Natural history and therapeutic options for COVID-19

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Natural history of COVID-19 and therapeutic options

Abstract

Introduction

COVID-19 presents benign forms in young patients who frequently present with anosmia. Infants are rarely infected, while severe forms occur in patients over 65 years of age with comorbidities, including hypertension and diabetes. Lymphopenia, eosinopenia, thrombopenia, increased lactate dehydrogenase, troponin, C-reactive protein, D-dimers, and low zinc levels are associated with severity.

Areas covered

The authors review the literature and provide an overview of the current state of knowledge regarding the natural history of and therapeutic options for COVID-19.

Expert opinion

Diagnosis should rely on PCR and not on clinical presumption. Because of discrepancies between clinical symptoms, oxygen saturation or radiological signs on CT scans, pulse oximetry and radiological investigation should be systematic. The disease evolves in successive phases: an acute virological phase, and, in some patients, a cytokine storm phase; an uncontrolled coagulopathy; and an acute respiratory distress syndrome. Therapeutic options include antivirals, oxygen therapy, immunomodulators, anticoagulants and prolonged mechanical treatment. Early diagnosis, care, and implementation of an antiviral treatment; the use of immunomodulators at a later stage; and the quality of intensive care are critical regarding mortality rates. The higher mortality observed in Western countries remains unexplained. Pulmonary fibrosis may occur in some patients. Its future is unpredictable.

Keywords: SARS-CoV-2, COVID, Humans, Pathophysiology, Treatment, Hydroxychloroquine, Azithromycin, Tocilizumab, Remdesivir, Care

Highlights

- Diagnosis of COVID-19 should rely on PCR and not on clinical presumption.
- Because of "happy hypoxia", pulse oximetry and radiological investigation should be systematic.
- The disease evolves in successive phases: an acute virological phase, and, in some patients, a cytokine storm phase; an uncontrolled coagulopathy; and an acute respiratory distress syndrome.
- Massive screening allowing early diagnosis, care (oxygen, anticoagulants), and implementation of an antiviral treatment; the use of immunomodulators at a later stage; and the quality of intensive care are critical regarding mortality rates.
- Intravenous catheterization should be avoided, and early oral treatment in an outpatient basis should be preferred.
- Treatment with an oral combination of hydroxychloroquine, azithromycin and zinc may represent the best current therapeutic option in relation to its antiviral and immunomodulatory effects.
- Preventive anticoagulants should be prescribed in patients with coagulopathy (positive D-dimers).

1. Introduction

COVID-19 has a pleomorphic clinical presentation including asymptomatic individuals and patients with mild to severe involvement with several evolutionary stages [1-3]. Age and comorbidities including, notably, hypertension, diabetes and coronary heart disease are the main risk factors for evolving toward severe infections [1-3]. Schematically, after the incubation period, two main clinical presentations can occur: upper respiratory tract infections (URTIs) with severe headaches, anosmia, ageusia (or dysgeusia) and rhinitis, which are mainly observed in young patients who then have a good clinical outcome; and lower respiratory tract infections (LRTIs) with pneumonia symptoms that are observed more frequently in patients with comorbidities and can be severe to fatal in older patients [1-3]. At admission, prognosis can be assessed through the National Early Warning Score (NEWS-2), a simple aggregate scoring system including respiration rate, oxygen saturation, systolic blood pressure, pulse rate, level of consciousness or new confusion, and temperature. Age has been added in a modified version of this score [4].

During the onset of the COVID-19 outbreak, olfactory and gustative disorders, including anosmia and ageusia were described in infected patients [5]. In Marseille, 3,497 adults who underwent PCR between 24 March and 25 April 2020 were asked the following question prior to being tested for SARS-CoV-2: “Have you lost your sense of smell or taste in the past two months?” The prevalence of the loss of smell and/or taste in COVID-19 patients was 356/673 (53%), and the positive predictive value (PPV) for the diagnosis of COVID-19 by PCR was 67% when smell and taste disorders were reported (submitted). Asking patients and healthcare workers (HCWs) about loss of smell and taste could be useful in areas where testing for SARS-CoV-2 is politically or technically limited or impossible. Interestingly, “happy hypoxemia”, a hypoxia observed in patients who are SARS-CoV-2 positive yet comfortable and without dyspnea emphasizes the need to perform a low-dose CT-scan on

most patients to detect pneumonia at an early stage [6]. Most COVID patients are definitively cured, but extreme caution is needed in patients with comorbidities and/or biological parameter abnormalities such as lymphopenia, eosinopenia, increased D-dimers, troponin, lactate dehydrogenase (LDH) or C-reactive protein (CRP) [1, 3]. Venous thromboembolism is relatively common [7], is mainly characterized by pulmonary embolism, and is found in up to one-third of critical cases [8]. Acute respiratory distress syndrome (ARDS), pulmonary embolism and bacterial superinfection may result in a fatal evolution [9, 10]. Finally, delayed pulmonary fibrosis may occur in an as yet unknown proportion of patients [11].

At the beginning of the health crisis, the use of chest X-rays was restricted to patients in intensive care units due to its low value in detecting ground-glass opacities. However, low-dose chest computed tomography (LDCT) appears to be a useful tool in the management of patients with regards to diagnosing, assessing and quantifying disease severity and for differential diagnosis. LDCT might be of interest in predicting lung fibrosis during healing [12-15]. The main findings of COVID-19 pneumonia on chest CT include ground-glass opacities, consolidation, and a crazy-paving pattern. These features are not specific, but the distribution of lesions during COVID-19 pneumonia is more likely to be peripheral, asymmetric and located in the lower lobes [16]. CT features revealed a good sensitivity and specificity for COVID-19 diseases in centers where CT was used as a diagnostic tool [17]. Furthermore, Li *et al.* developed a deep learning algorithm able to discriminate COVID-19 pneumonia from community-acquired pneumonia, with good results (**Figure 1**) [18]. COVID-19 pneumonia is also characterized by the high prevalence of lung involvement in paucisymptomatic patients. In our center, we decided to perform LDCT on all patients with a positive PCR for COVID-19. Of the 2,065 LDCTs that were performed on COVID-19 patients, more than 70% revealed pneumonia. Of the 1,043 patients with a NEWS-2 score=0 who underwent LDCT, 628 (60.2%) had radiological abnormalities, including 494 (47.4%)

with minimal lung lesions, 118 (11.8%) with intermediate lesions and 11 (1%) with severe lesions. Moreover, of the 1,370 LDCTs performed on patients without perceived dyspnea, 937 (68%) had pneumonia [3].

Symptoms at the time of diagnosis of COVID-19 pneumonia do not appear to be related to prognosis [1]. A meta-analysis of 1,558 patients found that significant risk factors for mortality in COVID-19 were hypertension, diabetes, chronic obstructive pulmonary disease, cardiovascular disease, and cerebrovascular disease [19, 20]. A study on 1,591 patients in the intensive care unit showed that the mortality rate was higher in patients over the age of 64 than in younger patients [20]. Furthermore, we showed that the percentage of lung involvement quantified using the deep learning algorithm is an independent prognostic marker, and the addition of lesion quantification significantly enhances the prediction model based on comorbidities and NEWS-2 score (unpublished).

Reports of an increased incidence of acute pulmonary embolisms or intravascular coagulopathy associated with COVID-19 have emerged in the literature [21]. The prevalence of pulmonary embolism in COVID-19 has been reported to be approximately 20% in patients with severe disease. Leonard-Lorant *et al.* found that a D-dimer threshold higher than 2,660 µg/L could detect all patients with a pulmonary embolus on chest CT after contrast injection [8].

COVID-19 might lead to sequelae such as lung fibrosis during the healing phase [22]. The real prevalence and clinical impact of COVID-19 sequelae on the lungs, as well as on the myocardium, requires further study.

Literature search methodology

A literature search was performed using the following keywords: SARS-CoV-2, COVID, coronavirus, pathophysiology, natural history, treatment, and humans without restriction of

the date or language. Medline, Google, and Google Scholar were used alongside crossreferencing.

2. SARS-CoV-2 epidemiology

The first COVID-19 cases were identified in late December 2019 in Wuhan, China, and the disease turned into a pandemic within a few weeks. As of 21 July 2020, more than fourteen million cases have been reported globally, with more than 600,000 deaths. (https://gisanddata.maps.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6). The SARS-CoV-2 epidemic exhibits a bell-shaped incidence curve [23, 24] (https://coronavirus.jhu.edu/data/new-cases; https://www.mediterranee-infection.com/covid-19/; **Figure 2**), which is a typical epidemic curve. In Western countries in the Northern hemisphere, the outbreak decreased dramatically during the spring, as is the case for epidemics arising from other respiratory viruses. Moreover, it affected people differently according to their age. Very few cases and less severe outcomes were observed in children [25-31], while SARS-CoV-2 infections have been more frequent and severe in the elderly. For instance, a large study conducted in Iceland on the general population found that children under the age of 10 years were half as likely to be diagnosed with SARS-CoV-2 than children over the age of 10 years or adults [31]. In addition, targeted screening did not diagnose infections in children under the age of 10 years. This predominance of cases among adults differs from the age distributions observed with other respiratory viruses, including endemic coronaviruses [32]. Interestingly, several studies have detected immune responses to SARS-CoV-2 in unexposed individuals. Thus, Grifoni *et al.* detected circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in ≈20-60% of SARS-CoV-2-unexposed individuals sampled between 2015 and 2018 [33], and 10% of uninfected pregnant women exhibited IgG to SARS-CoV-2 [34]. Conversely, increased IgG reactivity to endemic coronaviruses was reported in SARS-CoV-2 infections, further suggesting crossimmunity [35]. As the four

coronaviruses 229E, NL63, OC43 and HKU1 circulate endemically worldwide and massively infect humans during the first years of life [32], exposure to these viruses may have conferred crossimmunity to SARS-CoV-2 in a preferential way to children.

3. Natural history of the disease (Figure 3)

3.1 Transmission route

The main transmission route for SARS-CoV-2 is human-to-human transmission via respiratory droplets and skin contact, with incubation times of 2–14 days (mean incubation time of approximately 6 days) [36, 37]. In addition to its presence in nasopharyngeal swabs, bronchoalveolar lavage, and sputum, SARS-CoV-2 has been detected using molecular tools in saliva, stools, urine, blood, tears, and conjunctival secretions [36, 38-40]. Live viruses have been detected in feces, including nondiarrheal feces, implying that SARS-CoV-2 could be transmitted through feces [36]. Its molecular detection around the toilet (doorknob, surface of the toilet bowl, internal sink bowl) in the room of a patient who did not have diarrhea but who did have positive stool samples for SARS-CoV-2, supports the hypothesis that fecal viral shedding may be involved in transmission [41]. Live viruses have also been observed in saliva [38]. Since saliva can be released through coughing and the influenza virus can be present in respiratory droplets even during normal breathing, SARS-CoV-2 could be transmitted by saliva directly or indirectly, even in patients without a cough or other respiratory symptoms [38]. It has already been reported that asymptomatic carriers can spread SARS-CoV-2 [42]. Although SARS-CoV-2 has been detected in the tears and conjunctival secretions of COVID-19 patients with conjunctivitis, its transmission through the conjunctival route is debated [39].

SARS-CoV-2 can survive outside the body for long periods of time; it can remain viable in aerosols for up to three hours [43]. Viable SARS-CoV-2 was detected up to 72 hours after experimental application to plastic and stainless steel. On copper and cardboard, no viable SARS-CoV-2 was measured after four hours and 24 hours, respectively. Thus, transmission of

SARS-CoV-2 by aerosols and fomites is likely. Investigations of a COVID-19 cluster in a shopping mall support the hypothesis that the rapid spread of SARS-CoV-2 could result from spread via fomites (elevator buttons, bathroom taps) or virus aerosolization in a confined public spaces (bathrooms, elevators) [44].

Nosocomial transmission including outbreaks of SARS-CoV-2 have occurred. Although direct transmission is the most common route of transmission, contaminated surfaces that are frequently contacted in healthcare facilities are a potential source. An extensive environmental study was performed in an intensive care unit (ICU) and general ward (GW) at Wuhan Huoshenshan Hospital [45]. Nucleic acids from SARS-CoV-2 were observed on surfaces that were frequently touched, such as computer mice, rubbish bins, and handrails on patients' beds, but were also found on floors and were sporadically observed on doorknobs and on the sleeve cuffs and gloves of medical staff. Nucleic acids were also detected in the air from a patient's room, with an estimate that the transmission distance of SARS-CoV-2 could be around four meters in a GW. However, since the detection of nucleic acids does not indicate the amount of viable virus and the minimum infectious dose is unknown, the distance of aerosol transmission cannot be strictly determined. These data imply a risk of infection for medical personnel and other close contacts. However, appropriate precautions and adherence to hand and environmental hygiene can effectively prevent infection since no member of the hospital staff was infected with SARS-CoV-2 as of 30 March 2020 [45].

