ACCADEMIA NAZIONALE DEI LINCEI

Drugs for the Prevention and Treatment of COVID-19 and its Complications: Fall 2020 Report

Statement by the Lincei Committee on Covid-19[i]

1. 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 (May 1st), followed by approval for use in adults and pediatric patients requiring hospitalization (October 22nd). 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 C3 or 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. The benefit of corticosteroids in the treatment of COVID-19 has been established in large clinical trials in hospitalized critically ill patients, showing a significant reduction of mortality as compared to those allocated to usual care. The usefulness of dexamethasone in patients with severe pulmonary complications of COVID-19 infection has been confirmed by a recent WHO meta-analysis.

2. 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 date our first Report was issued) 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. Today (November 22nd) we dismayingly count 58,287,853 cases and 1,382,835 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); 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; and the place of corticosteroids in the treatment of critically ill patients with severe pulmonary complications of SARS-CoV-2 infection.

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.

References

1. J. Cui, F. Li & Z. Shi. Origin and evolution of pathogenic coronaviruses. Nature Rev. Microbiol. 2019, 17:181-192.
2. T.S. Fung & D.X. Liu. Human Coronavirus: host-pathogen interaction. Annu. Rev. Microbiol. 2019, 73:529-557.

3. 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 analyses based on protein structures predict that transmembrane dipeptidyl peptidase-4 (DPP4), which is the receptor for MERS-CoV, could also interact with SARS-CoV-2 (3), however DPP4 was unable to mediate virus entry in cells lacking ACE2 (1). Another tissue protease, the proprotein convertase furin, is involved 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.

An alternative approach to block the interaction between the SARS-CoV-2 and the ACE2 receptor is the use of picomolar miniprotein inhibitors that bind with high affinity to the SARS-CoV-2 spike protein and compete with ACE2 binding. Two of these recently designed inhibitors were found to prevent infection in cultured cells more efficiently than the most potent monoclonal antibodies described to date (9).

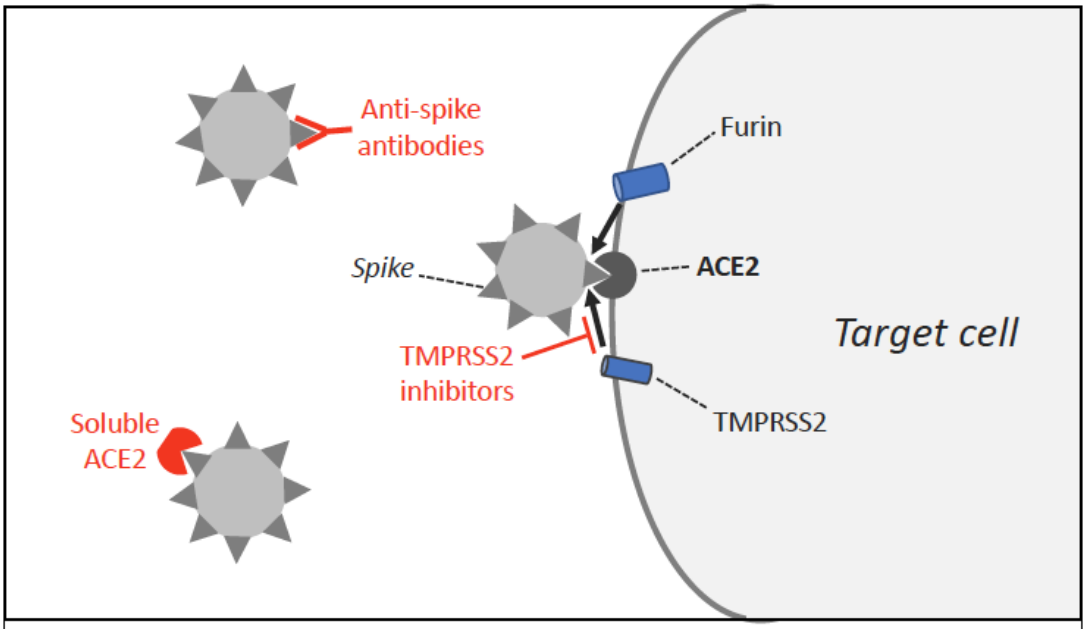
Administration of a large amount of soluble ACE2 may neutralize the virus and slow viral entry into cells. Interestingly, a recombinant human soluble ACE2 (rhsACE2), corresponding to the extracellular domain of ACE2, was developed several years ago and found to be safe in healthy volunteers and in a small cohort of patients with acute respiratory distress syndrome (ARDS) in completed Phase I and Phase II clinical trials (*ClinicalTrials.gov identifier: NCT00886353*) (10,11). This rhsACE2 was recently found to inhibit SARS-CoV-2 infection in cultured cells and in human blood vessel and kidney organoids (12) 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 (13, 14, 15). 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 (16, 17). 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 (18). 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 (19). 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*) (20).

