Accademia Nazionale dei Lincei

Commissione Salute

COVID-19: Summer 2020 report

Maurizio Cecconi, Guido Forni, Alberto Mantovani

The first version of this Report was issued on March 25, 2020. The Lincei Health Committee (Commissione Salute) plans to update and upload this report as new data significantly change our perception of the disease. The opinions here expressed fall within the sole responsibility of the above mentioned Commissione.

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INDEX

1. Introduction.

This past winter, Italy and the entire world were severely hit by the spread of SARS-Cov-2 infection. In the face of this unexpected pandemic, which is putting many aspects of human civilization in great difficulty, the *Commissione Salute* of the Accademia Nazionale dei Lincei felt that it was its social responsibility to provide the society at large with an updated summary of the current knowledge on the features of this infection and treatments available to tackle this new virus.

This Report does not intend to provide a comprehensive state-of-the-art review, but rather a snapshot of this rapid evolving situation, a field undergoing rapid evolution, with a daily flood of scientific publications and non-peer reviewed reports. The preparation of a COVID-19 report in this context is therefore a risky undertaking and the drafters of this document are well aware of their limits.

With the limits of the metaphor, we have been experiencing *wartime medicine* and *wartime scientific research*. We are too often called upon to respond to the plight of patients with empirical approaches. Despite these conditions, a rigorous assessment of the data remains and increasingly becomes an absolute obligation especially at a time when the pressure of the disease seems to ease and almost disappear. Finding a balance between emergency and methodological rigor represents a major challenge [Baden and Rubin, 2020]. Hopefully, with the above mentioned cautionary remarks, this report will provide for the moment the tools to better understand and respond to the unprecedented challenge we are facing.

2. Graphic summary.

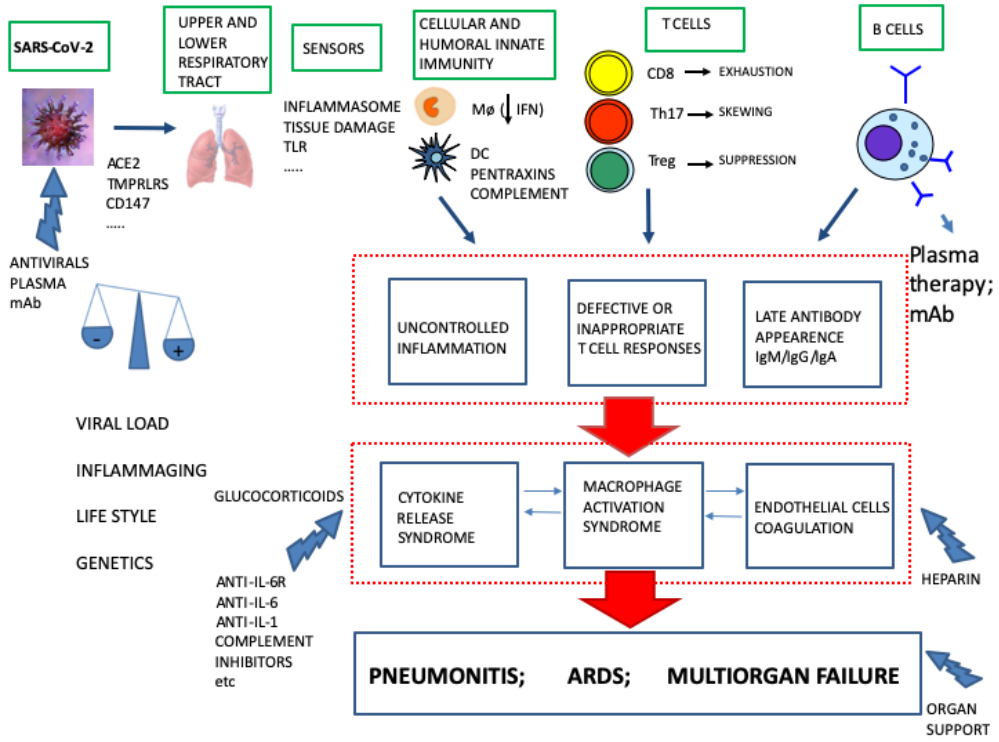

Fig. 1. Schematic representation of the pathogenesis of COVID-19. ACE2, Angiotensin Converting Enzyme-2; TLR, Toll-like receptors; Mϕ, macrophages; DC, dendritic cells; IFN, interferon; , low IFN; ARDS, acute respiratory distress syndrome; …. dots indicate more receptors involved (e.g. CD147 for viral entry). Modified with permission from Cecconi et al, 2020.

3. SARS-CoV-2.

The virus. Coronavirus disease 2019 (COVID-19) is initiated by the infection of the SARS-CoV-2 virus, a coronavirus. Coronaviruses are a large family of viruses that cause illness ranging from the common cold which usually occurs in the winter months to more severe diseases such as Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS) and COVID-19.

- *The structure.* SARS-CoV-2 is a spherical particle of about 0.125 μm in diameter. The virus membrane (the pericapsid) is made by three glycoproteins, Spike, Envelope and Membrane, and an external and a lipid coating. It is therefore an *enveloped virus*. On the SARS-CoV-2 surface, three Spike proteins aggregates to form homotrimers. Several Spike homotrimers protrude outside the pericapsid giving rise to a sort of crown, hence the name Coronavirus. Inside the pericapsid, the Nucleoprotein, a fourth SARS-CoV-2 structural protein, interacts with a single stranded positive sense RNA of ~ 30 kb which contains 30,000 bases, a very large genome for an RNA virus (Fig. 2). A proofreading mechanism keeps this large genome from accumulating frequent mutations [Corum and Zimmer, 2020].

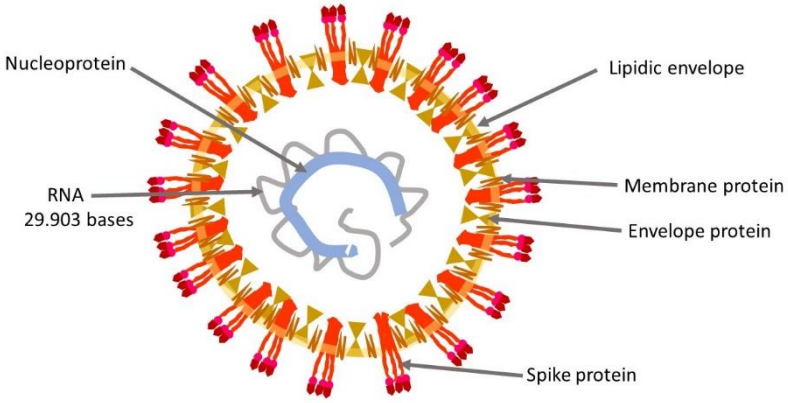

Fig.2. *From Seminara et al, 2020*

- *Entry into human cell.* The Receptor-Binding Domains (RBD) on the external portion of Spike homotrimers interact at high affinity with an ectoenzyme normally expressed by several human cells, the Angiotensin-Converting Enzyme 2 (ACE2) (Fig. 3 ; *see also 8. Predisposing factors*). After the Spike-ACE2 binding, two proteases normally present on human cell membrane (Furin and TMPRLRS) cleave Spike proteins and their exposed fusion peptides are instrumental for the fusion of the virus membrane with the membrane of human cells [Hoffman et al, 2020]. The virus RNA enters the cell and is immediately translated into viral proteins. The cell dies releasing millions of new viruses that infect other cells and other individuals.

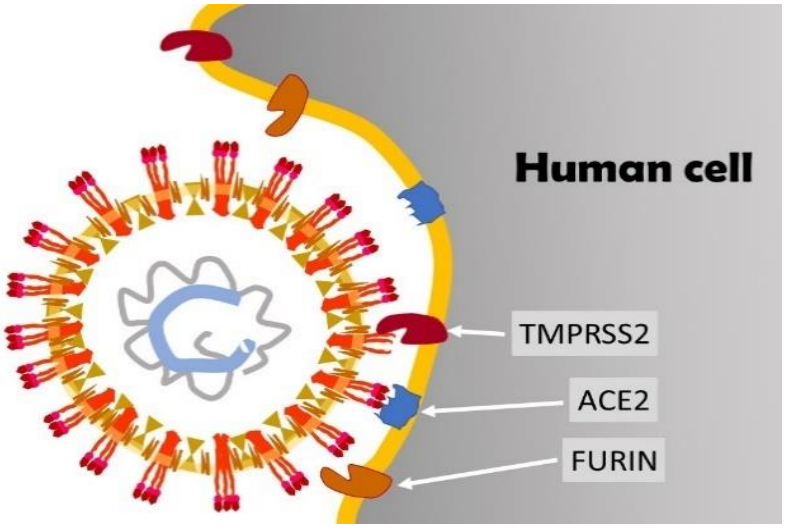

Fig.3. *From Seminara et al, 2020*

● *Alternative entry.* Bioinformatics analyses based on the protein crystal structures indicate that the transmembrane dipeptidyl peptidase-4 (DPP4), which is a receptor for MERS-CoV, might also bind SARS-CoV-2 [Li et al, 2016]. However, direct evidence that DPP4 is used by SARS-CoV-2 for cell entry is still missing.