3.2 Cellular life cycle of SARS-CoV-2 (Figure 4)

The SARS-CoV-2 is an enveloped RNA surrounded by spike glycoproteins [46-49]. The virus enters cells through membrane fusion. The first step of the SARS-CoV-2 replicative cycle is the attachment of the virus to the angiotensin-converting enzyme 2 (ACE2) glycoprotein. The receptor-binding domain (RBD) amino acid sequences present in the S1 spike protein interact with the N-terminal region 30-41 and 82-93 of ACE2 that contains

several sites for N-glycosylation. A cell surface protease, TMPRSS2, is responsible for spike cleavage allowing the appropriate conformation for the S2 spike to expose the hidden fusion peptide for insertion into the cellular membrane lipid bilayers. The viral nucleocapsid is thus delivered into the cytoplasm through the endocytic vesicle. After acidification of the late endosome, the action of cathepsin enables the uncoating of the genomic RNA and the enzymes necessary for its replication. The genomic RNA is used as a template by the replicase to synthesize the negative sense genomic RNAs (anti-genome), which are used as templates to synthesize the progeny positive sense genomes and subgenomic RNAs. Similarly to SARS-CoV, the 5'-proximal two-thirds of the SARS-CoV-2 viral genome are translated into polyproteins that give rise to several nonstructural proteins (Nsps) following autoproteolytic processing. Among the Nsps, Nsp12 is an RNA-dependent RNA polymerase, Nsp3 and Nsp5 are proteinases, Nsp13 is a helicase, Nsp14 and Nsp15 are ribonucleases, and Nsp14 is a methyltransferase (involved in RNA cap formation). The 3'-proximal third sequence serves as template for several subgenomic mRNAs that encode the viral structural (the spike/S, the envelope/E, the membrane/M, and the nucleocapsid/N) and accessory proteins. The S, E, and M proteins are synthesized and anchored on the endoplasmic reticulum (ER) with the N protein translated in the cytosol. Posttranslational modifications of viral proteins occur within the endoplasmic reticulum and trans-Golgi network vesicles. After assembly in the ER-Golgi intermediate compartment (ERGIC), where the E protein plays an essential role in virus assembly and the mature M protein shapes the virus, mature virions are released from smooth-walled vesicles by exocytosis.

3.3 Immune response

Immune responses shape the clinical course of COVID-19. The hallmark of the disease is the occurrence, in 10–20% of patients, of a sudden deterioration 7–10 days after the onset of symptoms, increasing the risk of acute respiratory failure, organ support and ultimately a

fatal outcome [27]. Early publications described lymphopenia mostly affecting T and B cells, neutrophilia, and decreased eosinophil and monocyte counts [10, 27]. The degree of eosinopenia, lymphopenia and neutrophilia, the latter sometimes expressed as the neutrophil-to-lymphocyte ratio, was associated with and was predictive of clinical severity. In severe cases, deficient induction of type I interferons at the initial stage, increased levels of some but not all pro-inflammatory cytokines, most notably interleukin (IL)-6, increased levels of the regulatory cytokine IL-10, an autoimmune signature, and an inconsistent specific antibody response to SARS-CoV-2 were reported [10, 27, 50-52]. Tissue investigations showed that SARS-CoV-2 pulmonary tropism targeted alveolar cells. Severe pulmonary lesions were associated with interstitial mononuclear infiltrates dominated by lymphocytes, $CD4^+$ and $CD8^+$ T-cells, pulmonary edema, hyaline formation and pneumocyte desquamation without histopathological evidence of sequestrated eosinophils [53]. Resident or recruited immunosuppressive and inflammatory monocytes and macrophages ($CD14^+HLA\text{-}DR^{lo/neg}$) tend to replace protective resident alveolar macrophages. Severe respiratory failure may arise through two distinct pathways: either an atypical macrophage activation syndrome, or an immune dysregulation status characterized by impaired monocyte activation and antigen presentation [54]. The systemic passage of high levels of cytokines produced in the lungs may contribute to a multivisceral failure syndrome [10]. Neutrophilia is associated with the increased formation of extracellular traps (NETosis), contributing to inflammation, cytokine dysregulation and autoimmune and thrombotic manifestations [55, 55]. SARS-CoV-2 was reported to infect T lymphocytes and macrophages, resulting in impaired antigen presentation, increased IL-6 production, and possibly in lymphocyte apoptosis in lymphoid organs [54, 56]. Th1 polarization is involved in the efficient control of SARS-CoV-1 but not SARS-CoV-2 infection [57]. Crossreactive T-cell recognition between circulating seasonal human coronavirus (HCoVs), the SARS-CoV and SARS-CoV-2 and crossreactive antibodies

between SARS-CoV-2 and SARS-CoV have been reported [58-60]. Interestingly, $CD4^+$ T cells from COVID-19 patients targeted the N and C terminal regions of the S protein equally, whereas $CD4^+$ T cells from noninfected patients only targeted the C terminal region, which is highly homologous with the seasonal HCoVs S protein [59]. Recently, Grifoni *et al.*, used HLA class I and II and predicted peptide megapools detected circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells in 100% and ≈70% of patients with resolved COVID-19, respectively [33]. Interestingly, such cells were also detected in ≈20–60% of SARS-CoV-2-unexposed individuals collected between 2015 and 2018. It should also be noted that all 20 SARS-CoV-2-unexposed individuals exhibited IgG to HCoV-OC43 and HCoV-NL63. Another study showed the presence of anti-SARS-CoV-2 humoral responses elicited by patients previously infected with endemic coronaviruses [34].

It is thus hypothesized that past infection with HCoVs causing upper respiratory infection may confer SARS-CoV-2 cellular immunity as the result of $CD4^+$ T cellular crossreactivity. Regarding the antibody response, between 47% and 100% of COVID-19 patients seroconvert within fifteen days after the onset of symptoms [61, 62, 62]. Recovery after mild or moderate forms is associated with neutralizing antibodies, whereas patients with severe forms of the disease present early and high levels of non-neutralizing and possibly deleterious antibodies [61, 63-65].

Thrombotic phenomena and lung vessels obstructive thrombo-inflammatory syndrome play a role in severe and critical COVID-19 presentations. Similar to SARS-CoV-1, an autoimmune signature including antiphospholipid antibodies, e.g., anticardiolipin IgA, anti-β2 glycoprotein 1 IgA, IgG and IgM, is present in SARS-CoV-2 infected patients [65]. It is assumed that autoimmune markers are associated with the occurrence of thromboembolic phenomena, but direct evidence is lacking. Lupus anticoagulant has also been reported as a

possibly frequent finding in up to 25 of 56 patients (45%), but again, the causative or predictive relation to thrombotic events during COVID has not been assessed [50].

To summarize, our current understanding of immune responses to SARS-CoV-2 is that of an early choice between two distinct pathways. An efficient monocyte-macrophage, $CD4^+$ and $CD8^+$ T cellular response accompanied by a controlled inflammatory response enables virus control and swift recovery. Conversely, a status of SARS-CoV-2-induced immune dysregulation associated with low levels of type I interferons, an IL-6-driven inflammatory status with immunosuppressive and inflammatory monocytes and macrophages, a defective antigen presentation, an extensive organ immunopathogenesis and a prominent anti-SARS-CoV-2 and autoimmune antibody response is associated with severe forms of the disease. Investigation of the SARS-CoV-2 immune response has already given insights into multiple immune modulating therapies, from repurposed molecules (antimalarials, chlorpromazine, antibiotics) to antivirals to monoclonal antibodies directed at cytokines (anti-IL-6, anti-IL-1) or innate pathways (C5/C5a). Whether the crossreactivity of $CD4^+$ T-cell lymphocytes epitopes and antibodies with seasonal HCoVs can protect against SARS-CoV-2 and SARS-CoV needs to be further explored.

3.4 Immunopathogenic phase: the cytokine storm

On approximately the tenth day of infection, COVID-19-associated pneumonia may evolve toward acute respiratory failure due to ARDS requiring ICU admission and high-flow oxygen or mechanical ventilation, with a severe prognosis [2]. The underlying mechanisms of these complications are immunological rather than due to the virus itself, which in most cases, is no longer detectable at this stage.

Persistence of viral RNA detection in respiratory specimens has been reported even three weeks after disease onset [66] and in 10-20% of patients at day 10 [3]. However, disease in some patients became aggravated while the virus was no longer detectable using

conventional detection methods. It cannot be ruled out that, although not detected, the virus was still present in cellular reservoirs not accessible to detection and can continue to activate a pro-inflammatory response. However, it is likely that the virus is at the origin of the triggering of a pro-inflammatory reaction that then self-amplifies.

Elevated circulating inflammatory cytokine concentrations have been reported in patients, notably interleukin (IL)-1β, IL-6, MCP-1, IP-10, MIP-1α, IL-2, IL-10, revealing a so-called "cytokine storm", described in other inflammatory diseases such as macrophage activation syndrome (MAS) or systemic inflammatory response syndrome [67, 68]. Strikingly, and consistently with immunopathophysiology, IL-6 levels are a near perfect predictor of subsequent acute respiratory failure [69].

In vitro experiments and animal models of other SARS-CoV infections have shown that the virus induces alveolar epithelial cell necrosis, the release of viral particles and of cytoplasmic proinflammatory danger-associated molecular patterns (DAMPS), including ATP, nucleic acids, or IL-1α [70]. In the meantime, SARS-CoV inhibits or delays IFNα/β production by alveolar epithelial cells, which normally constitute the initial anti-viral defense [71]. Alternatively, SARS-CoV induces a robust but delayed IFNα/β response by the plasmacytoid dendritic cells and macrophages, which, together with DAMPS, trigger chemokine production and the recruitment of inflammatory monocytes/macrophages into the lungs [71]. In inflammatory monocytes/macrophages, the virus induces NF-κB activation and the transcription of several pro-inflammatory cytokines, notably IL-1β and IL-18 precursors, and the constituents of the NLRP3 (for NOD-like receptor family, pyrin domain containing 3) inflammasome [72, 73]. NLRP3 assembles in the cytoplasm after a second danger cell signal, consisting in K+ efflux, ATP, lysosome degradation or production of mitochondrial reactive oxygen species (ROS) [74]. Once assembled, NLRP3 activates caspase 1 and the processing of biologically inactive IL-1β and IL-18 precursors into biologically active cytokines [74].

Importantly, SARS-CoV behaves as a membrane K+ channel and thus directly activates NLRP3 assembly [73, 75]. In SARS-CoV animal models, the excessive NLRP3 activation and IL-1β or IL-18 production induce a cytokine storm, ARDS and death, whereas blocking NLRP3, the IL-1 receptor type 1 or IL-18 are protective [72]. IL-1β is a major pro-inflammatory cytokine known to induce fever via prostaglandin E2 secretion, neutrophilia via GCSF production, liver acute-phase protein synthesis and a Th-17 immune response through IL-6 secretion [76]. IL-18 is known to induce IFNγ production by Th-1 lymphocytes and NK cells [77]. By binding to its receptor on inflammatory monocytes/macrophages, IL-1β induces a harmful cytokine loop consisting of excess NLRP3, IL-1β/IL-6, IL-18/IFNγ synthesis, initiating the cytokine storm [57]. NLRP3 also induces pyroptosis, a programmed cell death process leading to the uncontrolled release of IL-1β, IL-18 and SARS-CoV2 particles [74].

At this stage of COVID-19, patients usually present with high-grade fever (>38.5°C), moderate increased neutrophils, and elevated CRP concentrations (>150 mg/L), which are the hallmarks of an IL-1/IL-6 signature [78]. Later, hyperferritinemia, diffuse coagulopathy and cytopenia may appear, constituting the hallmarks of an IL-18/IFNγ signature [78]. Thus, the absence of or delayed primary immune defenses against SARS-CoV2 encourages the persistence of the virus, which in some individuals stimulates an uncontrolled self-stimulating inflammatory loop and a cytokine storm that are initially located in the lungs but may diffuse systemically and induce multi-organ failure.

3.5 Coagulopathy and thrombosis

Evidence of abnormal coagulation parameters associated with COVID-19 appeared in early reports from China [1]. The most common hemostatic abnormalities observed are mild thrombocytopenia and increased fibrinogen and D-dimers. Elevated D-dimers upon admission are associated with increased mortality [79, 80].

Although the SARS-CoV-2 virus does not appear to have intrinsic procoagulant effects, the

significant increase in cytokines, also known as a cytokine storm, induces the activation of coagulation and thrombin generation leading a state of major hypercoagulability. Initial pulmonary findings during autopsy showed the presence of diffuse alveolar lesions with infiltration of macrophages and $CD4^+$ T lymphocytes around thrombosed small vessels with significant hemorrhages. The formation of microthrombi in the pulmonary microvessels appears to be involved in the pathogenesis of the respiratory picture observed in patients with SARS-CoV-2 infection [9]. This form of thrombosis involving the immune cells is referred to as immunothrombosis [81]. In addition, hypoxia induced by respiratory impairment may cause thrombosis not only by increasing blood viscosity but also by increasing hypoxia-inducible transcription factors [82]. Moreover, the tropism of the virus for ACE2 receptors could induce the activation of endothelial cells, disruption of their natural antithrombotic properties, and apoptosis. Endothelialopathy may also contribute to the pathophysiology of microcirculatory thrombosis [83]. Based on the literature currently available, the coagulopathy and vasculopathy mechanisms are uncertain. Furthermore, patients with severe COVID-19 present a hypercoagulability that predisposes them to thrombotic events. This condition explains the presence of high levels of D-dimer. Despite coagulopathy, bleeding manifestations have not been described. In later stages of COVID-19, sepsis-induced coagulopathy and disseminated intravascular coagulation have been reported [83].

