (179). Furthermore, similar to other viral types of pneumonia, patients with SARS-CoV-2 infection are at a higher risk of acute pulmonary embolism. Patients with COVID-19 and pulmonary embolism have higher D-dimer levels compared to infected patients without pulmonary embolism (180, 181). Additionally, arterial and venous thromboembolic events are quite common cardiovascular manifestations among COVID-19 patients, which indicates a crucial role of COVID-19-associated coagulopathy (182, 183). A significant elevation of D-dimer and high levels of fibrin/fibrinogen degradation products are the most prevalent presentations of COVID-19-associated coagulopathy during the initial stages and altered coagulation parameters might be associated with poorer clinical outcomes (184). It was indeed showed that coagulation dysfunctions represent the major cause of death in severely ill patients with COVID-19 (185). Other non-respiratory COVID-19 clinical symptoms include neurological manifestations (headache, languidness, malaise, acute cerebrovascular disease, conscious disturbance, skeletal muscle injury, encephalopathy, prominent agitation and confusion, acute ischaemic stroke, epileptic seizures (105, 186-189)) and dermatologic manifestations (erythematous rash, urticaria, chickenpox-like vesicles, herpetiform lesions, petechial rash, maculopapular exanthem, papulovesicular rash, livedo reticularis lesions (190-197)).

Siddiqi H.K. and other authors proposed the use of a 3-stage classification system for clinical manifestations of SARS-CoV-2 infection, recognizing that COVID-19 illness exhibits 3 grades of increasing severity, which correspond with distinct clinical findings and clinical outcome (Figure 13) (198). The initial stage (Stage I, early infection) occurs at the time of inoculation. For most people, this involves an incubation period associated with mild and often non-specific symptoms. During this phase, SARS-CoV-2 replicates and establishes residence in the host, primarily focusing on the respiratory system. At this stage, the diagnosis includes respiratory

sample polymerase chain reaction (PCR), serum testing for SARS-CoV-2 IgM and IgG and chest imaging. Complete blood count may reveal lymphopenia and neutrophilia without other significant abnormalities. In the second stage (pulmonary phase without [IIA] or with [IIB] hypoxia), viral multiplication and localized inflammation in the lungs are the norm. At this stage, patients develop a viral pneumonia, with cough, fever, end possibly hypoxia. Imaging techniques, namely chest radiography computerized tomography (CT), show bilateral infiltrates or ground glass opacities. Blood tests reveal increasing lymphopenia, along with increase of transaminases. Markers of systemic inflammation can be elevated, but not remarkably so. At this stage, most patients with COVID-19 may need to be hospitalized for close observation and clinical management. In early stage II (without significant hypoxia) the use of corticosteroids may be avoided (199). If hypoxia persists and worsen, it is likely that patients will progress to requiring mechanical ventilation. In this situation, the use of anti-inflammatory therapy such as with corticosteroids may be useful (200). A minority of patients with COVID-19 will progress and transit into the third and most severe stage of the disease (hyperinflammation phase), which manifests as an extrapulmonary systemic hyperinflammation syndrome. In this stage, markers of systemic inflammation are elevated. SARS-CoV-2 infection results in a decrease in helper, suppressor and regulatory T cell counts (201). Studies showed that inflammatory cytokines and biomarkers such as IL-2, IL-6, IL-7, granulocyte colony-stimulating factor, macrophage inflammatory protein 1-α, tumor necrosis factor-α, CRP, ferritin and d-dimer are significantly elevated in those patients with more severe disease (202, 203). At this stage, shock, vasoplegia, respiratory failure, and even cardiopulmonary collapse are discernible. Systemic organ involvement, including myocarditis, can manifest during this stage. Overall, the prognosis and recovery from this clinical stage are poor.

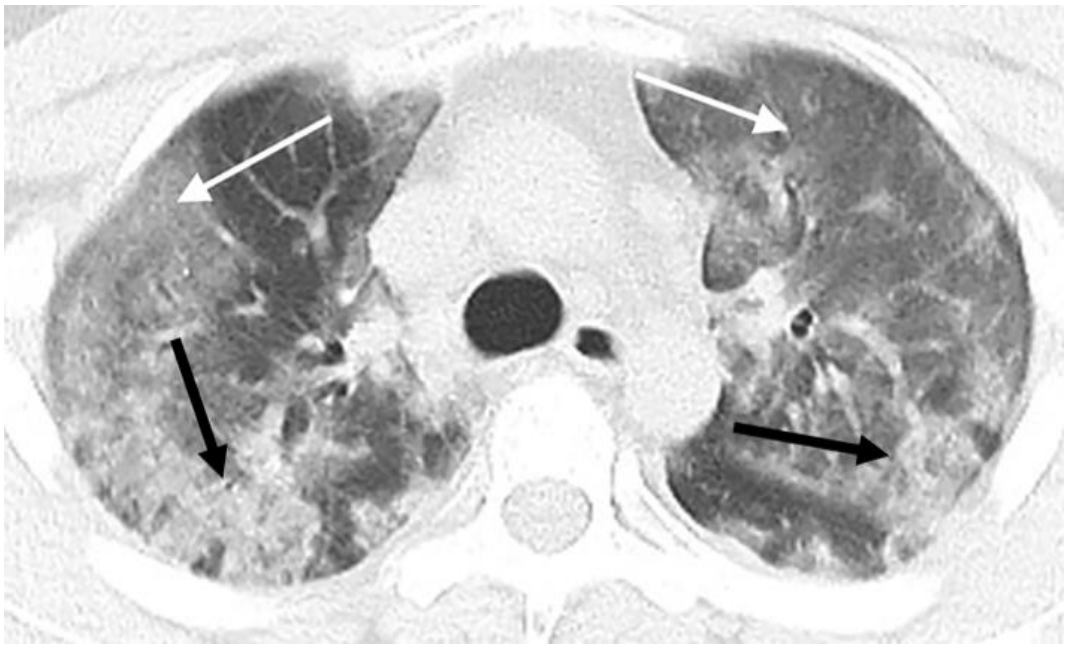
Figure 13: Classification of COVID-19 disease states. *Taken from Siddiqi HK et al J Heart Lung Transplant 2020 (198)*



Regarding the radiological findings, most patients show bilateral pneumonia and only a small percentage of patients with COVID-19 show unilateral pneumonia. The most frequent CT findings are bilateral patchy shadows and ground glass opacities (GGO) (Figure 14). Multilobe involvement and focal lesions (patches, stripes, or nodules) are also very characteristic (204-206). Less characteristics CT findings include centrilobular nodules, tree-in-bud sign, cystic change, pleural effusion, interstitial fibrosis, or lymphadenopathy. CT exams show that lesions are more likely to be localized in the periphery than in the centre of the lungs and the lesions are more patchy than oval (207, 208). Other CT findings include either pure GGO or GGO with reticular and/or interlobular septal thickening, GGO with consolidation, or pure consolidation (209, 210). Less commonly, although characteristic, CT findings include GGO followed by irregular or halo sign, air bronchogram, broncho-vascular bundle thickening, grid-form shadow, and hydrothorax (204). Ground grass-like shadows, fibrous stripes, patchy shadow, and pleural thickening are observed both in common-type and severe or critical-type patients, independent to the severity of COVID-19 (211). Single or multiple lobes of a single lung or both lungs can be affected. Chung M et al. developed radiological score ranging 0 to 20 depending on the degree of involvement for each of the five lung lobes (212). In each lobe, the absence of pathological involvement

corresponded to a lobe score of 0, minimal involvement to a lobe score of 1, mild involvement to a lobe score of 2, moderate involvement to a lobe score of 3, and severe involvement to a lobe score of 4. An overall total severity score is reached by the sum of the five lobe scores.

Figure 14: CT image in a 29-year-old man with COVID-19. Axial thin-section unenhanced CT scan shows diffuse bilateral confluent and patchy ground glass (white arrows) and consolidative (black arrows) pulmonary opacities. *Taken from Chung M et al Radiology 2020 (212)*



Generally, patients with COVID-19 tend to have normal or decreased white blood cell counts, lymphopenia, or thrombocytopenia (213, 214). Patients with high leukocyte count, higher neutrophil count, and lower lymphocyte count (< 0.4 x 10^9/L) showed to be at higher risk for severe COVID-19 pneumonia and composite endpoint (admission to intensive care unit, mechanical ventilation, or death) (215). Also, higher levels of CRP (> 150 mg/L) and increased D-dimer levels (> 1 mg/L) were strongly associated with an increased risk of COVID-19 pneumonia and the composite endpoint (146).