References

1. Zhou P et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273.
2. Hoffmann M et al. SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020; 181:271-280.e8.
3. Li Y et al. The MERS-CoV receptor DPP4 as a candidate binding target of the SARS-CoV-2 spike. iScience. 2020 May 13:101160.
4. Hoffmann M et al. A Multibasic Cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. Mol Cell. 2020; 78:1-6.
5. Casadevall A & Pirofski L-A. The convalescent sera option for containing COVID-19. J Clin Invest. 2020; 130:1545.
6. Wang C et al. A human monoclonal antibody blocking SARS-CoV-2 infection. Nat Commun 2020; 11:2251.
7. Andreano E et al. Identification of neutralizing human monoclonal antibodies from Italian Covid-19 convalescent patients. BioRxiv 2020. https://doi.org/10.1101/2020.05.05.078154.
8. Wu Y et al. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science 2020:eabc2241. DOI: 10.1126/science.abc2241
9. Cao L et al. De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. Science 2020; 10.1126/science.abd9909
10. Haschke M et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. Clin Pharmacokinet. 2013; 52:783-792.
11. Khan A et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Crit Care. 2017; 21:234.
12. Monteil et al, Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell. 2020; 181:905-913.
13. Yamamoto M et al. The anticoagulant nafamostat potently inhibits SARS-CoV-2 infection in vitro: an existing drug with multiple possible therapeutic effects. BioRxiv 2020. https://doi.org/10.1101/2020.04.22.054981
14. Hoffmann M et al. Nafamostat mesylate blocks activation of SARS-CoV-2: New treatment option for COVID-19. Antimicrob. Agents Chemother. 2020. doi:10.1128/AAC.00754-20.
15. Ko M et al. Comparative analysis of antiviral efficacy of FDA-approved drugs against SARS-CoV-2 in human lung cells: Nafamostat is the most potent antiviral drug candidate. BioRxiv 2020. https://doi.org/10.1101/2020.05.12.090035
16. Maggio R, Corsini GU. Repurposing the mucolytic cough suppressant and TMPRSS2 protease inhibitor bromhexine for the prevention and management of SARS-CoV-2 infection. Pharmacol Res. 2020; 157:104837.
17. Habtemariam S et al. Possible use of the mucolytic drug, bromhexine hydrochloride, as a prophylactic agent against SARS-CoV-2 infection based on its action on the Transmembrane Serine Protease 2. Pharmacol Res. 2020; 157:104853.

18. Stopsack KH et al, TMPRSS2 and COVID-19: Serendipity or opportunity for intervention? Cancer Discovery, 2020, April 10. DOI: 10.1158/2159-8290.CD-20-0451.
19. Montopoli M et al, Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study (n=4532). Ann Oncol, 2020. https://doi.org/10.1016/j.annonc.2020.04.479
20. Wadman M. Why coronavirus hits men harder: sex hormones offer clues. Science 3 June, 2020. doi:10.1126/science.abd1288



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.

4. 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).

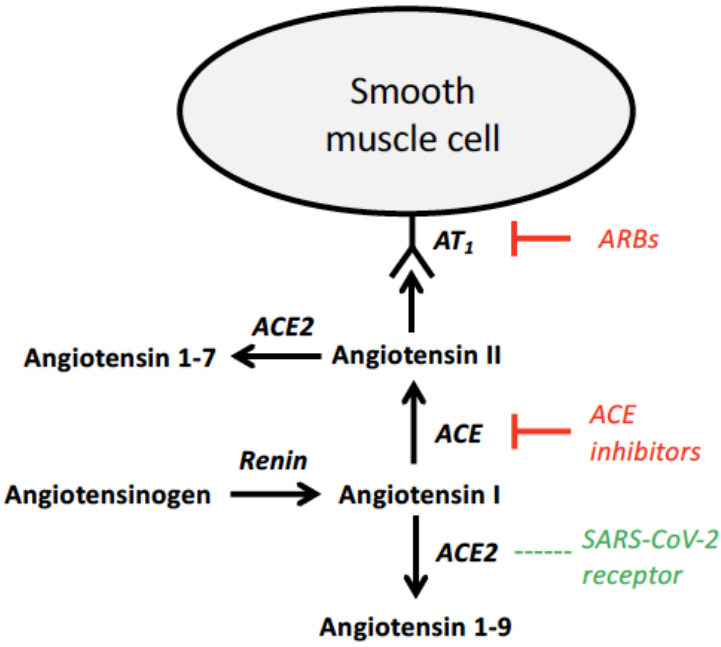
In the interim, according to the recently released US National Institutes of Health (NIH) COVID-19 Treatment Guidelines, persons with COVID-19 who are prescribed ACE inhibitors or ARBs for cardiovascular disease (or other indications) should continue these medications (10). Because abrupt withdrawal 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 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).

