ACCADEMIA NAZIONALE DEI LINCEI

Drugs for the Prevention and Treatment of COVID-19 and its Complications

Statement by the Lincei Committee on Covid-19*

Executive Summary

The COVID-19 Committee of the Lincei Academy has reviewed the evidence for the efficacy and safety of repurposed and new drugs for the prevention and treatment of COVID-19 and its complications, as well as the safety of some concomitant medications. A number of pharmacological strategies could theoretically prevent the entry of SARS-CoV-2 into target cells and are currently being evaluated for efficacy and safety. These include neutralizing antibodies against the SARS-CoV-2 spike protein, a soluble recombinant form of the SARS-CoV receptor angiotensin-

converting enzyme (ACE)2, and drugs inhibiting the activity or expression of the transmembrane protease serine 2 (TMPRSS2) required for the spike protein proteolytic cleavage. Although ACE inhibitors and angiotensin-receptor blockers (ARBs) may enhance ACE2 gene expression, an effect that would increase the availability of receptor molecules for SARS-CoV-2 entry, there is no evidence that these commonly used drugs might be harmful (or even beneficial) in patients with COVID-19. Therefore, persons with COVID-19 who are prescribed ACE inhibitors or ARBs for cardiovascular disease (or other indications) should continue these medications; moreover, these drugs are not recommended outside of the setting of a randomized clinical trial (RCT). Remdesivir was identified early as a promising therapeutic candidate for COVID-19 because of its ability to inhibit SARS-CoV-2 in vitro. A recent double-blind, placebo-controlled RCT of intravenous remdesivir in 1,063 adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement demonstrated that remdesivir was superior to placebo in shortening the time to recovery in this setting. Based on these findings the US Food and Drug Administration (FDA) has made remdesivir available under an emergency-use authorization (EUA) for the treatment of adults and children with severe COVID-19 disease. Earlier, the FDA had also issued an EUA allowing the temporary use of hydroxychloroquine (HCQ) and chloroquine (CQ) during the COVID-19 pandemic for treatment of the virus in hospitalized patients when clinical trials are not available, or participation is not feasible. This decision was largely based on mechanistic considerations and political pressure. Subsequent observational studies and a limited number of RCTs have not substantiated the clinical efficacy of these antimalarial drugs, while confirming their dose-dependent cardiac toxicity. At present, the US National Institutes of Health (NIH) COVID-19 Treatment Guidelines recommend against the use of CQ or HCQ for the treatment of COVID-19, except in a clinical trial. Early in the course of the SARS-CoV-2 pandemics, it was claimed that nonsteroidal anti-inflammatory drugs (NSAIDs), like ibuprofen, could aggravate the infection by masking its symptoms. However, after review of the evidence, the WHO and EMA advisories have been withdrawn. Therefore, until we have robust evidence, patients in chronic pain should continue to take their NSAIDs rather than turn to opiates. Complement inhibition has been proposed as a potential target in limiting tissue inflammation associated with COVID-19. The results of ongoing RCTs are needed to establish the therapeutic potential of C5 inhibition in COVID-19, and to characterize which patients may benefit the most. Finally, dysregulation of the

coagulation cascade and fibrinolytic system is emerging as an important pathophysiologic component of COVID-19. Largely based on observational studies, the International Society on Thrombosis and Haemostasis (ISTH) suggested measuring D-dimer, prothrombin time and platelet count in all COVID-19 patients. ISTH also recommends that all COVID-19 patients admitted to hospital be treated with prophylactic doses of low-molecular-weight heparin, unless contraindicated. Additional RCTs of several antithrombotic agents are currently ongoing.

1. Introduction

Coronaviruses (CoV), a group of enveloped positive-strand RNA viruses, were discovered in the 1960s and were originally thought to cause only mild disease in humans, with several strains being responsible for the common cold (1). This view changed in 2003 with the SARS (severe acute respiratory syndrome) pandemic and in 2012 with the MERS (Middle East respiratory syndrome) outbreak, two zoonotic infections that resulted in mortality rates greater than 10% and 35%, respectively (2).

At present, the newly discovered (2019) SARS-CoV-2 coronavirus continues to spread rapidly. On 30 January 2020, the WHO labelled it a public health emergency and on 25 May 2020 the total number of laboratory-confirmed COVID-19 cases stood at over 5,470,900, having spread to at least 177 countries and caused over 346,000 deaths. Given the unprecedented proportions of the pandemic in many countries, and the rise in the associated global death toll, over the past few months we have witnessed a race to find drugs/biologic treatments to save the lives of hospitalised, severely ill patients, as well as to develop vaccines. To this end, randomised clinical trials are underway to test experimental drug candidates or repurposed medicines. Therapeutic approaches to the early, mild phase of COVID-19 are also being debated and here, too, there is an emphasis on the need for randomised clinical trials. However, in times like the present, Regulatory Authorities occasionally issue emergency use authorisations (EUAs) for drugs, as the US Food and Drug

Administration (FDA) recently did for chloroquine and hydroxy-chloroquine for COVID-19. The documentation for this FDA authorisation, however, did not report or cite specific trials on which this decision was based, making it difficult to assess the scientific evidence for it. Nonetheless, physicians and healthcare providers interpreted the EUA for hydroxy-chloroquine as an instruction to incorporate this drug into therapeutic protocols for treating COVID-19 patients. However, on June 15th the FDA informed that it was revoking EUA of the two drugs, saying that they are "unlikely to be effective" and that current national treatment guidelines don't recommend using them outside of clinical trials (see below Section 5).

Indeed, it is necessary to conduct rigorous studies on COVID-19 drug candidates that will provide sufficient scientific data that can be evaluated meticulously, which will make it possible to differentiate between anecdotes and evidence. Otherwise, there is a high risk of sowing confusion among physicians caring for COVID-19 patients under these high-pressure circumstances.

Working Group 1a of the COVID-19 Committee of the Lincei Academy has prepared a brief review of the available scientific evidence about the efficacy and safety of existing and new drugs for the prevention and treatment of COVID-19 and its complications. The focus is on drugs that prevent the entry of SARS-CoV-2 into target cells, and the use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs); evidence of the benefits of the new anti-viral drug remdesivir; the debate about chloroquine and hydroxy-chloroquine; evidence of the risks/benefits of using non-steroidal anti-inflammatory drugs (NSAIDs); and whether complement inhibitors, as well as anticoagulants and other antithrombotic agents, have a place in the prevention and/or treatment of inflammatory and vascular complications of the disease.

This report does not intend to recommend any experimental drug, but to review the evidence supporting the efficacy and safety of these pharmacological treatments,

highlight the official position of health authorities and panels of experts with regard to each drug or class of drugs considered, and briefly mention the ongoing trials registered with clinicaltrials.gov or the WHO register.

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2. Drugs preventing SARS-CoV-2 entry into target cells

SARS-CoV-2 spike protein binds to its receptor, angiotensin-converting enzyme (ACE2), and is proteolytically activated by the transmembrane protease serine 2 (TMPRSS2), thus enabling the fusion of the virus with the cell membrane (1, 2). Bioinformatics uhn n ved in the cleavage of the spike protein, possibly promoting the subsequent cleavage by TMPRSS2 (4). However, furin inhibitors, unlike TMPRSS2 inhibitors, can interfere with important cell functions, thus furin is not an attractive drug target. Current approaches aimed at blocking SARS-CoV-2 cell entry are based on i) treatments inhibiting the SARS-CoV-2 spike-ACE2 interaction or ii) TMPRSS2 inhibitors.

