SARS-CoV-2 and COVID-19: from the bench to the bedside

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Call Out Box

1) Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causes Coronavirus Disease 2019 (COVID-19). Its clinical evolution is characterized by three main phases (early infection phase, pulmonary phase, and hyperinflammation phase), with clinical features ranging from mild or no symptoms to acute respiratory distress syndrome and multi-organ failure.
2) Numerous, mainly observational, retrospective studies are contributing to improve understanding of COVID-19. Besides provision of supportive medical care, much uncertainty remains as to the real efficacy of the proposed treatments that have been tested under dramatic clinical circumstances.
3) Antiviral drugs, antimalarial drugs, immunomodulators, agents affecting hemostasis, and other drugs have been administered in an unselected fashion, overlooking aspects of disease progression.
4) We are now entering into a new stage of the pandemic with many ongoing randomized controlled trials aimed at the more precise identification of patient tailored treatments and drugs better suited to the specific phase of the disease.

Abstract

First isolated in China in early 2020, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the novel coronavirus responsible for the ongoing pandemic of Coronavirus Disease 2019 (COVID-19). The disease has been spreading rapidly across the globe, with the largest burden falling on China, Europe, and the United States. COVID-19 is a new clinical syndrome, characterized by respiratory symptoms with varying degrees of severity, from mild upper respiratory illness to severe interstitial pneumonia and acute respiratory distress syndrome, aggravated by thrombosis in the pulmonary microcirculation. Three main phases of disease progression have been proposed for COVID-19: an early infection phase, a pulmonary phase, and a hyperinflammation phase. Although current understanding of COVID-19 treatment is mainly derived from small uncontrolled trials that are affected by a number of biases, strong background noise, and a litany of confounding factors, emerging awareness suggests that drugs currently used to treat COVID-19 (antiviral drugs, antimalarial drugs, immunomodulators, anticoagulants, and antibodies) should be evaluated in relation to the pathophysiology of disease progression. Drawing upon the dramatic experiences taking place in Italy and around the world, here, we review the changes in the evolution of the disease, and focus on current treatment uncertainties and promising new therapies.

Introduction

At the end of 2019, many cases of pneumonia of unknown origin were detected in the Chinese province of Wuhan. In early January 2020, they were confirmed to be caused by a novel coronavirus (CoV), later named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). SARS-CoV-2 has been recognized as the causal virus of Coronavirus Disease 2019 (COVID-19). Thanks to its remarkable capacity for asymptomatic transmission, SARS-CoV-2 possesses ideal attributes to reach pandemic levels. Since the identification of the first case in China, COVID-19 has rapidly spread all over the world, with about four million confirmed cases to date (https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). Compared with other major viral outbreaks in contemporary history, like the 2002–2003 Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS; 2012–ongoing), COVID-19 appears to have a lower case-fatality rate (CFR, 3-4%) but a significantly higher basic reproductive ratio (R_0) (2.68, 95% confidence interval: 2.47–2.86) (74) (TABLE 1). The main reasons why COVID-19 spreads more efficiently than SARS and MERS are its higher level of transmissibility and the number of freely circulating asymptomatic COVID-19 carriers (3, 27). A large study of 44,672 patients with COVID-19 in China, published by the Chinese Center for Disease Control and Prevention (CCDC), reported a CFR of 2.3% (61), which is significantly lower than that of SARS (9.5%) and MERS (34.4%) (14) (TABLE 1). Noteworthy, COVID-19 CFR is highly variable across the globe and increases substantially in people aged 60 and more, as observed in the Italian population. Relative susceptibility to symptomatic infection also increases with age (children are rarely infected), raising questions about the underlying biology of host responses in relation to age (73).

From virus transmission to systemic hyperinflammation: the three phases of the disease

Human-to-human transmission occurs primarily through respiratory tract droplets expelled from an infected person's cough or sneeze and capable of travelling up to 2 m/6 feet, as well as via respiratory secretions and direct contact (40). The average incubation period has been calculated to range from 1 to 14 days (40). Presymptomatic or asymptomatic transmission during the incubation period has been considered the Achilles' heel of COVID-19 pandemic control, since a considerable number of people testing positive for COVID-19 may show no symptoms (3, 27). The virus may

cross the mucous membranes, especially nasal and laryngeal mucosa, reach the lungs through the respiratory tract, and enter the circulatory system, causing viremia (see below for mechanisms of viral attachment and entry). Worsening of clinical conditions occurs at around 7 to 14 days after onset (42). It has been proposed that COVID-19 infection in the lungs encompasses three main phases (FIGURE 1): an initial phase involving viral replication and relatively mild symptoms (early infection phase); a second phase characterized by adaptive immunity stimulation and predominance of respiratory symptoms (pulmonary phase); and, in some cases, a third and last phase with progress to a hyperinflammatory condition (hyperinflammation phase) (59). Although substantial overlap among the three phases can occur in individual patients, recognition of each phase is crucial for tailored therapies (FIGURE 1). For instance, immunosuppressive regimens, including anti-interleukin (IL)-6 or corticosteroids, are more likely to be beneficial in the second and third phases, when the immune processes are critical for pathogen eradication, rather than in the early stage of infection, when use of antiviral and antimalarial agents is more appropriate to limit virus spread. Accordingly, the efficacy and safety profiles of drugs currently used to treat COVID-19 (antiviral drugs, antimalarial drugs, anti-IL-6, corticosteroids, immunoglobulins, heparin) should be scrutinized on the basis of the specific phase of disease progression and the corresponding pathophysiological processes (FIGURE 2).