References

1. Hoffmann M et al. SARS- CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271-80.
2. Ferrario CM et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 2005;111:2605-10.
3. Sanders JM et al. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19). A Review. JAMA 2020;323:1824-36.
4. Vaduganathan M et al. Renin–Angiotensin–Aldosterone System Inhibitors in Patients with Covid-19. N Engl J Med 2020;382:1653-9.

5. Jarcho JA et al. Inhibitors of the Renin–Angiotensin–Aldosterone System and Covid-19. N Engl J Med 2020;382:2462-4.
6. Mancia G et al. Renin–angiotensin–aldosterone system blockers and the risk of Covid-19. N Engl J Med 2020;382:2431-40.
7. Reynolds HR et al. Renin–angiotensin–aldosterone system inhibitors and risk of Covid-19. N Engl J Med 2020;382:2441-8.
8. Mehra MR et al. Cardiovascular disease, drug therapy, and mortality in Covid-19. N Engl J Med 2020;382:e102.
9. Alhenc-Gelas F and Drueke TB. Blockade of SARS-CoV-2 infection by recombinant soluble ACE2. Kidney Int 2020;97:1091-3
10. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 30 September, 2020.

* 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".



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.

5. Remdesivir

Remdesivir, an adenosine analog prodrug originally developed for the treatment of Ebola virus (EBOV), was found to inhibit the replication of human and animal coronaviruses *in-vitro* and in preclinical studies (1). Upon diffusion into the cell, remdesivir is metabolized into the nucleoside monophosphate form and ultimately into the active nucleoside triphosphate derivative, which is misintegrated into viral RNA by the viral RNA-dependent RNA polymerase, resulting in chain termination (1).

Remdesivir was identified early as a promising therapeutic candidate for COVID-19 because of its ability to inhibit SARS-CoV-2 in different types of cells, including primary human airway epithelial cells (2,3). In addition to *in-vitro* studies, remdesivir treatment initiated early during infection had a clinical benefit in a rhesus macaque model of SARS-CoV-2 infection, and reduced both pulmonary infiltrates and virus titers in bronchoalveolar lavages (4).

These findings, along with the safety profile of remdesivir in the clinical trial assessment against EBOV (5), supported the evaluation of remdesivir as a potential therapeutic drug for repurposing against the SARS-CoV-2 pandemic.
Reports of clinical improvement of a limited amount of patients seriously ill with COVID-19 treated with remdesivir under compassionate use access raised initial hopes (6,7). Over the past months a large number of studies have been launched to investigate the effectiveness of remdesivir, alone or in combination with other drugs, against COVID-19 (see *ClinicalTrials.gov*); however, these studies have produced conflicting results.
No significant benefit was found in a randomized placebo-controlled trial of intravenous remdesivir conducted in China starting with 236 patients with COVID-19 (8); 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 the US National Institute of Allergy and Infectious Diseases (NIAID) announced preliminary results from the Adaptive COVID-19 Treatment Trial (ACTT-1, NCT04280705), a double-blind, randomized, placebo-controlled phase 3 trial to evaluate the safety and efficacy of remdesivir compared with a placebo-control in 1,062 adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement. The final report of the study, published on November 5 (9), concluded that remdesivir was superior to placebo in shortening the time to recovery in patients: a median of 10 days in hospital (95% confidence interval [CI], 9 to 11), as compared with 15 days (95% CI, 13 to 18) for those assigned to the placebo group (rate ratio for recovery: 1.29; 95% CI, 1.12 to 1.49; P<0.001). A different open-label, randomized multi-center clinical trial (NCT04292899) comparing two remdesivir courses in patients with severe COVID-19 not requiring mechanical ventilation at baseline found that the outcomes of 5-day and 10-day regimens of remdesivir were not significantly different (10).