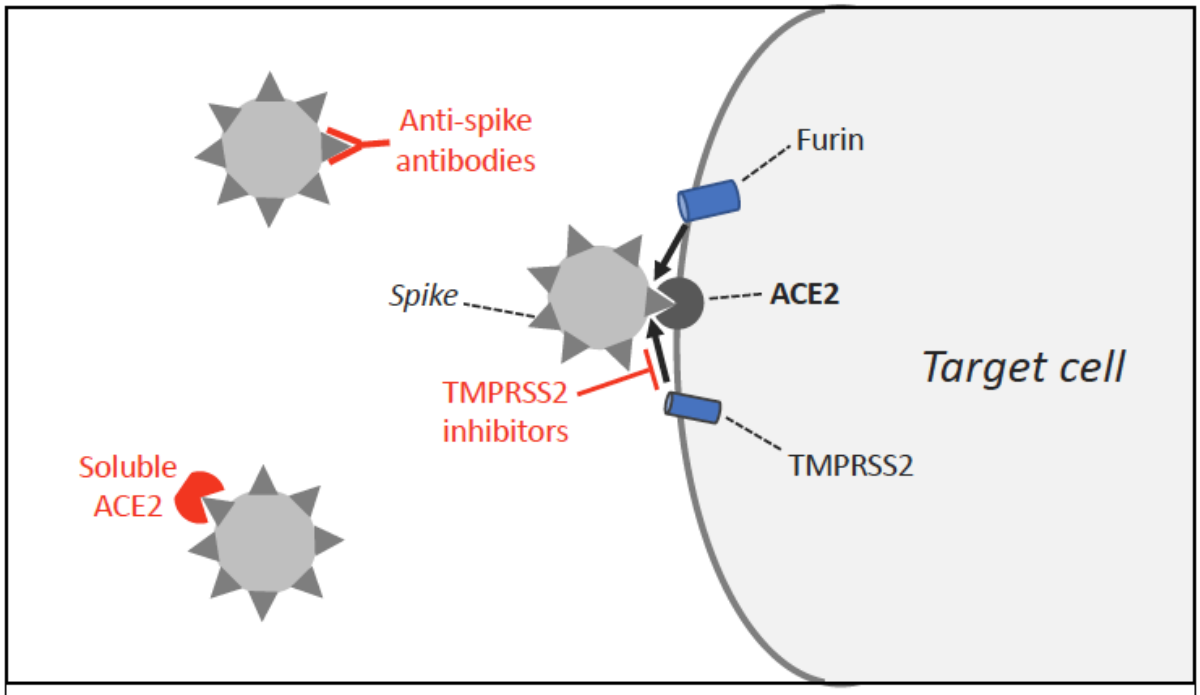
Anti-spike antibodies and soluble ACE2 can block the interaction between the virus spike protein and ACE2. Passive immunization with convalescent plasma is presently used in different countries for the therapy of COVID-19 with the view that neutralizing antibodies could both inhibit the binding of the virus to the cell and promote the clearance of the virus by immune cells. Neutralizing antibodies are thus promising candidates for prophylactic and therapeutic treatment of COVID-19. Previous experience with other viral diseases indicates that donors with high serum titers of neutralizing antibody should be identified (a proportion of those who recover from COVID-19 have low titers) and the risk related to antibody-dependent

enhancement of infection (ADE) should be considered (5). Randomized clinical trials are required to evaluate the efficacy and safety of anti-SARS-CoV-2 convalescent plasma, and at least two trials are ongoing (*EudraCT Number: 2020-001310-38; ChiCTR Number: ChiCTR2000030010*). Monoclonal antibodies against SARS-CoV-2, some of which were derived from COVID-19 patients B-cells, were found to neutralize the virus in cultured cells (6, 7) and a recent study in a transgenic mouse model bearing human ACE2 confirmed that specific monoclonal antibodies can reduce virus titers in infected lungs (8). The latter report was complemented by a detailed structural analysis of the interaction between antibody, receptor binding domain (RBD) of the spike protein and ACE2, thus providing important information for the development of vaccines and small molecule or peptide inhibitors.

Administration of a large amount of soluble ACE2 may neutralize the virus and slow viral entry into cells. Interestingly, a recombinant human soluble ACE2 (rhsACE2), corresponding to the extracellular domain of ACE2, was developed several years ago and found to be safe in healthy volunteers and in a small cohort of patients with acute respiratory distress syndrome (ARDS) in completed Phase I and Phase II clinical trials (*ClinicalTrials.gov identifier: NCT00886353*) (9, 10). This rhsACE2 was recently found to inhibit SARS-CoV-2 infection in cultured cells and in human blood vessel and kidney organoids (11) and a clinical trial has been launched to use rhsACE2 as a treatment for patients with COVID-19 (*ClinicalTrials.gov identifier: NCT04335136*).

TMPRSS2 protease inhibitors could be used to block a crucial step required for the fusion of the virus with the cell membrane. The TMPRSS2 protease inhibitor, camostat mesylate, was reported to inhibit SARS-Cov-2 entry into lung cell lines (2). This drug is approved in Japan and Korea for use in chronic pancreatitis and has been repurposed in a clinical trial for COVID-19 (*ClinicalTrials.gov number NCT04353284*). Nafamostat mesylate, another drug used for many years in Japan for

acute pancreatitis and disseminated intravascular coagulation (DIC), was recently reported to inhibit SARS-CoV-2 infection of Calu3 human lung cells in the nanomolar range, with 10-15 higher efficiency than camostat mesylate (12, 13, 14).



The entry of Sars-Cov-2 into target cells requires the binding of the spike protein present at the surface of the virus with cell membrane bound angiotensin converting enzyme 2 (ACE2), which acts as a receptor of the virus. Spike protein cleavage by the host cell proteases, transmembrane protease serine 2 (TMPRSS2) and furin, is also required for the fusion of the viral membrane with the plasma membrane of the cell. Ongoing clinical trials aim to determine whether cell entry of the virus can be prevented by 1) anti-spike antibodies, 2) soluble ACE2 extra-cellular domain or 3) inhibitors of TMPRSS2.

The efficacy of nafamostat in COVID-19 patients is presently evaluated in clinical trials (*ClinicalTrials.gov identifier: NCT04352400; Japan Registry of Clinical Trials: jRCTs031200026; Korea CRIS: KCT0005003*). It has been suggested that another TMPRSS2 inhibitor, bromhexine, presently used as mucolytic cough suppressant, could be used for the treatment of COVID-19 (15, 16). Finally, since TMPRSS2 expression is controlled by androgens, which could explain the greater frequency of severe COVID-19 in males, it is possible that androgen receptor antagonists might

reduce susceptibility to develop serious COVID-19 infection (17). This possibility is supported by epidemiological studies, showing that prostate cancer patients treated with anti-androgens are much less frequently affected by COVID-19 compared with those untreated (18). This study is supported by new results from different labs and the effect of testosterone suppression in COVID-19 patients is investigated in clinical trials, including a trial using Degarelix, a FDA-approved drug for prostate cancer (*ClinicalTrials.gov identifier: NCT04397718*) (19).

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3. Angiotensin-Converting Enzyme Inhibitors and Angiotensin Receptor Blockers

As reviewed above, human cell entry of SARS-CoV-2 depends on the SARS-CoV receptor angiotensin-converting enzyme 2 (ACE2), an enzyme that physiologically counters activation of the renin–angiotensin–aldosterone system (RAAS) (Figure) (1). Studies in animals have suggested that ACE inhibitors and angiotensin-receptor blockers (ARBs) may enhance cardiac ACE2 gene expression (2), an effect that would increase the availability of receptor molecules for SARS-CoV-2 entry (3-5). However, even if RAAS inhibitors modify ACE2 levels and/or activity in target tissue cells, clinical data are lacking to indicate whether this would in turn facilitate cellular entry of SARS-CoV-2. Despite this basic lack of knowledge, various considerations have led to the speculation that ACE inhibitors and ARBs might be harmful (or even beneficial) in patients with COVID-19 (3-5). However, there is currently no evidence in humans establishing a link between the use of these drugs with an increased risk of SARS-CoV-2 infection or disease severity. In fact, three recently published