Phase-1. During the early phase of infection, the virus infiltrates the lung parenchyma and begins to proliferate. SARS-CoV-2 RNA encodes four principal structural proteins: one nucleocapsid protein surrounding the RNA genome and three membrane proteins, the spike glycoprotein (S), the matrix glycoprotein, and the envelope protein (44). Neutralizing antibodies from immune patients who have recovered from COVID-19 have been used to decrease viral burden (16, 25, 58). In addition to vaccines, the possibility of generating recombinant immunoglobulins that mimic the neutralizing properties of endogenous antibodies is an additional immune strategy actively being pursued (https://www.activemotif.com/blog-covid19-abs). Virus entry into a cell is mediated by S glycoprotein recognition of the angiotensin converting enzyme (ACE2) receptor (64), with the S1 and S2 domains responsible, respectively, for virus-receptor binding on the host cell and fusion of the viral RNA with the cell membrane (38). Importantly, the CoV S protein is cleaved by a series of serine proteases, including trypsin, cathepsins, elastase, the host type 2 transmembrane serine protease (TMPRSS2) (36), and plasmin, which promote virus entry into epithelial cells (38, 39) (FIGURE 2). On this basis, interventional randomized clinical trials are recruiting patients to test efficacy and safety of agents with antiprotease activity, including the plasmin(ogen) inhibitor, tranexamic acid (NCT0433812), or camostat mesylate (used for many years for treating pancreatitis) and nafomastat, both of which inhibit TMPRSS2 (56) (FIGURE 2)

(NCT04321096; NCT04338906). Nevertheless, an interesting correspondence that was recently published underlines that the complexity of the coagulation disorder induced by SARS-CoV-2 can lead to both bleeding and thrombosis (4). The authors consider that many patients requiring plasmin(ogen) inhibitors (tPA), aimed at dissolving pulmonary microvascular clots and improving blood flow through pulmonary circulation, have severe ARDS and "may be more likely to have additional coagulopathies" and related risks for bleeding (4). Moreover, tPA is commonly used for large artery vascular occlusions, and may not be able to adequately re-perfuse small (large surface) pulmonary vessels. On the other hand, tranexamic acid, which is not pro-thrombotic and prevents the conversion of plasmin(ogen) into plasmin, thereby preventing fibrinolysis, is considered potentially useful in COVID-19 patients, even in the phase in which new clots are formed (4). Among the variety of actions ascribed to chloroquine and hydroxychloroquine, their ability to increase endosomal pH, thereby preventing ACE2 separation from SARS-CoV-2 (23), may limit intracellular virus diffusion. Canonical antiviral drugs (remdesivir, lopinavir/ritonavir, ribavirin, favipiravir, umifenovir) reduce viral replication by interfering with various steps of RNA processing (6, 10, 15, 28, 53, 61) (https://www.niaid.nih.gov/diseases-conditions/coronaviruses) (FIGURE 2). Clinically, this stage is characterized by mild constitutional symptoms and marks the initial response by the innate immune system driven by monocyte/macrophage infiltration (FIGURE 1).

Phase-2. Inflammatory response (vasodilation, endothelial permeability, leukocyte recruitment) and tissue damage lead to the following phase (pulmonary phase), with lung injury and hypoxemia as underlying causes of the respiratory dysfunction. The respiratory failure characterizing the second phase of the disease shows different features from the typical acute respiratory distress syndrome (ARDS). Although matching with the ARDS Berlin definition of severe state (52), pulmonary compliance in intubated COVID-19 patients is slightly decreased, and therefore patients appear relatively "easy to ventilate" (29) and receive some benefits from low-to-moderate levels of positive end expiratory pressure (PEEP) (8-10 cmH_2O) and prone positioning. The combination of severe hypoxemia without significant reduction in compliance is rarely observed in severe ARDS. In typical ARDS the alveolus is primarily involved, while COVID-19 is a systemic disease that elicits marked disruption of the pulmonary vascular endothelium. A rapidly activated coagulation cascade, with widespread micro- and macro-thromboses in the lungs and other organs, and very elevated serum D-dimer levels, has frequently been reported in association with adverse outcomes. In this regard, treatment with nebulized or intra-venous tissue plasminogen activator (rt-PA) may be an effective therapeutic option. Two studies with rt-PA are ongoing and currently recruiting patients (NCT04356833, NCT04357730). Progression to ARDS in COVID-19

patients implicates multiple pathophysiologic mechanisms, including disproportionate endothelial damage, which affects pulmonary vasoregulation and favors ventilation-perfusion mismatch (failure of hypoxic pulmonary vasoconstriction due to an endothelial involvement), hypoxemia, and thrombogenesis (65). Intense vasodilation and endothelial dysfunction with pulmonary shunting have been reported to be associated with vascular enlargement on CT scans (10). Lung vascular thrombosis from thrombotic microangiopathy and/or pulmonary embolism results in increased respiratory dead space. It is only in the following phase, when edema increases and lung damage progresses, that some patients develop a phenotype more consistent with ARDS (28, 29, 47), thus requiring supportive respiratory treatment. COVID-19-ARDS manifests itself unpredictably, due to the different disease phases and the variable contributions of the host response, physiological reserve, and comorbidities. Patients with hypoxemia or remarkable dyspnea can breathe normally, can have deep hypocapnia or normo/hypercapnia, and can be responsive or not-responsive to prone positioning (28). Drugs preventing SARS-CoV-2 entry into the host cell and inhibiting virus replication maintain their efficacy during this second phase. Additional drugs capable of reducing inflammation, and thus more appropriate in the third phase, may also be introduced at this stage.