Many reports describe a high risk of venous thromboembolism. Notably, a high frequency of pulmonary embolism (20 from 27%) was reported in ICU patients while patients were receiving a standard dose of venous thromboembolism (VTE) prophylaxis [84, 85]. However, a discrepancy between pulmonary embolism and deep venous thrombosis has been observed. In view of these data, the use of anticoagulants could reduce the vicious circle of inflammation-coagulation observed in patients with a severe form of the infection [86]. Moreover, a protective effect of heparin in patients with most severe COVID-19 infections

and increased D-dimers has recently been reported [87].

4. Therapeutic options (Tables 1 & 2)

Therapeutic options include antiviral and nonantiviral molecules. Potential antiviral drugs according to *in vitro* results and chemical structure are detailed in Table 1. Three chemical classes are particularly represented: 4-aminoquinolines, phenothiazines, which are chemically related to methylene blue, and ribonucleic analogues. Zinc is of particular interest because it is a nontoxic micronutrient with direct antiviral activity on RNA-dependent RNA polymerase of *Nidovirales* with demonstrated activity *in vitro* and in a clinical study with the synergistic association combining hydroxychloroquine (HCQ) and azithromycin (AZ). Interferon has been administered in several Chinese studies, but its efficacy remains unclear. Nonantiviral molecules are mainly represented by immunomodulators with corticosteroids, anti-interleukin 6, and hydroxychloroquine since the latter molecule has both antiviral and anti-inflammatory effects. Increasing evidence stresses the critical importance of early anticoagulants in patients with coagulopathy (positive D-dimers), and this is further supported by the fact that lung embolism may be the direct cause of death in up to 30% of cases at autopsy [9].

4.1 Drugs active against SARS-CoV-2

Testing for molecules that are potentially active against SARS-CoV2 has been based on different approaches. These included the selective testing of molecules previously shown to be active against SARS-CoV and/or MERS-CoV or suspected to have a broad enough range of activity to merit testing on SARS-CoV-2; high throughput testing of drug libraries; and *in silico* prediction followed by confirmation of drug activity *in vitro.* Based on previous studies on SARS-CoV, chloroquine (CQ) and hydroxychloroquine were among the earliest tested molecules against SARS-CoV-2 [88-91]. Both compounds were shown to be efficient but HCQ exhibited a less toxic profile [90]. Of 20 drugs previously demonstrated to have *in vitro* antiviral activity against SARS-CoV and MERS-CoV, several were found to be effective *in*

vitro on SARS-CoV-2, including antitumoral drugs, which are likely not to be easily applicable to treatment in humans, and antimalarial drugs such as amodiaquine and, again, CQ and HCQ [91]. Of the tested drugs that are suspected to display a broad enough range of activity to be active on SARS-CoV-2, there were two antimicrobial agents, ivermectin and teicoplanin [92, 93] and the antiviral remdesivir [89] (Table 1). In another study using a more systematic approach, testing of anti-SARS-CoV-2 activity in 1,520 approved drugs [94], identified 90 molecules with potential efficacy. Of these, opipramol, quinidine and omeprazole showed significant activity, and this was again the case for CQ and HCQ. From the antibacterial agents, these authors also identified several fluoroquinolones and the macrolide azithromycin (AZ). Interestingly, in a unique study associating AZ with HCQ (both at 5 µM), a relative viral inhibition of 99% was observed, suggesting a synergistic effect [95]. In another study, the authors cloned, tagged and expressed 26 of 29 SARS-CoV-2 proteins and studied their interactions with human proteins as inhibitors of these interactions [96]. Of the 69 suggested compounds, 29 FDA-approved drugs were identified as being able to inhibit SARS-CoV-2 replication *in vitro*, including HCQ. In conclusion, many drugs have been identified as efficient, including many FDA-approved and well known drugs that have been in use for decades. However, it would be illusory to imagine using psychotropic, antitumoral or anabolic steroids that were identified in high throughput screenings. Therefore, drugs such as CQ, HCQ, antibiotics or antihistamines used for allergies, and proton pumps inhibitors used to treat gastroduodenal ulcers, alone or in association with other treatments are likely to represent the most promising drugs to be tested in clinical trials against COVID-19 (Table 1). Finally, another approach uses *in silico* structure-based virtual drug screening. This consists of identifying candidate drugs potentially active on SARS-CoV-2, explaining the activity of drugs or trying to explain observed activities [97, 98]. This approach does not require, as is the case of *in vitro* testing of antiviral activity, a cell culture platform, viral strains, or a P3-

security level laboratory. It only predicts interactions and the inhibition of viral replication using bioinformatic approaches based on structures, biochemical interactions, structural and molecular modeling analyses, *in silico* docking models, or protein-protein interaction networks. For instance, 69 compounds including HCQ were identified as targeting 66 druggable human proteins or host factors [96]. Compounds were also described as interacting with the viral S-protein and angiotensin-converting enzyme 2 (ACE2)-host cell receptor [99] while three viral polymerase inhibitors, zidovudine, tenofovir and alovudine, were suspected to inhibit SARS-CoV-2 RNA polymerase [100]. In addition, structural and molecular modeling analyses made it possible to propose a mechanism of action of CQ and HCQ on SARS-CoV-2, by inhibiting the binding of the viral S protein to gangliosides, which are present on the host cell surface and are linked with sialic acids that are used by the virus for its entry, in addition to ACE2 [101].

4.2 Antiviral properties of chloroquine

CQ is a synthetic 4-aminoquinoline related to quinine. As far back as the mid-1960s, it was demonstrated that CQ inhibited the mouse hepatitis virus 3 and the encephalomyocarditis virus. Since these pioneering results, a large number of *in vitro* studies have confirmed that CQ and HCQ appear to be large spectrum bioactive agents, which possess antiviral activities against numerous viruses including rabies virus, poliovirus, human immunodeficiency virus, hepatitis viruses, influenza viruses, arboviruses and Ebola virus among others [102]. However, the demonstration of *in vitro* activity cannot anticipate the *in vivo* efficacy of CQ. Although there was evidence of an antiviral effect of CQ/HCQ in humans infected by hepatitis C virus, more disappointing results were reported on other infectious diseases, in particular in the treatment of Chikungunya virus, where CQ seemed to worsen symptoms [103]. With regard to human coronaviruses, CQ was reported to inhibit the *in vitro* replication of HCoV-229E, SARS-CoV, HCoV-O43 coronavirus, and MERS-CoV coronavirus [104].

Recently, it was reported that CQ/HCQ actually inhibited the *in vitro* replication of SARS-CoV-2 [89]. With HCoV-O43 it was reported that lethal infections in newborn mice could be prevented by administering CQ through the mother's milk [105]. However, CQ apparently failed to cure MERS-CoV *in vivo* [106].

CQ is known for its multiple *in vitro* mechanisms of action on viruses. Coronavirus cell entry occurs mainly through the endolysosomal pathway. A recent study reported the antiviral activities of CQ/HCQ against SARS-CoV-2 in Vero E6 cells treated for one hour with the drugs before exposure to the virus [90]. The virus yield in the cell supernatant was quantified by qRT-PCR, while vesicle-containing virion was investigated by confocal microscopy. The authors reported that CQ/HCQ significantly inhibited viral entry. Indeed, CQ/HCQ may possibly inhibit at least five steps of SARS-CoV-2 replication. Recently, the results of a molecular modeling approach suggesting that CQ binds to sialic acids and gangliosides with high affinity were reported [101]. They hypothesized that both CQ and HCQ inhibit the attachment of the amino acid region 111-158 of the viral spike of SARS-CoV-2 to gangliosides. A second mechanism of action of CQ/HCQ on the same step requires the drug to enter target cells to modulate the activity of cellular enzymes. CQ is likely to inhibit the biosynthesis of sialic acid found at the extremity of sugar chains of glycoproteins. The potent effects of CQ observed *in vitro* in cultures of Vero cells exposed to SARS-CoV was considered attributable to a deficit in the glycosylation of cell surface receptor ACE2, which is the first target of the virus [107]. The second step of the SARS-CoV-2 replicative cycle that could possibly be inhibited by CQ is the pH-dependent viral endocytosis. This was previously demonstrated for several viruses. The protonated form of CQ increased the pH of endosomal compartments, thereby blocking the release of the infectious nucleic acid and enzymes necessary for its replication [108, 109]. The third step of the SARS-CoV-2 that could be a target for CQ/HCQ is transcription. Indeed, CQ could modulate the activity of the SARS-

CoV-2 RNA-dependent RNA-polymerase through its function as an ionophore [110] favoring the intracellular transport of the mineral zinc, which inhibits the activity of the polymerase [111, 112]. The fourth step of the SARS-CoV-2 replicative cycle that is likely to be impaired by CQ/HCQ deals with the posttranslational modifications of viral proteins within the endoplasmic reticulum and trans-Golgi network vesicles, possibly by impairing the maturation of its M protein [113]. A fifth level of CQ action could be its effect on cell signaling, in particular through MAPK [114].

Since CQ/HCQ have well-characterized immunomodulating activities, in particular anti-inflammatory properties [115], the use of these drugs in the treatment of COVID-19 was also suggested to possibly protect patients from the cytokine storm that marks the most severe forms of COVID-19 disease.

4.3 Clinical use of chloroquine derivatives in COVID-19 patients (Table 2)

An early observational prospective controlled open label trial was performed in France at the very beginning of the French epidemic and reported a dramatic beneficial effect on viral shedding [116], although no clinical outcomes were investigated. In this context, we recently sought to clarify its clinical efficacy through a meta-analysis of comparative studies conducted on COVID-19 patients [117]. The first findings were that no meta-analysis could be performed due to major discrepancies in the direction of effect. We therefore investigated which moderator variables could explain such significant heterogeneity while *in vitro* studies consistently evidenced an activity of the drug on SARS-CoV-2. Several parameters were tested, including CQ versus HCQ, dosages, duration and timing of treatment, severity of the disease (mild versus severe), combination with an antibiotic (notably AZ), design of studies (prospective or retrospective, multicentric or monocentric, randomized controlled trials, clinical studies or big data analyses conducted on medical records, etc.), comparable groups at baseline, diagnostic approach (PCR, CT scan, clinical), and combination with other antivirals

(notably oseltamivir, lopinavir/ritonavir, ribavirin, umifenovir and alpha-interferon). Strikingly, we observed that the main parameter determining the direction of effect was a study design of big data versus clinical studies, and big data studies were associated with country (USA), a conflict of interest and the absence of a detailed treatment protocol. When analyzing big data separately (data were extracted from electronic record files by analysts who did not treat COVID-19-infected patients) and clinical studies (performed by physicians who treated COVID-19-infected patients), heterogeneity was controlled, and the directions of the effects became consistent in each group. In big data studies, CQ derivatives were associated with either a null [118-121] or a deleterious effect [122]. Strikingly, the latter big data study reporting a deleterious effect in 96,000 electronic medical records was subsequently retracted [123], confirming that clinical studies could be more reliable than big data studies.

In clinical studies, a favorable effect was observed for the duration of fever, duration of cough, clinical cure, death and/or ICU transfer, and viral shedding [117]. In clinical studies, three [124-126] out of four [124-127] randomized controlled trials reported a significant beneficial effect. Overall, CQ derivatives were beneficial and improved survival in COVID-19 infection. However, a standardized therapeutic protocol is required with an adequate dosage (between 400 and 1,000 mg/d). Studies with higher dosages were associated with significant toxicity [128]. In our experience, a dosage of 600 mg/day for 10 days is adequate. Indeed, we previously proposed this dosage in Q fever endocarditis for at least 18 months and in Whipple's disease for 12 months. This has been subsequently recommended by the American CDC [129]. We recently updated this meta-analysis [130] confirming a significant beneficial effect on mortality and viral shedding including two large observational studies from the USA [131, 132] and two from China [133]. Since then, two studies [134, 135], including the RECOVERY trial [134], have been published with a diagnosis not confirmed by

PCR, which does not allow for a conclusion to be drawn. In the RECOVERY trial, cases could be "clinically suspected" without laboratory confirmation (approximately 10% negative tests and 10% without any testing), and 2,400 mg of hydroxychloroquine was administered during the first 24 hours, which corresponds to a toxic dose. Here, we reported a last update of our meta-analysis after the inclusion of three very recent studies from Brazil (RCT) [136], Spain [137] and Taiwan [138] and focused on mortality (Figure 5) and persistent viral shedding (Figure 6). Notably, Cavalcanti *et al*. reported a better effect with the HCQ-AZ combination than with HCQ alone, demonstrating the importance of the synergy demonstrated *in vitro* [136]. Despite substantial heterogeneity, a significant beneficial effect could be confirmed for both mortality and viral shedding.

