Accademia Nazionale dei Lincei

Commissione Salute

COVID-19: An executive report

April 2020 update

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in alphabetical order

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1. Introduction

Italy and the entire world are currently facing the dramatic challenge of the SARS-Cov-2 virus. 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 Executive Summary of the current knowledge on the origin, mechanisms treatment 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 are 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. Finding a balance between emergency and methodological rigor represents a major challenge [1].
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. SARS-CoV-2

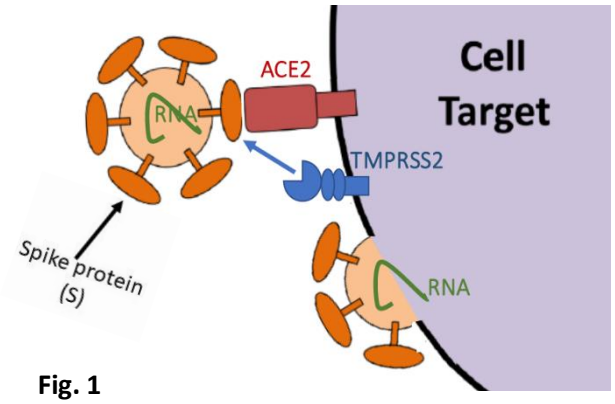

Fig. 1

The virus. Coronavirus disease 2019 (COVID-19) is caused 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 SARS-CoV-2 virion is made up of four structural proteins known as Spike, Envelope, Membrane and Nucleocapsid.
The large Spike protein (S) that forms a sort of crown on the surface of the viral particles acts as an *anchor* allowing the virus to bind to the Angiotensin-Converting Enzyme 2 (ACE2) receptors on the host cell [2]. After the binding, the host cell protease TMPRLRSS2 (Transmembrane Protease Serine) cuts the Spike protein, allowing the virus surface to approach the cell membrane, fuse with it and enter the cell [3] (Fig.1 [4]; see also 5. Predisposing factors). Then, the virus hijacks the cell machinery and the cell die releasing millions of new viruses.

Virus infection. COVID-19 starts with the arrival of SARS-CoV-2 virions on respiratory mucosal surfaces of the nose and throat that express on the surface high levels of ACE-2 receptors. Innate immunity mechanisms and the mucus secreted by goblet cells form a first effective reaction [5]. When

the virus manages to overcome this barrier, a rapid release of danger signals activates the reaction of innate immunity. We do not know yet if and how many SARS-CoV-2 viruses are eliminated by these initial inflammatory reactions, however it is reasonable to assume that the effectiveness of the immune reaction mechanism may play a crucial role in determining whether the infection will be benign or will have major consequences [5]. Once the virus has entered the target cell, viral RNA is immediately translated by the host cell that die by releasing millions of new viruses that invade new cells.

Virus spreading and containment. 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 [6].

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[7]. The SARS-CoV-2 outbreak was declared a Public Health Emergency of International Concern on 30 January 2020 [8].

On 20 February, a patient in his late thirties with no risk factors for SARS-CoV-2 was found positive to the virus while already admitted in an Intensive Care Unit in Codogno, 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 COVID-19 spread, first China, then South Korea, Italy and, progressively many countries around the world have imposed lockdowns and closed borders [8, 9]. The largest quarantine in the history of mankind is taking place.

Currently, USA and Europe are the epicenters of this pandemic: case counts and deaths are soaring in Spain, England, France, and Germany. The estimation of the prevalence of COVID-19 is made difficult due to the rapid spread of the infection and the different methods by which countries detect the disease. However, despite these uncertainties, it is undeniable that Italy has been one of the European countries affected with particular intensity.