Phase-3. As the host inflammatory response increases, even in the presence of diminishing viral loads, the patient enters the third phase of the disease, characterized by systemic inflammation (hyperinflammation phase) and damage of distant organs, resulting in multiorgan failure (MOF) (12, 59). During this phase, increased production of a series of cytokines, including IL-6, IL-2, IL-7, tumor necrosis factor (TNF)-α, interferon-γ inducible protein (IP)-10, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP) 1-α, granulocyte-colony stimulating factor (G-CSF), C-reactive protein (CRP) (37, 49, 55, 66, 78), may affect the prognosis. On this basis, treatment with tocilizumab, sarilumab and monoclonal antibodies against the IL-6 receptor has been proposed to attenuate the severity of the inflammatory storm, and a number of clinical trials are ongoing (https://clinicaltrials.gov). Direct viral injury, uncontrolled cytokine release, and damage-associated molecular patterns promote localized-systemic microvascular inflammation, which triggers endothelial activation and further emphasizes pro-thrombotic conditions. In line with these pathophysiologic mechanisms, symptomatic acute pulmonary thrombosis, ischemic stroke, myocardial infarction, and systemic arterial thrombosis have been reported in a large proportion of hospitalized COVID-19 patients (75). Vascular disease may also explain massive D-dimer elevations. Augmented fibrin degradation products and D-dimers have been detected predominantly in patients who developed the most severe forms of the disease, with MOF, ARDS, septic shock, hemorrhage/coagulopathy (disseminated intravascular coagulopathy), acute heart/liver/kidney injury, and secondary bacterial infections (17, 35, 37, 38). Under these circumstances, heparin

administration has been proposed as a promising step in the multitherapy approach to treat COVID-19 patients (63). In view of the close relationship between the immune and coagulation systems, thrombin inhibition by heparin may dampen the inflammatory response. In addition, the more general anti-inflammatory properties of heparin may be relevant in the setting of COVID-19 (63). The endless repurposing of thalidomide in cancer and infectious diseases could be applied to COVID-19 if its ability to inhibit TNF and other proinflammatory cytokines (24) can be demonstrated (NCT04273529; NCT04273581; NCT04361422).

Therapies against SARS-CoV-2/COVID-19: between pathophysiology and uncertainty

Although knowledge and understanding of COVID-19 is rapidly evolving, besides provision of supportive medical care, much uncertainty remains as to the real efficacy of the proposed treatments. Currently published data generally consisting of small uncontrolled trials, observational studies, descriptive reports, and case series, mainly deriving from the initial Chinese outbreak, are affected by a number of biases, strong background noise, and a litany of confounding factors. Not infrequently, contradictory therapeutic recommendations (antiviral drugs, steroids, anti-IL) and guidelines emerge as a consequence of clinical research conducted under emergency conditions and characterized by an overwhelming number of severe patients requiring rescue respiratory, renal, and cardiovascular support (2) (https://www.idsociety.org/globalassets/idsa/practice-guidelines/covid-19/treatment/idsa-covid-19-gl-tx-and-mgmt-v1.0.4.pdf). The current Centers for Disease Control and Prevention (CDC) guidelines for clinical care of patients with COVID-19 (as of April 25, 2020) underline that no specific treatment for COVID-19 is currently available or recommended, and World Health Organization (WHO) highlights that no evidence is currently available to recommend any specific anti-COVID-19 treatment for patients with confirmed disease. Both organizations emphasize the role of supportive care based on severity of illness (https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). However, it should be underlined that guidelines and recommendations are constantly evolving as new trials are concluded.

Nevertheless, due to the urgent need for effective treatments, a number of drugs with variable targets have been and continue to be unceasingly proposed for treatment of COVID-19 patients worldwide. While figure 2 includes drugs with a mechanistic rationale that supports their possible efficacy in COVID-19, Table 2 reports a selection of these medicines either more commonly used, or with at least one published report. While drugs proposed for COVID-19 could be better evaluated in the context of disease progression, to date, little phase-specific distinction has

been made, and medications have mostly been used in an non-specific fashion, as further described below.

Antimalarial and antivirals drugs

Chloroquine, hydroxychloroquine and azithromycin. Chloroquine and hydroxychloroquine have been previously used for the prevention and treatment of malaria and treatment of chronic inflammatory diseases. More recently, they have been used to treat cases of SARS and MERS, although only low-quality evidence of benefits is available. Used alone, or in combination with azithromycin, which has been shown to be active *in vitro* against Zika and Ebola viruses (30, 46, 53), hydroxychloroquine has been widely administered to COVID-19 patients due to its *in vitro* inhibitory activity against SARS-CoV-2 (67, 76). A French study reported encouraging results (30). However, this study was burdened with several major shortcomings and was not confirmed by a small Chinese randomized trial reporting no difference in virologic outcomes (15). Recently, a small systematic review on the use of chloroquine concluded that clinical research on this topic is justified by sufficient pre-clinical rationale, initial evidence on the effectiveness of chloroquine for COVID-19 treatment, and safety data from long-term use in clinical practice (20). Nevertheless, the Surviving Sepsis Campaign (SSC) guidelines on the management of critically ill adults with COVID-19 indicate that insufficient evidence exists for recommending the use of chloroquine or hydroxychloroquine in critically ill adults with COVID-19 (2). The "Infectious Diseases Society of America (IDSA) Guidelines on the Treatment and Management of Patients with COVID-19" recommend use of hydroxychloroquine/chloroquine, alone or in combination with azithromycin, only in the context of clinical trials for hospitalized COVID-19 patients, thus evidencing a persistent knowledge gap and the risk of inadequate monitoring of outpatients (5). As hydroxychloroquine and chloroquine cause serious adverse effects, including QTc prolongation, hypoglycemia, neuropsychiatric effects, and retinopathy (56), on April 23, 2020 the European Medicines Agency (EMA) released a warning by the COVID-19 EMA pandemic Task Force concerning the side effects of chloroquine and hydroxychloroquine when taken in high doses or in combination with azithromycin (https://www.ema.europa.eu/en/news/covid-19-reminder-risk-serious-side-effects-chloroquine-hydroxychloroquine). Proof of efficacy of chloroquine and hydroxychloroquine still warrants further evaluation in larger randomized trials (18, 31). A large multinational registry analysis has shown that the use of hydroxychloroquine or chloroquine with or without a macrolide for treatment of 96,032 COVID-19 patients (14,888 in the treatment group and 81,144 controls) was associated with decreased in-hospital survival and increased frequency of ventricular arrhythmia (48). On this

basis, the WHO has temporarily halted the hydroxychloroquine arm of the ongoing SOLIDARITY megatrial, which includes additional arms with remdesivir, lopinavir/ritonavir, and lopinavir/ritonavir plus interferon-β (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments).