● *The contagion*. The contagion by the SARS-CoV-2 occurs when breathing respiratory droplets released by an infected person via coughing, sneezing or speaking. The epithelial cells of mucosal surfaces of the nose and throat express on their surface high levels of ACE-2 receptors. Respiratory droplets finally land on various surfaces where the virus maintains its infective capacity for various times. Therefore, SARS-CoV-2 can also be transmitted by contact when a susceptible person touches the mucosa of the mouth, nose or eye after capturing the virus. For a detailed analysis of the mechanisms by which the respiratory droplets released by infected persons transmits the contagion see Seminara et al [2020].

4. Immune resistance to SARS-CoV-2 infection.

With the arrival of the SARS-Cov-2 virions on mucosal surfaces a quantitative and time-sequence competition between the virus ability to infect and host defense mechanisms is put into motion. The final outcome (inhibition of the viruses, minor and asymptomatic infection, a severe and fatal disease) rests on a series of confrontations between the virus infective ability and distinct immune defense mechanisms [Matricardi et al, 2020; Moryiama et al, 2020].

● *The mucociliary barrier.* The viruses arriving on mucosal surfaces have to deal with the effective barrier made by the mucus. Mucus is a viscoelastic gel continuously produced by goblet cells in the mucosal membranes and by particular glands. It contains several glycoproteins, salts, lactoferrin, enzymes, Complement components and antibodies (secretory IgA and IgM) [Matricardi et al, 2020]. It covers the mucosa lining the nose, throat and lungs. By harnessing and neutralizing viral particles, it prevents a direct contact of the viruses with the surface of the epithelial cell of the mucosa. Mucus production is regulated mainly by lymphokines secreted by sentinel lymphocytes associated with the mucous membranes [*See Fig. 4*, Seminara et al, 2020].

● *Cilia beats.* Mucus is continually transported by the coordinate beats of the cilia of the hairy cells in the mucous membranes, then it is swallowed and destroyed in the stomach. Mucus transport is fundamental for its protective action. The intensity of cilia beats is negatively regulated by cytokines and is lowered by environmental pollutants present in the breathed air, by the humidity and by the low temperature [NCBI, 2020].

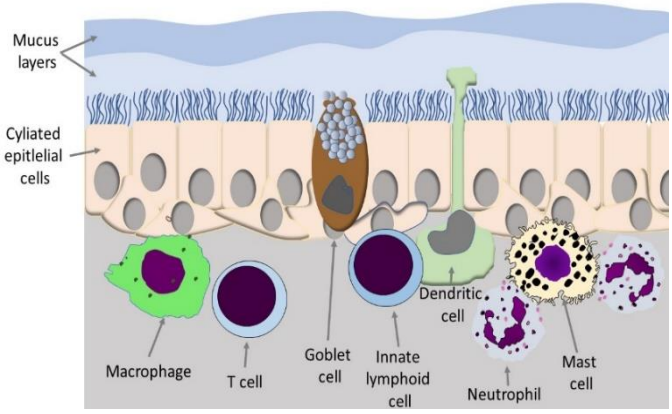

Fig. 4. Inhalation of cold, dry and polluted air affects the upper airway mucosa, impairs mucociliary clearance, increases mucus production and impairs antiviral innate immune responses. [Moryiama et al, 2020]. *From Seminara et al, 2020.*

● *Efficacy of the first barrier.* We do not know yet how many SARS-CoV-2 are eliminated by the mucociliary barrier,

although the influence that environmental factors (humidity, temperature, air pollution…) have on the defense against coronavirus infections that cause winter colds is well known. It is thus probable that in the great majority of cases, the mucus and the beats of cilia clear the invaders. Nevertheless, it is reasonable to assume that the effectiveness of the immune reaction mechanism may play a crucial role in determining whether the infection will end, be benign or will have major consequences [Mantovani et al, 2019; Matricardi et al, 2020].

- *The binding to ACE2.* If the SARS-CoV-2 particles manage to overcome the mucus barrier, they infect the cells of the respiratory mucosa that express high levels of ACE2 receptors. The invaded cell is endowed with several mechanisms to sense and block viral invasions. SARS-CoV-2 RNA recognized and destroyed by RNA helicases. The peculiarities of viral RNA are also recognized by RIG-1-like receptors and Toll like receptors (TLR) present in the cell cytosol that induce signaling cascades leading to the production of Type I interferon and other cytokines. Interferon activates antiviral programs in the invaded cell and induces the expression of families of transmembrane proteins that inhibit virus entry in nearby cells. In mucosal associated leukocytes signals transduced by RIG-1-like and Toll like receptors trigger the assembly of the inflammasomes, cytoplasmatic multimeric complexes which permit the production of high amounts of important pro-inflammatory cytokines (IL1, IL18…). The secretion of the mature form of these cytokines promotes the release of additional cytokines and the induction of an innate immunity inflammatory response. Moreover, inflammatory caspase 1 cleaves gasdermin D cause cytokine release and pyroptotic cell death, a kind of programmed cell death that occurs most frequently upon viral infection. The death of the virus infected cell is an effective way to block the progression of the viral infection [Vabret et al, 2020].

- *SARS-Cov-2 escape mechanisms.* Faced with this series of reaction mechanisms, SARS-CoV-2 has elaborated various ways to avoid viral RNA recognition or to antagonize with receptor signal transduction. Several proteins coded by the SARS-Co-V2 interfere with intracellular reaction mechanisms: NSP1 protein prevents the cell from assembling antiviral proteins; NSP3 alters the regulation of cell protein, thus reducing cell ability to put in motion the antiviral mechanisms; NSP10 and NSP16 protect viral RNA from destruction; ORF6 interfere with the signals activating cell reaction to viral RNA; ORF9b suppresses intracellular signaling thus limiting antiviral defenses nonspecifically. Other viral proteins interfere with the signaling downstream of interferon release: ORF3a, ORF6 viral proteins alter various steps of the signal transduction pathway that bridge the interferon receptor to the STAT proteins that activate transcription of interferon-stimulated genes [Zhou et al, 2020b; Corum and Zimmer, 2020].

- *Innate immunity.* If the infecting viral load is enough to overcome the mucosal barrier and sneak through intracellular defense mechanisms, the spread of SARS-Cov-2 RNA becomes evident in nostril, pharynx and eye mucosal surfaces. Here, viral infection is confronted by multiple reaction mechanisms of innate immunity. The intensity, efficacy and features of this reaction determines whether the virus spread will be efficiently blocked in upper airways and how many viruses will reach the lungs [Matricardi et al, 2020; Zhou et al, 200b]. Humoral elements of innate immunity, including interferons, the Complement and coagulation systems, soluble proteins of innate immunity such as the Mannose Binding Lectin, natural antibodies and cross-reactive antibodies

induced by previous infections by different viruses are immediately confronted with the virus spreading and the local cell damages. The damages associated with the viral invasion, alarm signals and cytokines induce the dilatation of local post capillary venule with the subsequent exit of plasma and of reactive leukocytes. The reactivity of leukocytes will be driven by multiple alarm signals, cytokines and interferons released by infected and dying epithelial cells [Vabret et al, 2020].

- *Cytokine production.* Metatranscriptomic sequencing performed to profile the inflammatory reaction shows a markedly elevated expression of several chemokine genes. CXCL8 chemokine gene overexpression is directly connected with the recruitment of neutrophils into the inflamed tissues, while the upregulation of CCL2 and CCL7 chemokine genes plays a central role in the recruitment of monocytes/macrophages [Zhou et al, 2020b]. Consonant with this pattern of gene expression, neutrophils, activated dendritic cells, monocytes are the most abundant cells present in the reactive infiltrate [Vabret et al, 2020]. SARS-CoV-2 infection triggers the expression of several interferon-inducible genes. While this suggest that a robust interferon response is ongoing, the interferon gene is not upregulated. This discrepancy is probably due to the interference exerted by SARS-CoV-2 proteins on the interferon gene expression and on the signaling cascade of interferon receptors, has a crucial importance in shaping the efficacy of the inflammatory reaction and the evolution of the viral infection [Vabret et al, 2020; Zhou et al, 2020b; Hadjadj et al, 2020]. The confrontation between the host's innate immunity and SARS-CoV-2 will determine whether infection will be blocked in upper airways or the virus will reach the lungs, causing the patient to be sick or very sick [Matricardi et al, 2020]. Epidemiological data suggest that this reaction is extremely effective as the vast majority of SARS-CoV-2 infected people are asymptomatic or minimally symptomatic [Day, 2020].

- *Suppression of cellular immunity during COVID-19.* T cells play a central role in the healing of numerous infectious diseases. CD4 T cells are required for the activation of the production of high affinity antibodies by B cells. Moreover, they modulate and guide a more efficient inflammatory response. Progress has been made in defining the viral epitopes recognized by T cells [Grifoni et al, 2020]. CD8 T cells are able to find and kill virus infected cells before they become factories of millions of new viral particles. Given the central role that T cells have in the healing of viral infections, it is not surprising that SARS-CoV-2 manages to reduce T cell number in peripheral blood. There is also evidence for skewing of the adaptive response in a Th17 direction [Xu et al, 2020]. Th17 cells drive neutrophil-mediated resistance against extracellular bacteria but are ineffective as anti-viral effectors. Interestingly, IL-6 and IL-1 which are candidate therapeutic targets (*see below*) are important drivers of Th17 differentiation [Mantovani et al, 2019]. In addition, there is evidence that innate and adaptive lymphocytes undergo exhaustion, which likely results in defective anti-viral immune responses. Not only NK cells are decreased in the peripheral blood of COVID-19 patients, but there is also evidence of an impaired cytotoxicity, as well as an impaired production of chemokines, interferon-gamma and TNF-α [Wilk et al., 2020; Zheng et al., 2020b; Maggi et al, 2020; Hadjadj et al, 2020].