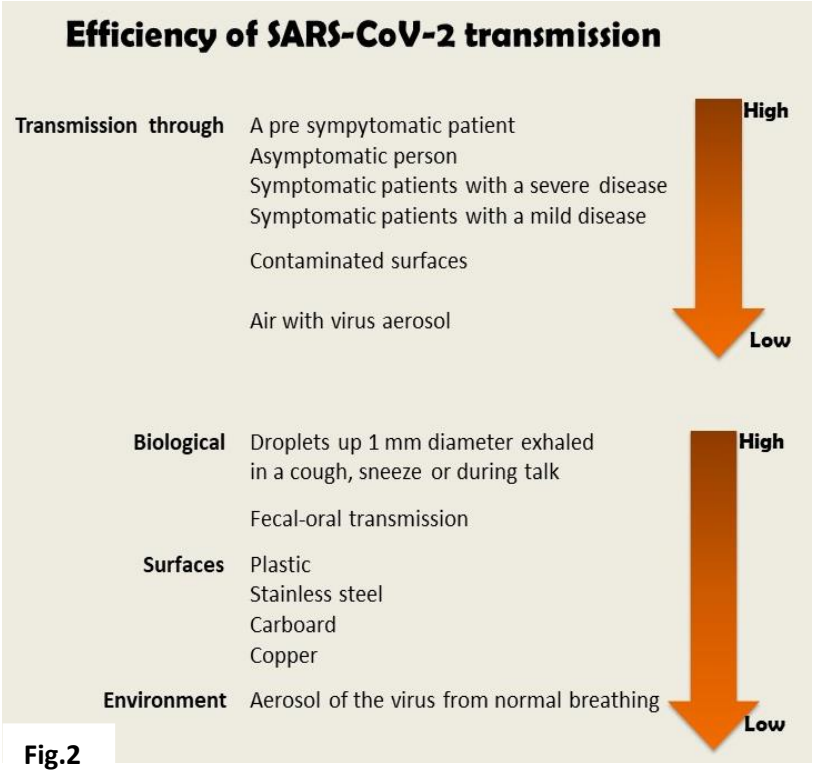

Fig.2

Virus transmission. SARS-CoV-2 is mainly transmitted through the respiratory route [10-12] via respiratory droplets, up to 1 millimetre across, that an infected person expels when she/he coughs or sneezes. 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 [11, 12]. However, the importance of SARS-CoV-2 transmission from infected but asymptomatic people is not clear since various data from China [13], from studies in Vo'Euganeo, Italy [14] and from Iceland [15] show that a large proportion of the population has SARS-CoV-2 infections that do not result in COVID-19 symptoms.

A very recent study sheds new unexpected light on virus replication and shedding [16]. 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 [10]. 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 [17].

Aerosolized SARS-CoV-2 can persist a few hours in the air [18]. 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 [10,18].

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 [19]. Dogs have low susceptibility SARS-CoV-2 [19]. It replicates poorly in pigs, chickens, and ducks, while ferrets are highly susceptible [20]. COVID-19 pandemic is also a threat to great apes [21].

3. 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 [22, 23].

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_t 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 cannot reproduce itself. By contrast, if R_t returns to be greater than 1, the infection persists or will increase (dependent on how much greater than 1 the R_t is) until the epidemic peak is reached. Eventually, the infection declines when the acquisition of herd immunity reduces the availability of susceptible individuals [24].

The Hubei 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 [24]. Effective reduction of infection is crucial to enable more effective patient care and a reorganization of the healthcare system, which has been put in great difficulty by an unexpected high number of patients.

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) [25]. 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 [26]. 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 subsequent and perhaps periodical lockdowns also in other nations and it may result in complex social, economic and political problems [23,24].

The different outcomes that are expected are schematically displayed in Fig. 3 taken (with permission) from a report prepared by the Leopoldina National Akademie der Wissennchaften, Germany [27]. The figure. 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 measures 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.