However, debate is currently rife in the scientific community and among government decision makers as to the risk-benefit of using HCQ in the treatment of COVID-19 patients. Recently, the WHO decided to ban the use of HCQ for COVID-19 patients, although various clinical trials suggest that the benefits of using this molecule in combination with azithromycin outweigh any harmful effects [126].

4.4 Azithromycin and COVID-19

AZ is a well-known and safe macrolide antibiotic with immunomodulatory properties. It has a long half-life and a large volume of distribution [139]. It has excellent tissue penetration in the lung and is widely prescribed for the treatment of respiratory infections. Its mechanism of immunomodulation includes decreased production of pro-inflammatory cytokines (IL-6, IL-8 and TNFα) and inhibition of neutrophil activation [140]. Although AZ has not been labeled for the treatment of antiviral infections, it has been studied *in vitro* and in clinical trials for activity against several viruses [139]. Numerous investigations have reported the *in vitro* antiviral activity of AZ against viral pathogens, including SARS-Cov-2, at concentrations that are physiologically achievable with the usual doses used for the treatment

of bacterial respiratory infections [139]. The precise mechanism of antiviral activity is not known [139]. However, the intracellular accumulation of AZ, a weak base in endosomal vesicles and lysosomes intracellularly may result in an increase in endosomal and/or lysosomal pH and limit viral replication, through the lack of an optimal acidic environment in the intracellular milieu. Interestingly, HCQ is also a weak base, and this could explain how the two drugs work together to inhibit viral replication. Recently, our group demonstrated that the combination of HCQ and AZ had a synergistic effect *in vitro* on SARS-CoV-2 at concentrations compatible with that obtained in the human lung [95].

Many clinical studies on the efficacy of AZ alone or in combination with other drugs against various viral infections have been observational, single-arm, non-randomized studies or retrospective evaluations and have mainly focused on viral load as an end point. Collectively, however, they present preliminary evidence that the inclusion of AZ in various treatment regimens can influence the course of viral infection and clinical outcomes [139, 141, 142].

4.5 Clinical use of zinc in COVID-19 patients

Zinc has both antiviral and immune properties [143]. Known to inhibit the multiplication of several viruses, *in vitro* models have demonstrated that low zinc concentrations combined with ionophores block the elongation of the SARS-CoV-1 RNA-dependent RNA polymerase. [112]. Interestingly HCQ and CQ are ionophores that enhance zinc uptake, thereby increasing its concentration into the lysosomes [110]. The potential synergistic effect of a combination with CQ/HCQ on SARS-CoV-2 replication remains, however, to be demonstrated. Zinc is also involved in antiviral immunity through several mechanisms including the modulation of interferon response [143]. As zinc supplementation over a short period is not harmful to health, it has been proposed in combination with HCQ in COVID-19 patients, with promising results [144].

4.6 Clinical uses of remdesivir on COVID-19 patients

To date, eight studies (1,773 patients) have reported the use of remdesivir in COVID-19 patients. Case reports showed that remdesivir has no clinically relevant efficacy. A compassionate uncontrolled study supported and funded by Gilead reported that 68% of treated patients saw clinical improvements, but there was considerable missing data, and the outcomes of 9/61 patients were still under evaluation at the time of publication and were not reported [145]. One randomized controlled study (RCT) included 236 patients (158/78) from 10 hospitals in Wuhan. The mean age, sex ratio, delay from onset to enrolment, comorbidities, enrolment criteria (O2<95%), and radiologically confirmed pneumonia, were comparable in the two arms. The primary clinical endpoint was the time to clinical improvement within 28 days after randomization, and 100% of patients enrolled were evaluated in the intention-to-treat analysis. No significant differences were noted between the two groups. Serious adverse events or events leading to administration of the drug being stopped were reported in 18% and 12% of patients, respectively, in the remdesivir group compared to 6% and 5%, respectively, in the placebo group, demonstrating the poor safety profile of the drug [146]. Another RCT conducted on 1,063 patients (541/522) argued for a significant benefit of remdesivir on time-to-recovery and mortality in the intention-to-treat analysis; however, at the time of publication, only one-third of enrolled patients had received complete treatment and had been analyzed. Such a loss to follow-up poses serious threats to the validity of this study [147]. The last released paper compares five days to 10 days of treatment with remdesivir with no significant difference in mortality rates or improvement in clinical status. Serious adverse events were reported in 27.7% of treated patients, including 4.7% acute kidney injuries. In 7.3% of patients, adverse events led to the treatment being stopped [148]. In addition, remdesivir is currently only available intravenously, making early treatment at home

impossible and exposing patients to the risk of intravenous catheter complications (lymphangitis, thrombophlebitis, endocarditis).

4.7. Use of nonantiviral therapy in COVID-19 patients

During the immunopathogenic phase of COVID-19, serum IL-6 concentrations have been found to be consistently elevated (90 to 160 pg/ml) and correlated with severity [1, 67]. IL-6 is produced by various cells, mainly in response to IL-1, and is the main inducer of acute phase-protein synthesis by the liver, notably CRP. It plays a role in B lymphocyte differentiation and encourages mononuclear cell inflammation and Th-17 response [149, 150]. IL-6 acts through binding to a ligand receptor, IL-6R, then complexes with signaling receptor gp130. Although gp130 is widely expressed, IL-6R expression is limited to leukocytes and hepatocytes but can be shed from these cells in a soluble form, which binds to IL-6 and then to gp130, rendering IL-6R-negative cells sensitive to IL-6, a mechanism named *trans*-signaling [151]. Through this mechanism, IL-6 has been involved in various inflammatory diseases, such as rheumatoid arthritis (RA) and lung fibrosis [149] [152].

Tocilizumab is a humanized anti-IL-6R monoclonal antibody (mAb) that is able to bind both to the membrane and soluble IL-6R and to inhibit IL-6 functions. It is currently used in the treatment of various chronic diseases, notably rheumatoid arthritis, giant cell arteritis, and systemic juvenile idiopathic arthritis (sJIA), and has a good safety profile despite its long half-life [149]. sJIA is often complicated by macrophage activation syndrome characterized by a cytokine storm, and tocilizumab has been shown to be effective in the treatment of this disease [153]. Moreover, cancer treatments using CAR-T cells may be complicated by a cytokine release syndrome, which is efficiently treated by tocilizumab [154]. Tocilizumab was, therefore, used to treat 21 severe or critical COVID-19 patients in China and was retrospectively reported to be effective [155]. The patients received a single 400 mg infusion. Their body temperature returned to normal within 24 hours, oxygen saturation improved

rapidly in 75% of cases, and CRP returned to normal in 80% of the patients after five days. This observation was confirmed in Italy [156]. Tolerance to treatment appeared to be good in all studies, but acute hypertriglyceridemia and pancreatitis were recently reported in two patients [157].

Together, these results are promising and have justified several ongoing trials in Italy, France and the US (using sarilumab, another anti-IL-6R mAb) [158]. However, other therapeutic strategies may be effective in order to treat the cytokine storm associated with COVID-19, such as IL-1 receptor antagonists (anakinra), which act upstream of IL-6 and have a short half-life, anti-IFNg (emapalumab), NLRP3 pharmacological inhibitors (dapansutrile) or JAK kinase inhibitors, which may block IL-6 and IFNs signaling downstream of their receptors. Recent retrospective case series or small cohorts conducted in Italy [159] and in France [160] have shown that anakinra may be an effective treatment in arresting inflammatory respiratory deterioration.

In addition, preliminary results from the RECOVERY trial suggest that dexamethasone either by mouth or by intravenous injection improves prognosis in patients on oxygen and those in intensive care [161]. However, final detailed results are not published. Moreover, no benefit was observed among those patients who did not require respiratory support. In this context, hydroxychloroquine, with its strong anti-IL6 activity [162], remains an attractive option as an early nonantiviral therapy.

4.8 Prophylactic use of hydroxychloroquine against COVID-19

4.8.1 Pre-exposure prophylaxis

In a case-controlled study in India on symptomatic healthcare workers (HCWs) including 378 positive cases (symptomatic HCWs with SARS-CoV-2 positive PCR, including 172 with HCQ prophylaxis) and 373 controls (symptomatic HCWs with SARS-CoV-2 negative PCR including 193 with HCQ prophylaxis), the administration of at least four HCQ

doses was associated with a significant, fifty percent lower risk of COVID-19 (adjusted odds ratio [aOR] 0.44, 95% confidence interval 0.22–0.88) [163]. A dose-dependent relationship was observed with an adjusted odds ratio decreasing from 0.44 (0.22–0.88, p = .02) for four–five doses and 0.04 (0.01–0.16, p < .001) for six doses and more. One to three doses had no effect while six or more prophylactic doses had a remarkably high (>80%) protective effect. Of the 365 HCWs who reported taking HCQ, nausea, headache and diarrhea were reported in approximately 5%; only individual case reported palpitations.

4.8.2 Postexposure prophylaxis

In South Korea, a COVID-19 exposure event occurred in a long-term care hospital, and HCQ postexposure prophylaxis was provided to 211 individuals [164]. None of them developed the disease, and none had a positive PCR during follow-up. However, since no subjects who did not receive prophylaxis became positive, effectiveness could not be truly evaluated. In the USA, a double-blinded randomized controlled trial was performed with 414 subjects receiving HCQ and 407 receiving folate as a placebo within four days of exposure [165]. No arrhythmias or deaths occurred. Furthermore, 49 (11.8%) developed a disease compatible with COVID-19 in the HCQ group compared to 58 (14.3%) in the control group (p = 0.35). The authors thus concluded that HCQ did not prevent the disease. However, most of the people included did not have a definitive diagnosis of COVID-19 by PCR. In addition, the delay in postexposure chemoprophylaxis strongly impacts the efficacy, with a fifty percent decrease in the risk of infection when HCQ was administered on day 1 and decreasing efficacy over time. For influenza, postexposure chemoprophylaxis has only been recommended when antivirals can be initiated within 48 hours of exposure [166]. This highlights the need to initiate chemoprophylaxis rapidly after exposure.

4.9. Global care of COVID-19 patients

4.9.1 Outpatient care

In the context of a brutal and deadly pandemic (global mortality rate of approximately 6% and up to 18% in France), no randomized trial investigating early versus delayed treatment has been conducted. Barbosa Esper *et al.* reported that early telemedicine treatment with HCQ and AZ was associated with a significant decrease in hospitalization rates [167]. Zev Zelenko, a general practitioner in the suburbs of New York, USA, reported on the early treatment of 405 patients with HCQ 200 mg 2/d, AZ 500 mg 1/d and zinc sulphate 220 mg 1/d for five days at a total cost of $20 [168]. All patients with dyspnea or with risk factors (regardless of clinical status) were treated. As of 26 April, he had reported one death in a patient with leukemia (1/405 (0.2%)). The only adverse events reported were nausea and diarrhea in 10% of cases but no cardiac toxicity. Guerin *et al.* reported on a French study on outpatient treatment of 88 patients with HCQ-AZ, HCQ and controls [169]. Early HCQ or HCQ-AZ treatment was associated with a significantly shorter time to clinical recovery. There was no significant difference between HCQ alone and HCQ-AZ. No cardiac toxicity was observed.

Finally, massive screening and early treatment have been implemented in our center (Marseille, France), with more than three-quarters of the 1,061 reported cases managed in a day care hospital for initial evaluation and treatment [141]. The therapeutic protocol included at least an ECG, potassium measurement, D-dimers, low-dose CT scan, HCQ (200 mg three times per day in the absence of ECG repolarization disorder and abnormal kalemia), and AZ (500 mg on the first day then 250 mg per day for four additional days). Correction of hypokalemia was needed in approximately 15% of our cohort and was associated with severity [170]. This is critical since hypokalemia could increase the risk of cardiac arrhythmia. After confirming in our cohort that zinc deficiency was associated with an unfavorable prognosis, and in view of a comparative study in favor of zinc [144], 15 mg of zinc three times daily was added. Any patient with positive D-dimers was evaluated for

pulmonary embolism, and treatment with low molecular weight heparin was systematically discussed on an outpatient or inpatient basis. At least one patient was diagnosed with a pulmonary embolism in our day care hospital after systematic D-dimer assessment without any clinical signs. In the presence of a clinical (NEWS score ≥ 5) or biological sign of severity, or if the treatment became difficult, patients were systematically hospitalized.