- *T cell reactivity.* A persistent and robust T reactivity to the peptides of Nucleocapsid, Membrane and Spike SARS-CoV-2 proteins is evident in patients recovering from SARS-CoV-2 infection. Often a preferential specific reaction against Spike peptides was found [Vabret et al, 2020]. While the

induction of a robust T cell reactivity is essential for virus control, a too high CD4 T cell reactivity may facilitate the development of lung immunopathologies and exacerbate the cytokine storm [Zhao et al, 2016; Leslie 2020].

● *Antibody.* Recent studies on large numbers of patients are leading to overcome the limits of initial observations that were due to both the small number of studied patients and technical issues. All of the 285 COVID-19 patients studied in China by Long et al [2020] developed antibody response to Spike protein within 19 days after symptom onset. In this study, three types of serum conversion were observed: a first onset of IgM followed by the production of IgG; a synchronous IgM and IgG production; IgG production before IgM production. The latter two patterns of seroconversion, often observed in COVID-19 patients, are intriguing as they contrast what is reported in immunology textbooks [Lou et al, 2020; Xiang et al, 2020]. In New York, a study on 1343 COVID-19 patients which recovered from a mild illness showed that over 99% of them developed anti-Spike IgG over a period of 7 to 50 days from symptoms onset [Wainberg et al, 2020]. Another study showed that COVID-19 patients not only produce antibodies to the Spike protein, but also to other structural and non-structural SARS-CoV-2 proteins. A stronger response was observed against Nucleoprotein and Surface protein. Through the combination of these antigens, it was possible to ide]ntify a SARS-CoV-2 specific seroconversion in all COVID-19 patients tested, including those that did not produce anti-Spike antibodies [Hachim et al, 2020] . Other studies identified also the production of antibodies to the Spike Receptor Binding Doman (RBD, *see also 11, Entry into human cell*) [Okba et a, 2020; Brouwer et al, 2020]. These antibodies appear to be of particular interest since they should block the interaction of SARS-CoV-2 and the ACE2 receptor on the human cell membrane. These results have obvious implications for serology and for antibody based therapeutic approaches as discussed below (*See 11, Therapeutic antibodies*). IgA antibodies appear in the circulation and in saliva [Wu et al, 2020; Guo et al, 2020] and may play a key role in the defense at the mucosal surfaces. There is evidence that circulating antibodies can have neutralizing activity in vitro. However, it is unclear whether there is a different antibody response in individual recovering from severe, mild and asymptomatic COVID-19 and how long antibody response will last [Mandavilli, 2020].

● *Immune memory.* With regards to policy and public health implications, a key issue is the occurrence and duration of a protective immune memory in patients who have recovered. Currently available data show that, in most cases, recovered patients do not get sick again. Ralph Baric recently stated that immune response and resistance should last at least 6-12 months [Baric, 2020]. Evidence from SARS suggests persistence of memory for 2-3 years [Prompetchara et al, 2020]. Spike-specific memory B cells can be isolated from the peripheral blood of most convalescent patients [Andreano et al, 2020; Wec et al, 2020; Leslie, 2020]. However, it is yet not clear whether the presence of antibodies to the Spike molecule or to the RBS of the Spike molecule correlate with virus neutralization.

It has been proposed that individuals with robust antibody response could safely return to normal life. However, WHO recently warned about the possibility of reinfection and emphasized the fact that the presence of antibodies is no guarantee against reinfection [WHO, 2020a].

5. SARS-CoV-2 spreading and containment.

● *The proximal origins of SARS-CoV-2*. Coronaviruses are zoonotic, meaning they are transmitted horizontally between animals and vertically between animals and humans. In the past twenty years a coronavirus has made the so-called "inter-species jumps" three times, passing from its natural host to humans: in 2003 in China the SARS virus; in 2015 the MERS virus in the Middle East; in late 2019 in Wuhan, again in China, the SARS-CoV-2. It is likely that, as already happened for the other coronaviruses, even in the case of SARS-CoV-2 the original host was the bat. There are more than 1,200 bat species worldwide, which account for 20% of the mammalian species: a huge virus reservoir. The passage to humans is believed to require an intermediate host: in the case of SARS it was the civet, for MERS the camel, for SARS-CoV-2 it is unknown, but probably the pangolin. Pangolins are an endangered species commercialized for its keratin scales, which are an important ingredient in traditional Chinese medicine while the meat is considered a delicacy in China and Viet Nam [Cyranosky 2020]. The hypotheses of the creation of SARS-CoV-2 in the laboratory seems to be improbable.

● *The spread of COVID-19.* In the fall of 2019, a pneumonia of unknown etiology was diagnosed in individuals connected with the seafood and live animal market in the city of Wuhan, in the province of Hubei, China. The new variant beta-coronavirus (SARS-CoV-2) was then isolated from the bronchoalveolar lavage fluid from these patients and the virus genome was quickly sequenced and made public by Chinese scientists [NCBI, 2020]. The SARS-CoV-2 outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 [WHO, 2020b]. On 20 February, a patient in his late thirties with no risk factors for SARS-CoV-2 was found positive to the virus while being treated in the Intensive Care Unit of the Codogno hospital, Lodi (Italy). The following day, 36 other cases with no link to the first patient were found in Codogno. The identification of this second cluster of infected persons marked the beginning of the largest SARS-CoV-2 outbreak outside China. In the following weeks clusters emerged in most Western Countries. On 11 March 2020, the World Health Organization (WHO) upgraded the state of SARS-CoV-2 infection from epidemic to pandemic. To try to limit the COVID-19 spread, first China, then South Korea, Italy and, progressively many countries around the world have imposed lockdowns and closed borders [WHO, 2020b; Fauci et al, 2020]. While the number of new infections in Europe is decreasing, easing restrictions to the largest quarantine in the history of mankind, the number of new cases is growing faster than ever worldwide, with more than 100,000 reported each day.

● *Virus transmission.* SARS-CoV-2 is mainly transmitted through the respiratory route [Wang et al, 2020c] via respiratory droplets, up to 1 millimetre across, that an infected person expels when she/he coughs or sneezes [Seminara et al, 2020]. As the virus multiplies, an infected person may shed copious amounts of it. The viral loads of asymptomatic infected people is much less than that of sick people [Seminara, 2020]. However, the large spread of COVID-19 is likely to rests with the infected people before they notice the symptoms of the infection. The importance of SARS-CoV-2 transmission from infected people that remain asymptomatic is not clear since various data from China [Day, 2020], from studies in Vo'Euganeo, Italy [Lavezzo et al, 2020] and from Iceland [Gudbjartsson et al, 2020] show that a large proportion of the population has SARS-CoV-2 infections that do not result in COVID-19 symptoms.

- *Seasonality of the SARS-CoV-2 infection.* The influence of the seasons on epidemic respiratory infectious diseases has been recognized by Hippocrates in 400 BC. Changes in temperature, relative humidity and UV radiations markedly affects virus stability. Temperature, relative humidity, transition between warm and cold environments markedly change the efficacy and integrity of the first mucociliary barrier and innate immunity reactions. Moreover, seasonal changes of human behavior change the probability of contacts between infected and susceptible individuals [Moryiama et al, 2020]. Currently, seasonal factors are likely to be influencing SARS-CoV-2 spread in the various areas of the world. However, only over time will it be possible to realize the importance of seasonal factors on COVID-19 pandemic, its possible reduction during the hottest months and its eventual rampant return with the arrival of the cold season.

- *Persistence of SARS-CoV-2 infectivity.* Aerosolized SARS-CoV-2 can persist a few hours in the air [van Doremalen et al, 2020]. Respiratory droplets and aerosolized virus may land on various surfaces where the virus remains infectious for hours or a day. People can pick it up the virus from the infected surfaces and infect themselves by touching their mouth, nose, or eyes [Wang et al, 2020c; Seminara, 2020].

- *Spread of SARS-CoV-2 in the human body.* A very recent study sheds new unexpected light on virus replication and shedding [Wölfel et al, 2020]. It has long been held that SARS-CoV-2 only replicates deep in the lungs. It is now apparent that a major site of viral replication is the upper respiratory tract. Interestingly, the virus replicates also in the gastrointestinal tract and in some patients diarrhea occurs, while apparently in the stools it is not infectious. Viral RNA is present in faeces of sick people [Wang et al, 2020c]. This is an important issue since the analysis of sewage water that goes from households to the wastewater treatment plant could reveal the true scale of SARS-CoV-2 outbreak [Mallapaty, 2020b].

- *SARS-CoV-2 ability to infect animals.* Cats are susceptible to airborne SARS-CoV-2 infection. The virus replicates efficiently and can be transmitted to naïve cats. However, the viral load spread by experimentally infected cats seems to be too little to pass the infection on to people and there is no evidence of infection in companion cats even in situations of repeated contacts and close proximity to infected humans. Dogs have low susceptibility to SARS-CoV-2 [Temmam et al, 2020]. It replicates poorly in pigs, chickens, and ducks, while ferrets are highly susceptible [Shi et al, 2020]. COVID-19 pandemic is also a threat to great apes [Gillepsie and Leendertz, 2020].