1.5 Clinical Management

The cornerstone of COVID-19 management is represented by oxygenation therapy among patients with dyspnoea in order to obtain an optimal oxygen saturation

(SpO_2) of 92-96% (216). In adults with COVID-19 and acute hypoxemic respiratory failure, conventional oxygen therapy may be insufficient to meet the oxygen needs of the patient. Options for providing enhanced respiratory support include high-flow nasal canula (HFNC) oxygen, non-invasive positive pressure ventilation (NIPPV), intubation and invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO). HFNC is preferred over NIPPV in patients with acute hypoxemic respiratory failure. In fact, in a trial comparing HFNC oxygen, conventional oxygen therapy and NIPPV, patients in the HFNC oxygen arm showed more ventilator-free days (median: 24) than those in the conventional oxygen therapy arm (median: 22) or in the NIPPV arm (19 days; p=0.02). Moreover, 90-day mortality was lower in the HFNC oxygen arm than in either the conventional oxygen therapy arm (hazard ratio [HR]: 2.01; 95CI: 1.01 to 3.99) or the NIPPV arm (HR: 2.50; 95CI: 1.31 to 4.78) (217). In addition, in the subgroup of more severely hypoxemic patients, the intubation rate was lower for the HFNC oxygen arm than for the conventional oxygen therapy or NIPPV arms (HR: 2.07 and 2.57, respectively). These findings were endorsed by a meta-analysis of eight trials with 1084 participants that was conducted to assess the effectiveness of oxygenation strategies prior to intubation. Compared to NIPPV, HFNC oxygen reduced the rate of intubation (OR: 0.48; 95CI: 0.31 to 0.73) and ICU mortality (OR: 0.36; 95CI: 0.20 to 0.63) (218). In patients in whom non-invasive oxygenation cannot provide adequate SpO_2 level, mechanical ventilation may be required. There is no evidence that ventilator management of patients with hypoxemic respiratory failure due to COVID-19 should differ from ventilator management of patients with hypoxemic respiratory failure due to other causes. However, there is evidence that the use of a higher positive end-expiratory pressure (PEEP) strategy (over a lower PEEP strategy) is beneficial in adult patients mechanically ventilated for ARDS related to COVID-19. In fact, PEEP prevents alveolar collapse, improves oxygenation, and minimizes atelectotrauma. A meta-analysis of individual patient data from the three largest trials that compared lower and higher levels of PEEP in patients with COVID-19 found lower rates of ICU mortality and in-hospital mortality with higher levels of PEEP in those with moderate or severe ARDS (219).

Apart from supportive oxygen therapy, several drugs have been used in patients with COVID-19. Some of them, namely hydroxychloroquine, azithromycin, and anti-HIV drugs such as lopinavir/ritonavir, were used in the first months of the pandemic, but they did not show any benefit in patients with SARS-CoV-2 and are not currently recommended (220-222). Convalescent plasma has been used as passive immunotherapy for prevention and treatment of infections for over 100 years (223). In the current pandemic, convalescent plasma obtained from individuals who recovered from COVID-19 has been used in over 75,000 patients with moderate to severe infection as part of an expanded access program (224). However, convalescent plasma transfusion failed to show or to exclude a beneficial or detrimental effect on mortality based on the body of evidence from randomized clinical trials (RTCs) (RR: 0.86; 95CI: 0.69 to 1.06) and it is not currently recommended (225). Currently recommended drugs for the management of patients with COVID-19 are corticosteroids, tocilizumab, low-molecular-weight heparin (LMWH), remdesivir and monoclonal antibodies.

1.5.1 Corticosteroids

During the initial phase of the pandemic, a vast majority of patients received system steroids due to the established evidence of the efficacy of steroid use in systemic inflammatory response syndrome and patients mechanically ventilated due to respiratory illness. Multiple studies showed the benefits associated with the use of corticosteroids, including a decrease in mortality, as observed in a trial at the University of Oxford including 6,000 patients receiving 6 mg of dexamethasone daily (226). Results from the CoDEX trial showed that patients receiving dexamethasone (20 mg or 10 mg daily for 5 days or until discharge) had a mean 6.6 ventilator-free days (95CI: 5.0 to 8.2) during the first 28 days, versus 4.0 ventilator-free days (95CI: 2.9 to 5.4) in patients in the standard care group (difference: 2.26; 95CI: 0.2 to 4.38; p=0.04) (227). In addition, a meta-analysis showed that the administration of systemic corticosteroids, compared with usual care or placebo, was associated with lower 28-day all-cause mortality in critically ill patients with COVID-19 (OR: 0.64; 95CI: 0.50 to 0.82, p<0.001) (228). Currently, corticosteroids are recommended in patients with severe COVID-19 or those

critically ill, while they are not recommended in non-severe patients with COVID-19 not requiring supplemental oxygen therapy (225). In fact, in the RECOVERY trial, dexamethasone (6 mg daily for up to 10 days) compared with the standard of care, resulted in lower 28-day mortality among patients who were receiving either invasive mechanical ventilation (29.3% vs 41.4%; rate ratio: 0.64; 95CI: 0.1 to 0.81) or oxygen alone (23.3% vs 26.2%; rate ratio: 0.82; 95CI: 0.72 to 0.94) at randomization, but not among those receiving no respiratory support (17.8% vs 14.0%; rate ratio: 1.19; 95CI: 0.92 to 1.55) (229).

1.5.2 Tocilizumab

Drugs such as tocilizumab work by binding to the cell-related and soluble IL-6 receptors, inhibiting classic signalling and trans-signalling, which results in improved outcomes of patients with significant pneumonia. In fact, a single dose of tocilizumab 400 mg improved lung function in 91% of patients and decreased the length of hospitalization in a large single-centre trial (230). Several studies on the use of tocilizumab (tocilizumab 8 mg/kg vs placebo or standard of care) among patients with COVID-19 were conducted, although different inclusion criteria were applied (e.g., patients on mechanical ventilation or standard oxygen supplementation) (231-235). The most robust evidence on the effect of tocilizumab in patients with COVID-19 were probably obtained by the RECOVERY study group (236). In this trial, 4,116 adult patients, including 3,385 patients receiving systemic corticosteroids, were included. Overall, 621 (31%) of the 2,022 patients allocated in the tocilizumab arm, and 729 (35%) of the 2,094 patients allocated to standard of care died within 28 days (rate ratio 0.85; 95CI: 0.76 to 0.94; p=0.0028). Moreover, patients allocated to tocilizumab were more likely to be discharged from hospital within 28 days (57% vs 50%; rate ratio: 1.22; 95CI: 1.12 to 1.133, p<0.0001). Among those not receiving mechanical ventilation at baseline, patients allocated in the tocilizumab arm were less likely to reach the composite endpoint of invasive mechanical ventilation or death (35% vs 42%; risk ratio 0.84; 95CI: 0.77 to 0.82, p<0.0001). Based on these evidence, tocilizumab is currently recommended in hospitalized adult patients with severe, or critical COVID-19 who have elevated markers of systemic inflammation (225).

1.5.3 Low-molecular-weight heparin

In severe forms of COVID-19 infection, activation of the coagulation cascade and consumption of clotting factors occurs. Reports from Wuhan indicated that up to 71% of patients who died met the criteria for diffuse intravascular coagulation (237). Inflammation of the lung tissue and dysfunction of its endothelium may lead to a microthrombic phenomenon causing deep venous thrombosis, pulmonary embolism, and thrombotic arterial complications. Data from a retrospective study conducted in China showed that anti-coagulant therapy with LMWH is associated with a reduction in 28-day mortality in patients with COVID-19 and sepsis-induced coagulopathy score ≥ 4 or D-dimer more than 6-fold the upper limit of normal (238). Results from a large US cohort of COVID-19-hospitalized patients confirmed that systemic anticoagulant treatment could be associated with improved outcomes (239). A study conducted on 4,389 hospitalized COVID-19 patients found an approximately 50% reduced hazard of in-hospital mortality and a 30% reduced hazard of intubation compared to patients without anticoagulant therapy (240). Recently, the results of at least 2 clinical trials on heparin therapy among patients with COVID-19 became available: the HEP-COVID trial and the RAPID trial. Results from the HEP-COVID trial (241), showed that in COVID-19-hospitalized patients treated with therapeutic-dose of LMWH, the risk of thromboembolic events and death was significantly reduced compared with patients treated with a standard-dose heparin treatment (composite RR: 0.68; 95CI: 0.49 to 0.96, p=0.03). However, the treatment effect was not seen in ICU patients. However, in the RAPID trial (242), which was conducted in moderately-ill patients with COVID-19 (namely, patients not immediately requiring mechanical ventilation or intensive care), therapeutic heparin was not significantly associated with a reduction in the primary outcome (death or any mechanical ventilation), when compared to prophylactic heparin treatment. Results from this trial suggest that patients with mild COVID-19 disease could benefit from therapeutic heparin treatment, while in patients with severe-critical illness a prophylactic heparin dosage may be suggested.

1.5.4 Remdesivir

Antiviral drugs are thought to work in different phases of viraemia, including in the prevention of viral entry into the host cell and in the prevention of both viral activation and replication. Remdesivir works by inhibiting viral replication via an adenosine analogue that becomes incorporated into the viral RNA, resulting in the inhibition of further viral replication and in early termination of the viral cycle (243). Three RCTs were conducted among COVID-19-hospitalized patients with oxygen saturation >94% and without supplemental oxygen (244-246). Overall, treatment with a five- or ten-day course of remdesivir (200 mg i.v. on the first day, then 100 mg i.v. daily) failed to show or to exclude a reduction in mortality when compared with no remdesivir (RR: 0.69; 95CI: 0.36 to 1.34). A five-day course of remdesivir may increase clinical improvement over no remdesivir (RR: 1.16; 95CI: 1.00 to 1.34) but a ten-day course was not associated with improved clinical status as compared with no remdesivir. For what concerns hospitalized COVID-19 patients with SpO2 $\leq$ 94% on room air, the best available evidence on the effectiveness of remdesivir derive from three RTCs comparing treatment with remdesivir (200 mg at day one, 100 mg daily days 2-10) against no remdesivir treatment (244, 245, 247), and one RCT comparing five days of treatment against ten days of treatment (248). A pooled analysis of these studies failed to show a mortality benefit at 28 days (RR: 0.92; 95CI: 0.77 to 1.10). However, patients receiving treatment with remdesivir trend toward greater clinical improvement at 28 days than patients not receiving remdesivir (RR: 1.13; 95CI: 0.91 to 1.41). In addition, treatment with remdesivir was associated with a shorter median time to recovery (median 11 vs 18 days; rate ratio: 1.31; 95CI: 1.12 to 1.52) and with a decreased need for mechanical ventilation (RR: 0.57; 95CI: 0.42 to 0.79). Finally, subgroups analysis from the SOLIDARITY and the ACTT-1 trials reported outcomes of mortality, time to recovery and serious adverse events among patients on invasive ventilation or ECMO (244, 245). Results from these analyses showed that treatment with remdesivir was not associated with a reduction in mortality among patients on invasive ventilation and/or ECMO (RR: 1.23; 95CI: 0.99 to 1.53). Similarly, remdesivir failed to show or exclude a reduction in time to recovery among these patients (HR: 0.98; 95CI: 0.70 to 1.36). Given the results

obtained from the above-mentioned RCTs, a five-day course of Remdesivir is currently recommended in hospitalized-COVID-19 patients needing oxygen therapy, but not requiring mechanical ventilation and/or ECMO (225).