Based on these findings, on May 1 2020 remdesivir (VEKLURY®) was made available in the US under an emergency-use authorization (EUA) for the treatment of adults and children with severe COVID-19 disease by the FDA (11), followed by the drug's authorization in the EU (*https://www.ema.europa.eu/en/medicines/human/EPAR/veklury#authorisation-details-section*).
On October 22 2020, the FDA has approved VEKLURY for use in adults and pediatric patients (12-years and older) requiring hospitalization (12).

However, the mortality rate recorded in the ACTT-1 study, even if lower in the patients treated with

remdesivir, remained high: Kaplan-Meier estimates of mortality at day 15 after enrollment were 6.7% with remdesivir and 11.9% with placebo (hazard ratio, 0.55; 95% CI, 0.36 to 0.83); at day 29 they were 11.4% and 15.2% in the two groups respectively (hazard ratio, 0.73; 95% CI, 0.52 to 1.03). The differences in mortality between the two groups varied considerably according to baseline severity (9).

Encouraging results relative to remdesivir-treated patients' survival, including a comparative analysis of the Phase 3 SIMPLE-Severe trial and a real-world retrospective cohort of patients with severe COVID-19 (NCT04292899 and EUPAS34303) were published recently (13). In this analysis, by day 14, remdesivir was associated with both an improvement in clinical recovery, and a 62% reduction in the risk of mortality compared with standard-of-care treatment [7.6% remdesivir *vs* 12.5% standard-of-care (adjusted odds ratio 0.38, 95% CI: 0.22–0.68, p=0.001)]. On the other hand, remdesivir was recently reported to be less effective in patients hospitalized with moderate COVID-19 pneumonia in an open-label multinational study (NCT04292730) (14).

Finally on October 15, 2020, the interim results of the WHO SOLIDARITY trial, a four-arm trial comparing remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta-1a, and chloroquine or hydroxychloroquine (ISRCTN83971151/NCT04315948), were made public in preprint form (15). They described a global, open-label, multicentric randomized trial on 11,266 adults, with 2750 allocated remdesivir and 4088 no study drug. The study concluded that none of the four drugs produced any measurable benefit in mortality or disease course. In the case of remdesivir, the study concluded that intravenous remdesivir had little or no effect on duration of hospital stay (the proportion still hospitalized at day 7, remdesivir *vs* control was: 69%v59%), or on mortality [RR=0.95 (0.81-1.11, p=0.50; 301/2743 remdesivir *vs* 303/2708 control)].

Although it will be necessary to wait for the appearance of the final results of the trial, these interim negative results are likely to increase uncertainty in the medical community, as there are now several randomized clinical trials of remdesivir in hospitalized patients with differing results; this raises the question of whether the drug is less efficacious than initially hoped or whether the discrepancies are artifacts of study design. In particular the timing of treatment during the course of the disease appears to be an important variable for clinical improvement; therefore, understanding the dynamic of SARS-CoV-2 infection is essential for selection of the optimal patient population and optimal duration of therapy in order to maximize the drug potential. The possible emergence of remdesivir-resistant strains should be also considered.

Based on the actual information, on the recent FDA approval of VEKLURY (12), and on the fact that drug supplies are limited, the NIH COVID-19 Treatment Guidelines (h*ttps://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/)* recommend:
A. for hospitalized patients with COVID-19 who require supplemental oxygen, but who do not require oxygen delivery through a high-flow device, noninvasive or invasive mechanical ventilation, or ECMO, the use of remdesivir 200mg IV for 1 day, followed by remdesivir 100mg IV for 4 days or until hospital discharge, whichever comes first; *or* a combination of remdesivir (dose and duration as above) plus dexamethasone (6mg IV or orally) for up to 10 days or until hospital discharge;

B. for hospitalized patients with COVID-19 who require delivery of oxygen through a high-flow device, noninvasive and/or invasive mechanical ventilation or ECMO, a combination of dexamethasone plus remdesivir at the doses and durations indicated in A. Combination of remdesivir and dexamethasone has not been studied in clinical trials. Despite the lack of clinical trial

data, the NIH Panel recognizes that there are theoretical reasons to use dexamethasone and remdesivir in combination, including that, although remdesivir might not have an impact later in the disease course due to the possible decrease in the viral replication rate, antiviral therapy, by reducing viral shedding, may prevent the harmful clinical outcomes that were observed in patients with other viral infections who have received steroids;

C. there are insufficient data for the Panel to recommend either for or against the use of remdesivir in patients with mild or moderate COVID-19.