6. Strategies to control COVID-19 spread.

On 23 January 2020, the Chinese government isolated and locked down tens of millions of people in the Hubei province. People were banned from working, going to school and all forms of aggregation, while shops were closed with the exception of those selling food or medicine. As a result of the lockdown, new cases started to slow down. On 19 March 2020, no new cases were reported in Hubei province.

- *Lockdowns.* Following the Chinese experience, lockdowns of various degree of population mobility are currently being carried out in several countries around the world. Lockdowns are based on closing borders, isolating infected persons, contact tracing and social distancing. Social distancing is the backbone of the lockdown strategy, which is playing a central role in slowing the spread of

the virus by reducing the virus reproduction number (R_0). But it also comes at a great economic and social cost. The choice of the social distancing measures that are applied rests on both the peculiar situation of the various countries and the stage of the epidemic spread [Flaxman et al, 2020; Ferguson et al, 2020]

- *Virus reproduction number.* A virus reproduction number (R_0) is the number of healthy people contaminated by each infected person. SARS-CoV-2 R_0 is around 3.87. The key aim of lockdown measures is to reduce the effective reproduction number (R_t) below 1. If R_0 is maintained at less than 1, the incidence of new infections decreases, ultimately resulting in having control of the epidemic. The epidemics disappears when the virus no longer reproduce itself. By contrast, if R_0 returns to be greater than 1, the infection persists or will increase until the epidemic peak is reached. Eventually, the infection declines when the acquisition of herd immunity reduces the number of susceptible individuals. Experience demonstrates that it is possible to block the spread of the virus and arrive at a R_t lower than 1 in a relatively short period of time [WEF, 2020]. Effective reduction of infection is crucial to enable more effective patient care and a reorganization of the healthcare system, which is put in great difficulty by an unexpected high number of patients.

- *The medical meaning of lockdowns.* As we will illustrate in this report, in a significant proportion of cases SARS-CoV-2 infection can give rise to a serious acute respiratory syndrome, requiring hospitalization in Intensive Care Units (ICU) [Cao et al, 2020]. In most countries around the world ICU beds are very limited. In Italy there were roughly 5,000 ICU beds available before the outbreak. Recent data shows that 12% of SARS-CoV-2 positive cases require ICU admission. In practice, if 42,000 people are infected at the same time the total ICU capacity of the country would be saturated. While the availability of beds in ICU varies from country to country, no healthcare system in the world could withstand an unlimited increase of patients in need of intensive care. For this reason, in the face of the outbreak of COVID-19, it is not possible to think only of increasing the number of beds in ICU, but it becomes absolutely necessary to put in place measures to contain the spread of the infection in order to avoid overloading the healthcare system.

- *Easing the lockdowns.* If prolonged, lockdowns open harsh social and economic issues, but their too hasty ending can lead to a rampant resurgence of the epidemic bringing even more devastating consequences. Currently, several governments around the world are announcing a gradual lifting of the lockdown trying to triangulate the health of their citizens, the freedoms of their population, and economic constraints, in a long haul, marked by trial and error [Kupferschmidt, 2020a]. The intense interconnection between European nations should impose coordination in the lockdown removal, an issue so far not particularly pursued. The rebound of new cases in one nation may impose the reintroduction of

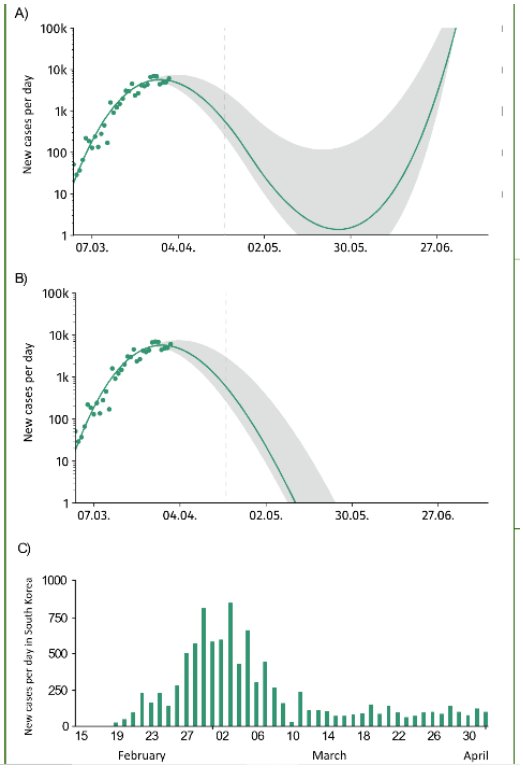

Fig.6

subsequent and perhaps periodical lockdowns also in other nations and it may result in complex social, economic and political problems. The different outcomes that are expected following a lockdown are schematically displayed in Fig. 6 taken (with permission) from a report prepared by the Leopoldina National Akademie der Wissennchaften, Germany [Leopoldina, 2020]. Fig. 6 shows a statistical modelling of new COVID-19 cases following lockdown removal without implementation of efficient protective measures (*upper panel*) and with a gradual and appropriate ease of restrictive measures (*middle panel*). It is evident that containment should be relaxed gradually and with very close monitoring and surveillance. In most areas, when lockdown is removed, the number of people infected and immune to COVID-19 will probably be much less than 10%, which means that a great majority of people is still vulnerable to the infection, a situation very far from a protective herd immunity. Aggressive testing, isolation of those infected, sophisticated tracing systems to quarantine the contacts of infected, social distancing and border control should be enforced to reduce the mingling of infected people with others. This is not an easy task.

7. The dark side of the reaction: Inflammation and Thrombosis.

- *Inflammation.* COVID-19 progression is characterized by uncontrolled inflammation driven by the production of inflammatory cytokines as a consequence of the interaction of the immune system with the virus and with virus-infected target cells, and of an uncontrolled and inappropriate immune activation [Huang et al, 2020; Cecconi et al, 2020]. Sensors of viral infection and cellular damage (e.g. Inflammasomes; Toll Like Receptors) trigger myeloid cell-dependent production of inflammatory cytokines (e.g. IL-1; IL-6; chemokines). Macrophages and inflammatory cytokines amplify local and systemic inflammation and are major drivers of organ failure. Thus, the cytokine storm is likely a reflection of the reaction of the immune system against the virus with target cells and of uncontrolled activation of inappropriate innate and adaptive immunity [Channappanavar and Perlman, 2020]. In analogy to what was observed with SARS-CoV-1, there is now evidence that COVID-19 is associated with defective early activation of Interferon production, resulting in defective antiviral immunity [Blanco-Melo et al, 2020; Hadjadj et al, 2020]. In addition, a late and prolonged release of interferons can lead to harmful proinflammatory effects aggravating viral infection by interfering with lung repair and epithelial cell proliferation [Major et al, 2020; Broggi et al, 2020].

- *Role of Complement.* The innate immune system includes a humoral arm with complement and fluid phase pattern recognition molecules [Bottazzi et al, 2010]. Evidence suggests that Complement may act as an amplifier of uncontrolled inflammation [Gao et al, 2020]. For instance, recognition of virus infected target cells can lead to activation of the lectin pathway. In addition, the pentraxin C Reactive Protein serves as a biomarker of disease severity. A systematic analysis of humoral innate immunity in resistance to COVID-19 and disease pathogenesis is warranted [Ristano et al, 2020].

- *Thrombosis.* Endothelial cell activation by inflammatory cytokines as well as a direct interaction with the virus may set the vascular bed in a prothrombotic setting. In addition, COVID-19 is characterized by endothelial disfunction and formation of microthrombi in the lungs [Ackermann et al, 2020; Klok, 2020; Zhou et al, 2020a; Lodigiani et al, 2020]. Microthrombi are present in lungs and alterations of the coagulation cascade can be measured at a systemic level.

8. Predisposing factors.

Vulnerable persons are those who are disproportionally exposed to the risks of COVID-19. However, those included in this category can change dynamically depending on the evolution of the pandemic and policy responses [Editorial, 2020]. Fig. 7 schematically shows a few biological, social and occupational factors that increase both the vulnerability to SARS-CoV-2 infection and the predisposition to become critically ill. The severity of the prognosis increases with the increasing of patient's age.

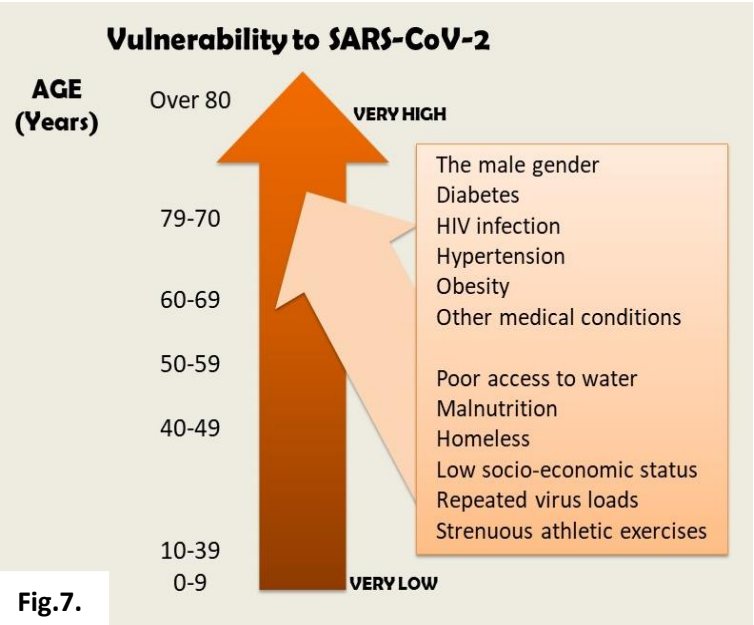

Fig.7.