Lopinavir/ritonavir. Lopinavir/ritonavir is an oral combination agent for treating HIV that has demonstrated activity against SARS-CoV and MERS-CoV (19, 21). An open-label randomized controlled trial (RCT), however, failed to meet the efficacy endpoint in COVID-19 patients (8). Adverse drug reactions (gastrointestinal symptoms such as nausea and diarrhea and hepatotoxicity), associated with poor efficacy outcomes, have raised concerns about the use of lopinavir/ritonavir in COVID-19 patients. The SSC guidelines do not recommend routine use of lopinavir/ritonavir (2), while IDSA guidelines limit the combination of lopinavir/ritonavir in the context of clinical trials (5).

Remdesivir. Initially developed to treat Ebola, remdesivir has also proven effective against SARS, MERS-CoV, SARS-CoV-2 *in vitro*, and SARS-CoV-2 replication in murine and non-human primate models (22, 57, 67). It thus holds promising therapeutic potential for the treatment of COVID-19 (34). Remdesivir was administered to 61 hospitalized patients with COVID-19 on a compassionate-use basis (non-randomized) (53 patients were analyzed): 36 patients (68%) had an improvement in oxygen-support class, 25 patients (47%) were discharged, and 7 patients (13%) died; mortality was 18% (6 of 34) among patients receiving invasive ventilation and 5% (1 of 19) among those not receiving invasive ventilation (34). Clinical trials are ongoing to evaluate the safety and antiviral activity of remdesivir in patients with COVID-19 (NCT04292899, NCT04292730, NCT04257656, NCT04252664, NCT04280705). However, a recent randomized, double-blind, placebo-controlled, multicenter trial conducted at ten hospitals in Hubei (China), including 237 patients (158 remdesivir and 79 placebo), did not show significant improvements in time to clinical improvement, mortality (22 (14%) deaths in the remdesivir group vs. 10 (13%) in the placebo group), or time to clearance of virus. Moreover, remdesivir was stopped early because of adverse events (68). Most recently, preliminary data from a randomized trial (remdesivir vs. placebo), sponsored by the United States National Institute of Health and involving 1,063 hospitalized patients with advanced COVID-19 and lung involvement, were reviewed by an independent data and safety monitoring board. The interim analysis showed that patients who received the antiviral drug recovered faster than similar patients who received placebo (https://www.niaid.nih.gov/diseases-conditions/coronaviruses).

Ribavirin. Because of antiviral activity against SARS-CoV, ribavirin is a candidate for COVID-19 treatment. In the absence of clinical data on the use of ribavirin for treating SARS-CoV-2, its potential role must be extrapolated from data obtained from other CoV studies. A systematic review of clinical experience with ribavirin in the treatment of SARS did not show conclusive results, and possible harm due to adverse effects, such as hematologic complications (hemolytic anemia in more than 60% of patients) and liver toxicity, have emerged (60).

Favipiravir. Recently tested in an open-label randomized trial, favipiravir showed faster resolution of fever and cough, but similar rates of respiratory failure, compared with the control group receiving umifenovir (or Arbidol) (13). In an open-label non-randomized control study of 80 COVID-19 patients, the favipiravir arm showed better responses in terms of disease progression and viral clearance compared with the control arm (7).

Umifenovir. A non-randomized study of 67 patients with COVID-19 reported that umifenovir treatment for a median duration of 9 days was associated with lower mortality rates and higher discharge rates compared with patients who did not receive the agent (69). While an open study of 50 COVID-19 patients showed that umifenovir was superior to lopinavir/ritonavir in treating COVID-19 (79), an observational retrospective study, carried out in a non-ICU setting, did not find any association with improved prognosis or acceleration of SARS-CoV-2 clearance in COVID-19 patients (41).

Others

Heparin. Commonly used to prevent deep venous thrombosis in ICU and non-ICU patients, heparin has not been included among anti-COVID medications in current guidelines so far (2). According to a Chinese study of 449 COVID-19 patients, no difference in 28-day mortality was found between heparin users and nonusers; however, 28-day mortality of heparin users was significantly lower in patients with high score sepsis-induced coagulopathy (62). This finding would favor the hypothesis of a major contribution of the anticoagulant treatment during the last phase of COVID-19. However, since deep venous thromboses leading to pulmonary embolism are marginally present, the microangiopathy observed in COVID-19 patients is more likely to be due to thrombi formation than hypercoagulation (11). Therefore, the use of high doses of low molecular weight heparin in severe patients should be regarded with caution, and antiplatelet treatment should be considered instead (11).

Tocilizumab. Increased levels of IL-6 in severely ill patients (77) have justified the use of treatments that attenuate the cytokine storm caused by the SARS-CoV-2 infection. The monoclonal antibody against the IL-6 receptor, tocilizumab, had been previously approved for the treatment of

rheumatoid arthritis and cytokine release syndrome, following chimeric antigen receptor T-cell therapy. Tocilizumab treatment in COVID-19 patients has been explored in small studies with few cases (45). However, several RCTs are underway in China (NCT04310228, ChiCTR200002976), Italy (NCT04317092), the United States (NCT04320615), and in many other countries all over the world.

Immunoglobulins/convalescent plasma/hyperimmune immunoglobulins. Antibodies contained in plasma collected from recovered patients and screened for virus-neutralizing antibodies may act against both free viral particles and infected cell immune clearance, when administered in a prophylactic or therapeutic manner (9, 16). Regrettably, only few, very small, promising reports describing the use of high-dose immunoglobulins (9) or antibodies collected from recovered patients (25, 58) have been published so far. However, an outstanding number of trials (over 100) on this topic are ongoing (https://clinicaltrials.gov).