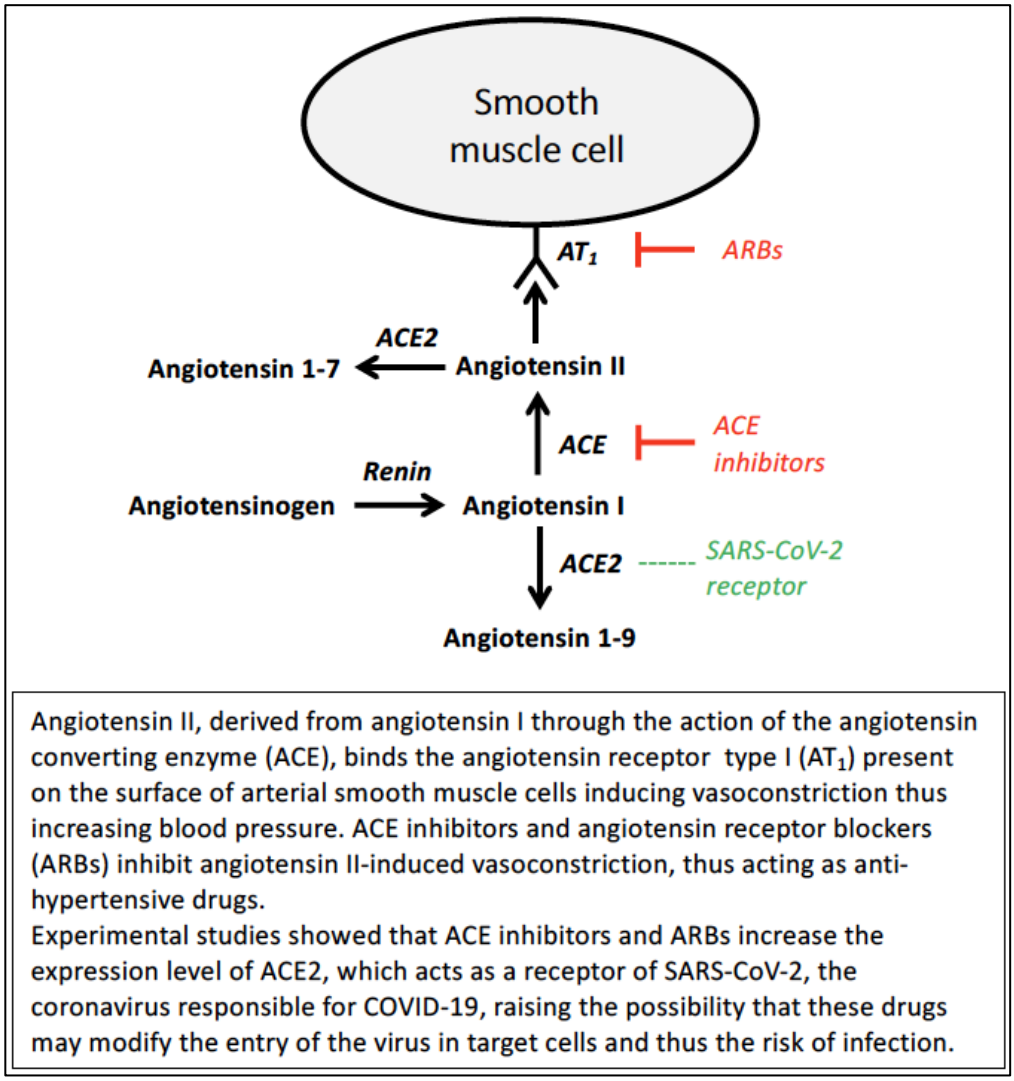
observational studies* in 27,776 COVID-19 patients, diagnosed or admitted to hospitals in different countries and continents, do not provide evidence to support the hypothesis that the use of ACE inhibitors or ARBs is independently associated with the risk of COVID-19 (6,7), the risk of severe illness among those who tested positive (7), or the risk of in-hospital death among hospitalized patients with COVID-19 (8). Observational studies have inherent limitations due to residual confounding but, despite different study designs, the main findings are consistent with each other in showing no evidence of harm with continued use of ACE inhibitors and ARBs (5-8).

It has been hypothesized that the administration of recombinant ACE2 protein may be beneficial in restoring balance to the RAAS network and potentially preventing lung injury (9). However, an investigator-initiated trial of the human recombinant ACE2 protein for the treatment of patients with COVID-19 in China (ClinicalTrials.gov number, NCT04287686) was recently withdrawn. In addition, two trials of losartan (an ARB) as a treatment for COVID-19 are currently recruiting US patients who have not previously received treatment with a RAAS inhibitor and are either hospitalized (NCT04312009) or not hospitalized (NCT04311177) (4).

In the interim, according to the recently released US National Institutes of Health (NIH) COVID-19 Treatment Guidelines, persons with COVID-19 who are prescribed ACE inhibitors or ARBs for cardiovascular disease (or other indications) should continue these medications (10). Because abrupt withdrawal of RAAS inhibitors in high-risk patients (e.g., those who have heart failure or have had myocardial infarction) may result in clinical instability and increased mortality, major institutions and medical societies, including the Centers for Disease Control and

* After completion of this report, on June 4, 2020 one of these papers (ref. 8) was retracted "Because all the authors were not granted access to the raw data and the raw data could not be made available to a third-party auditor, we are unable to validate the primary data sources underlying our article".

Prevention, the European Society of Cardiology, the American Heart Association, and the American College of Cardiology recommend continuation of ACE inhibitors or ARB medications for all patients already prescribed those medications for another indication (3-5). Furthermore, the NIH COVID-19 Treatment Guidelines Panel recommends against the use of ACE inhibitors or ARBs for the treatment of COVID-19 outside of the setting of a clinical trial (10).



Angiotensin II, derived from angiotensin I through the action of the angiotensin converting enzyme (ACE), binds the angiotensin receptor type I (AT_1) present on the surface of arterial smooth muscle cells inducing vasoconstriction thus increasing blood pressure. ACE inhibitors and angiotensin receptor blockers (ARBs) inhibit angiotensin II-induced vasoconstriction, thus acting as anti-hypertensive drugs.
Experimental studies showed that ACE inhibitors and ARBs increase the expression level of ACE2, which acts as a receptor of SARS-CoV-2, the coronavirus responsible for COVID-19, raising the possibility that these drugs may modify the entry of the virus in target cells and thus the risk of infection.

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4. Remdesivir is not a wonder drug, but....

Despite the pathogenic importance of coronaviruses, no approved antiviral drugs for the treatment of human coronavirus infections exist. However, remdesivir, a drug candidate originally developed as broad-spectrum Filovirus inhibitor (1,2), turned out to inhibit the replication of a wide range of human and animal coronaviruses in vitro (3), and to be effective in preclinical studies, including in a nonhuman primate model of MERS-CoV infection (4,5). It is therefore not surprising that when the novel highly pathogenic coronavirus SARS-CoV-2 emerged in late 2019 in Wuhan, China, causing a global health concern due to the virus strong capacity for human-to-human transmission, remdesivir was one of the first clinical candidates that has received attention.

Remdesivir and SARS-CoV-2

Remdesivir (GS-5734) was developed by Gilead Sciences and emerged from a collaboration between Gilead Sciences, the U.S. Centers for Disease Control and

Prevention (CDC) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to identify therapeutic agents for treating RNA viruses with global pandemic potential, and in particular Ebola virus (EBOV), driven by the EBOV outbreak in 2014 (2). During the recent Ebola virus epidemic in the Democratic Republic of Congo, remdesivir was included in a randomized, controlled trial of selected therapeutics in EBOV patients (NCT02818582); in midstudy primary analyses, remdesivir treatment was found inferior to antibody-based therapy with respect to mortality, and the remdesivir intervention arm was terminated (6); however, this study provided an initial insight into the safety profile of the drug.

Remdesivir is a phosphoramidate adenosine analogue prodrug that is metabolized within cells into an alanine metabolite (GS-704277), further processed into the monophosphate derivative and ultimately into the active nucleoside triphosphate derivative that can then be misintegrated into viral RNA by the viral RNA-dependent RNA polymerase (7-9).