● *The male gender*. Epidemiological data from around the world have confirmed that men face a greater risk of severe illness and death from COVID-19 than women. In the Lombardy Region, men comprises 82% of 1581 patients admitted to the intensive care units [Grasselli et al, 2020] and male mortality exceeded that of women in every adult age group [Richardson et al, 2020]. In addition, preliminary observations from Spain suggest that a disproportionate number of men with male pattern baldness—which is linked to a powerful androgen—end up in hospitals with COVID-19. Furthermore, Montopoli et al [2020] reported that prostate cancer patients on androgen-deprivation therapy, which slashes the levels of testosterone, were only one-quarter as likely to contract COVID-19 as men with prostate cancer not on androgen-deprivation therapy. The lower risk of SARS-CoV-2 infection in of women may be due to a greater reactivity of the female immune system or, more directly, to an up modulation by male hormones of the expression of the *TMPRSS2* enzyme, involved in the penetration of SARS-CoV-2 into human cells [Wadman, 2020].

● *Infection in children*. Children face the same risk as the general population of becoming infected with SARS-CoV-2. However, a systematic review of 18 studies with 1065 pediatric patients with SARS-CoV-2 infection showed that most children presented with mild symptoms if any. No deaths were reported in children aged 0 to 9 years [Castagnoli et al, 2020]. This different sensitivity to COVID-19 could be due to a more effective immune system, to a different regulation in the production of cytokines, to a lower expression of the ACE2 receptors or to other reasons. Somewhat connected with the lower susceptibility to COVID-19 of children is the decision to reopen schools or not. If children are driving the spread of the virus, infections will probably spike in the next few weeks in countries where children have already returned to school [Mallapaty, 2020a]. For the emergence of children Multisystem Inflammatory Syndrome see 9, Symptoms.

● *Superspreading.* Some people shed far more virus, and for a longer period of time, than others, perhaps because of differences in their immune system or the distribution of virus receptors in their body. Some situations may be particularly risky [Kupferschmidt, 2020b]. Situations where people grouped together speak loudly, scream, sing, e.g. choir, soccer games or perhaps where low

temperature and humidity helps the virus to survive seem to greatly facilitate the spread of SARS-CoV-2. The strenuous exercise of athletes which implies a different type of breathing facilitate both the infection and the direct arrival of the virus in the lungs.The repeated exposure to virus loads, as happens to doctors and nurses, are additional risk factors [Matricardi, 20205].

● *Gene polymorphisms.* Beside these predisposing factors, there are ongoing studies to identify gene polymorphisms that may underscore a particular susceptibility to the disease.

- *ACE2.* The protein coded by this gene is the surface receptor which the Spike protein of SARS-CoV-2 exploit to enter airway cells (Fig. 3). Polymorphisms of the ACE2 gene could make it easier or harder for the SARS-CoV-2 to enter the cell [Reineri et al, 2020]. While several ACE2 gene variants with potential impact of receptor stability were identified, no significant association with COVD-19 severity was found [Asselta et al, 2020] .

- *TMPRSS2*.The transmembrane protease serine coded by this gene cleaves the Spike protein of SARS-CoV-2 and allows the fusion of viral and cellular membranes (Fig. 3). TMPRSS2 is an androgen responsive gene that may improve the ability of the virus to enter male airway cells. Several variants of this gene with different frequency in various human population were found. Some of these variants may be involved in the modulation of COVID-19 severity [Asselta et al, 2020] (*See also 11. Inhibiting SARS-CoV-2 entry into target cells and Fig. 9*).

- *IFTM3.* This gene encodes interferon-induced transmembrane protein 3. A possible risk variant of this gene (*IFITM3-rs12252-C/C*) has been found linked to more severe forms of COVID-19 [Thevarajan et al, 2020]. Also in this case, further investigations are needed to confirm this observation.

- *HLA*. The ability of Human Leukocyte Antigen (HLA) genes to present peptides to T cells has a paramount importance for the elicitation of an effective immune response to viruses. In silico studies show that there are not real holes in the ability of HLA alleles to present SARS-CoV-2 derived peptides, while the various HLA alleles binds to SARS-CoV-2 peptides with different affinity [Baruah and Bose, 2020; Nguyen et al, 2020]. When the distribution of HLA allele frequencies was evaluated in Italian transplant recipients and in patients on waiting lists, the frequencies of a few alleles were found significantly different in those SARS-CoV-2 positive and negative [Amoroso et al, 2020].

- *ABO*. In two large recent analysis in Wuhan and Europe [Ellinghaus et al, 2020; Zhao et al, 2020c], blood group A was associated with an increased risk whereas blood group 0 was associated with a decreased risk. A similar association is also evident in the Italian population [Amoroso et al, 2020]. Guillon et al [2020] showed that anti-A antibodies inhibit the adhesion of SARS-CoV Spike protein to ACE2 cell receptor. Given the similarity between SARS-CoV and SARS-CoV-2, the same mechanism may impair the susceptibility to COVID-19. Further studies are needed to verify this hypothesis.

- *MBL*. The Mannose Binding Lectin (MBL) interact with surface sugars of several microorganisms and constitutes an important element in innate immune defence. Serum MBL levels decline with age [Tomaiulo et al, 2012]. Previous studies have shown that *MBL*

gene polymorphisms were associated with different outcomes of SARS infection [Zang et al, 2005]. It is therefore reasonable that MBL polymorphism play a role in modulating SARS-CoV-2 infection [Matricardi et al, 2020] (*See also 7, Role of Complement*).

However, the ability to implement an adequate immune response to a microbe rests on countless genetic traits and environmental factors. It can be expected that variants of predisposition and resistance of a single gene alone cannot explain, if not to a small extent, the variability observed in in the prevalence of the infection and in the clinical evolution of COVID-19.

9. Clinical aspects.

SARS-CoV-2 infection presents a variety of symptoms: it can be completely asymptomatic or present severe symptoms. In Italy, although there has been a very high daily incidence of new cases, about 67% of patients show mild symptoms and about 30% have symptoms that require hospital admission.

- *Symptoms.* The most common symptoms are fever and cough. A small percentage of cases reports gastrointestinal symptoms before the onset of the respiratory symptoms [Guan et al, 2020].]The first reports from China showed that only 5% of infected patients required ICU admission, while less than 3% needed mechanical ventilation [Huang et al, 2020]. Recent data from the Lombardy Region in Italy showed that the rate of ICU admissions is much higher, in the range of 12% of all positive cases, or 16% of all hospital cases [Grasselli et al, 2020].

In England, pediatricians described the appearance of an inflammatory syndrome similar to Kawasaki disease. Subsequent studies have instead led to the identification of the emergence a new disease of children, the Multisystem Inflammatory Syndrome, a serious and life-threatening illness which appears around two weeks after infection and which requires hospitalization in an intensive care unit [Levin et al, 2020].

- *Case fatality rate* (CFR) varies in countries across the world. In Italy, the overall CFR is 8.5%. CFR varies significantly among age groups. With almost no reported deaths among people aged 29 or younger, CFR goes from 0.3 % to 24.1% in the over 90 years old. Patients with comorbidities are more likely to be severely affected and die [Grasselli et al, 2020] (*See also Fig. 7*).

- *Pathology*. Pathological lesions are primarily affecting distal lung, causing a Diffuse Alveolar Damage. However, at least in the late stages of the disease, several other organs (liver, heart, brain) are affected as well. Consistently with the radiological picture in dying patients, the most prominent gross anatomical feature is a diffuse or focal hardening of the lung parenchyma, with edematous and with focal hemorrhages, which are the hallmark features of Acute Respiratory Distress Syndrome (ARDS) [Fox et al, 2020].

While available reports on a limited number of biopsies and autopsies of COVID-19 patients do not allow to draw definite conclusions [Fox et al, 2020; Hanley et al, 2020; Franks et al, 2020; Barton et al, 2020] the matching histological data with extensive reports on SARS-CoV epidemic in China (2002-2003) allows us to portray a pathological evolution of COVID-19 [Guo et al, 2008; Franks et al, 2003]. ACE2-rich lung alveolar pneumocytes appear to be the prime target of SARS-CoV-2. The alveolar damage is followed by interstitial edema and vascular congestion. The inflammatory

exudate is comprehensive of mononuclear inflammatory cells, CD8 T cells, monocyte/macrophages, a few granulocytes and multinucleated giant cells. Apoptosis of epithelial cells, monocytes/macrophages, lymphocytes, and pneumocytes, together with plasma exudate proteins and fibrin fragments leads to the formation of hyaline membranes, further aggravating the gas exchange with capillary vessels. Alveolar capillaries are enlarged with focal thrombi clots. Diffuse intra-vascular coagulation in the lung and elsewhere is a late event. Only in some instances, thrombosis it might occur earlier. However, the high incidence of thromboembolic events suggests an important role of COVID-19 –induced coagulopathy [Wichmann et al, 2020] (*See also 7. Thrombosis*).