4.9.2 Inpatient care

Under this global management strategy, based on early massive nonselective screening, day care hospitals, inpatient and early resuscitation care, no deaths under the age of 60 years have been observed. In the 60+ age group, the mortality regardless of treatment was 5.0%, which was similar to the mortality in the same age group reported in China (6.0%) but lower than that reported in Italy (12.3%) [171]. On the other hand, among patients aged 60 years and over who had at least three days of dual HCQ-AZ therapy in our center, the mortality was 3.1%, which was much lower than that reported in China and Italy for the same age group. Based on the currently available literature, we would recommend measuring D-dimers, prothrombin time, and platelet count in all patients with COVID-19 infection. The optimal dosage of low molecular weight heparin is currently the subject of much discussion within the medical community. Although the majority suggests prophylactic daily LMWH, a minority considers intermediate or therapeutic doses [83]. In all cases, the optimal doses for each patient need to take account specific patient thrombotic risk factors, such as active cancer (treatment within the last six months), recent personal history of thromboembolic events (<two years) and an elevated body mass index (BMI > 30 kg/m^2) [172].

Regarding possible drug interactions, oral anticoagulants prescribed for long-term use should be replaced by curative heparin therapy.

4.9.3 Intensive care

Approximately 15% of COVID-19 patients require admission to an ICU. The biggest reason for admission is acute respiratory failure [173]. Gattinoni *et al.* recently described two phenotypes of ICU patients: phenotype L and phenotype H. Phenotype L corresponds to a clinical picture of ARDS with *low elastance*, which is unexpected in this syndrome. Phenotype H for *high elastance* is in line with the common picture of ARDS, representing 20-30% of COVID-19 patients [174]. A CT scan is a perfect tool to differentiate the two phenotypes: the nonaerated lung is close to 0 in phenotype L patients, while posterior condensations are found in phenotype H patients [174]. Most patients evolve from phenotype L to phenotype H, which may be due to COVID-19 pneumonia evolution or to the adverse effects of high-pressure mechanical ventilation. The hospital mortality rate of COVID-19 patients admitted to the ICU ranged from 26% [20] to 50% [175] and was even higher in elderly patients [173]. However, in our center, the in-ICU mortality was approximately 18% (unpublished data) in a context of a global strategy including early testing, early care and early treatment [141].

While COVID-19 was initially documented as isolated acute respiratory failure, patients admitted to the ICU develop multiple organ failure in approximately 50% of cases [173]. Among organ failures, an exacerbated activation of coagulation in COVID-19 patients resulted in a large number of thromboses and pulmonary embolisms. The activation of coagulation is a well-known host response in patients with septic shock. Specific treatments were developed to counteract this response, but successive studies failed to demonstrate efficiency. However, disruptions of coagulation strongly affect the outcomes of ICU patients with COVID-19. In a cohort of 184 ICU patients, 27% of patients developed deep vein thrombosis, and pulmonary embolism was found in 80% of cases [84]. Anticoagulation treatment should be widely administered to reduce the risk of pulmonary embolism, and protocols should include curative anticoagulation in most mechanically ventilated patients.

Monitoring coagulation factors, including D-dimers and fibrinogens, provides relevant information to decide between prophylactic or curative anticoagulation. Those with an increased body mass index are at a very high risk of thrombosis.

In ICU patients, an intense inflammatory response has been described in the late phase of the disease. The production of pro-inflammatory cytokines has been reported to be exacerbated in patients with negative SARS-CoV-2 PCR. This response profile encouraged a few teams to use steroids, notably for patients developing ARDS [68]. In a cohort series, the use of steroids in ARDS patients was associated with improved outcomes, within the limitations of this study based on multivariate analysis [176]. However, the pro-inflammatory profile of septic patients in the ICU was previously described, while recent trends revealed profound immunosuppression. Recent data do not confirm the pro-inflammatory response in COVID-19 patients in the ICU (personal data) as the profile is time dependent. Introducing steroids to patients with viral infections and immunosuppressed patients can be high risk, resulting in an increase in superinfections. Thus, it seems prudent to restrict the use of steroids in those severe patients who usually have a severe lymphopenia.

4.10 Traditional Medicine

Traditional Chinese Medicine (TCM) therapy has played a role in treating epidemic diseases in China's long history [177]. In combination with Western medicine, TCM therapy was widely used in China for the management of COVID-19, with up to 91.5% of patients with a confirmed diagnosis having reportedly received TCM [178]. Several studies support that the current practice of TCM has a favorable impact in the management of COVID-19 and can shorten of the course of fever, course of the disease, and length of hospital stay, and reduce the number of severe patients and death rate [177, 179]. Three patented TCM medicines (Lianhua Qingwen capsules, Jinhua Qinggan granules, and Xuebijing injection) and three TCM decoctions (Qingfei Paidu, Huashi Baidu, and Xuanfei Baidu) have mainly

been highlighted for the management of COVID-19 [177, 180]. Lianhua Qingwen is a formulation prepared from the classic compounds of ancient China, commonly used in the treatment of colds and flu. Jinhua Qinggan is a formulation developed for the treatment of influenza A (H1N1) in 2009. The use of Jinhua Qinggan and Lianhua Qingwen is suggested for medical observation of COVID-19 [181]; they could also be used for patients with mild and moderate symptoms. Xuebijing injection was developed for the treatment of SARS in China and is indicated for systemic inflammatory response syndrome induced by infections [180]. Xuebijing is used for severe and critical COVID-19 infection [181]. Qingfei Paidu, Huashi Baidu, and Xuanfei Baidu decoctions are three new prescriptions specifically designed for COVID-19 [181]. Their use is suggested in the prevention and mild and moderate infections of SARS-CoV-2 [181]. The action mechanisms of Chinese medicines are still unclear. Most of them are founded on pharmacology-based predictions [177]. The composition, potential active ingredients, predicted targets, signaling pathways and mechanisms of these Chinese herbal medicines have been recently detailed by Huang *et al.* in a review article [177]. The use of TCM at different stages of COVID-19 is among the recommendations in China and South Korea [177, 182]. Ho *et al.* have summarized the guidelines of the National Health Commission and the National Administration of Traditional Chinese Medicine of the People's Republic of China for the management of COVID-19, which, in addition to the abovementioned formulas, includes compositions adapted according to the patient's condition [182]. The reported duration of COVID-19 treatment with herbal medicines usually varied from 5 to 15 days [179]. Regarding potential side effects, no severe discomfort or abnormal liver or kidney function has been identified [179]. Although basic and clinical research is still needed to understand the mechanisms of action and lead to evidence-based medicine, it is worth paying attention to TCM. Indeed, it must be remembered that the use of herbal medicine against malaria has allowed to the discovery of artemisinin, a plant

extract of *Artemisia annua* used as a standard treatment against *Plasmodium falciparum* worldwide [183].

5. Conclusions

COVID-19 is a newly emerging disease that requires us to adjust our medical approach as we receive new observational evidence about the virus and its behaviors. Indeed, the observation of symptoms usually rarely associated with respiratory infections, such as anosmia or a lack of perceived dyspnea with documented hypoxemia and severe radiological impairment on CT scan, and viral thrombotic disease with up to 30% of critical cases developing pulmonary embolism, led us to adapt our clinical approach toward SARS-CoV-2-associated pneumonia. Early treatment of patients through the rapid implementation of specific PCR diagnostic tools has been, in many cases, key to successfully controlling the disease. In most affected countries, the epidemic appears to spontaneously resolve over time, but COVID-19 morbidity and mortality indicators are, paradoxically, worse in the rich countries of Western Europe and the US than in developing countries. The debate about the usefulness of chloroquine derivative-based treatment, initially proposed by Chinese physicians, and that of new antivirals such as the orphan drug remdesivir, has reached unprecedented levels of aggressiveness. This culminated in the recent retraction of COVID-19 publications from the world's two most prestigious medical journals, The Lancet and The New England Journal of Medicine. Thus, lessons from the COVID-19 pandemic go far beyond the disease itself and question our responsiveness toward a disease with medical, societal, economic, and political consequences that may lead to major editorial pitfalls challenging the credibility of the main actors in the field of medical literature.

6. Expert opinion (Figure 7)

The COVID-19 epidemic is an unprecedented crisis, not in terms of mortality, but in terms of emotion and the measures taken to fight it. It is not the worst epidemic we have experienced

in recent decades, but it has led to changes in the organization of care, including lockdown measures, which are unprecedented. However, it is likely that in most countries, COVID-related mortality will go unnoticed in the annual mortality statistics. This is particularly because it has mostly affected older people. The epidemic has also revealed the disorganization of political decisions with totally different strategies from one country to another and paradoxically higher mortality in the richest countries, especially those in Western Europe and the US. No consensus has been reached around the implementation of basic strategies including diagnostic tests, isolation of contagious patients, patient care and potentially safe and effective therapies. Finally, unsupported decisions taken by governments and the WHO have increased confusion. This has led to doubtful publications being retracted from the best journals in the world and has given rise to a previously unknown state of nervousness. When studying the disease seriously, a few things are worth noting. It does not present itself as common flu but commonly presents with anosmia. The patient may be free of fever, cough, and shortness of breath, despite significant hypoxia and CT lesions. This warrants an expanded strategy of diagnostic testing, measuring oxygen saturation and performing low-dose CT scans to detect specific lesions. Early therapeutic management in terms of care (anticoagulants, oxygen therapy) and drugs that have proven or will prove to be effective at this stage (HCQ and AZ and possibly remdesivir in the early stages) will prevent progression to respiratory failure, resuscitation and death. A number of markers have been associated with this pejorative evolution including a lymphopenia below 500 being be the most predictive, decreased zinc levels, eosinopenia, increase in polymorphonuclear cells and increase in D-dimers. Subjects with hypertension are more at risk as a result of hypertension or antihypertensive drug use. The disease appears to unfold in three stages: a purely virological stage, a stage associating the virus with the immune response, and a final stage in the most severe forms, which appears essentially as an immune response without the virus.

This explains why antiviral treatment attempts in patients with very severe disease are usually ineffective, and samples taken to look for the viral load at this stage are often negative. A cytokine storm occurs against which no drug has proven to be effective. This is something that is common in adult acute respiratory distress syndromes, where treating the cause is less critical than properly managing it. Immunity as evidenced by antibodies against the virus appear around the tenth day and is extremely marked in the most seriously affected subjects. In our experience, deceased subjects had the highest antibody levels. Concerning the epidemiology of the disease, it presents in the form of a bell curve, which is highly typical of respiratory viral infections. It appears that part of the population is naturally immune, which may be related to endemic circulating coronaviruses, which cause 10 to 20% of respiratory infections, especially in children. Indeed, it is remarkable that the incidence of both the virus and the disease in children has been low. Under these conditions, the use of a vaccination before this point is likely to present more risks than benefits. The future of this disease is unknown.

What lessons has this crisis taught us? First, in 2020, infectious disease diagnosis can no longer be based solely on clinical criteria without microbiological testing. Indeed, at the peak of the epidemic in our center, only 22% of symptomatic patients were PCR positive. Many viruses have cocirculated, including endemic coronaviruses. Second, general recommendations cannot be made until the disease is observed and known. It is only recently that the four stages of the disease have been identified: 1) viral, 2) viral and dysimmune, 3) dysimmune, and 4) lesional (ARDS) stages. Each stage corresponds to a specific treatment (Figure 3). Then, when evaluating the efficacy of a treatment, the molecule should not be considered in isolation. A treatment is not a molecule, it is a therapeutic protocol with one or more molecules (synergistic effect of the HCQ+AZ+zinc combination [144]), indications, contraindications, precautions for use, and a precise dosage and duration. For example, for

several decades we have been administering HCQ at 200 mg three times a day for 18 to 24 months in *Coxiella burnetii* endocarditis in association with doxycycline [184]. This dosage is necessary to achieve therapeutic blood levels [184]. This protocol has been used according to the recommendations of the American CDC for the same indication [129] and in the most well-known professional sites (https://www.uptodate.com/contents/q-fever-endocarditis). In rheumatic diseases such as lupus or rheumatoid arthritis, dosages of 400 to 600 mg/d are used (www.uptodate.com). In COVID-19, extreme dosages of 2,400 mg/d were used in the RECOVERY trial, i.e., four times the usual dose, while in the Discovery trial, low doses of 400 mg/d were used. Overall, standardized therapeutic protocols associated with a comprehensive strategy including early massive screening would appear to be critical in responding to the COVID-19 pandemic.

Declaration of interest statement

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. CD declares owning SANOFI shares.

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Figure Legends

Figure 1: LDCT image showing ground glass opacities and consolidation in A. The deep learning algorithm provides automatic segmentation of the consolidation in B (yellow label), ground glass opacities in C (green label) and the lung in D (purple label).

Figure 2: Number of newly diagnosed SARS-CoV-2 patients per week at the IHU Méditerranée Infection by reverse transcription PCR

Figure 3: Natural history of COVID-19 and treatment options (adapted from reference [3])

Figure 4: Schematic representation of SARS-CoV-2 replication cycle. ACE2: angiotensin-converting enzyme 2, NSPs: nonstructural proteins, ERGIC: endoplasmic reticulum Golgi intermediate compartment

Figure 5: Meta-analysis of chloroquine and COVID-19 mortality

CI: confidence interval, HCQ: hydroxychloroquine, CQ: chloroquine, (H)CQ: chloroquine derivatives (hydroxychloroquine (HCQ) or chloroquine (CQ)). This meta-analysis was performed with a random-effects model using Comprehensive Meta-Analysis v3 (Biostat, Englewood, NJ, USA).