Corticosteroids. Corticosteroids previously used during outbreaks of SARS and MERS have been frequently administered to COVID-19 patients to limit lung inflammation and treat and prevent the development of ARDS, despite lack of evidence for their clinical efficacy (72). Furthermore, a remarkable delay of viral clearance and increased risk of secondary infection have been evidenced, which may represent serious drawbacks (2). Absence of proven benefits and the potential for harm call for caution in the routine use of corticosteroids in patients with COVID-19.

COVID-19 in Italy and regional differences

By the end of March 2020, Italy had the second highest number of COVID-19 infections worldwide, and the largest number of deaths (https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). After the identification of the first severe case of COVID-19 on February 20, 2020 – a young man with no history of possible exposure abroad, diagnosed with COVID-19 in Codogno, Lombardy – the outbreak rampaged through various areas of northern Italy. Within two weeks, an exponential increase in new cases of COVID-19, including many critically ill patients (33), was reported in the surrounding areas, and new clusters were identified in the nearby regions of Piedmont and Veneto. Since then, COVID-19 infection has spread throughout the country with a somewhat lower impact from north to south (http://opendatadpc.maps.arcgis.com/apps/opsdashboard/index.html#/b0c68bce2cce478eaac82fe38d4138b1). It should be underlined that, although the village of Vo' Euganeo (Veneto) was hit as early as Codogno (Lombardy), regional health officials in Veneto markedly limited the impact of the COVID-19 outbreak compared to Lombardy, probably due to the timely and extensive policy of

reverse transcriptase–polymerase chain reaction (RT-PCR) swab testing and efforts in identification of asymptomatic carriers (http://opendatadpc.maps.arcgis.com/apps/opsdashboard/index.html#/b0c68bce2cce478eaac82fe38d4138b1). Notably, CFR in Italy (7.2%, (1,625 deaths/22,512 cases) (43) has been reported to be markedly higher than that previously recorded in China (2.3%) (46) (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200306-sitrep-46-covid-19.pdf?sfvrsn=96b04adf_2). Approximately half of the COVID-19 deaths in Italy occurred in Lombardy. Two main factors have been identified that might explain this discrepancy in CFR between Italy and other countries.

The mean age of the Italian population is higher than that of many other countries (including China). In 2019, approximately 23% of the Italian population was aged 65 years or older. Age is an independent risk factor for mortality in patients with COVID-19 (72, 78). Interestingly, CFRs in patients aged 60-69 yrs were similar in Italy and China (3.5 and 3.6, respectively) (51) and, in both countries, CFR increased with increasing age (51). However, the median age (interquartile range) of COVID-19 patients in China was 49 yrs (41-58) (37) compared with 63 yrs in Italy (56-70) (33), similar to that reported in the United States (62 yrs, 49–74) (32). Both in Italy and China, increases in the median age were associated with higher CFR values. Nevertheless, while similar CFRs were reported for the middle-aged cohort, the CFR of patients in the highest age group (≥80-yr) was markedly higher in Italy compared with China (20.2 and 14.8, respectively) (51). CFR statistics in Italy are based on defining COVID-19-related deaths as those occurring in patients who test positive for SARS-CoV-2 via RT-PCR analysis, independently from preexisting diseases that may have caused death, thus leading to CFR overestimation. Moreover, shortly after the epidemic began, on February 25, the Italian Government issued more stringent testing policies, giving priority to patients with more severe clinical symptoms, suspected of having COVID-19 and requiring hospitalization, thus markedly reducing the denominator and increasing CFR. Other countries, like the Republic of Korea (CFR 1.0%) (https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200227-sitrep-38-covid-19.pdf?sfvrsn=9f98940c_2) decided to test for SARS-CoV-2 on a larger population, even those presenting mild or limited to no symptoms.

National data (51) on the COVID-19 epidemic (51) show that the disease has been spreading in a random fashion throughout Italy during the last two months (FIGURE 3). One sixth of the Italian population live in Lombardy where 37% of cases and 53% of deaths were reported as of April 15, 2020 (50). Lombardy has reported a much higher CFR compared with the rest of Italy (18.3% vs. 1.6%, respectively) (50). This remarkable difference can partially be explained by the

unexpected catastrophic outbreak that suddenly wrecked Lombardy and only later spread to other regions, thus giving health authorities in central and southern Italy more time to carry out interventions to curb similar situations (6). Overwhelmed emergency rooms concentrated within a short period of time put the otherwise highly performing Lombardy healthcare system under stress, which was then unable to control outpatient care, hospital admission, and, most importantly, cases of COVID-19 among elderly in nursing homes. All these factors favored an uncontrolled transmission of SARS-CoV-2. The number of positive cases in Tuscany was 9,231 (*vs.* 73,348 in Lombardy) with 811 deaths (*vs.* 13,575 in Lombardy) as of April 25, 2020 (http://opendatadpc.maps.arcgis.com/apps/opsdashboard/index.html#/b0c68bce2cce478eaac82fe38d4138b1). The advantage of facing the outbreak weeks after other areas (France, Germany, and Spain after Italy, or Tuscany after Lombardy) provided health systems with the time and experience to adjust healthcare infrastructure, to cope with the emergency by increasing ICU beds, recruiting doctors and nurses, suspending elective procedures, and optimizing resources. This critical advantage led to an increase in the level of care and an improvement of outcome (54).