Diffuse lesions affect the liver parenchyma, kidneys, gastro-intestinal tract. Myocardiocytes can undergo focal necrosis and evidence is accruing that the nervous system can also be affected, with focal necrosis in neurons [Clerkin et al, 2020; Fox et al, 2020; Asadi-Pooyaa et al, 2020].

10. Diagnostics tests: Virus and Antibodies.

- *SWABS.* The milestone in diagnostic tests is represented by by Polymerase Chain Reaction (RT-PCR) based assays that detect SARS-CoV-2 RNA in nasal swabs. The tests currently used must be carried out by specialized personnel and take approximately 4 hours. The tests have serious limitations, e.g. in advanced patients, nasal swabs may be negative while bronchoalveolar lavages are positive and the frequency of false negatives in asymptomatic patients may be higher [Service, 2020a].

A one-hour PCR-based assay (DiaSorin, Italy) recently approved by the USA Food and Drug Administration (US FDA) may improve the diagnostic output [DiaSorin, 2020]. At the end of March 2020 under the Emergency Authorization Procedure, US FDA has approved a diagnostic system based on isothermal amplification of *SARS-CoV-2 RdRp* gene. This system delivers result in as little as 5 minutes but for now it is only available in the USA [Abbott, 2020]. In the USA "home tests" have been approved by FDA: Kits are shipped to homes along with detailed instructions. The swab is inserted into a protective vial and subsequently mailed to one of the Everlywell diagnostic laboratories for PCR analysis [Service, 2020a, b].

In the US, scientists are working to validate the DETECTR and SHERLOCK diagnostic tests based on CRISPR machinery's ability to recognize specific SARS-CoV-2 genetic sequences and cut them. In the process, it also cuts a 'reporter' molecule added to the reaction, which reveals the presence of viral genetic material. The key advantage is that a CRISPR reaction is very specific and can be done in 5–10 minutes [Subbaraman, 2020].

- *Saliva sampling* is an appealing alternative to nasopharyngeal swabs, since collecting saliva is non-invasive and easier. Recently, A.L. Wyllie and colleagues [2020] reported that when the SARS-CoV-2 detection from patient-matched nasopharyngeal and saliva sample was compared, saliva yielded greater detection sensitivity.

- *Serological tests.* The search for antibodies is an invaluable source for both individual diagnosis of an infectious disease and epidemiological studies [Amanat, 2020]. A number of assays have been developed in academic institutions and in small and large companies as illustrated by the joint effort developed by DiaSorin and San Matteo hospital in Pavia, Italy, now FDA approved [DiaSorin, 2020].

Unfortunately, it appears that many of the over 100 available serological tests are still poorly characterized in terms of fundamental properties including sensitivity, specificity and detection of neutralizing antibodies [Bastian and Waring, 2020; Clerici, 2020]. Appropriately validated assays are instrumental for epidemiological studies [Sandri et al, 2020], evaluation of plasma donations, assessment of memory and as an adjunct to diagnostic procedures under selected conditions.
As discussed above *(See 7. The dark side of the reaction*), there is no data showing that the presence of a given titer of antibodies is associated with protection against subsequent exposure to SARS-CoV-2. Therefore, there is no ground for issuing what have been referred as "Immunity Passports" or "Immunity Patents", as these terms imply assuring resistance to COVID-19. Patents or Passports of this kind could have vital ethical, social and legal implications because they would imply an immunity to COVID-19 which today cannot yet be ascertained. A false perception of being "immune" may encourage irresponsible behaviors. WHO has recently issued a warning along these lines [WHO, 2020a], with which we agree.
As soon as these validated tests are available, the World Health Organization plans to coordinate SOLIDARITY II, a study program to test blood samples for the presence of antibodies to the virus involving more than half a dozen countries around the globe [Voge, 2020].

11. Therapy.

A wide range of therapeutic approaches have been tested under uncontrolled conditions. These range from anti-retroviral and anti-viral agents, to immunomodulation agents and even Chinese traditional medicine preparations. A detailed report of all the compounds and strategies goes beyond the purpose of this executive report. As stated in the Introduction, while we understand the challenge posed by emergency medicine, we concur with the New England Journal of Medicine ("…rapidly initiated high quality clinical trials are possible in epidemic situations, even in the trying circumstances that prevailed in Wuhan") and the Journal of American Medical Association editorials calling for high quality rigorous clinical trials [Baden and Rubin, 2020; Kalil, 2020].
Since several drugs are claimed o be effective without high quality clinical trials, recently the WHO announced the launch of a large global trial called SOLIDARITY, which is designed to determine whether any of the drugs to be administered to COVID-19 patients are really effective. This is an unprecedented effort to collect robust scientific data including information on thousands of patients in dozens of countries [Kupferschmidt and Cohen, 2020; Cao et al, 2020].

● *The pillar of treatment: respiratory support and management of organ failure.* Currently, there is no specific treatment for SARS-CoV-2. Supportive therapy is the only treatment that can be offered to patients, to allow the time to regain their basic function. In the context of Severe Acute Respiratory Failure, supportive therapy could mean invasive mechanical ventilation and or non-invasive support (in the form of high flow oxygen, continuous positive airway pressure or non-invasive ventilation).
Patients that require invasive mechanical ventilation usually are very sick and in need of intense care resources, both in terms of nursing and medical time and technology. Many of these patients develop a form of acute respiratory failure called ARDS (Acute Respiratory Distress Syndrome). One of the cornerstones of ARDS treatment is the so-called "protective lung strategy". This method of

treatment consists of using the lowest possible ventilation pressures and volume required to oxygenate the blood without causing harm to the lungs with the ventilator itself.
In some cases, prone positioning is used as a therapy to maximise the gravity effect of blood flow towards the better-aerated parts of the lungs.
While protecting the lungs and allowing them time to heal, particular attention should also be paid to supporting the other organs. Vasopressors may be required to maintain adequate perfusion pressure; fluids have to be carefully titrated to avoid both hypovolemia and fluid overload. In some cases, acute kidney injury develops, and renal replacement therapy may be necessary.
In the most severe cases of ARDS, extracorporeal membrane oxygenation (ECMO) can be used to temporarily substitute the gas exchange function of the diseased lungs. This technique is very invasive, requires a lot of resources and is particularly challenging to perform during a pandemic in which the volume of critically ill patients to treat is particularly high.
While there is currently no convincing evidence as to the efficacy of any other drug for COVID-19 patients with acute respiratory failure, several clinical protocols based on antivirals, chloroquine, anti-inflammatory drugs, to name a few, have been developed [Accad Naz Lincei, 2020b]. The rationale and clinical evidence of some of these treatments is reported in this document.

- *Selected antivirals*

  - Lopinavir/ritonavir. This is a combination of agents used in the treatment of HIV and has been widely used. However, a recent randomized study in advanced patients showed no benefit [Cao et al, 2020]. Further carefully controlled and adequately powered studies are needed to assess the potential of this combination in early disease.

  - Remdesivir. This agent has potent antiviral activity in vitro and in animal model of MERS. Its potential in COVID-19 is undergoing clinical evaluation [Wang et al, 2020b]. A recent randomized controlled study just published [Wang et al, 2020d] has reported no mortality benefits (primary outcome) for remdesivir vs placebo in hospitalized patients. However, this study reports a faster time for recovery (secondary outcome). A press release from NIH states that similar results are also reported in a larger study that has just been completed. This study is yet unpublished. Remdesivir has been approved by FDA and EMA.

  - Chloroquine and hydroxychloroquine. Chloroquine and hydroxy-derivative have anti-viral activity as well as the capacity to suppress inflammation. Its potential for the treatment of COVID-19 needs to be investigated even if the majority of the studies show that these drugs lack efficacy and are potentially harmful [Accad Naz Lincei, 2020b] (*See below 11. Inhibition of excessive inflammation*). Azithromycin cardiovascular mortality and therefore its association chloroquine is not recommended

  - Interferons. The rational for considering interferon therapy, systemic or via lung aerosol, is mentioned in point 4. *The binding to ACE2.* It has been used in Ebola and SARS [Loutfy et al, 2003; Konde et al, 2017]. It will be important to assess its potential in COVID-19 in subsets of patients based on cytokine and immune cell profiles.

The four therapies that seem to be the most promising and will be included in the above mentioned WHO SOLIDARITY global trials are remdesivir, cloroquine, hydroxychloroquine, lopinavir and the same drugs plus interferon-beta [Kupferschmidt and Cohen, 2020].
Some important efforts are currently ongoing with the aim to produce high quality scientific evidence. A novelty compared to previous epidemics is the use of research platforms using Bayesian statistics and adaptive design. The novelty of these adaptive design trials is that they allow simultaneous testing of more interventions.
REMAP-CAP [2020] is a Randomized, Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia. It was developed as a joint multinational consortium to test different treatment during a pandemic. COVID-19 Pneumonia represents the first challenge for this innovative platform in which several treatments are currently being tested.
CORIMUNO [2020] uses a similar principle to study different immunomodulation therapies in COVID in France.