Figure 6: Meta-analysis of chloroquine derivatives and SARS-CoV-2 persistent shedding

CI: confidence interval, HCQ: hydroxychloroquine, CQ: chloroquine, RCT: randomized controlled trial, (H)CQ: chloroquine derivatives (hydroxychloroquine (HCQ) or chloroquine (CQ)). This meta-analysis was performed with a random-effects model using Comprehensive Meta-Analysis v3 (Biostat, Englewood, NJ, USA).

Figure 7: Schematic representation of previous findings by multiple correspondence analysis.

Table Legend

Table 1: Level of evidence for the efficacy of a combination of hydroxychloroquine and hydroxychloroquine-azithromycin against COVID-19 based on clinical studies with a clear description of the therapeutic protocol.

1439 **Table 1. Anti-COVID-19 potential drugs according to *in vitro* results and chemical structure**

	EC50 (µM)	CC50 (µM)	Selectivity Index	Reference
4-aminoquinoleines[a]				
Chloroquine	1.13 (EC90 : 6.9)	>100	>88.5	[89]
	2.71-7.36	273	37-100	[90]
	42-46	>50	>1.07-1.19	[91]
	(Inhibition index 1.35)			[94]
	5.5			[88]
	1.38	238	172	[185]
Hydroxychloroquine[b]	4.06-13.00	249	14-61	[90]
	9-11	>>50	>>2.81	[91]
	4 (EC90 : 25)	>40	>10	[94]
	<10			[96]
	0.72			[88]
Mefloquine	7-8	18	2.3-2.6	[91]
Amiodaquine	2-5	>38	>6->13	[91]
Phenothiazine				
Chlorpromazine	3-4	12	3-4	[91]
Fluphenazine	6-9	20	2-3	[91]
Promethazine	9-10	>42	>4	[91]
Thiethylperazine	7-8	18	2-3	[91]
Tricyclic antidepressant				
Clomipramine	5-7	>30	>4-5	[91]
Ribonucleic analogs				
Ribavirin	109	>400	>3.65	[89]
	>10	>100		[185]
Remdesivir	0.77 (EC90 : 1.76)	>100	>129	[89]
	1.6			[94]
	0.99	275	278	[185]
Penciclovir	95	>400	>4.17	[89]
Other chemical classes				
Azithromycin[b]	2 (EC90 : 8)	>40	>19	[94]
Cycloserine	(inhibition index 1.4)			[94]
Umifenovir	10.7 (EC90 : 15.2)	>40	>3.7	[94]
	3.5	75	21	[185]
Omeprazole[a]	17 (EC90 : 38)	>40	>2	[94]

Teicoplanin	1.66			[92]
Levofloxacin	(inhibition index 1.18)			[94]
Ivermectin	2.8			[93]
Favipiravir	62	>400	>6.46	[89]
	>100	631	<6.3	[185]
Nafamostat	22	>100	>4.44	[89]
Nitazoxanide	2.12	>35.53	>16.76	[89]

EC50: drug concentration needed to inhibit 50% of viral spread, CC50: Cytotoxic concentration causing death to 50% of viable cells, SI: selectivity index. A molecule is considered potentially effective when EC_{50}<20 µM [94]. [a]Molecules demonstrated to increase the pH of the endosomal pathway. [b]Synergy demonstrated *in vitro* [95].

Table 2. Level of evidence for efficacy of hydroxychloroquine and hydroxychloroquine-azithromycin combination against COVID-19 based on clinical studies with a clear description of the therapeutic protocol.

Level of evidence	Type of evidence *	Available studies	References
Ia	Systematic review (with homogeneity) of RCTs	A meta-analysis of four RCTs conducted in China evidenced a significantly favorable effect of hydroxychloroquine in three out of four studies. An update of this meta-analysis including a new Brazilian RCT confirmed such an effect (Figure 5 & 6).	[117, 130]
		An RCT conducted on 62 COVID-19 patients showed significantly shortened body temperature recovery time and cough remission time and a larger proportion of improved pneumonia as assessed by CT scan in patients treated with 400 mg hydroxychloroquine per day over five days (N=31) than in controls (N=31). In addition, a nonsignificant favorable direction effect was observed regarding overall clinical improvement.	[124]
		An RCT conducted on 150 COVID-19 patients showed a significant effect on the alleviation of symptoms in post hoc analysis and C-reactive protein reduction in patients treated with hydroxychloroquine 1,200 mg per day for three days then 800 mg for two to three weeks (N=75) than in controls (N=75). It is notable that these results were not included in the version published in the British Medical Journal because these endpoints were not prespecified in the study protocol and due to an underpowered sample size, arguments that we consider fallacious. In addition, a nonsignificant favorable direction effect was observed regarding lymphocyte count decrease and shortened viral shedding duration.	[126, 186]
		An RCT conducted on 373 COVID-19 patients showed a significantly shortened length of hospital stay in patients treated with chloroquine 500 mg or 1,000 mg per day for no more than 10 days (N=197) than in controls (N=176). In addition, a nonsignificant favorable direction effect was observed regarding clinical and radiological improvement and shortened viral shedding duration.	[125]
		An RCT conducted on 48 COVID-19 patients who were randomized to chloroquine (N = 18), hydroxychloroquine (N = 18) or controls (N = 12). The chloroquine and the hydroxychloroquine groups achieved shorter time to clinical recovery (TTCR) than the control group. The time to reach viral RNA negativity was significantly faster in the chloroquine group and the hydroxychloroquine group than in the control group.	[133]

		An RCT conducted in Brazil on 504 patients with PCR-confirmed COVID-19 infection reported no significant effect, but mortality was lower in patients treated by the HCQ-AZ combination (1.7%) versus control (2.9%) or patients treated by HCQ alone (3.1%). This correspond to a prevented fraction of 40% in the treated population (odds ratio = 0.60). By contrast, a Chinese RCT conducted on 30 COVID-19 patients showed no significant differences between patients treated with 400 mg hydroxychloroquine per day for five days (N=15) and controls (N=15) regarding the pharyngeal carriage of viral RNA at day 7, clinical and radiological improvement; however, patients received multiple additional treatments, including antivirals.	[136] [127]
Ib	Individual RCT (with narrow confidence interval)	A preliminary French (nonrandomized) clinical trial conducted on 36 COVID-19 patients showed a significant reduction in viral nasopharyngeal carriage at day 6 in patients treated with hydroxychloroquine at 600 mg per day for 10 days (N=20, 70% testing negative) compared with untreated controls (N=16, 12.5% testing negative). In addition, of the twenty patients who were treated with hydroxychloroquine, six received azithromycin for five days (for the purposes of preventing bacterial superinfection) and all (100%) were virologically cured at day 6, compared to 57.1% of the remaining 14 patients.	[116]
Ic	All or none study	-	
2a	Systematic review (with homogeneity) of cohort studies	A meta-analysis of ten cohort studies conducted in Iran, Brazil, France, China, South Korea, Spain and Saudi Arabia evidenced (depending on studies) a significant favorable effect of hydroxychloroquine on death or transfer to an intensive care unit, the need for hospitalization, overall clinical cure, body temperature recovery time, shortened viral shedding duration, length of hospital stay, cough remission time and interleukin-6 levels. In addition, a nonsignificant favorable direction effect was observed regarding death and/or transfer to an intensive care unit, overall clinical cure, length of hospital stay, radiological improvement, and body temperature recovery time. By contrast, a nonsignificant deleterious direction effect was observed on overall clinical cure, length of hospital stay, and shortened viral shedding duration in three of these studies.	[117]
2b	Individual cohort study (including low	Clinical results were reported in a news briefing by the Chinese government revealing that the	[187]

	quality RCT; e.g., <80% follow-up)	treatment of over 100 patients with chloroquine phosphate in China had resulted in significant improvements of pneumonia and lung imaging, with reductions in the duration of illness.	
		An uncontrolled French noncomparative observational study was conducted on a cohort of 80 relatively mildly infected inpatients treated with a combination of hydroxychloroquine and azithromycin over a period of at least three days; all patients improved clinically with the exception of one 86-year-old patient who died, and one 74-year-old patient who remains in intensive care. A rapid fall in nasopharyngeal viral load was noted, with 83% negative at day 7, and 93% at day 8. Virus cultures from patients' respiratory samples were negative in 97.5% of patients at day 5. Consequently, patients were able to be rapidly discharged with a mean length of stay of five days.	[142]
		Another similar study by the same team was conducted on 1,061 patients under the same therapeutic protocol. Good clinical outcome and virological cure were obtained in 973 patients within 10 days (91.7%). A poor clinical outcome was observed for 46 patients (4.3%) and eight died (0.75%) (74–95 years old). All deaths resulted from respiratory failure and not from cardiac toxicity.	[117]
		A large study by the same team involved 3,119 patients treated with the same therapeutic protocol and 628 with other regimens. Combined treatment was associated with decreased risk of transfer to an ICU or death (Hazard ratio (HR) 0.18 0.11-0.27), decreased risk of hospitalization ≥10 days (odds ratios 95% CI 0.38 0.27-0.54) and shorter duration of viral shedding (time to negative PCR: HR 1.29 1.17-1.42). QTc prolongation (>60 ms) was observed in 25 patients (0.67%) leading to the cessation of treatment in 12 cases including three cases with QTc> 500 ms. No cases of torsade de pointe or sudden death were observed.	[3]
2c	"Outcomes" research; ecological studies	Three studies have demonstrated that chloroquine phosphate inhibits SARS-CoV-2 and two have [88] demonstrated that hydroxychloroquine sulfate inhibits SARS-CoV-2 *in vitro*. In addition, one study showed that the combination of hydroxychloroquine and azithromycin inhibits SARS-CoV-2 *in vitro*.	[88-90, 188]
3c	Systematic review (with homogeneity) of case-control studies	-	

3b	Individual case-control study	-	
4	Case-series (and poor quality cohort and case-control studies)	-	
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"	The National Health Commission of the People's Republic of China published their recommendation mid-February, suggesting treating patients with 500 mg chloroquine phosphate twice a day, for a maximum of 10 days.	[188]
		In Italy, the L. Spallanzani National Institute for Infectious Diseases published their recommendations for treatment on 17 March, which included the provision of 400 mg of HCQ per day or 500 mg CQ per day, in combination with another antiviral agent.	[189]

* https://www.cebm.net/2009/06/oxford-centre-evidence-based-medicine-levels-evidence-march-2009/