Conclusions

The SARS-CoV-2 pandemic can be considered the greatest global public health tragedy since the pandemic influenza outbreak of 1918. To date, the underlying mechanisms responsible for the severe form of the disease and death are not completely understood, and no specific therapies have been demonstrated to be effective against COVID-19. During the first phase of the SARS-CoV-2 pandemic, many drugs with undocumented efficacy have been used as a “last resort”, based on the unproven assumption that benefits will outweigh harm. Under the dramatic circumstances of the last 2-3 months, testing new drugs or repurposing older drugs for severely ill patients (like COVID-19) has been challenging. Among the almost four million individuals diagnosed with COVID-19, many have been offered unproven treatments. We are now entering a new era of the pandemic, with many ongoing RCTs aimed at identifying patient-tailored drugs, and drugs better suited to the specific phase of the disease with improved precision. Monoclonal antibodies against specific sites essential for viral function are increasingly recognized as a promising class of drugs. Early preprint reports describe preclinical development of a human monoclonal antibody directed against a common epitope to block SARS-COV-2 (and SARS-CoV) infection (70). One or more vaccines represent the most effective long-term strategy for prevention of possible further outbreaks of SARS-CoV-2 (71). However, vaccines eligible for prevention campaigns must guarantee protective immunity and induce prolonged generation of neutralizing antibodies. Although the extraordinary pressure on pharmaceutical and biotech companies has resulted in the

publication of the SARS-CoV-2 genome (26), key epitope identification (1), and first-in-man vaccine administration at an unprecedented speed (https://www.bbc.com/news/health-52394485?intlink_from_url&), a minimum of 12 to 18 months would be necessary for vaccine approval and for the enormous effort required to produce enough vaccine doses to protect the world population. Still, clinical and scientific communities, and the pharmaceutical industry, supported by public and private funds, are making a tremendous effort to support an unprecedented number of pathophysiological studies and clinical trials to face this highly unexpected pandemic.

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Figure legends

Fig. 1: Schematic representation of the symptoms that characterize the three different phases of COVID-19 progression and corresponding possible treatments.

Fig. 2: Schematic representation of the SARS-Cov-2 cycle in airway epithelial cells and inflammatory consequences of viral infection. Some of the mechanisms related to the possible therapeutic action of drugs used or proposed to treat COVID-19 are reported.

Fig. 3: The highly varied impact of the COVID-19 outbreak in the respective Italian regions.

Table 1 - Pathogenicity and Transmissibility of SARS-CoV, and MERS-CoV, and SARS-CoV-2.

	Pandemic	CFR (%)	Pandemic	R_0	Remarks
SARS-CoV	Yes	9.6	Yes	1.7-1.9	58% of cases result from nosocomial transmission (12, 16, 17)
MERS-CoV	No	34.4	No	0.7	70% of cases result from nosocomial transmission (12, 16, 17)
SARS-CoV-2	Yes	3-4*	Yes	2.68	Very high community transmission (12, 16, 17)

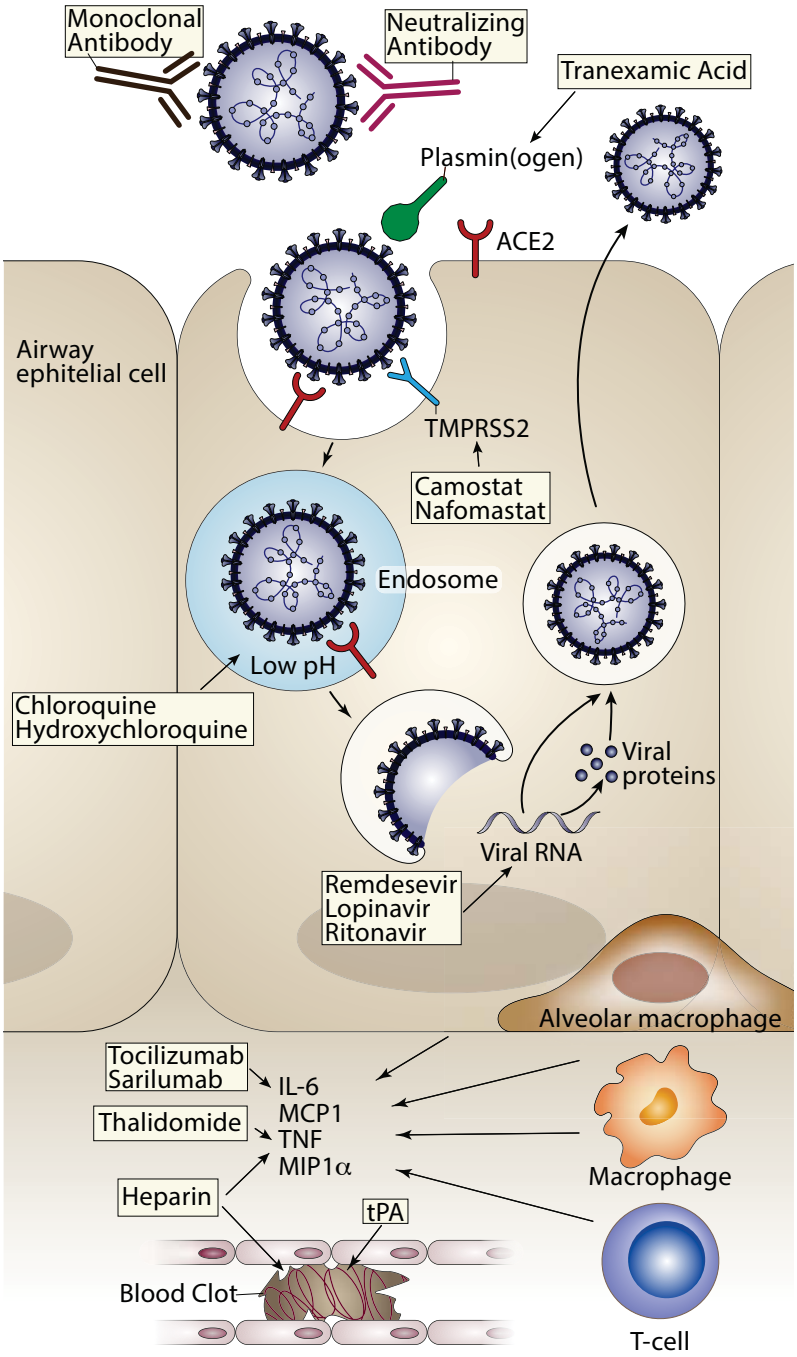
Abbreviations: CFR, Case Fatality Rate; MERS-CoV, Middle East Respiratory Syndrome-Coronavirus; SARS-CoV-(2), Severe Acute Respiratory Syndrome-Coronavirus-(2); R_0, basic reproductive ratio* World Health Organization as of 26 April 2020