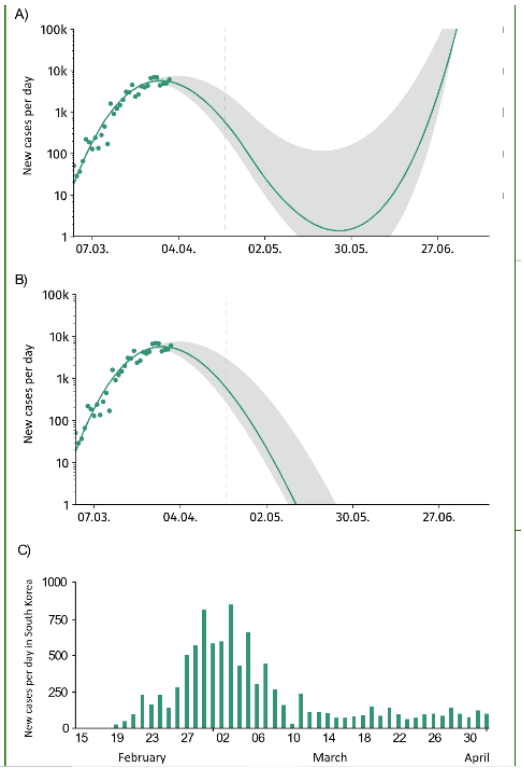

Fig.3

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 [28]. It is not an easy task, as shown in Fig. 3, lower bar chart that displays the daily occurrence of new infections in South Korea from 15 February to 1 April 2020. The particularly well-organized South Korean testing program to isolate infected people and trace and quarantine those who they have come into contact with, has allowed to manage the epidemic without the lockdown of entire cities or the whole country [29]. While new clusters of infection may emerge, so far, the South Korean lesson is that high tech preparedness may play a central role in the control of COVID-19 spread [29]. However, new infections cannot be avoided completely [27]. Lockdown removals in Singapore and Japan have been met with waves of new infections [28].

4. Immunity, Inflammation, Thrombosis

Innate immunity. Innate immunity represents a first line of resistance against microbes and evidence suggests that it can block over 90% of encounters with pathogens. Information on innate immunity in COVID-19 is scanty. After infection, the number of lymphocytes decreases

(lymphopenia) while the number of neutrophils increases. Inflammatory cytokines (e.g. IL-6, TNF, chemokines) generally increase [25, 30, 31]. SARS-CoV and MERS-CoV infect macrophages and lymphocytes, but this may not be the case with SARS-CoV-2. These viruses suppress the production of interferons, a group of anti-viral cytokines of crucial importance [32]. These findings, as shown below, have clinical implications.

Adaptive immunity and memory. As mentioned above, Coronaviruses are very successful at suppressing various mechanisms in immune response [33, 34]. They do so by suppressing interferon production in macrophages and by downregulating antigen presentation via Class I and Class II HLA glycoproteins. As far as adaptive immunity is concerned, although results are insufficient and mainly based on SARS and MERS [35], evidence suggests that, as is generally the case for antiviral resistance, T helper 1 (Th1) cells orchestrate protective immunity [36]. There is also evidence for skewing of the adaptive response in a Th17 direction [31]. 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 [37]. In addition, there is evidence that innate and adaptive lymphocytes undergo exhaustion, which likely results in defective anti-viral immune responses [33].

Antibody responses were identified in SARS, MERS and CO-19VID-19 patients and, as discussed below, there is evidence for antibody-mediated neutralization of the virus [35]. Studies conducted in China, USA and Europe suggest that the appearance of antibodies in the circulation occurs relatively late, with most subjects scoring positive as late as 20 days post exposure and 15 days after beginning of symptoms [38-43]. Intriguingly, in contrast to textbook immunology, there appears to be no distinct timing of appearance of IgM and IgG, the two classes of antibodies appearing with a minor delay and substantial overlap. It remains unclear whether this overlap reflects limitations of current technology or the actual immunobiology of COVID-19. The different kinetics in the development of antibodies displayed by each patient is another limitation to these conclusions [44]. IgA antibodies appear in the circulation and in saliva [40, 44] and may play a key role in defense at mucosal surfaces. There is evidence that circulating antibodies can have neutralizing activity in vitro. These results have obvious implications for serology and for antibody based therapeutic approaches as discussed below.

A key issue with policy and public health implications, is the occurrence and duration of immunological memory. Evidence suggests that coronavirus infections, including SARS-CoV-2, elicits memory. Ralph Baric recently stated that immune response and resistance should last at least 6-12 months [45]. At this stage there is evidence from Wuhan that patients who recovered may be resistant to reinfection for a few months and evidence from SARS suggests persistence of memory for 2-3 years [34]. However, WHO recently warned about the possibility of reinfection, although it may actually be a reflection of a persistent undiagnosed viral persistence. WHO also emphasized the fact that the presence of antibodies is no guarantee against reinfection [46].