SARS-CoV-2 is a positive-sense single-stranded RNA virus, sharing 79.6% sequence identity to SARS-CoV (10). Its replication is mediated by a multi-subunit replication/transcription machinery, containing several non-structural viral proteins (nsp). A key component of this complex is the RNA-dependent RNA polymerase (RdRp), also known as nsp12, which contains the canonical viral RdRp motifs in its C-terminal part and, employing a primer-dependent initiation mechanism, catalyzes the synthesis of viral RNA, thus playing a central role in SARS-CoV-2 replication and transcription (Introduction, ref. 1; 11). However, in order to function effectively nsp12 requires accessory factors including nsp7 and nsp8 that increase RdRp template binding and processivity (11,12). The replicase complex is quite unique to CoVs and it is used not only to transcribe full-length negative and positive strand RNAs, but also subgenomic negative strand RNAs, and a 3'-co-terminal set of nested subgenomic mRNAs (Introduction, ref. 1; 13). It is likely that additional viral nsp

subunits are necessary to carry out the full repertoire of transcription/replication activities; however, so far the nsp12-nsp7-nsp8 complex represents the minimal complex required for nucleotide polymerization (14).

Being distinct from the host transcriptional machinery, CoV RdRp is therefore considered a primary target for anti-coronavirus drugs, and has been a subject of intensive structural biology studies that provided the overall architecture of the RdRp complex (14-17). Recently the structure of the SARS-CoV-2 RdRp in complex with a template-primer RNA and remdesivir also provided critical insights into the mechanism of viral RNA replication (17). Finally, it should be noted that development of nucleoside-based therapeutics for CoV infections has been hampered by the presence of an exoribonuclease (ExoN, nsp14) that acts as a "proofreading" enzyme correcting errors in the RNA sequence, and potentially limiting the effects of analogues; however remdesivir was found to be able to evade this proofreading (9).

Remdesivir and COVID-19

Remdesivir was identified early as a promising therapeutic candidate for COVID-19 because of its ability to inhibit SARS-CoV-2 in vitro (18). Using qRT-PCR quantification of viral RNA in infected Vero E6 cells, Wang et al. found that remdesivir potently inhibits SARS-CoV-2 replication with an EC50 = 0.77 μM and a high selectivity index (SI> 129.87) (18). These findings, along with the safety profile of remdesivir in the clinical trial assessment against EBOV (6), supported the evaluation of remdesivir as a potential therapeutic drug for repurposing against the SARS-CoV-2 pandemic.

Over the past months a series of studies, recently summarized by Eastman et al. (8), have been launched to investigate the effectiveness of remdesivir, alone or in

combination with other drugs, against COVID-19, including the WHO SOLIDARITY trial, a four-arm trial comparing remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta-1a, and chloroquine or hydroxychloroquine (ISRCTN83971151), the INSERM DisCoVeRy trial (NCT04315948), and the multicenter retrospective REMDECO-19 trial (NCT04365725) in Europe; however, up to now, these studies have produced conflicting results.

On January 20, 2020, a 35-year-old man, later confirmed as the first positive case of COVID-19 in the USA, presented to urgent care clinic in Snohomish County, Washington and was given remdesivir under compassionate use access; the patient clinical condition improved the next day, but the interpretation of remdesivir impact was difficult, due to concurrent treatment with anti-inflammatory drugs and antibiotics (19). Following this first case, an early analysis of 53 people seriously ill with COVID-19 in the United States, Canada, Europe and Japan who were given remdesivir raised initial hopes, since 68% of the patients showed a clinical improvement when given the drug (20); however, the study did not include a control group (20).

No significant benefit was instead found in a randomized placebo-controlled trial of intravenous remdesivir conducted in China starting with 236 patients with COVID-19 (21); this study, though, could not exclude clinically meaningful differences following remdesivir treatment since the trial was halted early, due to the fact that the China outbreak subsided.

On April 29 Gilead Sciences released results from a study of 397 people (NCT04292899) showing that remdesivir diminishes to a modest degree the time to recovery for people hospitalized with COVID-19, raising new hopes; on the other hand, because the study lacked a control group, it was impossible to conclude with any certainty whether the drug had worked. On the same day, the US National

Institute of Allergy and Infectious Diseases (NIAID) announced preliminary results from a double-blind, randomized, placebo-controlled trial of intravenous remdesivir in 1,063 adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement (NCT04283481). A preliminary report of this study has been published on May 22nd (22) and concluded that remdesivir was superior to placebo in shortening the time to recovery in adults hospitalized with COVID-19: a median of 11 days in hospital (95% confidence interval [CI], 9 to 12), as compared with 15 days (95% CI, 13 to 19) for those given a placebo (rate ratio for recovery: 1.32; 95% CI, 1.12 to 1.55; P<0.001).

The authors indicate that the preliminary findings support the use of remdesivir for patients who are hospitalized with COVID-19 and require supplemental oxygen therapy (22). Based on these findings the Food and Drug Administration (FDA) has made remdesivir available under an emergency-use authorization for the treatment of adults and children with severe COVID-19 disease in the United States (22). However, despite the use of remdesivir, the mortality rate remained high in this study: Kaplan-Meier estimates of mortality by 14 days were 7.1% and 11.9% in the remdesivir and placebo groups, respectively, but the difference was not significant (hazard ratio for mortality: 0.70; 95% CI, 0.47 to 1.04). The Kaplan–Meier estimates of mortality by 28 days were not reported in the preliminary report, given the large number of patients that had yet to complete day 29 visits (22). These results indicate that treatment with an antiviral drug alone is not likely to be sufficient.

Future strategies are needed to evaluate whether cocktails of antiviral agents in combination with other therapeutic approaches will improve patient outcomes in COVID-19.

Finally, on April 30 the European Medicines Agency (EMA) has started a 'rolling review' of data on the use of remdesivir for the treatment of COVID-19. Although remdesivir is not yet authorized in the European Union, it is available through

clinical trials and 'compassionate use' programs through which patients can get access to unauthorized medicines in emergency situations.

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5. Is Hydroxychloroquine beneficial for COVID-19 Patients?

On March 28, 2020, the U.S. Food and Drug Administration (FDA) issued an Emergency Use Authorization (EUA) allowing the temporary use of hydroxychloroquine and chloroquine during the COVID-19 pandemic for treatment of the virus in hospitalized patients when clinical trials are not available, or participation is not feasible. However, on April 24, 2020, the FDA issued a drug safety communication cautioning against the use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems. Finally, on June 15th, based on a review of new information the FDA withdrew EUA for the two drugs, declaring that they are "unlikely to be effective".

Preliminary studies

In Peru, using the bark extracts of cinchona tree to treat malaria and babesiosis started almost 400 years ago. About 200 years ago quinine was found to be the key anti-malaria compound in the bark. The analogue of quinine, chloroquine (CQ) was made in 1934 and formally introduced into clinical practice in the United States in 1947 for the prophylactic treatment of malaria. In addition, CQ was also used to treat rheumatoid arthritis and lupus erythematosus. A safer derivative hydroxychloroquine (HCQ) was made available in 1955.

Preliminary studies have suggested HCQ may prove useful in fighting COVID-19 (1, 2). As until now no data indicate HCQ has any immunity boosting effect, here we will mainly discuss its putative anti-viral and anti-inflammatory effects. In addition,

the possible beneficial effects of HCQ/CQ on the vasculature will also be discussed (3, 4), since endothelial inflammation and endothelial cell injury have been reported in COVID-19 patients (5). CQ has anti-angiogenic, tumor vessel normalizing properties in murine models of melanoma, without inducing endothelial cell (EC) death; moreover, the EC effects induced by CQ included increased vessel barrier function, which alleviated tumor hypoxia (4). In *in vitro* assays, both CQ and HCQ have been shown to possess antiviral activity against various viruses, such as human immunodeficiency virus (HIV), hepatitis A virus, hepatitis C virus, influenza A and B viruses, influenza A H5N1 virus (6), as well as against SARS-CoV-2 (2, 8), thus suggesting that they are potentially applicable to COVID-19 patients. However, there is to date no convincing report of the *in vivo* anti-viral effects of HCQ/CQ.