1.5.5 Monoclonal Antibodies

Neutralizing antibodies directed against the receptor-binding domain of SARS-CoV-2 spike protein were evaluated either as prophylactic or therapeutic agents for COVID-19. Animal models showed that treatment with antibodies may more rapidly reduce viral load in the upper and lower airways of infected animals, resulting in reduced viral-induced pathology (249, 250). Among the available monoclonal antibodies for COVID-19, the Infectious Diseases Society of America (IDSA) currently recommends the use of casirivimab/imdevimab as prophylactic treatment of patients exposed to COVID-19 who are at high risk of progression to severe disease. Additionally, the use of casirivimab/imdevimab, bamlanivimab/etesevimab or sotrovimab as therapeutic agents in patients with mild to moderate COVID-19 at risk for progression to severe disease is currentle recommended by IDSA (225). One RCT reported results on post-exposure prophylaxis with casirivimab/imdevimab in patients exposed to COVID-19 who were at high risk of progression (251). In this study, 1,505 persons tested negative for SARS-CoV-2 infection within 96 hours following household contact with a diagnosis of SARS-CoV-2 infection were randomized 1:1 to receive 1200 mg of casirivimab/imdevimab subcutaneously or a placebo. Symptomatic SARS-CoV-2 infection developed in 11 of 753 participants in the casirivimab/imdevimab group (1.5%) and in 59 of 752 participants in the placebo group (7.8%) with a relative risk reduction of 81.4% ($p<0.002$). Among symptomatic infected participants, the median time of resolution of symptoms was 2 weeks shorted with casirivimab/imdevimab than with placebo (1.2 weeks and 3.2 weeks respectively), and the duration of a high viral load ($>10^4$ copies per millilitre) was shorter (0.4 weeks and 1.3 weeks respectively). One phase III RCT assessed a single infusion of either 1200 mg or 2400 mg of casirivimab/imdevimab in non-hospitalized participants with mild-to-moderate COVID-19 (252). Among 275 participants with risk factors for severe disease, the least-squares mean difference (combined

casirivimab/imdevimab dose groups vs placebo group) in the time-weighted average change in viral load from day 1 through day 7 was -0.56 $\log_{10}$ copies per millilitre (95CI: -1.02 to -0.11) among patients who were serum antibody-negative at baseline, and -0.41 $\log_{10}$ copies per millilitre (95CI: -0.71 to -0.10) in the overall trial population. In the overall population, 6% of the patients in the placebo group and 3% of the patients in the casirivimab/imdevimab groups reported at least one medically attended visit; among patients who were serum antibody-negative at baseline, the corresponding percentages were 15% and 6% (difference: -9 percentage points; 95CI: -29 to 11). The efficacy of bamlanivimab was analysed in at least two RCTs. One phase II RCT reported on non-hospitalized patients with recently diagnosed mild to moderate COVID-19 randomized to treatment with either a single infusion of bamlanivimab in one of three doses (700 mg, 2800 mg or 7000 mg) or placebo (253). The observed mean decrease from baseline in the log viral load for the entire population was -3.81, for an elimination of more than 99.97% of viral RNA. For patients in the 2800 mg dose group, the difference from placebo in the decrease from baseline was -0.53 (95CI: -0.98 to -0.08, p=0.02); smaller and non-significant differences from placebo in the change from baseline were observed among patients who received the 700 mg dose or the 7000 mg dose. On days 2 to 6, patients who received bamlanivimab had a slightly lower severity of symptoms than those who received placebo. The percentage of patients who had COVID-19-related hospitalization or visit to an emergency department was 1.6% in the bamlanivimab groups and 6.3% in the placebo group. In the BLAZE-1 phase II/III RCT, 613 patients who tested positive for SARS-CoV-2 infection and had 1 or more mild-to-moderate symptoms were randomized in one of four different groups: bamlanivimab 700 mg, 2800 mg or 7000 mg, or combination treatment with bamlanivimab 2800 mg plus etesevimab 2800 mg, or placebo (254). The change in log viral load from baseline to day 11 was -3.72 for 700 mg group, -4.08 for 2800 mg group, -3.49 for 7000 mg group, -4.37 for combination treatment group, and -3.80 for placebo group. Compared with placebo, the difference in the change in log viral at day 11 resulted significant only in patients in the combination treatment group (-1.00 to -0.14, p=0.01). The proportions of patients with COVID-19-related hospitalizations or emergency department visits were 5.8% (9 events) for placebo,

1.0% (1 event) for 700 mg group, 1.9% (2 events) for 2800 mg group, 2.0% (2 events) for 7000 mg group, and 0.9% (1 event) for combination treatment group. Finally, a phase III RCT reported results on non-hospitalized participants with mild-to-moderate COVID-19 who were at risk for severe disease and who were treated with a single infusion of sotrovimab 500 mg (255). Unlike other studies, participants with immunocompromising conditions were not excluded from this trial. The results showed that, among 583 participants (291 in the sotrovimab group, and 292 in the placebo group), the risk of COVID-19 progression was significantly reduced by 85% (97.24%; 95CI: 44% to 96%, p=0.002). All five patients enrolled in the study who were admitted to ICU, including one who died by day 29, received placebo. Recently, a meta-analysis conducted by the Cochrane review group showed the pooled results on the efficacy of monoclonal antibodies among persons with COVID-19 (256). Overall, the results showed that among non-hospitalized patients, casirivimab/imbdevimab may reduce hospital admission or death (2400 mg RR: 0.43; 95CI: 0.08 to 2.019; 8000 mg RR 0.21; 95CI: 0.02 to 1.79), bamlanivimab/etesevimab reduced the risk of death by day 30 (RR: 0.05; 95CI: 0.00 to 0.81) and hospital admission by day 29 (RR 0.30; 95CI: 0.16 to 0.59) and sotrovimab reduced the number of participants with oxygen requirement (RR: 0.11 95CI: 0.02 to 0.45), and hospital admission or death by day 30 (RR: 0.14; 95CI 0.04 to 0.48). However, the meta-analysis showed that, among hospitalized individuals with COVID-19, casirivimab/imdevimab has probably little or no effect on mortality by day 30 (RR: 0.94; 95CI: 0.87 to 1.02), invasive mechanical ventilation or death (RR 0.96; 95CI: 0.90 to 1.04) nor alive at hospital discharge by day 30 (RR: 1.01; 95CI: 0.98 to 1.04). Bamlanivimab also showed little or no effect on mortality by day 30 (RR: 1.39; 95CI: 0.40 to 4.83), development of severe symptoms at day 5 (RR 1.17; 95CI: 0.75 to 1.85), time to hospital discharge (HR: 0.97; 95CI: 0.78 to 1.20) and mortality by day 90 (HR: 1.09; 95CI: 0.49-2.43). The authors concluded that the current evidence on monoclonal antibodies is insufficient to draw meaningful conclusions regarding the treatment with SARS-CoV-2 neutralising monoclonal antibodies. The emergence of SARS-CoV-2 variants raised concerns regarding the efficacy of monoclonal antibodies in patients infected with VOIs or VOCs. Unfortunately, there is limited data from clinical

studies. Bamlanivimab alone and the combination of bamlanivimab/etesevimab had activity against pseudovirus expressing the del69-70 + N501Y mutation found in the alpha variant, but pseudovirus expressing spike protein from the beta variant had reduced susceptibility to bamlanivimab and etesevimab (257). *In vitro* neutralization studies showed that bamlanivimab lost activity against the delta variant, but etesevimab retained activity (258). On the contrary, casirivimab and imdevimab individually and together had neutralization activity against pseudovirus expressing all spike proteins substitutions found in the alpha and in the beta variants (259). In *in vitro* neutralization studies, casirivimab and imdevimab retained activity against the delta variant (258).