Inflammation and Thrombosis. 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 [47]. SARS-CoV-2 has been shown to trigger inflammasome activation leading to production of IL-1[47], a primary inflammatory cytokine upstream of a host of inflammatory mediators [37]. 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. In addition, COVID-19 is characterized by endothelial disfunction and formation of microthrombi in the lungs [48-50].

Complement and humoral innate immunity. The innate immune system includes a humoral arm with complement and fluid phase pattern recognition molecules [51]. Evidence suggests that Complement may act as an amplifier of uncontrolled inflammation [52]. For instance, recognition of virus infected target cells can lead to activation of the lectin pathway [53]. 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 [54].

5. 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 [55]. Fig. 4 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. Children have 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 [56].

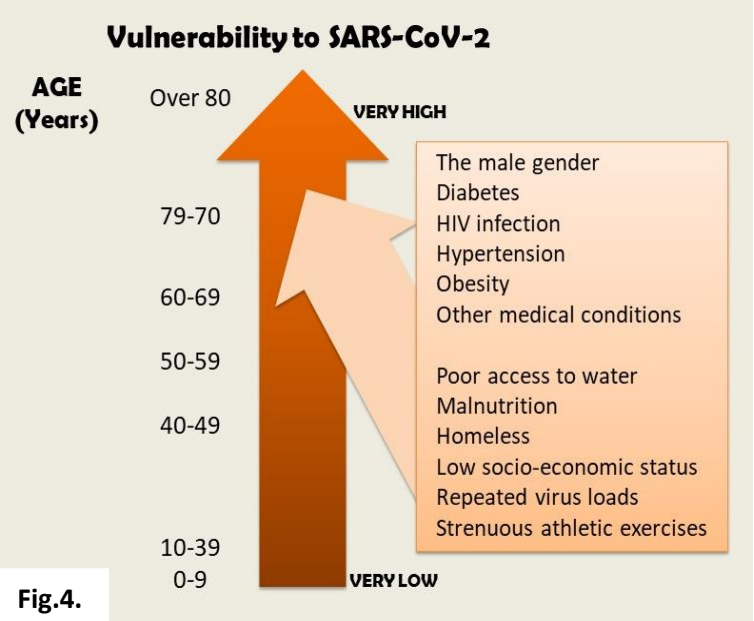

Fig.4.

The strenuous exercise of athletes and the repeated exposure to virus loads, as happens to doctors and nurses, are additional risk factors [5].

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. 1). Polymorphisms of the ACE2 gene could make it easier or harder for the SARS-CoV-2 to enter the cell [57]. While several ACE2 gene

variants with potential impact of receptor stability were identified, no significant association with COVD-19 severity was found [58].

- *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.1). 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 [58] (See also 8. Inhibiting SARS-CoV-2 entry into target cells and Fig. 7).

- *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 [59]. in this case too, 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 [60], while the various HLA alleles binds to SARS-CoV-2 peptides with different affinity [61, 62]. When the distribution of HLA allele frequencies was evaluated in Italian transplant recipients and in patients of the waiting list, the frequencies of a few alleles were found significantly higher in those SARS-CoV-2 positive and negative [60].

- *ABO*. In a recent analysis of 1775 patients infected with SARS-CoV-2 in Wuhan [63], 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 [60]. Guillon et al [64] 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. More studies need to be done 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 [65]. Previous studies have shown that *MBL* gene polymorphisms were associated with different outcomes of SARS infection [66]. It is therefore reasonable that MBL polymorphism play a role in modulating SARS-CoV-2 infection [5] (See also Complement and humoral innate immunity).

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.

6. Clinical aspects

SARS-CoV-2 infection presents a variety of symptoms: it can be completely asymptomatic or present severe symptoms. In Italy, while the country had a very high daily incidence of new cases, about

67% of patients show mild symptoms and about 30% have symptoms that require hospital admission.

The most common symptoms are fever and cough. A small percentage of cases reports gastrointestinal symptoms before the onset of the respiratory symptoms [67].
The first reports from China showed that only 5% of infected patients required ICU admission, while less than 3% needed mechanical ventilation [47]. Recent data from 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 [67].