- *Inhibition of excessive inflammation.* There is a strong rationale that an uncontrolled immune response and excessive inflammation may play a role in amplifying tissue damage in SARS and possibly in COVID-19. The high levels of inflammatory cytokines (e.g. IL-6, TNF, IL-1, chemokines) and the prognostic significance of IL-6 levels provide a rationale for these strategies [Liu et al, 2019]. These include monoclonal antibodies anti-IL-6 (e.g. sarilimab) or anti-IL-16 receptor (e.g. tocilizumab), anti-IL-1 (e.g. canakinumab); a recombinant IL-1 receptor antagonist (anakinra); complement targeting strategies; inhibitors of cytokine signaling pathways (JAK1,2) (e.g. baricitinib).

It is worth mentioning that chloroquine, proposed as an antiviral drug, has immunosuppressive and anti-inflammatory properties. Incidentally, the speculation that the use of chloroquine as an antimalarial drug underlies Africa's apparent resistance to COVID-19 does not take into account the fact that this agent, for some time now, has largely been abandoned for the treatment of malaria.
Much of the evidence seems to point to a lack of efficacy of this drug for the treatment of COVID-19. There are also signs of major side effects when administered at higher doses [Borba eta al, 2020; Fihn et al, 2020].
Tocilizumab, an anti-IL-6 receptor humanized monoclonal antibody is, to the best of our knowledge, the one agent in this field for which there is more available data. The rational stems from its limited use in rheumatoid arthritis and, most important, in controlling the cytokine release syndrome in CAR-T cell therapy. To the best of our knowledge, Prof. Haiming Wei in Hefei conducted the first experimental administration of tocilizumab in a limited series of patients followed by widespread use of this drug in accordance with the guidelines issued on 13/02/2020 in China [The Treatment Guideline, 2020]. It should be noted that studies are now ongoing in China and elsewhere, including Italy under the auspices of Agenzia Italiana del Farmaco (AIFA). From AIFA data it appears that the treatment with Tocilizumab does not change patient mortality.
The French CORIMUNO-TOCI study has just completed the enrollment of patients into a randomized study on tolicizumab vs standard care. No results are available yet.
Other immunomodulation therapies are currently being tested. On 27 April 2020, Sanofi and Regeneron [2020] deeming it futile stopped a study on sarilumab, a monoclonal antibody against human IL-6, due to potential harm in the severe arm group. *"The drug lowered C-reactive protein in*

both severe and critical patients, however, clinical outcomes in the severe arm of the study—including lowering the risk of ventilation and death—showed "negative trends".

- *Heparin.* According to evidence for a role of a thrombotic component in the pathogenesis of lung damage (*See 4. Immune resistance to SARS-CoV-2 infection; 7. Thrombosis and 9. Pathology*), heparin is part of current treatment protocols.

- *Steroids.* The usage of glucocorticoids has long been a matter of discussion and controversy. A large clinical trial has now shown that in a well-defined advanced disease window, dexamethasone significantly reduced the COVID-19 mortality [Hornby et al, 2020]. Therefore a glucocorticoid is the only drug proven to have an impact on COVID-19 mortality in a large controlled clinical trial.

- *Therapeutic Antibodies: plasma therapy and monoclonal antibodies*. Since the early days of immunology, plasma from recovered patients has been used as a source of antibodies. Plasma from recovered patients has been used in China and elsewhere, including Italy, as a source of antibodies, as already done for SARS and MERS. Initial pilot studies in China have been widely followed by widespread usage in the USA and elsewhere [Casadevall and Pirofski, 2020; Shen et al, 2020; Duan et al, 2020]. An unprecedented effort is ongoing in USA along this line [Bloch et al, 2020]. Randomized studies are ongoing or are planned and will address the fundamental issue of the efficacy of plasma therapy. In the only randomized trial conducted so far plasma therapy had no significant impact on mortality. The trial planned to enroll 200 patients was stopped at 100 subject due to lack of patients [Gharbharan et al, 2020]. Therefore, further large controlled trials are needed.

  Several academic and industrial laboratories are at various stages of developing human monoclonal antibodies against components of SARS-CoV-2 virions, such as the Spike protein [Wang et al, 2020a; Regeneron, 2020; Andreano et al, 2020; Wec et al, 2020]. It should be noted that both with SARS and with other viral infections, under particular conditions, the antibodies can enhance viral entry (Antibody-Dependent Enhancement, ADE) [Fu et al, 2020] and tissue damage [Liu et al, 2020a]. Therefore, as emphasized above, rigorous clinical evaluation will be mandatory also for antibody-mediated therapies.

● *Data Science, Artificial Intelligence and Modelling.* Big Data, Artificial Intelligence, Machine Learning, Data Science were the "buzz words" before the COVID-19 outbreak. Data science has been fundamental in mapping the evolution of the pandemic and planning capacity and forecasting resources, such as ICU capacity [Grasselli et al, 2020]. Furthermore, mathematical modelling has been used to project possible evolution of the pandemic in different scenarios.
However, it has unfortunately also been a wakeup call for this field; despite significant investments, it has not allowed SARS-COV2 to be better controlled. Policies and decisions lie in the hands of doctors, public health authorities and governments. In this most challenging moment good decision making is still needed by humans.

● *Inhibiting SARS-CoV-2 entry into target cells*.

One strategy to block the interaction between the virus Spike protein and ACE2 is to target the coronavirus virions by using the ACE2 extracellular domain as bait to bind to Spike protein (Fig. 8, down). A human recombinant soluble ACE2 receptor, which was previously tested in phase 1 and 2 clinical trials for acute respiratory distress syndrome, was shown to reduce SARS-CoV-2 viral growth in infected cultured cells and human blood vessel organoids [Monteil et a, 2020]. A phase 2 clinical trial for the treatment of COVID-19 using soluble ACE2 has been recently launched. Otherwise, as shown in Fig. 8 (up), an immunoglobulin Fc domain can be fused to the ACE2 extracellular domain to facilitate prolonged circulation of the complex [Kruse et al, 2020].

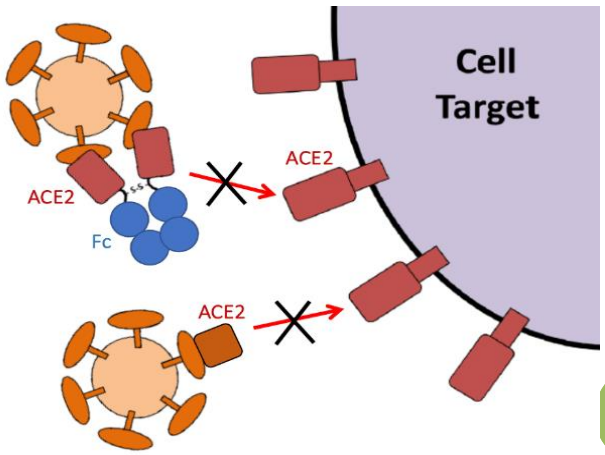

Fig. 8

An alternative approach is to use TMPRSS2 protease inhibitors. A protease inhibitor called camostat mesylate, which is active against TMPRSS2, was found to partially block SARS-Cov-2 entry into lung cell lines [Hoffmann et al, 2020] (Fig. 9). This inhibitor has been approved in Japan and South Korea to treat pancreatitis and is presently repurposed in clinical trials for COVID-19. The effect of another TMPRSS2 inhibitor, bromhexine, has not yet been explored. Interestingly, TMPRSS2 expression is controlled by androgen s and it has been suggested that this could contribute to the male predominance of COVID-19 [Stopsack et al, 2020]. The possibility that androgen pathway blockade might reduce susceptibility to COVID-19 pulmonary symptoms and mortality is endorsed by epidemiological studies, showing that prostate cancer patients treated with anti-androgens are much less frequently affected by COVID-19 compared with those untreated, and is currently tested in clinical trials [Montopoli et al, 2020].

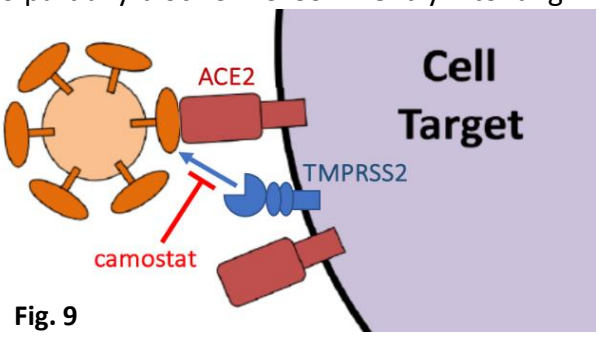

Fig. 9

12. Anti SARS-CoV-2 vaccines.

● *Rationale.* The hope and hype that the media and public at large are placing on having as soon as possible a vaccine that protects against COVID-19 is the result of the great triumphs that vaccines have had and are having in the control of infectious diseases [Accad Naz Lincei, 2020c].

In many cases, recovery from a viral disease rests on the combined action of antibodies in the biological fluids that neutralize viral particles and the killer activity of lymphocytes that track down and kill the body's cells infected with the virus, which are turning into factories of millions of new viral particles. However, there are viral diseases whose healing depends mainly, if not exclusively, on the antibody response and others where the destructive action of the killer lymphocytes is fundamental.