1.6 The Prognostic Role of CRP and LDH in Patients with COVID-19

It is known that CRP and LDH are often elevated in patients with SARS-CoV-2 infection. A meta-analysis showed that elevated CRP (73.6% of patients; 95CI: 65.0 to 81.3%), elevated LDH (46.2%; 95CI: 37.9% to 54.7%) and lymphopenia (47.9%; 95CI: 41.6% to 54.9%) were among the most prevalent laboratory findings in patients with COVID-19 (260). In the same study, a correlation between these laboratory abnormalities and the severity of COVID-19 was found. Namely, patients with increased CRP (OR: 3.0; 95CI: 2.1 to 4.4), increased LDH (OR: 6.7; 95CI: 2.4 to 18.9) or lymphopenia (OR: 4.5; 95CI: 3.3 to 6.0), were found to be at high risk for severe COVID-19. In a Chinese study conducted among 140 patients with COVID-19, CRP showed significant increase in patients with severe COVID-19 compared to those with non-severe disease (93.9% vs 56.1%, $p<0.0001$) (261). Moreover, patients with CRP > 41.8 mg/L showed to be at risk for severe complications (HR: 4.39; 95CI: 1.92 to 10.03, $p<0.0001$). In another study, CRP was found to be significantly higher in patients with severe or critical COVID-19 disease compared with patients with ordinary illness (median, 60.8 mg/L vs 7.7 mg/l) (262). Moreover, CRP was identified as independent predictors of death (OR: 1.02; 95CI: 1.01 to 1.03, $p<0.0001$) with a sensitivity of 90.5%, a specificity of 77.6%, a positive predictive value (PPV) of 61.3% and a negative predictive value (NPV) of 95.4% with a threshold of 41.4 mg/l being considered. Sharifpour M. et al. showed similar results (263) and they additionally showed that CRP levels

increased in a linear fashion during the first week of hospitalization, peaking on day 5, after which CRP levels decreased continuously. As compared to those who died, patients who survived had lower peak CRP levels and earlier decline in CRP levels compared with patients who survived. In fact, the slope of CRP change during the first 7 days resulted an independent predictor for mortality (OR 1.03 per unit change; 95CI: 1.01 to 1.05, p<0.001). Finally, a systematic review and meta-analysis showed that patients with LDH > 245 U/l had increased risk of critical COVID-19 disease or death (OR: 43.24; 95CI: 9.92 to 188.49, p<0.001) (264).

Dickens BSL et al elaborated a laboratory score, the "Rule-of-6", based on laboratory parameters collected within 48 hours from admission in patients hospitalized for COVID-19, namely CRP, ferritin and LDH (265). The authors found that CRP > 60 mg/l, ferritin > 600 µg/l, and LDH >600 U/l aided to early identify COVID-19 patients at risk of deterioration to the ICU, yielding notably high out-of-sample areas under the curve (AUCs) with the above CRP, ferritin and LDH cut-offs at 0.99, 0.88 and 0.90, respectively.

1.7 Aim of the present study

As discussed above, increased CRP and LDH levels showed to be correlated with death and severe disease in COVID-19 patients. However, it is not clear how high levels of CRP and LDH can influence the disease progression in patients with SARS-CoV-2 infection. It is reasonable to hypothesise that CRP levels reflect the triggering of cytokine storm in COVID-19 patients, which showed to be implicated in the genesis of COVID-19 complications, including ARDS (96). On the other hand, increased LDH is probably related to lung inflammation and damage due to SARS-CoV-2 infection that could anticipate clinical deterioration of the respiratory function. However, a direct correlation between CRP and LDH levels in COVID-19 patients and the worsening of the respiratory function has not been proven yet. Given such considerations, the aim of the present study was to analyse the correlation between laboratory parameters at admission (including CRP) in patients hospitalized for COVID-19 and the deterioration of the respiratory function in the subsequent days of the disease.

2.0 Methods

2.1 Study Design

A multicentre retrospective study was conducted among adult inpatients with COVID-19 hospitalized between January 2020 and April 2021 and referring to the following clinical centres:

- Unit of Infectious Diseases. University Hospital Federico II, Naples.
- Hospital "D. Cotugno". AORN "Dei Colli", Naples.
- Hospital "G. Rummo", Benevento.
- Hospital "Sant'Anna e San Sebastiano", Caserta;

All included patients had a diagnosis of SARS-CoV-2 infection performed with a molecular (PCR) nasal and oropharyngeal swab and were hospitalized for COVID-19-related symptoms. The following exclusion criteria were applied:

- Absence of respiratory symptoms related to COVID-19.
- No serum CRP performed at admission (within 48 hours).
- No serum LDH performed at admission (within 48 hours).
- No arterial blood gas (ABG) test performed at admission (within 48 hours).
- History of a previous SARS-CoV-2 infection or presence of positive SARS-CoV-2 molecular test antecedent 2 weeks from hospitalization.
- History of SARS-CoV-2 vaccination.
- Other hospitalizations in the previous 30 days.

Respiratory symptoms related to COVID-19 included: cough, dyspnoea, tachypnoea, and respiratory failure. Extra-pulmonary manifestations of COVID-19 were not considered for the inclusion in the present study.

The primary outcome of the study was to analyse the correlation between serum CRP at hospital admission and the worst partial pressure of arterial oxygen to fraction of inspired oxygen ratio (P/F ratio) observed during the hospitalization in patients with COVID-19-related respiratory symptoms. Secondary outcomes were:

- To analyse the correlation between serum LDH at hospital admission and the worst P/F ratio observed during the hospitalization.
- To analyse the correlation between blood lymphocyte count at admission and the worst P/F ratio observed during the hospitalization.

- To analyse the presence of risk factors for a worst P/F ratio < 200 during the hospitalization.
- To elaborate a score for prediction of respiratory function deterioration
- To investigate the presence of risk factors for intensive care need during the hospitalization.
- To investigate the presence of risk factors for death during hospitalization.

The clinical records of all included patients were revised, and the following data were collected and reported on an electronic dataset: demographic and clinical data, main comorbidities, laboratory parameters (including CRP, LDH, white blood count), ABGs, outcomes (ICU needs and death). All laboratory parameters were collected at admission (within 48 hours) and every 7 days from admission. All results from ABGs performed during hospitalization were collected and the P/F ratios were calculated. The lowest value of P/F ratio observed during the hospitalization for each patient were collected and reported as "worst P/F ratio".

2.2 Statistical Analysis

All the variables were tested for parametric/non-parametric distribution with the Kolmogorov-Smirnov test. Comparisons between categoric dichotomic variables were performed with the χ^2 test (or with Fischer's exact test when applicable), while comparisons between ordinary variables were conducted with the Mann-Whitney's U test (non-parametric variables). Comparisons of demographic and laboratory parameters were stratified according three different clinical outcomes: worst P/F ratio < 200 (meant as the lowest P/F ratio observed during the entire hospitalization for each patient), ICU admission during the hospitalization, and death. The Spearman's test and the linear regression analysis were used to correlate demographic (age) and laboratory parameters (CRP, LDH, lymphocyte count) with ordinary clinical parameters (namely, worst P/F ratio during hospitalization). The multivariate liner regression analysis was performed including all the parameters significantly correlated with the dependant variable at the univariate linear regression analysis with a $p<0.2$. In order to investigate the presence of risk factors for all the three clinical outcomes (worst P/F ratio <200 during hospitalization, ICU

admission, death), the logistic regression analysis was used. Parameters associated with the dependant variables ($p<0.2$) at the univariate logistic regression analysis were then included in a multivariate model. A predictive score for worst P/F ratio < 200 during hospitalization was elaborated according to the results of the logistic regression analysis. The age of patients was included in the predictive score based on the same criteria used for the Charlson's comorbidity index (266). The predictive score was correlated with the worst P/F ratio during hospitalization using the Spearman's test and logistic regression analysis (ordinary worst P/F ratio) and logistic regression analysis (worst P/F ratio < 200). The diagnostic accuracy for worst P/F ratio < 200 of the predictive score was evaluated with a ROC curve. For all the test, a p-value < 0.05 was considered for significance. IBM SPSS© version 27 was used for statistical analysis.

3.0 Results

Globally, 323 patients from the 4 participants centres were included in the study in accordance with the inclusion/exclusion criteria. Demographic and clinical characteristics of the included patients are reported in Table 3. Most of the included patients were male (63.2%), and half of them were aged > 60 years (median: 61 years, interquartile range [IQR]: 49-70). One-hundred thirty-six patients (42.1%) had at least one chronic comorbidity (cardiovascular disease, COPD, chronic kidney disease [CKD], malignancy, cirrhosis, diabetes). Most patients showed impaired laboratory parameters within 48 hours from hospital admission, with 35.9% and 50.8% of patients showing CRP values above 60 mg/l and a lymphocyte count below 1000 cell/µl, respectively. Only a minority of patients (4.6%) showed LDH values above 600 U/l, but 142 patients (44%) had LDH values above 300 U/l within 48 hours from hospital admission. The median worst P/F ratio observed during the hospitalization was 207 (IQR: 124-301) and about a half of all the included patients (47.4%) had a worst P/F ratio below 200 during the hospitalization. The ICU necessity rate and death rate were 15.8% and 6.8% respectively.