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 death in 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 [68] (See also Fig. 4).

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 oedematous and with focal hemorrhages, which are the hallmark features of Acute Respiratory Distress Syndrome (ARDS) [69].

While available reports on a limited number of biopsies and autopsies of COVID-19 patients do not allow to draw us definite conclusions [69-72] the matching of histological data with extensive reports on SARS-CoV epidemic in China (2002-2003) allows us to portray a pathological evolution of COVID-19 [73, 74]. 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.

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 [75-77].

7. 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 [78]. In addition, at present swabs which inactivate the virus are no longer available, at least in the Lombardy region, Italy.

A one-hour PCR-based assay (DiaSorin, Italy) recently approved by the USA Food and Drug Administration (US FDA) may improve the diagnostic output [79]. 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 [80]. 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 [81].

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 [82].

Saliva sampling is an appealing alternative to nasopharyngeal swabs, since collecting saliva is non-invasive and easier. Recently, A.L. Wyllie and colleagues [83] 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 [84]. A number of assays have been developed in academic institutions and in small and large companies [38-44] as illustrated by the joint effort developed by DiaSorin and San Matteo hospital in Pavia, Italy, now FDA approved [79].

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 [85-86]. Appropriately validated assays will be instrumental for epidemiological studies, evaluation of plasma donations, assessment of memory and as an adjunct to diagnostic procedures under selected conditions.

As discussed above (See 4. Immunity, Inflammation, Thrombosis), 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 [46] 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 [87].

8. Therapy

General introduction. 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 discussion of all 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 [1, 88].
Since several drugs are claimed to 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 [89, 90].

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. 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 [90]. Further carefully controlled 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 [91]. A recent randomized controlled study just published [92] and discussed [93] on Lancet 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 notifies that similar results are also reported in a larger study that has just been completed. This study is yet unpublished.

- Chloroquine and hydroxychloroquine. Chloroquine and hydroxy- derivative have anti-viral activity as well as the capacity to suppress inflammation (see below). Its potential for the treatment of COVID-19 needs to be investigated even if the general feeling is that these drugs lack of efficacy and are potentially harmful (See below Inhibition of excessive inflammation).

- Interferons. The rational for considering interferon therapy, systemic or via lung aerosol, is mentioned under 4. Immunity, Inflammation, Thrombosis. It has been used in Ebola and SARS [94, 95]. 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 trial are remdesivir, cloroquine, hydroxychloroquine, lopinavir and the same drugs plus interferon-beta [89].

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 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 [96].
CORIMUNO uses a similar principle to study different immunomodulation therapies in COVID in France [97].

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 rational for these strategies [98]. 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 usage of chloroquine as an antimalarial drug underlies the apparent resistance of Africa to COVID-19 does not take into account the fact that this agent, for some time now, has largely been abandoned in malaria.
Most evidence seems to point out to a lack of efficacy for this drug in treating COVID-19. There are also signs of major side effects when used at higher dosages [99, 100].

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 following the recommendations included in guidelines issued on 13/02/2020 in China [101]. It should be noted that studies are now ongoing in China and elsewhere, including Italy under the auspices of Agenzia Italiana del Farmaco (AIFA).

The French CORIMUNO-TOCI study has just completed the enrollment of patients into a randomized study tolicizumab vs standard of care. No results are available yet.
Other immunomodulation therapies are currently being tested. On the 27th of April 2020 Sanofi and Regeneron 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"* [102].

Heparin. According to evidence for a role of a thrombotic component in the pathogenesis of lung damage (See 4. Immunity, Inflammation, Thrombosis and 6. Pathology), heparin is part of current treatment protocols [48-50, 69-71, 75,76].

Steroids. In patients with ARDS there is still controversy around the use of corticosteroids. In patients that develop shock the use of hydrocortisone is associated with faster resolution of shock. On the other hand, evidence from other viral infections suggests that steroids can prolong viral shedding. For this reason, recent guidelines do not recommend the routine use of corticosteroids[103].

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 [104]. 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 [104-107]. An unprecedented effort is ongoing in USA along this line [105]. Randomized studies are ongoing or are planned and will address the fundamental issue of the efficacy of plasma therapy.