An interesting new approach for treatment of patients with early stage COVID-19 is a Phase 1b/2a clinical study to evaluate the safety, efficacy, and pharmacokinetics of remdesivir administered by inhalation (NCT04539262). Administering remdesivir by inhalation may also decrease adverse effects, as intravenous remdesivir can cause gastrointestinal symptoms, elevated transaminase levels, and an increase in prothrombin time (*https://www.covid19treatment guidelines.nih.gov/antiviral-therapy/remdesivir/)*.

Future strategies are needed to evaluate whether cocktails of antiviral agents in combination with other therapeutic approaches will improve patient outcomes in COVID-19. Currently the NIAID ACTT-2 trial (NCT04401579) is evaluating the activity of remdesivir in combination with modifiers of the immune response (e.g., the Janus-kinase inhibitor baricitinib), while the ACTT-3 trial (NCT04492475) is aimed at evaluating the combination of interferon beta-1a and remdesivir compared to remdesivir alone.

References

1. R.T. Eastman et al. Remdesivir: a review of its discovery and development leading to emergency use authorization for treatment of COVID-19. ACS Cent. Sci. 2020, 6:672–683.
2. M. Wang et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020, 30:269-271.
3. A.J. Pruijssers et al. Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARSCoV-2 RNA polymerase in mice. Cell Reports 2020, 32:107940.
4. B.N. Williamson et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 2020, 582:273–276.
5. S. Mulangu et al. A randomized, controlled trial of Ebola virus disease therapeutics. N. Engl. J. Med. 2019, 381:2293-2303.
6. M.L. Holshue et al. First case of 2019 novel Coronavirus in the United States. N. Engl. J. Med. 2020, 382:929-936.
7. J. Grein et al. Compassionate use of remdesivir for patients with severe Covid-19. N. Engl. J. Med. 2020. http://doi.org/ggrm99.
8. Y. Wang et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020, 395:1569-1578.
9. J.H. Beigel et al. Remdesivir for the treatment of Covid-19 - Final Report. N. Engl. J. Med. November 5, 2020, 383:1813-1826.
10. J.D. Goldman et al. Remdesivir for 5 or 10 days in patients with severe Covid-19. N. Engl. J. Med. 2020, 383:1827-1837.

11. US Food and Drug Administration. Coronavirus (COVID-19) update: FDA issues Emergency Use Authorization for potential COVID-19 treatment. Published May 1, 2020. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment.
12. US Food and Drug Administration. FDA Approves First Treatment for COVID-19. https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19.
13. S.A. Olender et al. Remdesivir for severe COVID-19 versus a cohort receiving standard of care. Clin. Infect. Dis. published on 24 Jul 2020. https://doi.org/10.1093/cid/ciaa1041.
14. C.D. Spinner et al. Effect of Remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. JAMA. 2020, 324:1048-1057.
15. WHO Solidarity Trial Consortium. Repurposed antiviral drugs for covid-19 - interim WHO Solidarity trial results. 15 Oct 2020. doi: 10.1101/2020.10.15.20209817.

6. Chloroquine and Hydroxychloroquine

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”.

Chloroquine (CQ) was initially used for the prophylactic treatment of malaria, but is presently used as a safer derivative, hydroxychloroquine (HCQ), to treat autoimmune diseases, such as rheumatoid arthritis and lupus erythematosus. The mechanism of action of CQ and HCQ is not completely understood: both drugs appear to act on several signaling pathways affecting the function of the immune system, and a major final effect is to reduce the production and release of pro-inflammatory cytokines (1).

An anti-viral effect of CQ and HCQ, presumably due to the ability of these drugs to increase the pH of the endosomal system, has been demonstrated in cultured cells and in animal models. However, this anti-viral potential has not been proved in clinical trials, therefore CQ and HCQ are not currently recommended as anti-viral drugs in humans (2).

The clinical use of both drugs is complicated by several common adverse effects reported in patients after long-term exposure, such as gastrointestinal disorders, skin rash, retinopathy, blurred vision, cardiac toxicity and others (3). 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 (4). The side effect of greatest concern is cardiotoxicity, including cardiomyopathy, arrhythmias, and conduction disorders which have been observed with both HCQ and CQ (5).

Preliminary descriptive studies have suggested HCQ may prove useful in fighting COVID-19 (6). However, this conclusion has not been supportd by subsequent randomized clinical trials. Boulware et al. (7) 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. Another recent randomized clinical trial showed that patients hospitalized with COVID-19, those who received hydroxychloroquine did not have a lower incidence of death at 28 days than those who received usual care (8). In fact, an opposite trend in the primary endpoint (rate ratio, 1.09; 95% confidence interval [CI], 0.97 to 1.23; P = 0.15) was confirmed by secondary endpoints: Patients in the HCQ group had a longer duration of hospitalization than those in the usual-care group (median, 16 days vs. 13 days) and a lower probability of discharge alive within 28 days (59.6% vs. 62.9%; rate ratio, 0.90; 95% CI, 0.83 to 0.98). Among the patients who were not undergoing invasive mechanical ventilation at baseline, the number of patients who had progression to the prespecified composite secondary outcome of invasive mechanical ventilation or death was higher among those in the HCQ group than among those in the usual-care group (8).