Table 3: Demographic and clinical characteristics of patients hospitalized for COVID-19 and included in the study (N=323)

Sex (M; n, %)	204 (63.2)
Age (median, IQR)	61 (49-70)
Age > 60 years (n, %)	163 (50.5)
Comorbidities (n, %)	
- Cardiovascular disease	55 (17.0)
- COPD	54 (16.7)
- CKD	15 (4.6)
- Malignancy	41 (12.7)
- Cirrhosis	3 (0.9)
- Diabetes	53 (16.4)
N° of comorbidities (n, %)	
- 0	187 (57.9)
- 1-2	112 (34.7)
- 3-5	24 (7.4)
Baseline CRP (mg/l; median, IQR)	41.15 (15.10-88.75)
Baseline CRP > 60 mg/l (n, %)	116 (35.9)
Baseline LDH (U/l; median, IQR)	288 (230-369)
Baseline LDH > 600 U/l (n, %)	15 (4.6)
Baseline LDH > 300 U/l (n, %)	142 (44.0)
Baseline Lymphocyte count (cell/µl; median, IQR)	990 (680-1432)
Baseline Lymphocyte count < 1000 cell/µl (n, %)	164 (50.8)
Worst P/F ratio (median, IQR)	207 (124-301)
Worst P/F ratio < 200 (n, %)	153 (47.4)
ICU admission (n, %)	51 (15.8)
Exitus (n, %)	22 (6.8)

COPD: chronic obstructive pulmonary disease. CKD: chronic kidney disease. CRP: c-reactive protein. LDH: lactate dehydrogenase; ICU: intensive care unit

Patients with a worst P/F ratio below 200 during the hospitalization were more frequently male (70.9% vs 58.1%, $p<0.05$) and older (63 years; IQR: 54-72 vs 58 years; IQR: 42-67, $p<0.001$) than those with P/F $\geq$ 200 (Table 4). Patients who needed ICU were more frequently male compared to those with no ICU necessity (78.4% vs 61.4%, $p<0.001$), while those who had a fatal outcome were older than those who survived (78 years; IQR: 71-84 vs 60 years; IQR: 48-68, $p<0.001$). Baseline CRP levels were found to be significantly higher among patients with a worst P/F ratio < 200 during the hospitalization ($p<0.001$) and those who died ($p<0.001$). Baseline LDH levels were also higher among patients with a worst P/F

ratio < 200 during the hospitalization ($p<0.001$) and those with a fatal outcome ($p<0.05$); they also found to be higher among patients who needed ICU admission ($p<0.001$). Finally, blood lymphocyte count at admission was lower among patients with a worst P/F ratio during the hospitalization ($p<0.001$), those who needed ICU ($p<0.05$) and those who died ($p<0.01$). Interestingly, the presence of comorbidities was not associated with a worst P/F ratio < 200 nor with ICU admission. However, among patients who survived, most had no comorbidities ($p<0.001$), while patients who had a fatal outcome, more frequently had 1-2 comorbidities (54.4% vs 33.2%, $p<0.05$) or 3-5 comorbidities (22.7% vs 6.3%, $p<0.01$) compared to those who survived. When literature-derived cut-offs for CRP, LDH and lymphocyte blood count were applied, it was found that CRP > 60 mg/l, LDH > 600 U/l, and lymphocyte < 1000 cell/µl were associated with all the three unfavourable outcomes (worst P/F ratio < 200 during hospitalization, ICU admission, death). Given the paucity of patients with LDH levels above 600 U/I, a cut-off of 300 U/l was also applied. The prevalence of patients with LDH > 300 was higher among patients with a worst P/F ratio below 200 during the hospitalization ($p<0.001$) and those who were admitted to the ICU ($p<0.05$), compared with patients with a worst P/F ratio above 200 and those who did not needed ICU, respectively. No differences in the rate of patients with LDH > 300 U/l were found among patients who had a fatal outcome when compared with those who survived.

Table 4: Differences in the demographic and laboratory parameters among included patients, stratified according to the presence or the absence of three unfavourable outcomes (Worst P/F ratio < 200 during hospitalization, ICU admission, Death)

	Worst P/F			**ICU**			**Death**		
	<200	***≥ 200***	***p-value***	***Yes***	***No***	***p-value***	***Yes***	***No***	***p-value***
Male Sex (n, %)	70.9	58.1	**<0.05**	78.4	61.4	**<0.001**	68.2	63.9	0.683
Age (median, IQR)	63 (54-72)	58 (42-67)	**<0.001**	65 (52-71)	60 (49-70)	0.132	78 (71-84)	60 (48-68)	**<0.001**
Age > 60 years (n, %)	56.4	37.7	**<0.001**	66.0	49.2	**<0.05**	90.9	49.0	**<0.001**
Comorbidities (n, %)									
- Cardiovascular disease	18.3	15.9	0.564	17.6	16.9	0.898	50.0	14.6	**<0.001**
- COPD	19.0	14.7	0.307	7.8	18.4	0.064	22.7	16.3	0.298
- CKD	3.9	5.3	0.558	3.9	4.8	0.568	13.6	4.0	**<0.05**
- Malignancy	14.4	11.2	0.388	7.8	13.6	0.257	27.3	11.6	**<0.05**
- Cirrhosis	0.7	1.2	0.625	0.0	1.1	0.596	0.0	1.0	0.638
- Diabetes	17.0	15.9	0.788	15.7	16.5	0.879	45.5	14.3	**<0.001**
N° of comorbidities (n, %)									
- 0	54.9	60.6	0.301	62.7	57.0	0.445	22.7	60.5	**<0.001**
- 1-2	37.9	31.8	0.247	35.3	34.6	0.919	54.4	33.2	**<0.05**
- 3-5	7.2	7.6	0.876	2.0	8.5	0.081	22.7	6.3	**<0.01**
Baseline CRP (mg/l; median, IQR)	60.0 (21.1-129.9	32.0 (14.30-60.10)	**<0.001**	77.4 (12.0-137.0)	39.0 (16.0-75.0)	0.059	87.15 (45.40-149.0)	38.5 (15.0-80.0)	**<0.001**
Baseline CRP > 60 mg/l (n, %)	49.0	25.5	**<0.001**	52.9	33.6	**<0.01**	68.2	34.4	**<0.01**
Baseline LDH (U/l; median, IQR)	342 (256-427)	269 (211-321)	**<0.001**	357 (258-479)	280 (220-351)	**<0.001**	337 (254-479)	287 (228-360)	**<0.05**
Baseline LDH > 600 U/l (n, %)	10.1	0.0	**<0.001**	16.0	2.7	**<0.001**	13.6	4.1	**<0.05**
Baseline LDH > 300 U/l (n, %)	59.7	32.3	**<0.001**	62.0	42.2	**<0.05**	59.1	44.3	0.180
Baseline Lymphocyte count (cell/µl; median, IQR)	861 (605-1220)	1100 (720-1550)	**<0.001**	880 (520-1150)	1000 (690-1450)	**<0.05**	670 (430-920)	1000 (690-1440)	**<0.01**
Baseline Lymphocyte count < 1000 cell/µl (n, %)	62.2	43.4	**<0.001**	64.7	49.8	0.051	76.2	50.5	**<0.05**

ICU: intensive care unit. IQR: interquartile range COPD: chronic obstructive pulmonary disease. CKD: chronic kidney disease. CRP: c-reactive protein. LDH: lactate dehydrogenase

At the correlation analysis, a significant and inverse correlation was found between the worst P/F ratio during hospitalization and: i) age (Spearman's ρ= -0.299, p<0.001), ii) basal CRP values (Spearman's ρ= -0.293, p<0.001), iii) basal LDH values (Spearman's ρ= -0.363, p<0.001). On the other hand, a direct correlation was found between the worst P/F ratio during hospitalization and basal lymphocyte count (Spearman's ρ=0.250, p<0.001). At the linear regression analysis, a significant and negative association was found between worst P/F ratio during hospitalization (dependant variable) and: i) age (*B*= -2.372, r^2=0.125, p<0.001), ii) baseline CRP (*B*=-0.504, r^2=0.084, p<0.001) (Figure 15), iii) baseline LDH (*B*=-0.256, r^2=0.116, p<0.001) (Figure 16). The lymphocyte count at admission was not significantly associated with the worst P/F ratio during the hospitalization at the regression analysis.

Figure 15: Linear regression analysis between worst P/F ratio during hospitalization (dependent) and CRP levels at admission.