HCQ exerts immunomodulatory effects

Despite widespread clinical use of CQ and HCQ in the treatment of inflammatory rheumatic diseases and virus infection, the underlying therapeutic effects and cellular mechanisms of these drugs remain largely unknown. Various modes of action have been proposed to explain the potential therapeutic and/or adverse effects of CQ and HCQ in COVID-19 patients, although most of the evidence is based on *in vitro* studies. *In vitro* experiments in tissue culture, have shown that CQ and HCQ can increase endosomal pH, prevent virus-cell fusion, and interfere with glycosylation of the ACE2 receptor and thus the binding of the SARS-CoV-2 S protein to ACE2 (9). On the other hand, others have proposed that the anti-inflammatory properties of CQ and HCQ prevent autoimmune flare-ups and organ damage in diseases like lupus (10), and hence, might have relevance to controlling SARS-CoV-2 induced immune dysregulation. It has been suggested that the anti-inflammatory effects of CQ and HCQ are based on preventing antigen processing and interrupting

molecular pathways involved in immune activation, thereby reducing pro-inflammatory cytokine secretion (11).

Various studies have shown that HCQ increases the intracellular pH and inhibits lysosomal activity in antigen presenting cells (APCs), including plasmacytoid dendritic cells (pDCs) (12, 13) and B cells (14), and also blocks major histocompatibility complex (MHC) class II-mediated antigen presentation to $CD4^+$ T cells, thus preventing their differentiation to, for example, T follicular helper cells (15) with a consequent reduction in tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-1beta (16). The potential clinical relevance of these observations is evident in lupus where HCQ can effectively reduce symptoms by suppressing pDCs pro-inflammatory cytokine secretion induced by anti-dsDNA-associated immune complexes (16, 17, 18).

Is it justified taking HCQ against COVID-19?

HCQ and CQ have not been shown to be safe and effective for treating or preventing COVID-19. They are being studied in clinical trials for COVID-19, and the FDA authorized their temporary use during the COVID-19 pandemic for treatment of the virus in hospitalized patients when clinical trials are not available, or participation is not feasible, through an EUA.

SARS-CoV-2 infection can be generally divided into three stages: asymptomatic, mild and severe, with speculatively distinct levels of activation of the immune system (19, 20). At present, little information is available on the temporal immunophenotypes across the clinical course of COVID-19. Given that there is no in vivo evidence for an antiviral effect of CQ and HCQ in COVID-19, a concern might be that if given to asymptomatic patients or early in the disease it might interfere with the development of an immune response to the virus (16, 18).

At the severe stage of SARS-CoV-2 infection, inflammation leads to tissue damage, especially in the lungs (20). At this stage, suppressing inflammation is likely to have therapeutic benefits (see below for current clinical trials). At this stage, through unknown mechanisms, large amounts of cytokines are released and the patients develop cytokine release syndrome (CRS), or cytokine storm, an uncontrolled recruitment of immune cells and production of a unique combination of cytokines often in absence of T cells. These cytokines cause a special type of acute respiratory distress syndrome (ARDS) within a very short period of time, requiring intubation and mechanical respiratory support (20). This leads to severe damage to tissues of lungs, kidneys and heart, and eventually results in a multiple organ dysfunction (21).

Owing to the absence of solid evidence at this juncture, large scale, randomized controlled trials are necessary to assess the preventive and therapeutic effects of CQ and HCQ in COVID-19.

Indeed, very recent clinical trial results have led FDA to conclude that these drugs may not be effective to treat COVID-19 and that their potential benefits for such use do not outweigh known and potential risks. This new information has induced FDA to revoke its EUA for both CQ and HCQ on June 15th, 2020.

Is HCQ more suitable than CQ in treating COVID-19?

HCQ and CQ are similar in their structure except for the addition of a hydroxyl group to the side chain and β-hydroxylation of the N-ethyl substituent. These modifications decrease HCQ toxicity while preserving its efficacy in lupus (22, 23). HCQ is administered as a sulfate, whereas CQ as a phosphate, and both of them are absorbed in the upper intestinal tract. The half-lives of CQ and HCQ are relatively long (960–1440 h) after absorption, and both drugs distribute to aqueous cellular and intercellular compartments, leading to long mean residence times (~900 h for CQ and ~1,300 h for HCQ) (24). In general, both drugs are well tolerated. However, several

common adverse effects have been reported in patients after long-term exposure to CQ and HCQ, such as gastrointestinal disorders, skin rash, retinopathy, blurred vision, cardiac toxicity and others (25). A serious toxicity effect of HCQ and CQ is retinopathy: although it is rare, sight threatening may progress even to loss of vision and it is generally irreversible (26). Clinical studies indicate that HCQ is associated with a lower risk of retinopathy than CQ, which may be due to the lower distribution volume as compared to CQ (24). The side effect of greatest concern is cardiotoxicity, including cardiomyopathy, arrhythmias, and conduction disorders which have been observed with both HCQ and CQ (27, 28). Moreover, keratopathy appears to occur more frequently in patients under treatment with CQ than with HCQ (29). In addition, CQ exerts a number of severe side effects on fetal development, while HCQ can be safely used in patients with SLE during pregnancy and breastfeeding and provides protective effect for both mother and child (30). Another important issue is to determine whether CQ and HCQ are rendered particularly toxic in COVID-19 patients.

A cautionary note

A recent observational study in 1446 consecutive patients hospitalized with Covid-19 suggests that HCQ administration was not associated with either a greatly lowered or an increased risk of the composite end point of intubation or death (31). In a retrospective multicenter cohort study on 1438 hospitalized patients with a diagnosis of COVID-19 there were no significant differences in in-hospital mortality for patients receiving HCQ + azithromycin, HCQ alone, or azithromycin alone (32). However, the interpretation of these findings may be limited by the observational design of the studies.

More recently, Boulware et al. (33) reported a randomized, double-blind, placebo-controlled trial across the United States and parts of Canada testing HCQ in 821

asymptomatic participants who had household or occupational exposure to someone with confirmed Covid-19. HCQ did not prevent illness compatible with Covid-19 or confirmed infection when used as postexposure prophylaxis within 4 days after exposure. Side effects were more common with HCQ than with placebo (40% vs. 17%), but no serious adverse reactions were reported.

Additional randomized, controlled trials of HCQ in patients with Covid-19 are clearly needed and currently ongoing. The multifaceted actions of HCQ on several vital processes, including autophagy and lysosomal function, which have been proposed to be key organ repair mechanisms essential to survive critical illness, may ultimately oppose its potential benefits. In line with this cautionary approach, the US National Institutes of Health (NIH) COVID-19 Treatment Guidelines recommends against the use of chloroquine or hydroxychloroquine for the treatment of COVID-19, except in a clinical trial (COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 11 June, 2020).

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6. Nonsteroidal anti-inflammatory drugs and COVID-19

As the incidence of COVID-19 began to accelerate in Europe, the French Health Minister, Olivier Véran, claimed that nonsteroidal anti-inflammatory drugs (NSAIDs), like ibuprofen could aggravate the infection (1). This led to an advisory on the WHO and EMA websites. However, evidence has not emerged to substantiate this claim. The advisories have been withdrawn.

NSAIDs work by suppressing prostaglandin synthases 1 and 2, colloquially known as cyclooxygenase (COX)-1 and COX-2. These enzymes produce prostaglandins (PGs), lipids that can trigger pain and fever. COX-2 produces most of the PGs relevant to pain and inflammation. NSAIDs selective for inhibiting COX-2

include celecoxib, etoricoxib and diclofenac; ibuprofen is an NSAID that blocks both COXs.

Minister Véran advised people to take paracetamol (acetaminophen) instead of NSAIDs to treat a fever (1). However, acetaminophen is an NSAID (2). The commonest oral daily dose - 1000mg - inhibits prostaglandin (PG) formation by both COX-1 and COX-2 enzymes by about 50% (3). Common daily doses of drugs like ibuprofen hit ~100% at time of peak action (3).