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 [108, 109]. 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) [110, 111] and tissue damage [111]. Therefore, as emphasized above, rigorous clinical evaluation will be mandatory also for antibody-mediated therapies.

Inhibiting SARS-CoV-2 entry into target cells. Cell entry of SARS-Cov-2 depends on the binding of the viral Spike proteins to ACE2 cellular receptor and on Spike protein cleavage by the host cell protease TMPRSS2 (transmembrane protease serine 2, see also 1. SARS-CoV-2 and 5. Predisposing factors), which allows the fusion of viral and cellular membranes (Fig. 5 modified from [4]); for the sake of simplicity the virus is shown to fuse with the plasma membrane whereas viral and host membrane fusion actually occurs following endocytosis).

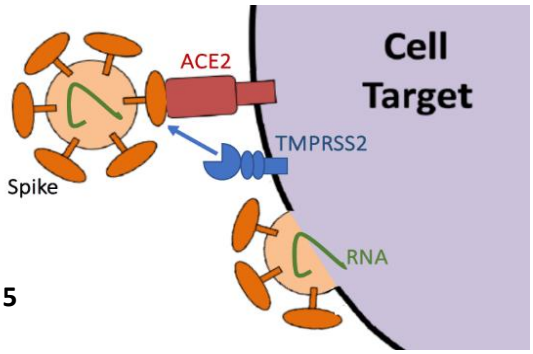

Fig. 5

Two approaches can thus be used to interfere with these processes.

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. 6, 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 [112]. A phase 2 clinical trial for the treatment of COVID-19 using soluble ACE2 has been recently launched.

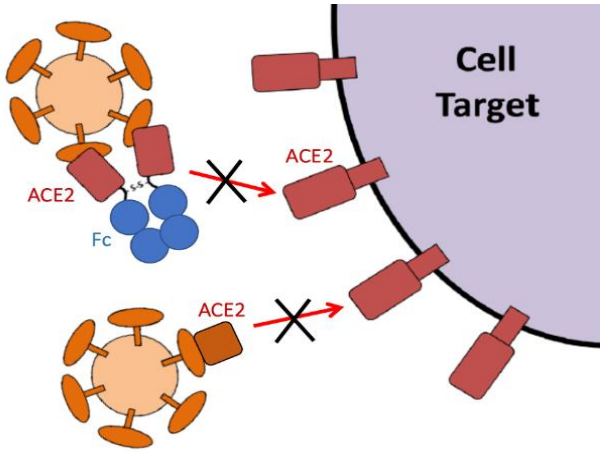

Fig. 6

Otherwise, as shown in Figure 6 (up), an immunoglobulin Fc domain can be fused to the ACE2 extracellular domain to facilitate prolonged circulation of the complex [4].

An alternative approach to interfere with virus entry into target cells 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

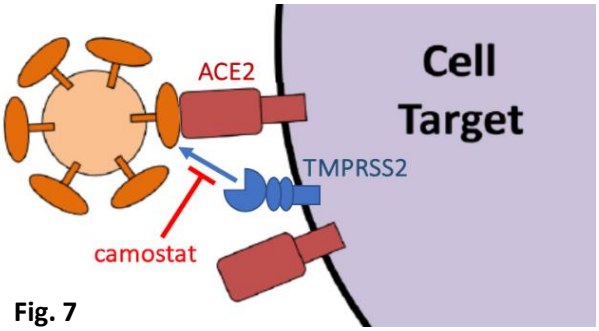

Fig. 7

cell lines [113] (Fig. 7). 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 androgens and it has been suggested that this could contribute to the male predominance of COVID-19 [114]. 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 [115].

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 [68]. Furthermore, mathematical modelling has been used to project possible evolution of the pandemic in different scenarios [22-27].
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. It shows us that in this moment we still need good decision making by humans.

9. 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 [34].

Preliminary issues. 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? [116]

There is a long series of serious infectious diseases in which vaccines are only partially effective and we have 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. On January 2017, during the World Economy Forum in Davos, 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 [117].

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, 6 are already in Phase 1 (first trial in humans) [118].