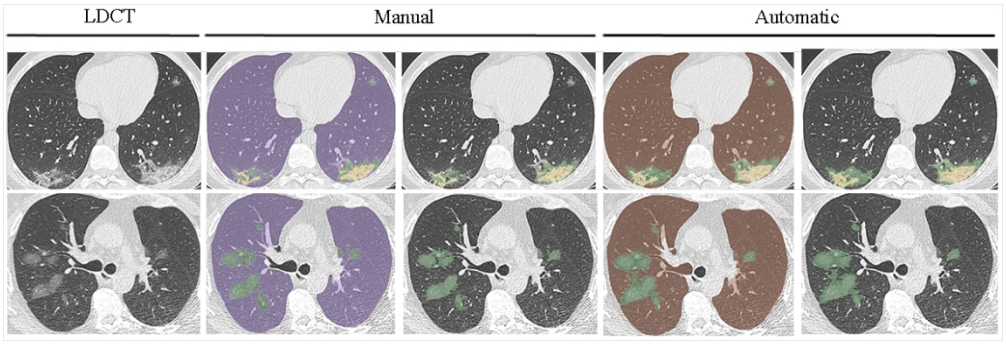
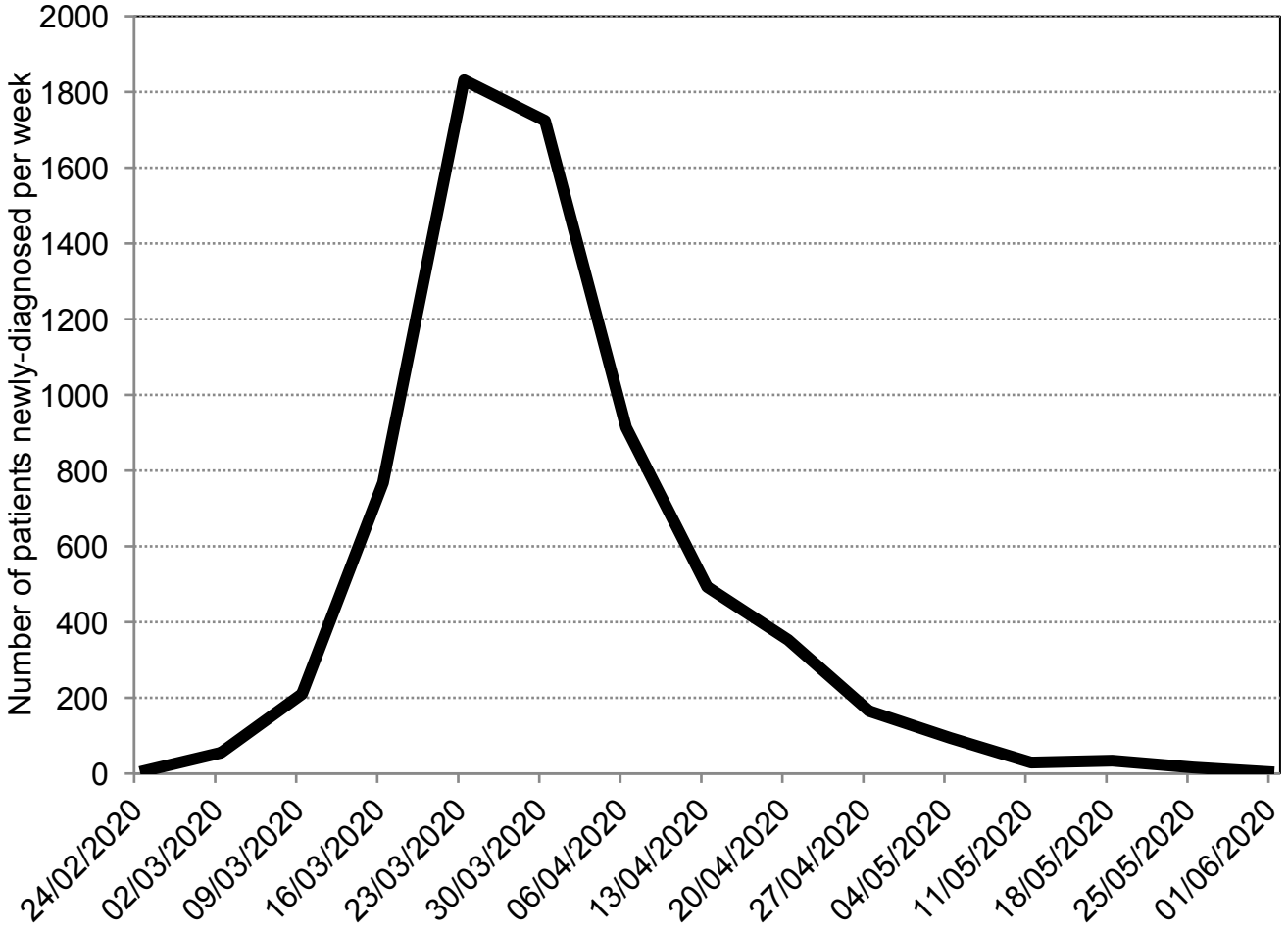


Figure 1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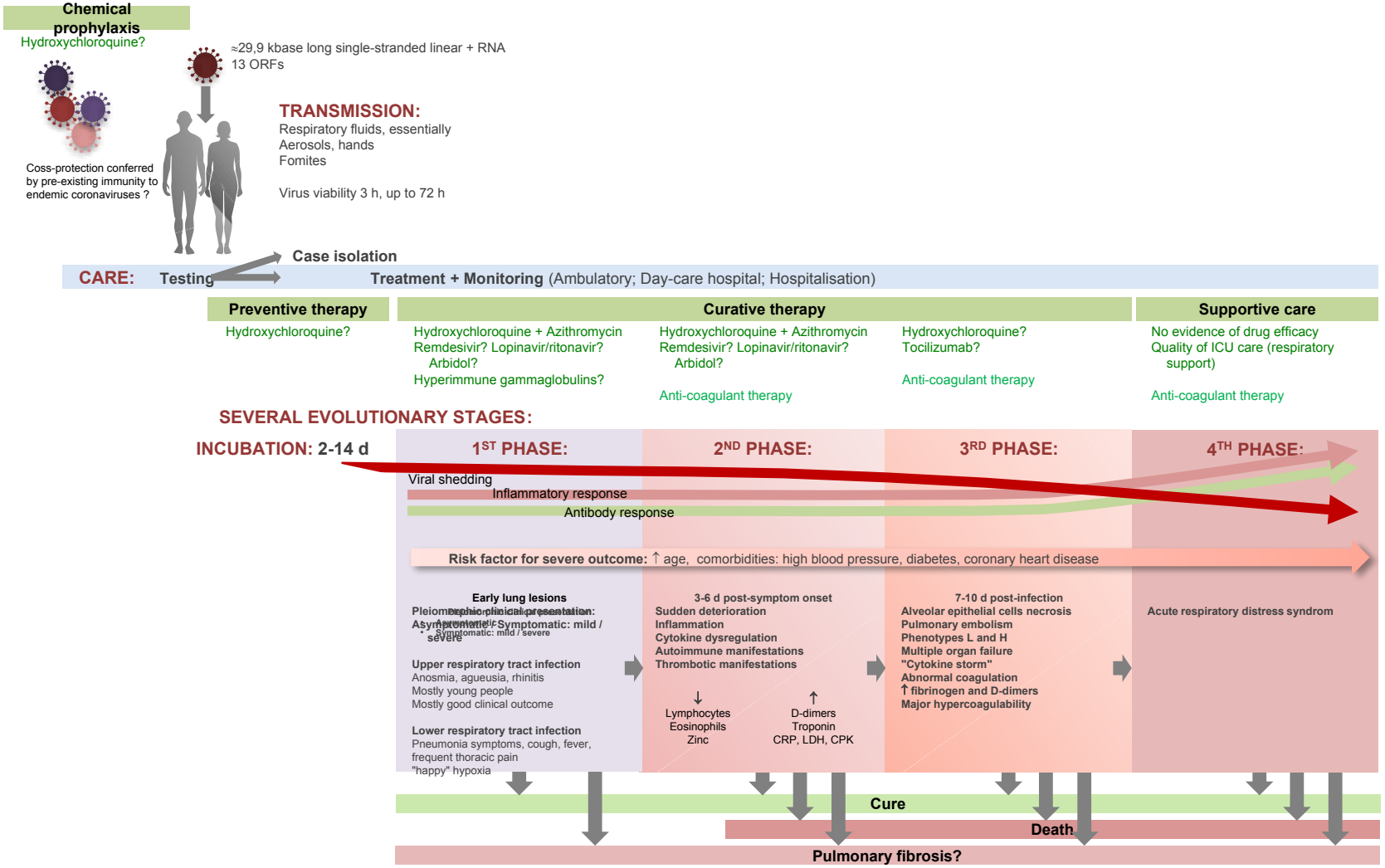
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Hydroxychloroquine?
≈29,9 kbase long single-stranded linear + RNA
13 ORFs
TRANSMISSION:
Respiratory fluids, essentially
Aerosols, hands
Fomites
Virus viability 3 h, up to 72 h
Coss-protection conferred by pre-existing immunity to endemic coronaviruses ?
Case isolation
CARE: Testing
Treatment + Monitoring (Ambulatory; Day-care hospital; Hospitalisation)
Preventive therapy
Hydroxychloroquine?
Curative therapy
Hydroxychloroquine + Azithromycin
Remdesivir? Lopinavir/ritonavir?
Arbidol?
Hyperimmune gammaglobulins?
Hydroxychloroquine + Azithromycin
Remdesivir? Lopinavir/ritonavir?
Arbidol?
Anti-coagulant therapy
Hydroxychloroquine?
Tocilizumab?
Anti-coagulant therapy
Supportive care
No evidence of drug efficacy
Quality of ICU care (respiratory support)
Anti-coagulant therapy
SEVERAL EVOLUTIONARY STAGES:
INCUBATION: 2-14 d
1ST PHASE:
2ND PHASE:
3RD PHASE:
4TH PHASE:
Viral shedding
Inflammatory response
Antibody response
Risk factor for severe outcome: ↑ age, comorbidities: high blood pressure, diabetes, coronary heart disease
Early lung lesions
Pleiomorphic clinical presentation:
Asymptomatic / Symptomatic: mild / severe
Upper respiratory tract infection
Anosmia, agueusia, rhinitis
Mostly young people
Mostly good clinical outcome
Lower respiratory tract infection
Pneumonia symptoms, cough, fever, frequent thoracic pain
"happy" hypoxia
3-6 d post-symptom onset
Sudden deterioration
Inflammation
Cytokine dysregulation
Autoimmune manifestations
Thrombotic manifestations
↓ Lymphocytes Eosinophils Zinc
↑ D-dimers Troponin CRP, LDH, CPK
7-10 d post-infection
Alveolar epithelial cells necrosis
Pulmonary embolism
Phenotypes L and H
Multiple organ failure
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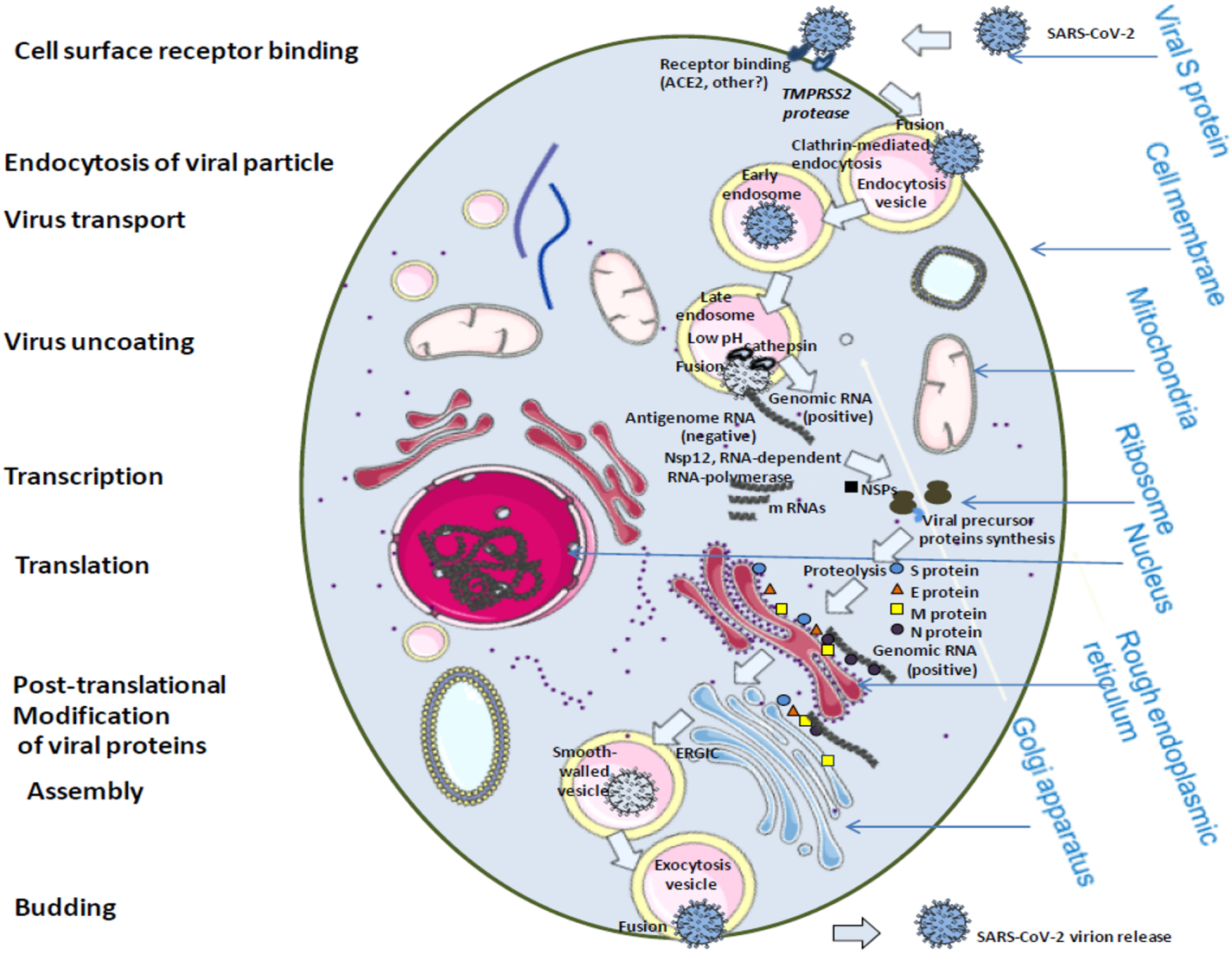
Abnormal coagulation
↑ fibrinogen and D-dimers
Major hypercoagulability
Acute respiratory distress syndrom
Cure
Death
Pulmonary fibrosis?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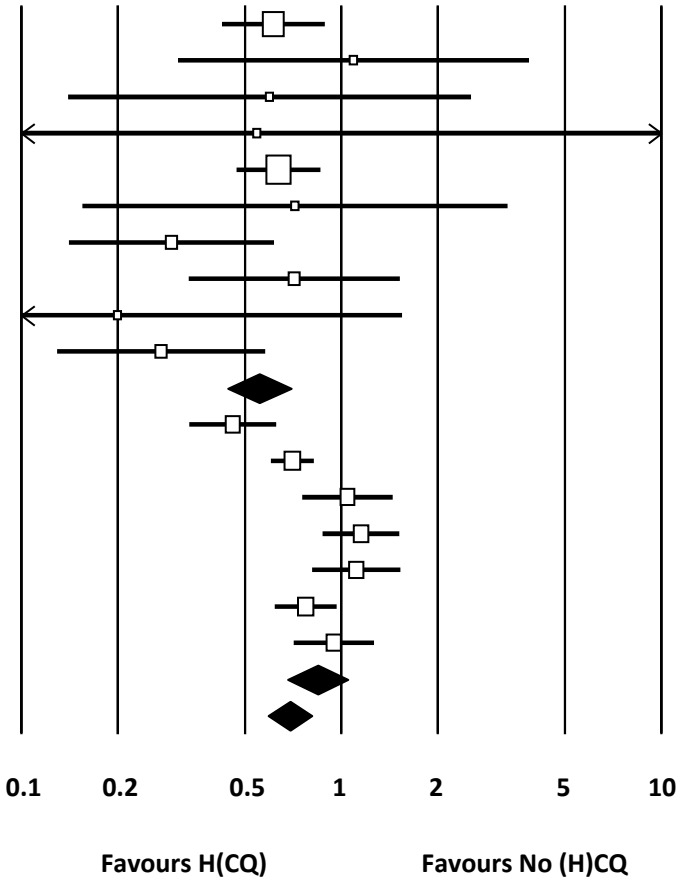
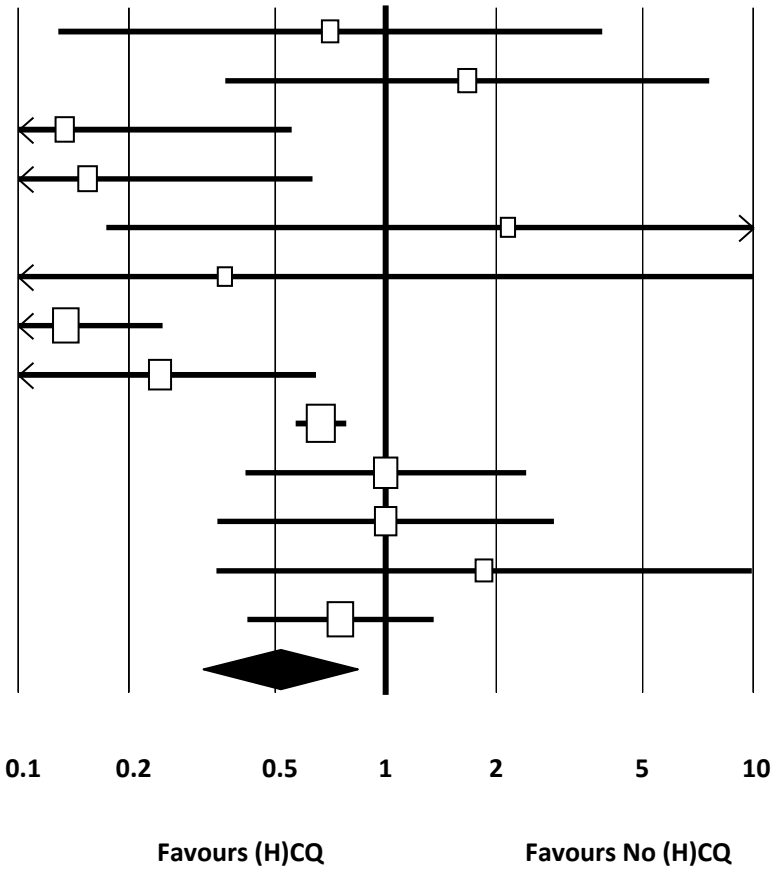
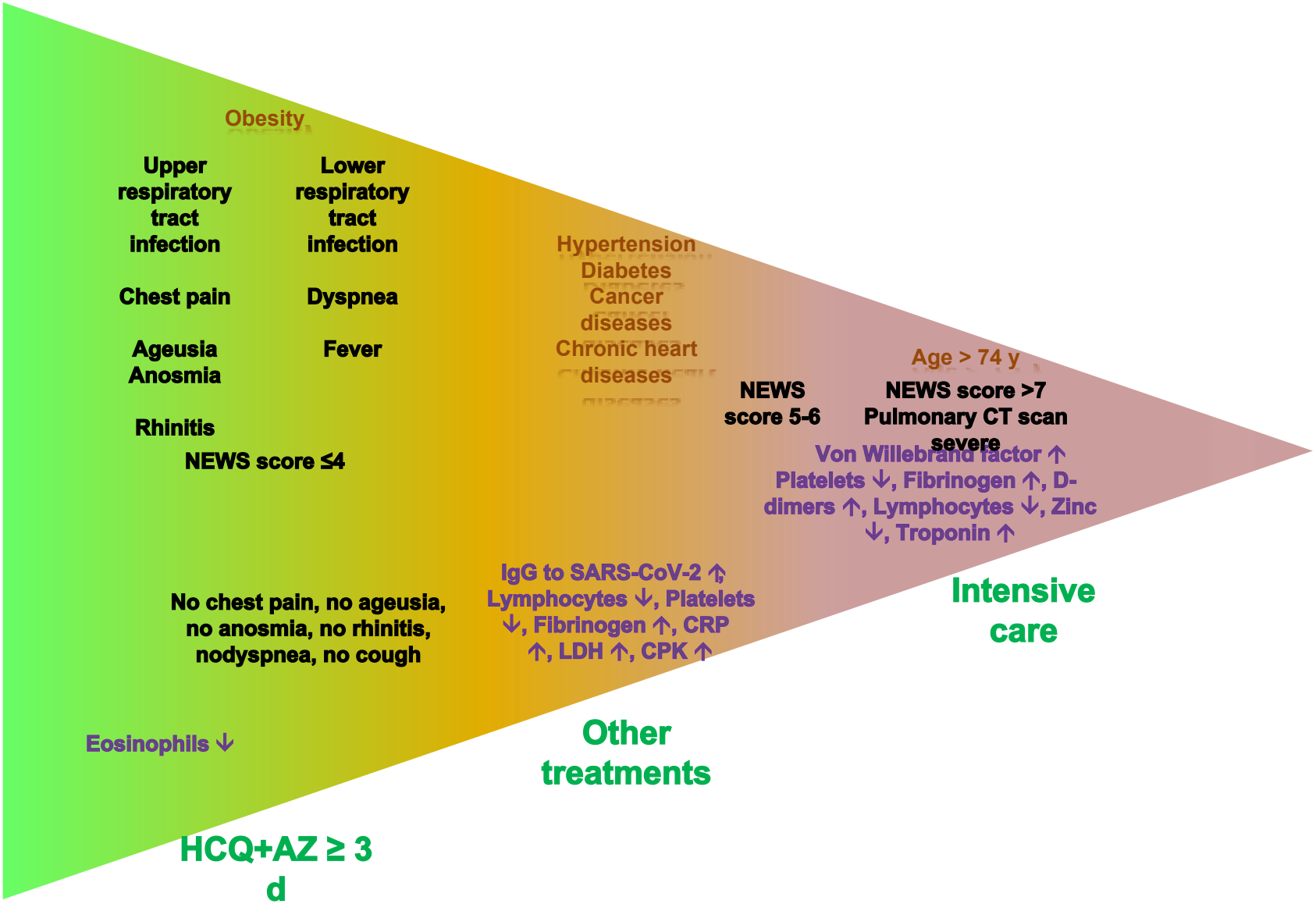
Virus transport
Virus uncoating
Transcription
Translation
Post-translational Modification of viral proteins
Assembly
Budding
SARS-CoV-2
Viral S protein
Receptor binding (ACE2, other?)
TMPRSS2 protease
Fusion
Clathrin-mediated endocytosis
Endocytosis vesicle
Early endosome
Cell membrane
Late endosome
Low pH
cathepsin
Fusion
Mitochondria
Genomic RNA (positive)
Antigenome RNA (negative)
Nsp12, RNA-dependent RNA-polymerase
m RNAs
NSPs
Ribosome
Viral precursor proteins synthesis
Nucleus
Proteolysis
S protein
E protein
M protein
N protein
Genomic RNA (positive)
Rough endoplasmic reticulum
Smooth-walled vesicle
ERGIC
Golgi apparatus
Exocytosis vesicle
Fusion
SARS-CoV-2 virion release