Acetaminophen and other NSAIDs reduce body temperature the same way - inhibition of central PGE2 dependent activation of EPr3 (4). They are also analgesic through the same mechanism, reduction of PGE2 dependent central and peripheral activation of EPrs (5). One must move up the dose response curve with NSAIDs to achieve maximal PG inhibition (as is achieved on common daily doses of other NSAIDs that inhibit both COXs like ibuprofen) to gain anti-inflammatory efficacy. Thus, at acetaminophen 3-4000mg/day, there is a similar GI (6) and hypertensive (7) adverse effect profile as with other NSAIDs.

However, acetaminophen has a particular risk of hepatotoxicity at higher doses which are avoided for that reason. The makers made a virtue of that necessity and marketed acetaminophen as an anti-pyretic, analgesic. They claimed that it was not an NSAID because it did not cause GI toxicity. At that time (before the discovery of COX-2) all NSAIDs competed in direct to consumer advertising in the US by claiming a safer GI profile. So, the myth that acetaminophen was not a NSAID was marketed and widely believed. Like other NSAIDs, acetaminophen has PG independent effects of unestablished relevance to their clinical profile. Most commonly, we also use aspirin, another NSAID, at doses that are not anti-inflammatory and take advantage of its particular action at low doses on the platelet, thereby minimizing its GI toxicity.

Membrane sphingolipids (8) and membrane cholesterol (9) modulate viral entry into cells. Furthermore, activation of phospholipases by viral attachment to its cellular receptors releases many bioactive lipids, including PGs, such as PGE2, PGD2, and prostacyclin (PGI2) can both promote and restrain inflammation. For example, the infection of certain immune cells (microglia) with a related coronavirus (not the one that causes COVID-19) activates a proinflammatory response (the inflammasome) to combat the pathogen; however, PGD2 increases the expression of PYDC3, a putative inflammasome inhibitor, in certain immune cells in mice (10). The SARS coronavirus responsible for the 2003 outbreak directly binds to the COX-2 promotor and increases its expression (11), boosting PG production capacity, and there is also evidence that PGE2 inhibits SARS coronavirus replication (12). Indomethacin, an NSAID, blocks coronavirus RNA synthesis, but independently of COX inhibition (13). By contrast, COX-2–dependent PGE2 attenuates the chronic antiviral lymphocyte response of unresolved viral infection (14). Thus, based on these findings, multiple contrasting possibilities are plausible, but evidence has yet to emerge of the relevance of these observations to the course or treatment of COVID-19. Some trials using NSAIDs have begun; for example, there is an open label study using indomethacin together with hydroxychloroquine and Zithromax in subjects with mild symptoms of COVID-19 (NCT 04344457).

Patterns of individual PG formation may turn out to reflect the intensity of disease and forecast its course but also signal the opportunity to intervene with potentially preventative therapies before patients progress to severe disease. For example, microangiopathy and hemostatic activation is a feature severe COVID-19 and roughly 30% of our patients have elevated d-dimers on hospitalization. As just one example, thromboxane (Tx) biosynthesis is markedly elevated in the acute respiratory distress syndrome (ARDS) and our preclinical studies have shown that Tx receptor (TPr) antagonism prevents evolution of a lipopolysaccharide (LPS)

induced syndrome of ARDS in sheep (15). Unlike NSAIDs that suppress the vasodilator PGs that maintain renal blood flow (RBF) in syndromes such as ARDS, TPr antagonism would be expected to sustain RBF even in renoprival syndromes such as ARDS where NSAIDs are precluded (16). Thus, serial analysis of PGs in patients with COVID-19 might suggest that modulation of individual PGs be considered for therapeutic intervention or to be biomarkers predictive of disease progression.

Summary

So, if there is no clear evidence of risk from NSAIDs, should patients with clinically complicated SARS-CoV-2 infections be administered them as a treatment? No. There is no evidence of benefit either. If such a patient were also to have poor kidney function, maintenance of renal blood flow becomes critically dependent on vasodilator PGs, such as PGE2 and PGI2 (16). Such a situation might also predispose the patient to the gastrointestinal and cardiovascular complications of NSAIDs. However, until we have robust evidence, patients in chronic pain should continue to take their NSAIDs rather than turn to opiates. Given that the elderly comprises an at-risk group for severe COVID-19, an association between NSAIDs and the disease may merely reflect reverse causality.

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7. Complement inhibitors

Pathophysiologic and pharmacologic rationale

The complement system is the host immune system's first response to clear pathogens (1) and evidence is accumulating that SARS-CoV-2 activates complement

(2,3,4). However, unrestrained complement activation contributes to acute and chronic inflammation, intravascular coagulation and cell injury and ultimately leads to multiple organ failure and death (1).

In a pre-print manuscript (2), an immunohistochemistry analysis of lung tissue from patients who died of COVID-19 revealed strong staining for the complement components mannose-binding lectin (MBL), C4, C3 and the terminal membrane attack complex C5b-9, in alveolar epithelial cells, as well as inflammatory cells, and some pneumocytes. In the same manuscript, the authors also described increased serum C5a levels in COVID-19 patients, particularly in severe cases. In another online report (5), an analysis of autoptic kidney tissues from 6 patients revealed strong C5b-9 deposition on tubules in all six cases and low levels of glomerular C5b-9 deposits in two of these, demonstrating that viral infection induced complement activation in the kidney as well, which could contribute to tissue injury and organ dysfunction. Collectively, these results indicate that complement is strongly activated in the lungs, in the circulation, as well as in the kidneys of COVID-19 patients.

Clinical evidence of potential efficacy in COVID-19 patients and registered clinical trials

Complement inhibition is being proposed as a potential target in limiting tissue inflammation associated with COVID-19 (6,7) but great care should be taken in choosing between the drugs that are currently available or in advanced clinical development (4, 8). Timing is also relevant in that shutting down complement activation components that restrict viral propagation may be harmful while preventing uncontrolled activation is desirable (9). In this context, C3 inhibitors do not appear to be a safe option for COVID-19 patients, since these drugs will prevent the activation of all 3 complement pathways in response to viral infections. Studies

to establish the relationship between the protective role of the lectin complement pathway in virus clearance vs. its potential pathogenetic roles in sustaining inflammatory response and tissue injury will be instrumental in the perspective of clinical studies with specific inhibitors such as anti-MASP2 antibodies (8), in COVID-19 patients.

On the other hand, C5 inhibitors could exert a favorable effect by blocking the proinflammatory and prothrombotic actions of the terminal products of the complement cascade (C5a and C5b-9) activated by SARS-CoV-2, while preserving the activity of early complement components that are important for viral clearance and activation of the adaptive immune response (1, 4, 10).

Complement C5 inhibition with eculizumab has been shown to be an effective therapeutic tool in thrombotic, hematological and inflammatory diseases (11). In particular, eculizumab blocked venous thromboembolic events in paroxysmal nocturnal hemoglobinuria and thrombotic microangiopathy in atypical hemolytic uremic syndrome (11). Thus, C5 inhibition could protect from COVID-19 associated vasculopathy as supported by a recent study (12) showing prominent deposition of C5b-9 within the microvasculature as well as in larger caliber vessels of the lung parenchyma, and in the microvasculature of the skin of COVID-19 patients (12).

Based on this background, Piero Ruggenenti and Giuseppe Remuzzi activated two compassionate use protocols for an expanded access programme to C5 blockade with eculizumab therapy in patients with mild/moderate (in need of high-flow nasal oxygenation) or advanced (in need of continuous positive airway pressure ventilation) COVID-19 pneumonia, with the objective of stopping complement-mediated lung damage and preventing thromboembolic events. In addition, three clinical trials have been registered for off-label compassionate use of eculizumab for the treatment of patients with COVID-19 (13,14,15). A preliminary update is

available online about a critically ill COVID-19 case who was enrolled in one of the above trials (13), and clinically improved after eculizumab treatment (9,16).