In line with these results, the US National Institutes of Health (NIH) COVID-19 Treatment Guidelines (https://www.covid19treatmentguidelines.nih.gov/.) recommends the following:

- The COVID-19 Treatment Guidelines Panel (the Panel) recommends against the use of chloroquine or hydroxychloroquine for the treatment of COVID-19 in hospitalized patients (AI).
- In non-hospitalized patients, the Panel recommends against the use of chloroquine or hydroxychloroquine for the treatment of COVID-19, except in a clinical trial (AI).
- The Panel recommends against the use of high-dose chloroquine (600 mg twice daily for 10 days) for the treatment of COVID-19 (AI).
- The Panel recommends against using hydroxychloroquine plus azithromycin to treat COVID-19, except in a clinical trial (AIII).

References

1. Nirk EL, Reggiori F Mario Mauthe M. Hydroxychloroquine in rheumatic autoimmune disorders and beyond. EMBO Mol Med 2020; e12476. DOI 10.15252/emmm.202012476

2. Rodrigo C, Fernando SD, Rajapakse S (2020) Clinical evidence for repurposing chloroquine and hydroxychloroquine as antiviral agents: a systematic review. Clin Microbiol Infect https://doi.org/10.1016/j.cmi.2020.05.016
3. Srinivasa A, Tosounidou S, Gordon C. Increased incidence of gastrointestinal side effects in patients taking hydroxychloroquine: a brand-related issue? J Rheumatol. 2017; 44(3):398.
4. Jorge A, Ung C, Young LH, et al. Hydroxychloroquine retinopathy - implications of research advances for rheumatology care. Nat Rev Rheumatol. 2018;14(12):693-703.
5. Chatre C, Roubille F, Vernhet H, et al. Cardiac Complications Attributed to Chloroquine and Hydroxychloroquine: A Systematic Review of the Literature. Drug Saf. 2018; 41(10):919-931.
6. Ferner RE, Aronson JK. Chloroquine and hydroxychloroquine in covid-19. BMJ. 2020; 369: m1432.
7. Boulware DR, Pullen MF, Bangdiwala AS ,et al. A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for Covid-19. N Engl J Med. 2020. 383(6):517-525. doi: 10.1056/NEJMoa2016638.
8. RECOVERY Collaborative Group, Horby P, Mafham M, Linsell L, et al. Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. N Engl J Med. 2020 Oct 8. doi: 10.1056/NEJMoa2022926. PMID: 33031652.

7. 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 of 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 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.

References

1. K. Willsher, Anti-inflammatories may aggravate Covid-19, France advises, The Guardian (2020).
2. Smyth E, Grosser T and FitzGerald GA Chapter 38: Anti-inflammatory, Antipyretic, and Analgesic Agents; Pharmacotherapy of Gout. In Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e Laurence L. Brunton, Bruce A. Chabner, Björn C. Knollmann Editors. 2018
3. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, Vyas SN, FitzGerald GA. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med. 2001 Dec 20;345(25):1809-17.
4. Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N, Narumiya S. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature. 1998 Sep 17;395(6699):281-4.
5. Grosser T, Theken KN, FitzGerald GA. Cyclooxygenase Inhibition: Pain, Inflammation, and the Cardiovascular System. Clin Pharmacol Ther. 2017 Oct;102(4):611-622.
6. García Rodríguez LA, Hernández-Díaz S. Relative risk of upper gastrointestinal complications among users of acetaminophen and nonsteroidal anti-inflammatory drugs. Epidemiology. 2001 Sep;12(5):570-6.
7. Sudano I, Flammer AJ, Périat D, Enseleit F, Hermann M, Wolfrum M, Hirt A, Kaiser P, Hurlimann D, Neidhart M, Gay S, Holzmeister J, Nussberger J, Mocharla P, Landmesser U, Haile SR, Corti R, Vanhoutte PM, Lüscher TF, Noll G, Ruschitzka F. Acetaminophen increases blood pressure in patients with coronary artery disease. Circulation. 2010 Nov 2;122(18):1789-96. doi: 10.1161/CIRCULATIONAHA.110.956490. Epub 2010 Oct 18.
8. Ynske P.M. van der Meer-Janssen, Josse van Galen, Joseph J. Batenburg, J. Bernd Helms Lipids in host–pathogen interactions: Pathogens exploit the complexity of the host cell lipidome. Prog Lipid Res. 49(1): 1–26 (2010). PMID: 19638285
9. Hu B., Höfer C., Thiele C., Veit M. Cholesterol Binding to the Transmembrane Region of a Group 2 Hemagglutinin (HA) of Influenza Virus Is Essential for Virus Replication, Affecting