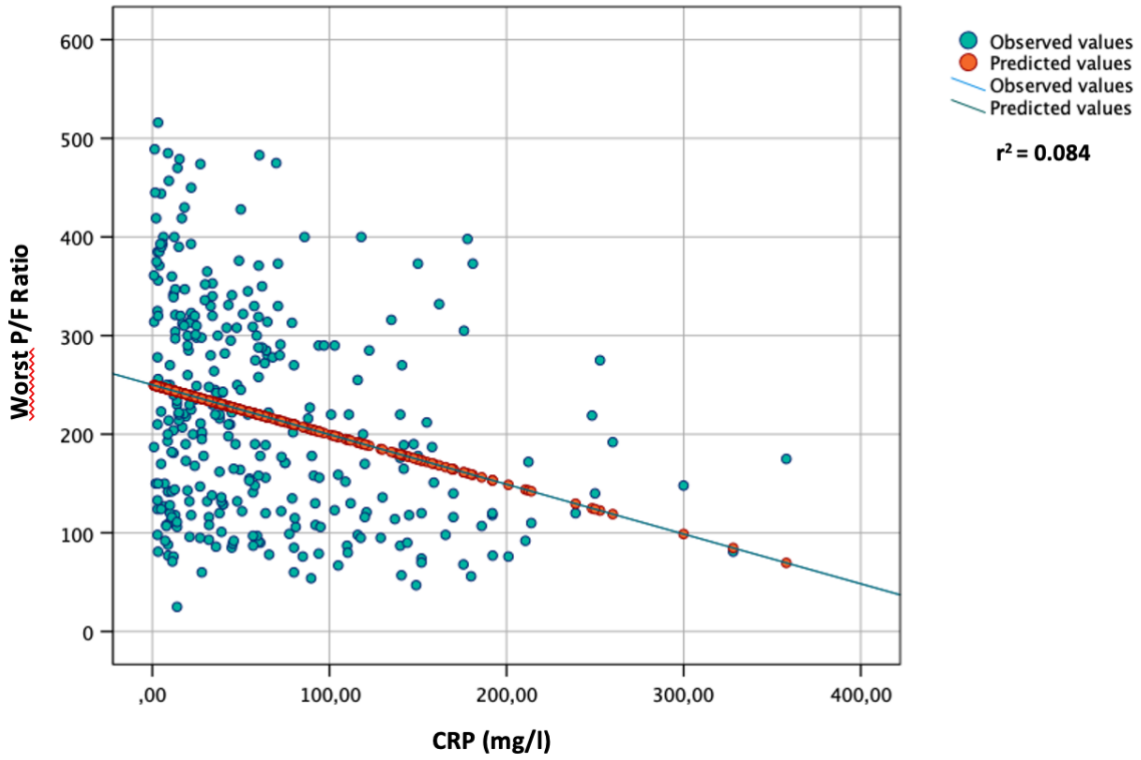
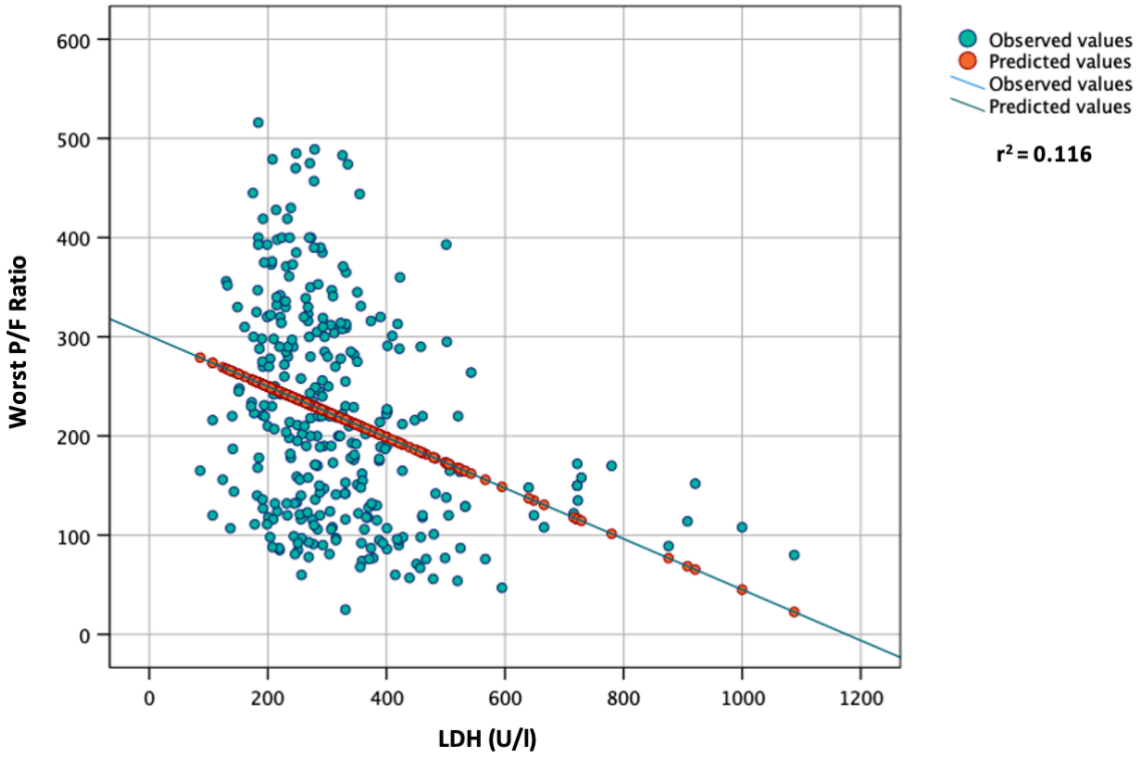


Figure 16: Linear regression analysis between worst P/F ratio during hospitalization (dependent) and LDH levels at admission



Interestingly, age, baseline CRP values, and baseline LDH values were significantly associated with the worst P/F ratio during the hospitalization at the multivariate linear regression analysis (all p<0.001) (Table 5).

Table 5: Univariate and multivariate linear regression analysis between the worst P/F ratio during hospitalization (dependent), age and laboratory parameters at admission

	Univariate Analysis			Multivariate Analysis		
	B	95CI	p-value	*B*	95CI	p-value
Worst P/F ratio[#]	-	-	-	-	-	-
Age	-2.372	-3.073 to -1.672	**<0.001**	-2.079	-2.724 to -1.433	**<0.001**
CRP	-0.504	-0.690 to -0.319	**<0.001**	-0.323	-0.497 to -0.149	**<0.001**
LDH	-0.256	-0.335 to -0.177	**<0.001**	-0.205	-0.279 to -0.130	**<0.001**
Lymphocyte	0.000	-0.005 to +0.006	0.862	-	-	-

[#]Worst P/F ratio was set as dependant variable.

B: B coefficient. 95CI: 95% confidence intervals. CRP: c-reactive protein. LDH: lactate dehydrogenase

At the logistic regression analysis, male sex, age above 60 years, baseline CRP above 60 mg/l, baseline LDH above 300 U/l and lymphocyte count below 1000 cell/µL, were found to be associated with a worst P/F ratio below 200 during hospitalization (Table 6). Male sex (aOR 1.73, p<0.05), age > 60 years (aOR: 1.80, p<0.05), CRP > 60 mg/l (aOR: 2.33, p<0.01) and LDH > 300 U/l (aOR: 2.47, p<0.001) also showed to be independently associated with a worst P/F ratio below 200 during the hospitalization.

Table 6: Univariate and multivariate logistic regression analysis for worst P/F ratio < 200

	Univariate Analysis			Multivariate Analysis		
	OR	95CI	p-value	aOR	95CI	p-value
Worst P/F ratio < 200[#]	-	-	-	-	-	-
Male sex	1.75	1.10 to 2.80	**<0.05**	1.73	1.03 to 2.91	**<0.05**
Age > 60 years	2.14	1.36 to 3.56	**<0.001**	1.80	1.10 to 2.94	**<0.05**
1-2 comorbidities	1.31	0.83 to 2.08	0.247	-	-	-
3-5 comorbidities	0.94	0.41 to 2.15	0.936	-	-	-
CRP > 60 mg/l	2.81	1.75 to 4.52	**<0.001**	2.33	1.37 to 3.94	**<0.01**
LDH > 300 U/l	3.11	1.95 to 4.93	**<0.001**	2.47	1.50 to 4.06	**<0.001**
Lymphocyte < 1000 cell/µl	2.14	1.36 to 3.37	**<0.001**	1.38	0.83 to 2.29	0.209

[#]Worst P/F ratio < 200 was set as dependant variable.

OR: Odds Ratio. 95CI: 95% confidence intervals. aOR: adjusted Odds Ratio. CRP: c-reactive protein. LDH: lactate dehydrogenase

Similarly, male sex, age > 60 years, CRP > 60 mg/l at admission and LDH > 300 U/l at admission were associated with ICU admission (Table 7), with only male sex (aOR: 2.31, p<0.05) and CRP > 60 mg/l at admission (aOR: 2.00, p<0.05) being independently associated with ICU admission at the multivariate analysis

Table 7: Univariate and multivariate logistic regression analysis for ICU admission

	Univariate Analysis			Multivariate Analysis		
	OR	95CI	p-value	aOR	95CI	p-value
ICU admission[#]	-	-	-	-	-	-
Male sex	2.28	1.12 to 4.65	**<0.05**	2.31	1.08 to 4.92	**<0.05**
Age > 60 years	2.00	1.06 to 3.77	**<0.05**	1.66	0.86 to 3.21	0.130
1-2 comorbidities	1.03	0.55 to 1.93	0.919	-	-	-
3-5 comorbidities	0.22	0.03 to 1.64	0.214	-	-	-
CRP > 60 mg/l	2.22	1.21 to 4.08	**0.01**	2.00	1.03 to 3.86	**<0.05**
LDH > 300 U/l	2.23	1.20 to 4.16	**<0.05**	1.74	0.89 to 3.41	0.107
Lymphocyte < 1000 cell/µl	1.85	0.99 to 3.44	0.054	1.18	0.60 to 2.33	0.628

[#]ICU admission was set as dependant variable.
OR: Odds Ratio. 95CI: 95% confidence intervals. aOR: adjusted Odds Ratio. CRP: c-reactive protein. LDH: lactate dehydrogenase

Finally, age > 60 years, the presence of chronic comorbidities, CRP > 60 mg/l at admission, LDH > 300 U/l at admission and lymphocyte < 1000 cell/µl at admission, were associated with fatal outcome (Table 8). At the multivariate analysis, age > 60 years (aOR: 8.65, p<0.01), the presence of 3-5 chronic comorbidities (aOR: 8.17, p<0.01) and CRP > 60 mg/l at admission (aOR: 5.45, p<0.01) were independently associated with death.