A preliminary report published recently describes four COVID-19 patients with pneumonia requiring oxygen supplementation who were treated off-label with eculizumab (17). The inflammatory markers dropped after eculizumab and the patients recovered in a mean of 13 days. However, the clinical course of the patients is poorly described, and the study is limited by a lack of comparison with COVID-19 controls with the same disease severity and taking the same medications, including anticoagulant and antiviral therapies, hydroxychloroquine and antibiotics, which could have demonstrated the add-on value of eculizumab in clinical outcomes.

The results of ongoing controlled studies are needed to establish the therapeutic potential of C5 inhibition in COVID-19, and to discover which patients may benefit the most.

Another therapeutic option could be avacopan, an orally-administered C5aR inhibitor that, in a randomized clinical trial in ANCA-associated vasculitis, was effective in replacing high-dose glucocorticoids (18). To the best of our knowledge no clinical trials involving avacopan for COVID-19 patients have been registered yet**.** However, a double-blind randomized study with the anti-C5aR antibody avdoralimab in patients with COVID-19 severe pneumonia has been recently initiated in France (19). Studies in a murine genetic model that develops both venous thromboembolism and renal and ocular thrombotic microangiopathy suggested that C5a mediates systemic thrombophilia, whereas microvascular injury depends on C5b-9 (20). In this model C5 inhibition rescued both phenotypes. Comparative studies with anti-C5 vs. anti-C5a drugs are required to evaluate their relative safety/efficacy advantages in COVID-19 patients (9).

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8. Anticoagulants and other antithrombotic agents

8a. Anticoagulant agents

Pathophysiologic and pharmacologic rationale

Dysregulation of the coagulation cascade and fibrinolytic systems are emerging as an important issue in patients with COVID-19 (1). In a recent Dutch report on severely ill patients hospitalized in intensive care units (ICUs), 31% experienced thrombotic complications (2). This finding is in line with a study showing that in Irish patients admitted to hospital with severe COVID-19 infection abnormal blood clotting occurred, causing micro-clots within the lung (3). These patients had a significantly worse prognosis and were more likely to require ICU admission (3). Similarly, a retrospective study of 191 adult COVID-19 patients in Wuhan, China, found that blood levels of D-dimer - a marker of coagulation activation - greater than 1 μg/ml on admission significantly increased the risk for in-hospital mortality (odds ratio 18.42, 95% CI 2.64-128.55; P=0.0033), even though the wide 95% confidence interval highlights the statistical uncertainty of the estimate (4). Others have reported that, compared to COVID-19 survivors, non-survivors had higher blood levels of D-dimer and longer prothrombin time on admission, and lower fibrinogen blood concentration 10 to 14 days after hospitalization, indicating a state of hyper-coagulability (5). Furthermore, in the autopsies of the first patients who died of COVID-19, microthrombi were found not only in the lungs, but also in other organs, including the liver, kidneys and heart (6,7). In addition, fibrin deposits are found in the lung parenchyma of patients with SARS-CoV-2 and acute respiratory distress

syndrome (ARDS) (8). Indeed, COVID-19 not only causes hypercoagulability, but also affects fibrinolysis. In this regard, a retrospective study showed that D-dimer levels higher than 2.6 μg/ml combined with lack of clot lysis at 30 minutes on thromboelastography reflected complete fibrinolysis shutdown, and markedly increased the risk of kidney failure and thromboembolic complications in critically ill patients with COVID-19 (9). These thrombotic/thromboembolic events are promoted by the inflammatory process underlying viral infections such as SARS-CoV-2. In these patients, inflammation induces excessive production of thrombin and a reduction in fibrinolysis caused by endothelial dysfunction due to the ongoing viral infection (10). Moreover, the hypoxia that characterizes SARS-CoV-2 infection also contributes to thrombosis by enhancing blood viscosity (10).

Heparin is a glycosaminoglycan with anticoagulant activity produced by basophils and mast cells in all mammals. It activates antithrombin III which, in turn, inhibits thrombin (Factor II), Factor X and other proteases involved in the blood coagulation cascade (11). Heparin and low-molecular-weight (LMW) heparins (derived from unfractionated heparin by depolymerization) are commonly used prophylactically to prevent post-surgical venous thromboembolism, as well as in non-surgical patients with heart failure or acute respiratory failure, conditions characterized by reduced mobility. They are also used in the pharmacological treatment of deep vein thrombosis, pulmonary embolism and acute coronary syndromes.

Preclinical evidence of efficacy

Heparin also displays anti-inflammatory properties which could be valuable in the context of COVID-19. According to the immune-thrombosis model, the formation of thrombi inside blood vessels, in particular in microvessels, induces an innate immune response (12). Thus, blocking thrombin by means of heparin may dampen the inflammatory response. Heparin elicits anti-inflammatory functions also through

mechanisms independent of its anticoagulant activity, which include binding to inflammatory cytokines, inhibiting neutrophil chemotaxis and leukocyte migration, neutralizing the positively charged peptide complement factor C5a, and sequestering acute phase proteins (13). In an animal model of acute lung injury, treatment with nebulized heparin reduced injury-mediated coagulation factors and inflammation in the alveolar space, without affecting systemic coagulation (14).

Heparin appears to protect the vascular endothelium. Apart from pathogens, histones released from damaged cells can also cause endothelial injury (15). Heparin can antagonize histones thereby protecting endothelial cells (16). This protective function seems to extend to the endothelial tight junctions as demonstrated in a sepsis animal model, where unfractionated heparin reduced lung edema and vascular leakage (17).

Finally, experimental evidence suggests an antiviral potential for heparin. Indeed, heparin structure highly resembles heparan sulfate, a linear polyanionic polysaccharide used by a large number of human viruses, including coronaviruses, for attachment to target cells (18). A recent online paper has used spectroscopic techniques along with molecular modeling to show that the SARS-CoV-2 Spike S1 protein receptor binding domain interacts with heparin (19). This observation raises the intriguing possibility that heparin could compete with heparan sulfate for binding to SARS-CoV-2, thereby preventing virus entry into cells. Nevertheless, this hypothesis remains to be demonstrated (Figure).

Clinical evidence of efficacy in patients with COVID-19

A retrospective study in Wuhan on 449 hospitalized COVID-19 patients with severe pneumonia, 99 of whom received prophylactic doses of heparin (mainly LMW heparin) for 7 days or longer, showed that among patients with markedly elevated D-dimer (> 6-fold of the upper limit of normal) or sepsis-induced coagulopathy (SIC)

criteria > 4, the 28-day mortality was significantly lower in heparin users than in non-users (20). Similarly, a retrospective analysis of 395 COVID-19 patients hospitalized within the Mount Sinai Health System in New York and requiring mechanical ventilation, found that in-hospital mortality was lower in those receiving systemic anticoagulation than in those who did not receive the treatment (29% versus 63%, respectively) (21). Moreover, a pre-print non-peer-reviewed evidence indicates that LMW heparin therapy in COVID-19 patients improved coagulation dysfunction (22). Based on available information, the International Society on Thrombosis and Haemostasis (ISTH) suggested measuring D-dimer, prothrombin time and platelet count in all patients who present with COVID-19 infection. ISTH has also recommended that all COVID-19 patients admitted to hospital be treated with prophylactic doses of LMW heparin, unless contraindicated (e.g., active bleeding or platelet count $<25 \times 10^9$/L) (23). Likewise, the American Society of Hematology stated that all hospitalized patients with COVID-19 should receive pharmacological thromboprophylaxis with LMW heparin, unless they are judged to be at increased bleeding risk (24). On 11th April 2020 the Italian drug agency (AIFA) included LMW heparin among the drugs available for the treatment of COVID-19 patients (25). In particular, in the initial phase of the disease, when patients present with pneumonia and are bedridden, LMW heparin at prophylactic doses is recommended to prevent venous thromboembolism. In the more advanced stage, in hospitalized patients with thrombotic events, LMW heparin is recommended at therapeutic doses.

Several issues remain to be addressed to establish the optimal anticoagulant approach in patients with COVID-19, such as the proper time of treatment start, dosage and administration schedule of drugs.