- RNA vaccines. On 17 March 2020, only 76 days since the sequence of SARS-CoV-2 was published, Dr. Michael Witte administered to volunteers the first shot of an RNA vaccine against the SARS-CoV-2 virus prepared by Moderna, a biotech company from Cambridge, MA [119]. RNA vaccines have been developed precisely to be produced in a very short time. The RNA specific for a particular protein is brought into cells by virus-like particles or into liposomes or bound to nanoparticles. Once the RNA has penetrated the cells of the organism, the cells use its genetic information to produce the target protein.

- DNA vaccines. Other biotech companies, including TAKIS Biotech, from Castel Romano, Italy, are experimenting DNA vaccines against SARS-CoV-2 on animals. DNA vaccines are also based on the possibility of inducing the body cells to temporarily produce the protein against which an immune response should be induced. DNA vaccination stimulate the production of antibodies but can lead to the activation of killer T cells. IGEA, a biotech in Carpi, Italy, is providing clinical grade electroporator devices to facilitate the cell entrance of a DNA vaccine produced by the Karolinska Instituted, Stockholm, Sweden. RNA and DNA vaccines have not yet been specifically tested on elderly people, the population with the greater need for an efficient vaccine for COVID-19 [120].

- Viral vaccines. Sinovac Biotech in Beijing, China has developed an attenuated variant of SARS-CoV-2 which induces a strong immune response in monkeys. A Phase 1 study has already been started. IRBM in Pomezia, Italy has engineered an adenovirus that will be

developed as an anti-COVID-19 vaccine by the University of Oxford and the Jenner institute in collaboration with AstraZeneca. In animals, this vaccine induces a strong immune response.

- Protein vaccines. Other laboratories are preparing COVID-19 vaccines using the "reverse vaccinology technique" developed by Rino Rappuoli, GSK in Siena. Starting from the virus RNA sequence, the proteins of the surface of the SARS-CoV-2 virions are identified. Crucial fragments of these proteins, produced in the laboratory with recombinant DNA technology, associated with new adjuvants of synthetic origin that most effectively induce an optimal immune response in the elderly. GlaxoSmithKine in Siena, Italy is making a few of these new adjuvants for use with novel SARS-CoV-2 vaccines developed by others.

Vaccine assessment. The administration of the new vaccine on a limited number of volunteers, as is already the case with the vaccine developed by Moderna, 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. Subsequently, the real evaluation of the effectiveness of the new vaccine 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, there is so much urgency for the vaccine that in order to quickly verify its efficacy it has been proposed to vaccinate human volunteers and then intentionally challenge them with that SARS-CoV-20 [121]. Support for this highly controversial human vaccine-challenge study, with ethical implications is growing [122].

Risks associated to fast track vaccine evaluations. It is likely that in the 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 [123].

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 [123] and have induced T helper 2-type immunopathology [124]. 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. To make the new vaccines available to the global population will be challenging [125]. The WHO is trying to make sure that vaccine stockpiles are shared equitably, a crucial challenge that must be collectively addressed by governments [125].

Hence the consideration that vaccines for COVID-19, if effective, will be very difficult to 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 [116, 125].

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 recommendation of 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, not yet peer-reviewed epidemiological studies endorse this hypothesis showing an inverse relationship between COVID-19 attributable mortality and country's policy concerning BCG vaccination [126, 127]. 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 [128]. As discussed above (see point 4, Innate Immunity) innate immunity plays a key role in controlling the first stage of SARS-CoV-2 infection [5]. Therefore, strategies which increase innate immunity ("training strategies") need to be carefully evaluated by epidemiologists and in carefully controlled clinical studies.

10. Preparedness

In the face of this COVID-19 tragedy, which is causing many deaths, suffering and social disaster, it is inevitable to ask how much the world as a whole, and Italy in particular were or should have been prepared for the pandemic.

According to the "2010 Global Health Security Index ranking" [129, 130], 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 has been placed in isolation because they are infected. 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 [131, 132]. On the other hand, many other countries and even 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 [133, 134].

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 [130].
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 have proven to be worth the investment of time and energy during "peace time" [96]. 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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