both Virus Assembly and HA Fusion Activity. J Virol. 93(15): e00555-19 (2019). PMID: 31118253
10. R. Vijay et al., Virus-induced Inflammasome Activation Is Suppressed by Prostaglandin D 2/DP1 Signaling Proc. Natl. Acad. Sci. U.S.A. 114, E5444 (2017).
11. W. Yan et al., Nucleocapsid Protein of SARS-CoV Activates the Expression of cyclooxygenase-2 by Binding Directly to Regulatory Elements for Nuclear Factor-Kappa B and CCAAT/enhancer Binding Protein. Int. J. Biochem. Cell Biol. 38, 1417 (2006).
12. W. J. Sander et al., Prostaglandin E 2 As a Modulator of Viral Infections. Front. Physiol. 8, 89 (2017).
13. C. Amici et al., Indomethacin Has a Potent Antiviral Activity Against SARS Coronavirus. Antivir. Ther. 11, 1021 (2006).
14. K. Schaeuble et al., Attenuation of Chronic Antiviral T-cell Responses Through Constitutive COX2-dependent Prostanoid Synthesis by Lymph Node Fibroblasts. PLOS Biol. 17, e3000072 (2019).
15. Kühl PG, Bolds JM, Loyd JE, Snapper JR, FitzGerald GA. Thromboxane receptor-mediated bronchial and hemodynamic responses in ovine endotoxemia. Am J Physiol. 254(2 Pt 2):R310-9 (1988). PMID: 2964208
16. Qi Z, Hao CM, Langenbach RI, Breyer RM, Redha R, Morrow JD, Breyer MD. Opposite effects of cyclooxygenase-1 and -2 activity on the pressor response to angiotensin II. J Clin Invest. 110(1):61-9 (2002). PMID: 12093889

8. 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 (2,3,4,5). 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 (6), 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 (7,8) but great care should be taken in choosing between the drugs that are currently available or in advanced clinical development (4, 9). Timing is also relevant in that shutting down complement activation components that restrict viral propagation may be harmful while preventing uncontrolled activation is desirable (10). In this context, C3 inhibitors may be inadvisable as early interventions, since these drugs will prevent the activation of all 3 complement pathways in response to viral infections. Nonetheless, a case report of a patient with COVID-19 associated ARDS and pneumonia showed that treatment with the C3 inhibitor AMY-101 was safe and associated with a favorable course of the disease (11). 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 (9), 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, 12).

Complement C5 inhibition with eculizumab has been shown to be an effective therapeutic tool in thrombotic, hematological and inflammatory diseases (13). In particular, eculizumab blocked venous thromboembolic events in paroxysmal nocturnal hemoglobinuria and thrombotic microangiopathy in atypical hemolytic uremic syndrome (13). Thus, C5 inhibition could protect from COVID-19 associated vasculopathy as supported by a recent study (14) 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 (14)..

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 (15,16,17). A preliminary update is available online about a critically ill COVID-19 case who was enrolled in one of the above trials (15), and clinically improved after eculizumab treatment (10,18).

A preliminary report published recently describes four COVID-19 patients with pneumonia requiring oxygen supplementation who were treated off-label with eculizumab (19). 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. Similarly, in three cases of

critical COVID-19 with respiratory failure and acute kidney injury, eculizumab led to a marked decline in D-dimers and neutrophil counts, and normalization of liver function and serum creatinine levels in two of them (20). Recently, preliminary evidence consistent with the efficacy of eculizumab indicates that the drug may improve survival and reduce hypoxia in 35 patients with severe COVID-19 in the intensive care unit (21).

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 (22). A study of avacopan in patients admitted with COVID-19 but not yet in the ICU will soon be initiated in the US. A double-blind randomized study with the anti-C5aR antibody avdoralimab in patients with COVID-19 severe pneumonia has been recently initiated in France (23). 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 (24). 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 (10).