Ongoing clinical trials registered on www.clinicaltrials.gov

At present, several clinical trials and observational studies registered on www.clinicaltrials.gov are testing the efficacy and safety of LMW heparin or unfractionated heparin in patients with COVID-19. In particular, 11 clinical trials are evaluating LMW heparin or unfractionated heparin (n=10; ClinicalTrials.gov identifiers: NCT04397510, NCT04344756, NCT04372589, NCT04345848, NCT04367831, NCT04377997, NCT04373707, NCT04366960, NCT04362085, NCT04359277) or dociparstat (a glycosaminoglycan derived from porcine heparin, n=1; ClinicalTrials.gov identifier: NCT04389840), while 2 observational studies are exploring low molecular weight heparin (ClinicalTrials.gov identifiers: NCT04393805, NCT04359212). Two additional randomized controlled trials registered on Chinese Clinical Trial Registry (ChiCTR) are exploring the efficacy and safety of intravenous enoxaparin in the treatment of hospitalized adult patients with COVID-19 (ChiCTR2000030700; ChiCTR2000030701). Moreover, on 22th April 2020 AIFA authorized the INHINACOVID study (EudraCT Number: 2020-001308-40), a phase 2 multicenter clinical trial aimed to assess the efficacy and safety of enoxaparin in hospitalized patients with moderate to severe COVID-19.

8b. Other antithrombotic agents

Pathophysiologic and pharmacologic rationale

Dipyridamole is an antiplatelet agent and acts as a phosphodiesterase inhibitor, eventually increasing intracellular cAMP and cGMP (26). Apart from the well-known antiplatelet function, dipyridamole may provide potential beneficial effects in patients with COVID-19. First, experimental and clinical evidence indicated that dipyridamole has a broad spectrum of antiviral activity (27,28), particularly against positive-stranded RNA viruses (29). Second, it suppresses inflammation and

promotes mucosal healing, as demonstrated in pediatric patients with colitis or inflammatory bowel disease (30). Third, as a pan-phosphodiesterase inhibitor, dipyridamole may prevent acute injury and progressive fibrosis of the lung, heart, liver and kidney (31).

Preclinical evidence of efficacy

In silico and *in vitro* evidence has shown that dipyridamole exhibits direct antiviral effects by binding and neutralizing the SARS-CoV-2 protease Mpro (32,33). Interestingly, dipyridamole was found to suppress SARS-CoV-2 replication in vitro at concentrations comparable to those reported in the blood of patients treated with this medication after ischemic stroke (32). These data suggest that the dosages of dipyridamole used to inhibit platelet aggregation could potentially suppress SARS-CoV-2 replication in infected patients.

Clinical evidence of efficacy in patients with COVID-19

Emerging evidence points to beneficial effects of dipyridamole adjunctive therapy in patients with COVID-19. In an open-label clinical trial involving 31 hospitalized patients with COVID-19, dipyridamole treatment for two weeks (150 mg/day) blunted the progressive increase in D-dimer levels, increased lymphocyte and platelet recovery, and markedly improved clinical outcomes compared to control patients (32,33).

However, to date available data are too scanty to support the use of dipyridamole in hospitalized COVID-19 patients. Beside this, it would be also useful to design trials in preventive mode, for example with antiplatelet drugs with good therapeutic ratios, to intercept COVID-19 patients with mild illness at home, before hospitalization.

Ongoing clinical trials registered on www.clinicaltrials.gov

At present 5 clinical trials registered on www.clinicaltrials.gov are investigating the efficacy and safety of antithrombotic agents other than heparin in patients with COVID-19, that is dipyridamole (n=1; ClinicalTrials.gov Identifier: NCT04391179), rivaroxaban (a Factor X inhibitor, n=2; ClinicalTrials.gov Identifiers: NCT04394377, NCT04333407) and defibrotide (n=2; ClinicalTrials.gov Identifiers: NCT04348383, NCT04335201).

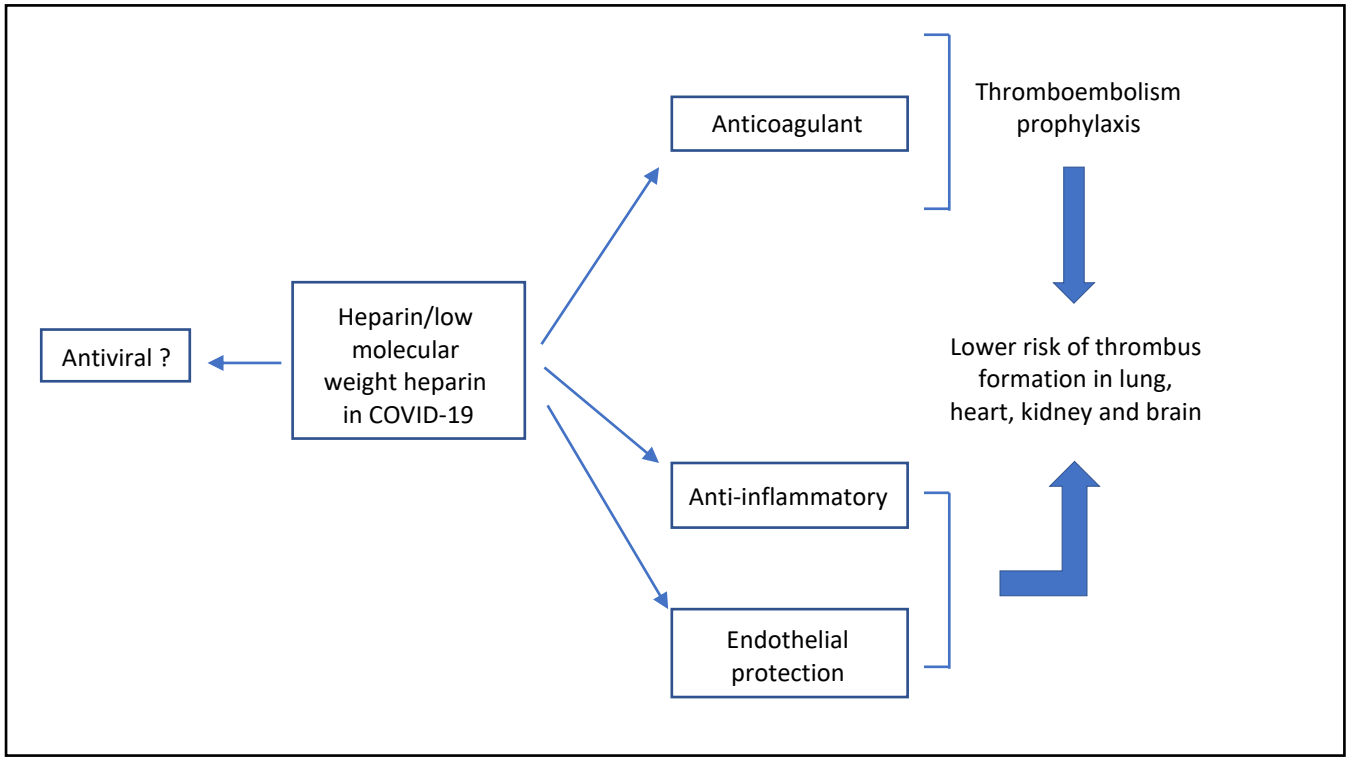

Figure. Possible effects of heparin and low molecular weight heparin in COVID-19

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Note added in proofs: According to a press release of 16th June by investigators at the University of Oxford, dexamethasone -a synthetic glucocorticoid with broad anti-inflammatory properties- was associated with reduced mortality among patients with severe COVID-19. Over 6,000 hospitalized patients were randomized to receive either dexamethasone (6 mg daily) or usual care for 10 days. Dexamethasone was associated with significantly lower risk for death among ventilated patients (rate ratio [RR], 0.65) and other patients receiving oxygen (RR, 0.80), compared with those given usual care. No benefit was found in patients who did not require respiratory support. The results have not been published in a scientific journal yet, and the present document will be updated upon publication of the full paper.

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* Responsibility for the information and views expressed in this document lies solely with the Covid-19 Committee