Table 8: Univariate and multivariate logistic regression analysis for death

	Univariate Analysis			Multivariate Analysis		
	OR	95CI	p-value	aOR	95CI	p-value
Death[#]	-	-	-	-	-	-
Male sex	1.21	0.48 to 3.07	0.683	0.93	0.33 to 2.60	0.885
Age > 60 years	10.42	2.39 to 45,39	**<0.01**	8.65	1.86 to 40.33	**<0.01**
1-2 comorbidities	2.41	1.01 to 5.77	**<0.05**	2.85	0.92 to 8.87	**0.07**
3-5 comorbidities	4.36	1.45 to 13.11	**<0.01**	8.17	1.72 to 38.71	**<0.01**
CRP > 60 mg/l	4.09	1.62 to 10.37	**<0.01**	5.45	1.82 to 16.34	**<0.01**
LDH > 300 U/l	1.81	0.75 to 4.38	0.185	1.02	0.36 to 2.90	0.969
Lymphocyte < 1000 cell/µl	3.13	1.12 to 8.78	**<0.05**	2.20	0.71 to 6.78	0.169

[#]Death was set as dependant variable.
OR: Odds Ratio. 95CI: 95% confidence intervals. aOR: adjusted Odds Ratio. CRP: c-reactive protein. LDH: lactate dehydrogenase

Given the results from the linear and logistic regression analysis for worst P/F ratio < 200, a 11-points numeric ordinary score based on age, sex, CRP at admission and LDH at admission (ASCL score) was elaborated, as shown in Table 9.

Table 9: The ASCL score, based on age, sex, CRP at hospital admission and LDH at hospital admission

Parameter	Points
Age	
- < 50 years	0
- 50-59 years	1
- 60-69 years	2
- 70-79 years	3
- ≥ 80 years	4
Sex	
- Female	0
- Male	2
CRP	
- ≤ 60 mg/l	0
- > 60 mg/l	2
LDH	
- ≤ 300 U/l	0
- > 300 U/l	3

OR: Odds Ratio. 95CI: 95% confidence intervals. aOR: adjusted Odds Ratio. CRP: c-reactive protein. LDH: lactate dehydrogenase

The median ASCL score among patients included in the study was 5 (IQR: 3-7). The highest the ASCL score, the highest was the risk for P/F<200 during the hospitalization (Table 10). At least the half of the patients with an ASCL score ≥ 5 had a worst P/F ratio < 200 during the hospitalization. An ASCL score of 0 was found to be a protective factor for P/F ratio < 200 during hospitalization (OR: 0.20; 95CI: 0.07 to 0.60, p<0.01), while patients with ASCL score of 7 (OR 4.59; 95CI: 1.81 to 11.65, p<0.01): , 8 (OR: 2.70; 95CI: 1.14 to 6.40, p<0.05) or 9 (OR: 2.53; 95CI: 1.11 to 5.79, p<0.05), were significantly at risk for P/F deterioration below 200.

Table 10: Logistic regression analysis for P/F ratio < 200 during the hospitalization according to the ASCL score

	P/F ratio < 200 (n=153)		
ASCL score	**%***	**OR**	**95CI**
0 (n=24)	16.7	0.20	0.07 to 0.60
1 (n=4)	0.0	#	#
2 (n=31)	32.3	0.50	0.23 to 1.09
3 (n=40)	30.0	0.43	0.21 to 0.88
4 (n=35)	31.4	0.47	0.22 to 0.99
5 (n=40)	52.5	1.26	0.65 to 2.45
6 (n=48)	47.9	1.03	0.55 to 1.90
7 (n=28)	78.6	4.59	1.81 to 11.65
8 (n= 26)	69.2	2.70	1.14 to 6.40
9 (n=28)	67.9	2.53	1.11 to 5.79
10 (n=5)	80.0	4.54	0.50 to 41.04
11 (n=5)	100.0	#	#

*Raw percentage
#Incalculable due to paucity of data (denominator equal to 0)
ASCL: age, sex, CRP, LDH. ICU: intensive care unit. OR: odds ratio. 95CI: 95% confidence intervals

The regression analysis for the worst P/F ratio (dependant variable) showed that for each 1-point increase of the ASCL score, a reduction of the worst P/F ratio of approximately 19 is expected (*B*=-18,98; 95CI: -22,93 to -15.02, r^2=0.222, p<0.001;) (Figure 17). The diagnostic accuracy of ASCL score for P/F ratio deterioration below 200 was almost good (AUC: 0.717, p<0.001) (Figure 18).

Figure 17: Linear regression analysis between worst P/F ratio during hospitalization (dependent) and ASCL score at admission

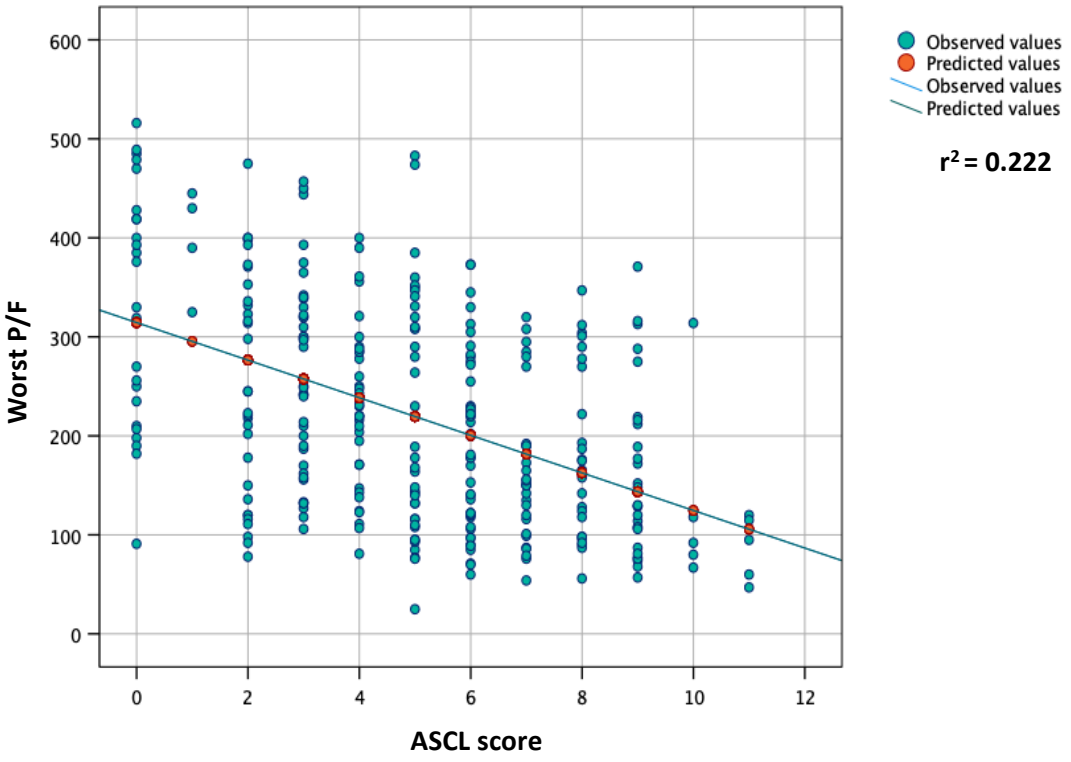
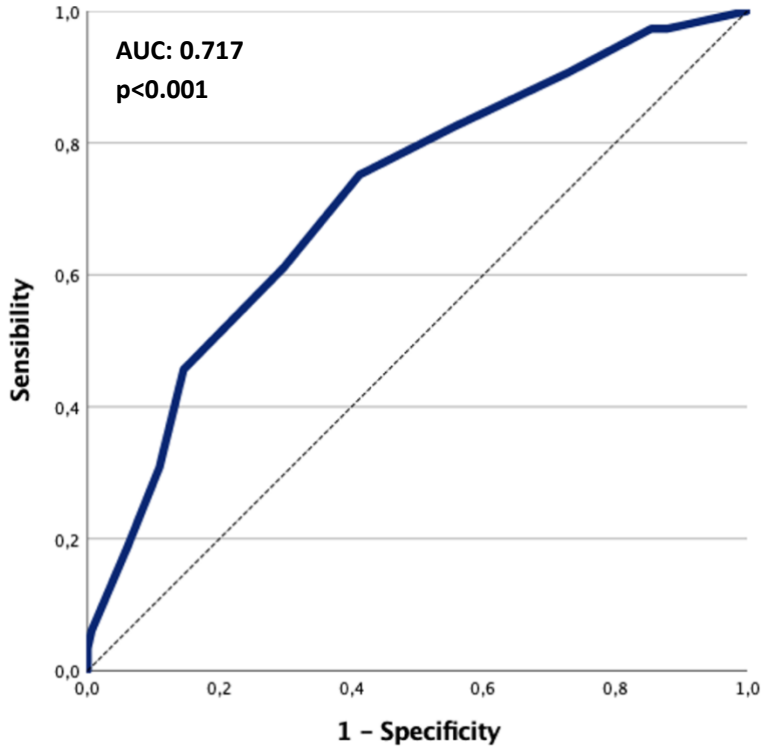


Figure 18: ROC curve for the diagnostic accuracy of the ASCL score in predicting P/F ratio deterioration below 200