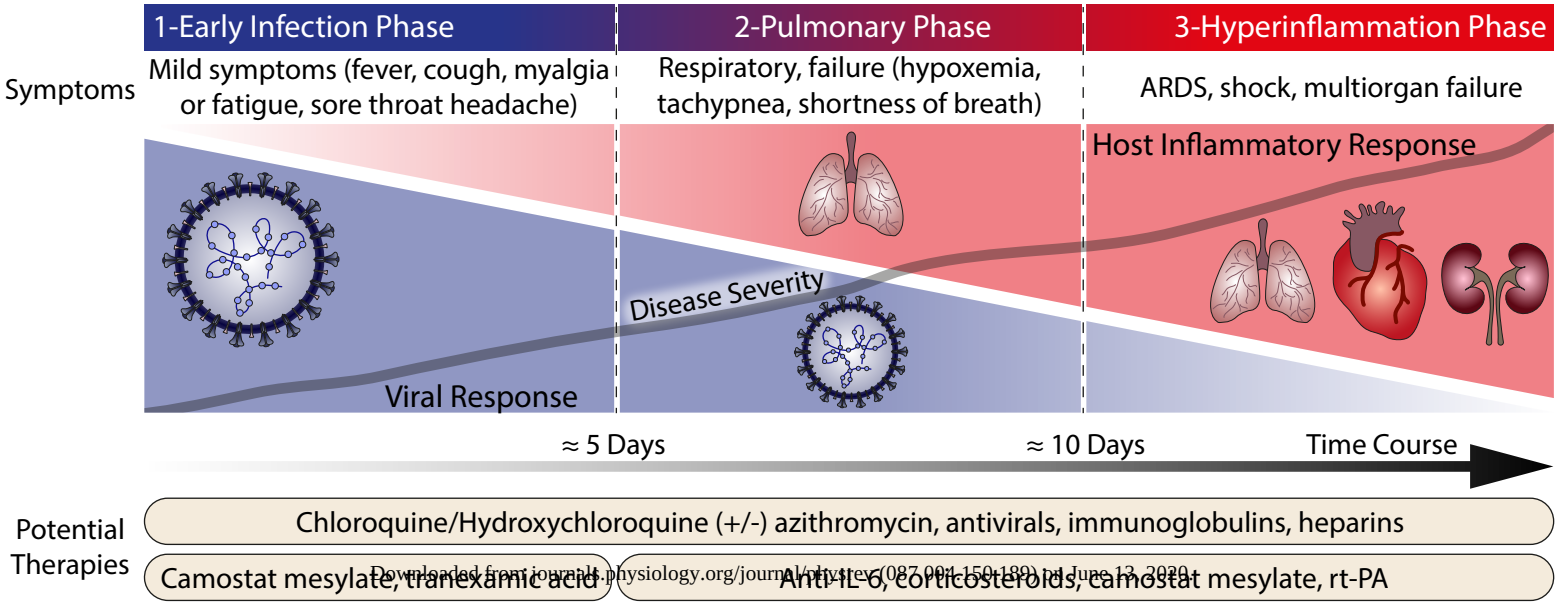
Table 2 – Clinical trials of medications currently used most for treating COVID-19 (13)