	Country	Study name	Odds ratio	Lower limit	Upper limit	p-Value
Clinical studies	USA	Arshad, Int J Infect Dis, 2020	0.61	0.43	0.88	0.008954
	Brazil	Cavalcanti, N Engl J Med, 2020 - HCQ alone RCT	1.09	0.31	3.84	0.892228
	Brazil	Cavalcanti, N Engl J Med, 2020 – HCQ-AZ RCT	0.60	0.14	2.54	0.484016
	France	Guerin, Asian J Med Health, 2020	0.54	0.02	14.01	0.713884
	France	Lagier, Travel Med Infect Dis, 2020	0.64	0.47	0.86	0.002990
	France	Mahevas, MedRxiv, 2020	0.72	0.16	3.30	0.668282
	Spain	Membrillo, Preprints, 2020	0.29	0.14	0.61	0.001115
	France	Paccoud, Clin Infect Dis, 2020	0.71	0.33	1.52	0.378609
	USA	Scholz, Preprints, 2020	0.20	0.03	1.54	0.122632
	China	Yu, MedRxiv, 2020	0.27	0.13	0.58	0.000647
			0.55	**0.44**	**0.70**	**0.000000**
Big data studies	Spain	Bernaola, MedRxiv, 2020	0.46	0.34	0.62	0.000001
	USA	Mikami, J Gen Intern Med, 2020	0.70	0.60	0.82	0.000006
	USA	Rosenberg, JAMA, 2020 - HCQ alone	1.04	0.76	1.44	0.789895
	USA	Rosenberg, JAMA, 2020 - HCQ-AZ	1.15	0.88	1.51	0.309873
	France	Sbidian, MedRxiv, 2020 - HCQ-AZ	1.11	0.81	1.53	0.502133
	France	Sbidian, MedRxiv, 2020 - HCQ alone	0.77	0.62	0.96	0.021751
	USA	Singh, MedRxiv, 2020	0.95	0.71	1.26	0.715438
			0.84	**0.68**	**1.05**	**0.123244**
			0.69	**0.59**	**0.81**	**0.000004**



Country	Study name	Odds ratio	Lower limit	Upper limit	p-Value
Taiwan	Chen CP, MedRxiv, 2020 RCT	0.71	0.13	3.87	0.688187
Taiwan	Chen CP, MedRxiv, 2020 - Retrospective study	1.67	0.37	7.57	0.508116
China	Chen L, MedRxiv, 2020 - CQ RCT	0.13	0.03	0.55	0.005506
China	Chen L, MedRxiv, 2020 - HCQ RCT	0.15	0.04	0.63	0.009252
China	Chen, J Zheijang Univ, 2020 RCT	2.15	0.17	26.67	0.550103
China	Huang, J Mol Cell Biol, 2020 RCT	0.37	0.01	9.98	0.550511
China	Huang, MedRxiv, 2020	0.13	0.07	0.25	0.000000
South Korea	Kim, MedRxiv, 2020	0.24	0.09	0.65	0.004518
France	Lagier, Travel Med Infect Dis, 2020	0.67	0.57	0.78	0.000000
Saudi Arabia	Shabrawishi, MedRxiv, 2020 - HCQ/CQ + AZ	1.00	0.42	2.40	1.000000
Saudi Arabia	Shabrawishi, MedRxiv, 2020 - HCQ/CQ	1.00	0.35	2.86	1.000000
Saudi Arabia	Shabrawishi, MedRxiv, 2020 - HCQ/CQ + antivirals	1.85	0.35	9.88	0.470789
China	Tang, MedRxiv, 2020 RCT	0.75	0.42	1.35	0.339175
		0.52	**0.32**	**0.84**	**0.007291**




Obesity
Upper respiratory tract infection
Lower respiratory tract infection
Chest pain
Dyspnea
Ageusia
Anosmia
Fever
Rhinitis
NEWS score ≤4
Hypertension
Diabetes
Cancer
diseases
Chronic heart diseases
Age > 74 y
NEWS score 5-6
NEWS score >7
Pulmonary CT scan severe
Von Willebrand factor ↑
Platelets ↓, Fibrinogen ↑, D-dimers ↑, Lymphocytes ↓, Zinc ↓, Troponin ↑
IgG to SARS-CoV-2 ↑
Lymphocytes ↓, Platelets ↓, Fibrinogen ↑, CRP ↑, LDH ↑, CPK ↑
Intensive care
No chest pain, no ageusia, no anosmia, no rhinitis, nodyspnea, no cough
Eosinophils ↓
Other treatments
HCQ+AZ ≥ 3 d
Evolutionary stages
Mild
Severe
Very severe (ICU, hospitalization ≥10 d, death)