4.0 Discussion

The pandemic of coronavirus disease 2019 (COVID-19) has caused an unprecedent global social and economic impact, and high numbers of deaths. The clinical features of COVID-19 are diverse and range from asymptomatic to critical illness and death, with severe and critical cases represented by 14% and 5% of laboratory-confirmed COVID-19 patients, respectively (267). This posed a high burden to the healthcare system as it consumed most of its medical resources during the first months of the pandemic and contributed to the majority of deaths. Severe patients present signs of dyspnoea, respiratory frequency ≥ 30/min, blood oxygen saturation ≤ 93%, P/F ratio < 300, and/or lung infiltrates > 50% within 24 to 48 hours (267). Critically ill cases may experience respiratory failure that requires mechanical ventilation, shock, disseminated coagulopathy, and other organs failure requiring admission to the ICU (60). A good understanding of the possible risk factors in combination to disease immunopathology associated with COVID-19 severity is helpful for clinicians in identifying patients who are at high risk and require prioritized treatment to prevent disease progression and adverse outcome (268). Risk factors range from demographic factors, such as age (36, 60, 61), sex and ethnicity (62, 269), diet and lifestyle habits (270, 271), to underlying diseases (65, 77, 79, 81, 84, 85) and complications (240, 272-274). Several laboratory abnormalities were also associated with increased risk of severe COVID-19 and disease progression (170, 275-282). There is a spate of literature showing the association between lymphopenia and COVID-19 severity (143, 283). The decreased lymphocyte counts might be caused by viral attachment, immune injuries from inflammatory mediators, or exudation of circulating lymphocytes into inflammatory lung tissues (284). Elevated serum LDH levels have been widely reported in COVID-19 cases and were predominantly higher in severe patients (285). A meta-analysis showed that the mean value of LDH in severe patients with COVID-19 was indeed 1.54 times higher than in non-severe cases (286). The positive correlation between increasing levels of LDH and disease severity makes it a valuable candidate biomarker for monitoring severe COVID-19 patient. In fact, using a mathematical modelling approach (287), LDH was identified to have the highest weight in both training and evaluation sets based on the AUC score, when

compared to other biomarkers (low lymphocyte counts and high-sensitivity CRP), which stressed that high level of LDH was the most valuable predictive factor for mortality. Since higher levels of LDH had been observed in non-survivors at the early stage of illness (288), measuring this parameter at admission will be of greater predictive value for patients' risk rather than during the ICU. Elevated LDH values showed to be correlated with the lung injury Murray score in patients with COVID-19 (289) and thus, elevated LDH values at the early stages of SARS-CoV-2 infection can likely predict a severe deterioration of respiratory function. Finally, high level of serum CRP is a key markers of disease progression and a risk factor for mortality of severe COVID-19 patients and it is indicative of developing cytokine storm in COVID-19 patients (60, 290). Out of 32 studies, 20 showed a nearly four-fold higher risk of poor outcomes in COVID-19 patients with elevated CRP (291). Moreover, laboratory analysis of patients admitted to the ICU showed an overall increase of CRP levels in the first seven days, peaking between days two and three (292), suggesting that CRP levels may be correlated with lung injury and respiratory function in patients with COVID-19. Although the role of CRP as predictive factor for disease progression and mortality in patients with SARS-CoV-2 infection has been widely established, a direct correlation between CRP levels and respiratory function is yet to be documented. As an indicator of triggered cytokine storm, elevated CRP levels in the early phases of the infection may predict a subsequent lung damage and respiratory function deterioration caused by the hyper-inflammatory status in patients with COVID-19, as conceivable from the results by *Dickens BSL et al*. (265). In their study, the authors indeed elaborated a score based on CRP, LDH and ferritin, using CRP > 60 mg/l, LDH > 600 U/l and ferritin 600 µL/l as cut-off, which showed good accuracy for disease progression. Aside from the limited number of patients included in this validation cohort, the score by *Dickens BSL et al.* did not consider the prognostic weight of demographic factors (such as sex, age, and ethnicity) which are known to heavily influence the prognosis of patients with COVID-19. Finally, the authors did not clarify whether their score was correlated with respiratory function deterioration or other clinical variables.

Given the above-mentioned considerations, the aim of this study was to analyze the correlation between laboratory parameters at admission and the respiratory function in patients hospitalized for COVID-19. The respiratory function was evaluated with the partial pressure of arterial oxygen to fraction of inspired oxygen ratio (P/F ratio) which takes into account either the lung capabilities in providing tissues oxygenation and the patients' oxygen demand. Patients who showed a P/F ratio < 200 were considered as having the most severe clinical picture and thus, this cut-off was chosen for correlation. The chosen laboratory parameters ad admission to correlate with the worst P/F ratio were CRP, LDH and blood lymphocyte count, according to literature evidence and their possible role in predicting worsening of the respiratory mechanics. Cut-offs for laboratory parameters at admission were chosen according to literature evidence. The initial cut-off chosen for LDH was > 600 U/l, in alignment with the "Rule-of-6" provided by *Dickens BSL et al.* (265). However, in consideration of the paucity of patients with such high levels of LDH at admission (15, 4.6%), this cut-off was lowered to 300 U/l, also in accordance with the results from a systematic review and meta-analysis (264). Demographic, clinical and laboratory parameters of included patients were stratified according to the worst P/F ratio observed during hospitalization (< or ≥ 200) as well as to other clinical outcomes, namely ICU admission and death (Table 4). We found that patients with respiratory deterioration had higher levels of CRP and LDH, and a lower lymphocyte count compared with patients with a P/F > 200 during the hospitalization. Similar results were obtained comparing laboratory parameters in patients requiring or not ICU admission and in patients who survived compared with those with a fatal outcome, as described in other studies (283, 288, 291). Interestingly, the presence of comorbidities was not associated with a P/F < 200 or with ICU admission. However, cardiovascular disease, CKD, malignancy, and diabetes, as well as the presence of at least 1 comorbidity, were significantly more frequent in patients with a fatal outcome. This result points up that, contrary to laboratory parameters and other demographic characteristics (e.g., age), the presence of comorbidities does not directly influence respiratory function and mechanics. In fact, at the correlation and linear regression analyses, we found an inverse and significant association between age ($p<0.001$) serum CRP ($p<0.001$,

Figure 15) and LDH (p<0.001, Figure 16) and the values of worst P/F ratio during hospitalization at the multivariate analysis (Table 5). Moreover, the logistic regression analysis showed that male sex (aOR: 1.73; 95CI: 1.03 to 2.91, p<0.05), age > 60 years (aOR: 1.80; 95CI: 1.10 to 2.94, p<0.05), CRP > 60 mg/l (aOR: 2.33; 95CI: 1.37 to 3.94, p<0.01) and LDH > 300 U/l (aOR: 2.47; 95CI: 1.50 to 4.06, p<0.001) were independently associated with respiratory deterioration (P/F below 200 during the hospitalization). CRP > 60 mg/l was found to be an independent risk factor for ICU admission (aOR: 2.00; 95CI: 1.03 to 3.86, p<0.05, Table 7) and death (aOR: 5.45; 95CI: 1.82 to 16.34, p<0.01, Table 8), while LDH > 300 only showed an association with ICU admission at the univariate logistic regression analysis (OR: 2.23; 95CI: 1.20 to 4.16, p<0.05). The blood lymphocyte count <1000 cells/µL was not associated with P/F < 200 at the multivariate logistic regression analysis, probably because the white blood count of patients with COVID-19 is considerably influenced by the inflammatory status and, thus, dependant to CRP values. Similarly, no associations were found between lymphocyte < 1000 cells/µL and ICU admission or death. Finally, the presence of at least 3 comorbidities was only found to be an independent risk for mortality (aOR: 8.17; 95CI: 1.72 to 38.71, p<0.01). In accordance with the results from the multivariate logistic regression analysis for P/F < 200 during hospitalization, we elaborated a score based on Age, Sex, CRP and LDH (ASCL score, Table 9). A progressive increase in the ASCL score was found to be significantly associated with disease progression and respiratory function (Table 10, Figure 17). In particular, an ASCL score > 6 was found to be associated with a high risk of P/F < 200 during the hospitalization. The regression analysis for P/F < 200 showed that for each 1-point increase in the ASCL score, the worst P/F is reduced by approximately 19 (*B*= -18.98, p<0.001). The diagnostic accuracy of ASCL score for P/F < 200 and, thus, for respiratory deterioration, was almost good (Figure 18).

This study had some limitations, especially in consideration of its retrospective nature that partially compromised the data collection. In fact, several patients were excluded from the study as they did not perform CRP/LDH nor ABG at admission. Only a minority of patients needed ICU admission (51, 15.8%) or had a fatal outcome (22, 6.8%) and this must be taken into account in interpreting the results.

Nevertheless, we believe that the sample size was sufficient to draw significant conclusions regarding correlations with the worst P/F. Moreover, it is known that serum CRP levels in patients with COVID-19 may be affected by the presence of bacterial co-infections. In this cohort of patients, the presence of bacterial co-infections at admission was not systematically evaluated. However, a systematic review and meta-analysis showed that the co-infection rate among patients with COVID-19 is relatively low (7%) (293) and this rate is even lower when considering the presence of bacterial co-infections at hospital admission (3%) (294). Having said that, a routine and systematic screening for bacterial infections at hospital admission in patients with COVID-19 is not recommended, and we believe that the possible presence of bacterial co-infections at hospital admission among patients included in this study cohort unlikely affected the results. Finally, the ASCL score must be validated in more numerous prospective cohorts in order to draw significant conclusion regarding its diagnostic accuracy in predicting respiratory deterioration in patients with COVID-19.

In conclusion, despite the above-mentioned limitations, the results from this study showed that CRP and LDH levels at admission well correlates with a respiratory function deterioration in patients with COVID-19. A score based on Age, Sex, CRP and LDH at admission seems to have a good predictive role in the progression of the respiratory clinical picture. Patients with CRP > 6 mg/l or LDH > 300 U/l at hospital admission, as well as patients with an ASCL score > 6 at hospital admission, should be prioritized for careful respiratory function monitoring and early treatment with specific drugs (i.e., remdesivir, monoclonal antibodies), when indicated, in order to prevent a progression of the disease.

5.0 Bibliography

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