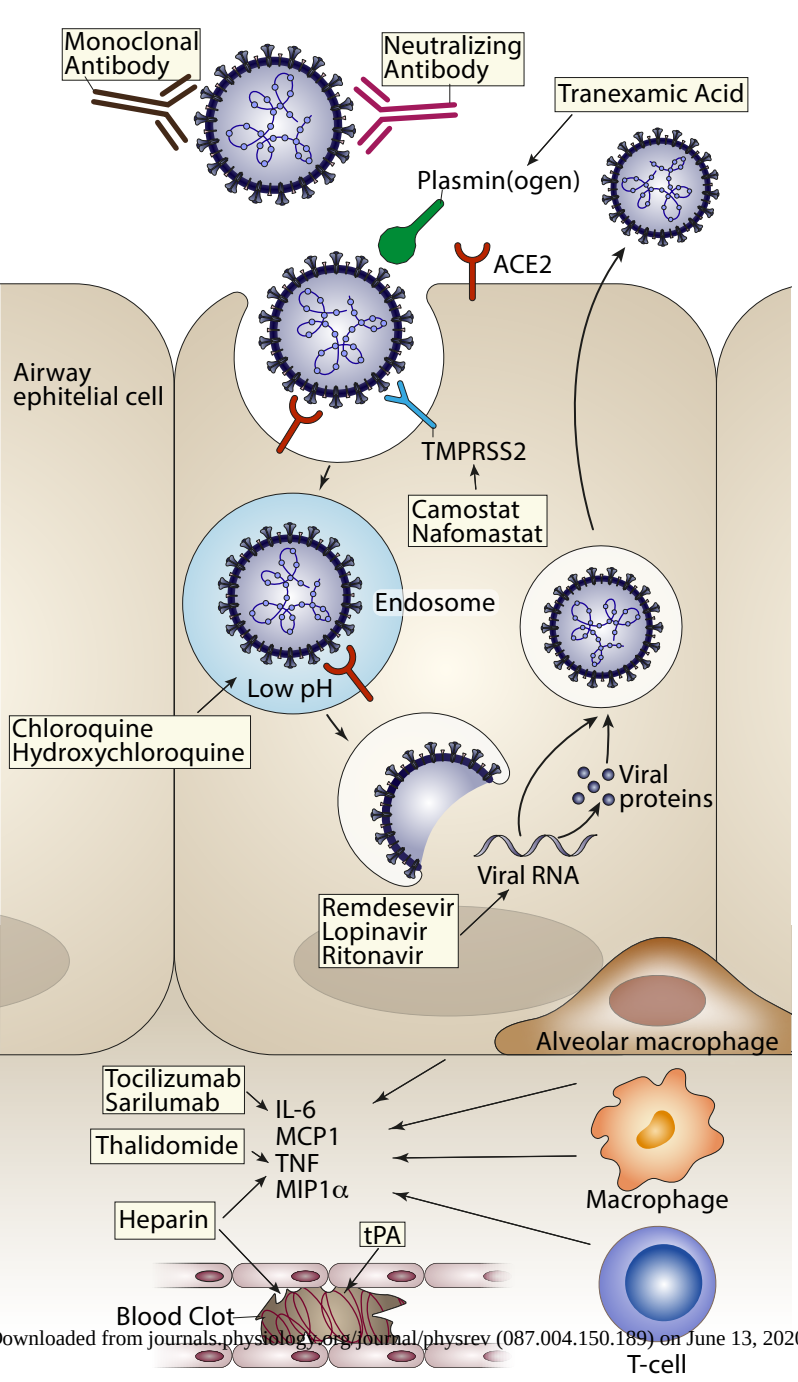
Medication*	**Therapeutic mechanisms**	**Remark**	**Surviving Sepsis Campaign guidelines** (1)	**Infectious Diseases Society of America Guidelines** (2)	**Studies registered on Clinicaltrial.gov**
Antivirals and antimalarial					
Chloroquine and hydroxychloroquine with or without azithromycin *	Inhibition of viral entry into cells by inhibiting glycosylation of host receptors, proteolytic processing, and endosomal acidification and of cytokine production, autophagy and lysosomal activity in host cells (8).	Approved use for malaria, systemic lupus erythematosus and rheumatoid arthritis and used to treat SARS and MERS (no high-quality evidence). Contradictory small studies available to date (6, 7, 9). A large multinational registry analysis showing a decreased in-hospital survival and an increased frequency of ventricular arrhythmias in the hydroxychloroquine/chloroquine group (11).	There is insufficient evidence to issue a recommendation on the use of chloroquine or hydroxychloroquine in critically ill adults with COVID-19.	Hydroxychloroquine/chloroquine (or hydroxychloroquine/chloroquine plus azithromycin) are recommended in the context of a clinical trial.	>200
Lopinavir/ritonavir *	Antiretroviral protease inhibitor 3-chymotrypsin-like protease (used for the treatment of HIV infection).	Negative results in one RCT (199 patients with COVID-19) could be due to delayed administration (3). Insufficient evidence, frequent side effects.	The use of lopinavir/ritonavir is not recommended.	The combination of lopinavir/ritonavir is recommended only in the context of a clinical trial.	>50
Remdesivir *	Premature termination of viral RNA transcription.	In a compassionate-use protocol 53 patients were treated. After a median follow-up of 18 days 68% of the patients improved with 13% mortality (10).	No recommendations	The drug should be used in the context of ongoing trials with limited availability for compassionate use and expanded access use.	12
Favipiravir *	Selective inhibition of viral RNA-dependent RNA polymerase	An anti-influenza medication approved in Japan that showed faster resolution of fever and cough but similar rates of respiratory failure compared to the control (umifenovir) (5).	No recommendations	No recommendations	11
Antibodies and immunomodulation					
Tocilizumab	Humanized immunoglobulin that blocks IL-6 receptor.	Approved for cytokines release syndrome, rheumatoid arthritis and juvenile idiopathic arthritis. Trials are ongoing to test the safety and efficacy of this therapy in	There is insufficient evidence to issue a recommendation on the use of tocilizumab in critically ill adults with COVID-19.	Tocilizumab is only recommended in the context of a clinical trial.	30

		COVID-19 (45).			
Convalescent plasma or hyperimmune immunoglobulins	Antibodies from recovered patients against both free virus and infected cell immune clearance.	To date only two studies with very small series of patients are currently available (4, 14).	The routine use of convalescent plasma in critically ill adults with COVID-19 is not recommended.	Undergoing evaluation – no recommendations.	65
Corticosteroids	To decrease the host inflammatory responses in the lungs, underlying cause of acute lung injury and ARDS.	A small uncontrolled retrospective study reported that methylprednisolone treatment was associated with a decreased risk of death in patients who developed ARDS (15).	The routine use of systemic corticosteroids in mechanically ventilated adults with COVID-19 and respiratory failure (without ARDS) is not recommended.	The use of corticosteroids in in-hospital patients with COVID-19 pneumonia is not recommended. Corticosteroids may be used in patients with ARDS due to COVID-19 in the context of a clinical trial.	23

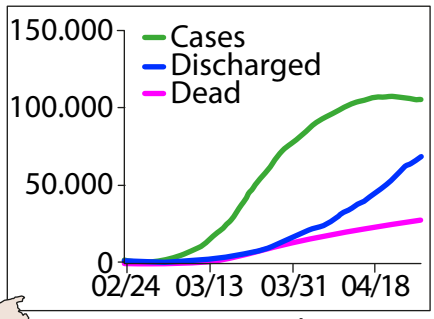
The asterisk indicates uncertain efficacy or negative trials. Abbreviations: ACE2, Angiotensin Converting Enzyme 2; IDSA, Infectious Diseases Society of America; HIV, human immunodeficiency virus; IL, Interleukin; TMPRSS2, Transmembrane Serine Protease 2; RCT, Randomized Controlled Trial; RNA, Ribonucleic Acid; SSG, Surviving Sepsis Guidelines.







Regions	Cases
Lombardy	73.348
Piedmont	25.450
Emilia Romagna	24.914
Veneto	17.708
Tuscany	9.231
Calabria	1.097



April 29, 2020