- What is the case with COVID-19?

- Patients who have recovered from COVID-19 are protected against a second infections?
- If they are protected, how long does the protection last?

There is a long series of serious infectious diseases in which vaccines are only partially effective and there have been a series of sensational defeats. Indeed, each disease is an immunological problem in itself: even today, with all the data in our possession, it is difficult to predict what vaccine can be truly effective. This difficulty is even greater for COVID-19, a young disease in which ongoing studies in laboratories worldwide are bringing new data. In addition, RNA viruses generally have a high mutation rate. This is one reason why it is difficult to develop effective vaccines to prevent diseases caused by RNA viruses.

● *Role of CEPI.* In January 2017, during the World Economy Forum in Davos, the Coalition for Epidemic Preparedness Innovations (CEPI) was established, an international organization aimed at promoting the development and storage of vaccines against those microbes that could cause new frightening epidemics: substantial funding was provided by the Bill & Melinda Gates Foundation, the Welcome Trust and the governments of numerous countries. The major multinational pharmaceutical companies have announced their collaboration. And it was precisely CEPI that, together with numerous other private and public initiatives, already at the very early stages of the epidemic, activated and coordinated numerous and different programs for the preparation of vaccines against COVID-19, following very different conceptual and technological strategies. This diversification appeared essential precisely because, for many diseases, but mainly in the case of a new disease as COVD-19, it is difficult to predict which type of immune response and therefore vaccine will be more effective [Lurie et al, 2020].

● *The race to find and produce anti SARS-CoV-2 vaccines*. As of 30 April 2020 there are at least 150 anti SARS-CoV-2 vaccine candidates based on different technological platforms: nucleic acids (RNA, DNA), virus like particles, replicating and non-replicating viral vectors, recombinant proteins, recombinant peptides, live attenuated viruses, and inactivated viruses. Of these vaccines, 10 are already in Phase 1-3 trial in humans [Acad Naz Lincei, 2020c; Tagliabue and Forni, 2020].

● *Vaccine assessment.* The administration of the new vaccine on a limited number of volunteers makes it possible to understand whether the vaccine induces a good antibody response and / or a response of the T killer cells and whether its administration causes clear adverse events. In several cases, “vaccine candidates” appear able to trigger a marked immune response. These data suggest that they are able to prevent SARS-CoV-2 infection or make the infection mild. Subsequently, the real evaluation of their effectiveness will be based on randomized controlled trials that will compare the incidence of COVID-19 in groups of vaccinated and non-vaccinated people. Only the extension of this evaluation to progressively larger groups and for longer periods will determine whether one, several or none of the new COVID-19 vaccines protects effectively or only marginally and if its administration is associated with important collateral events. However, in view of the great urgency for a vaccine to quickly verify its efficacy it has been proposed to vaccinate human volunteers and then intentionally challenge them with SARS-CoV-2 [Eyal, 2020]. Support for this highly controversial human vaccine-challenge study, with ethical implications is growing [Callaway, 2020].

● *Risks associated to fast track vaccine evaluation.* It is likely that in view of the enormous pressure exerted by the COVID-19 pandemic, surrogate markers are initially used, such as the evaluation of the amount of antibodies or the intensity of the reaction of the T killer cells induced by the vaccine on the volunteers to decide whether initially the new vaccine could reasonably be used for vaccination. However, the administration of a new vaccine must always be carefully associated with a rigorous study of its safety. This is particularly important because a vaccine is not a drug for sick people at risk of dying, but rather a treatment that is given to those who are well so as to prevent the risk of falling ill [Jiang, 2020].

The race to develop a COVID-19 vaccine is not only justified but absolutely necessary. However, the time required to evaluate the dangers and risks that may arise from a new vaccine must be included in its development. In some cases, vaccines prepared against other coronaviruses or other viruses have worsened the disease [Jiang, 2020] and have induced T helper 2-type immunopathology [Chien-Te Tseng et al, 2020]. These issues must be carefully evaluated and excluded before a new COVID-19 vaccine is distributed to combat the pandemic or its subsequent outbreak.

● *Production and economic issues.* Once the new vaccine has been validated, subsequent problems will be related to production and distribution. Technological, organizational, regulatory and economic problems will have to be overcome. The industrial technology needed to scale up the production to a billion doses will depend on which kind of vaccine work best. Initially it might not be physically possible to make enough vaccines for the world's population. In addition, political and economic constrains may limit vaccine access to the country that make it or to the countries that can afford to pay for it [Tagliabue and Forni, 2020]. Making the new vaccines available to the global population will be challenging [Accad Naz Lincei, 2020a]. The WHO is trying to make sure that vaccine stockpiles are shared equitably, a crucial challenge that must be collectively addressed by governments [Khamsi, 2020].

Hence the consideration that vaccines for COVID-19, if effective, will not be generally available before several months This long interval raises another problem of crucial importance: It is possible that by the time the vaccine arrives it will no longer be crucial or it will be exploited by only a small population in a particular area of the world. In fact, we cannot predict what the evolution of COVID-19 will be: the pandemic will end; the epidemic will continue to hit massively; it will only spread in some areas of the world; or there will be periodic outbreaks of new epidemics. In any case, the vaccine will be needed worldwide to boost COVID-19 immunity.

● *Recommended vaccines and BCG.* At present, no reliable data are available concerning the impact of seasonal influenza vaccination and anti-pneumococcus vaccines on the incidence and clinical progression of COVID-19. However, it should be underlined that we agree with the general recommendations for anti-pneumococcal vaccination in the elderly because of its effectiveness in protecting against super-infection by pneumococcus in the course of viral infections and in reducing the appearance of bacteria resistant to antibiotics.

Lastly, somewhat connected with vaccines, it is worth mentioning the hypothesis that the old anti-tuberculosis Bacillus Calmette Guerin (BCG) vaccine may reduce the risk of SARS-CoV-2 infection. Two independent epidemiological studies endorse this hypothesis showing an inverse relationship between COVID-19 attributable mortality and country's policy concerning BCG vaccination [Sala and

Miyakawa, 2020; Shet et al, 2020]. A team in the Netherlands has launched a clinical trial with 1,000 health care workers. Similar trials in other countries will evaluate whether BCG vaccine increases resistance to SARS-CoV-2 in elderly people [de Vrieze, 2020]. As discussed above (*See point 4, Innate Immunity*) innate immunity plays a key role in controlling the first stage of SARS-CoV-2 infection. Therefore, strategies which increase innate immunity ("training strategies") need to be carefully evaluated by epidemiologists and in carefully controlled clinical studies [Mantovani and Netea, 2020].

13. Preparedness.

In the face of this COVID-19 tragedy, which is causing many deaths, suffering and social disaster, it is inevitable to ask to what extent was the world as a whole, and Italy in particular prepared for the pandemic.

According to the "2010 Global Health Security Index ranking" [GHS, 2020; Kandel et al, 2020], Italy was not particularly aware of the problems posed by the spread of infectious diseases. Is this justified? In Italy, in just a few weeks over 120 doctors and 30 nurses (15 April 2020) lost their lives because of the pandemic and even a greater number of infected people were placed in isolation. This is a very serious loss that Italy cannot afford to repeat.

Certainly, much more could have been done regarding many aspects and a few of them even relatively simple [Jacobsen, 2020; WHO, 2020c]. On the other hand, many other countries and ven international agencies have taken action in an uncoordinated and sometimes contradictory manner.

We must consider, however, that only a few months ago the proposal to dedicate energy and resources to be better prepared for a possible, but still hypothetical pandemic lacked the necessary force to overcome indifference, skepticism, anti-scientific attitudes and suspicions of unclear interests and corruption. Italy, a country that has difficulty in convincing a large portion of its population on the importance of basic childhood vaccinations, would unlikely dedicate a significant share of resources for measures to face an unprecedent event such as a new pandemic.

A large majority of the countries in the world would face the same difficulty, declining it differently on the basis of their own culture [Ranney et al 2020; Hunter, 2020].

An assessment of how Italy and the world could have been better prepared can only be made when the pandemic is over. In the future, preparedness is likely to be much more at the center of public health policy [Kandel et al, 2020].

The lesson on the dangers of anti-scientific attitudes and errors in the allocation of resources that Italy and the world are experiencing is complex and very hard, so hard that today we do not even have a clear idea of the aftermath that awaits us.

Importantly, however, in this scenario it has emerged that preparing for a pandemic requires not only public health preparedness and health infrastructures for emergency response, but also "Research Preparedness". In this respect, research platforms such as REMAP-CAP [2020] have proven to be worth the investment of time and energy during "peace time". Different adaptive treatments are currently being tested that will allow us to acquire robust knowledge and hopefully pave the way for evidence based bedside practice to determine what works, what doesn't work or what may cause harm.

Today, like never before, we desperately need to bring back the concepts of precision medicine that took decades to develop. We must continue our efforts to get the right treatment to the right patient at the right time.
It is encouraging to see that since our first report we are starting to see randomized controlled trials, although results at present are inconsistent. Nonetheless, this should not discourage us; the pathway of research leads to new questions. We should not look for a "magic bullet", but we should praise the efforts to answer research questions, and if the answer brings new questions, we should praise them even more.

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