References

1. Noris M, Remuzzi G. Overview of complement activation and regulation. *Semin Nephrol* 2013; 33:479-492.
2. Gao T, Hu M, Zhang X*, et al.*: Highly pathogenic coronavirus N protein aggravates lung injury by MASP-2-mediated complement over-activation. In *medRxiv*, 2020.
3. Gralinski LE, Sheahan TP, Morrison TE*, et al.* Complement Activation Contributes to Severe Acute Respiratory Syndrome Coronavirus Pathogenesis. *mBio* 2018; 9.
4. Noris M, Benigni A, Remuzzi G.: The case of Complement activation in COVID-19 multiorgan Impact. *Kidney Int* 2020; 98:314-322.
5. Holter JC, Pischke SE, de Boer E, *et al.* Systemic complement activation is associated with respiratory failure in COVID-19 hospitalized patients. *Proc Natl Acad Sci USA,* 2020; Sept 17; 202010540. Doi: 10.1073/pnas.2010540117.
6. Diao B, Wang C, Wang R*, et al.*: Human Kidney is a Target for Novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection. In *medRxiv*, 2020.
7. Risitano AM, Mastellos DC, Huber-Lang M*, et al.* Complement as a target in COVID-19? *Nat Rev Immunol* 2020; 20:343-344.
8. Campbell CM, Kahwash R. Will Complement Inhibition be the New Target in Treating COVID-19 Related Systemic Thrombosis? *Circulation* 2020; 141: 1739-1741.
9. Mastellos DC, Ricklin D, Lambris JD. Clinical promise of next-generation complement therapeutics. *Nat Rev Drug Discov* 2019; 18:707-729.

10. Song WC, FitzGerald GA. COVID-19, microangiopathy, hemostatic activation, and complement. *J Clin Invest*. 2020; 130: 3950-3953.
11. Mastaglio S, Ruggeri A, Risitano AM, *et al.* The first case of COVID-19 treated with the complement C3 inhibitor AMY-101. Clinical Immunology 2020; 215: 108450.
12. Stoermer KA, Morrison TE. Complement and viral pathogenesis. *Virology* 2011; 411:362-373.
13. Wijnsma KL, Ter Heine R, Moes D*, et al.* Pharmacology, Pharmacokinetics and Pharmacodynamics of Eculizumab, and Possibilities for an Individualized Approach to Eculizumab. *Clin Pharmacokinet* 2019; 58:859-874.
14. Magro C, Mulvey JJ, Berlin D*, et al.* Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Transl Res* 2020; 220:1-13.
15. Eculizumab (Soliris) in Covid-19 Infected Patients (SOLID-C19). ClinicalTrials.gov Identifier: NCT04288713.
16. Trial Evaluating Efficacy and Safety of Eculizumab (Soliris) in Patients With COVID-19 Infection, Nested in the CORIMUNO-19 Cohort (CORIMUNO19-ECU). ClinicalTrials.gov Identifier: NCT04346797.
17. SOLIRIS® (Eculizumab) Treatment of Participants With COVID-19. ClinicalTrials.gov Identifier: NCT04355494.
18. A preliminary update to the Soliris to Stop Immune Mediated Death in Covid-19 (SOLID-C19) Compassionate Use Study. 2020. https://hudsonmedical.com/articles/soliris-stop-death-covid-19/.
19. Diurno F, Numis FG, Porta G*, et al.* Eculizumab treatment in patients with COVID-19: preliminary results from real life ASL Napoli 2 Nord experience. *Eur Rev Med Pharmacol Sci* 2020; 24:4040-4047.
20. Laurence J, Mulvey JJ, Seshadri M, *et al.* Anti-complement C5 therapy with eculizumab in three cases of critical COVID-19. Clinical Immunology 2020; 219: 108555.
21. Annane D, Heming N, Grimaldi-Bensouda L, *et al*. Eculizumab as an emergency treatment for adult patients with severe COVID-19 in the intensive care unit: A proof-of-concept study. EClinicalMedicine 2020; https://doi.org/10.1016/j.eclinm.2020.100590
22. Jayne DRW, Bruchfeld AN, Harper L*, et al.* Randomized Trial of C5a Receptor Inhibitor Avacopanin ANCA-Associated Vasculitis. *J Am Soc Nephrol* 2017; 28:2756-2767.
23. Avdoralimab an anti-C5aR antibody, in patients with COVID-19 severe pneumonia (FORCE). ClinicalTrials.gov Identifier: NCT04371367.
24. Ueda Y, Miwa T, Ito D, el al. Differential contribution of C5aR and C5b-9 pathways to renal thrombotic microangiopathy and microvascular thrombosis in mice carrying an atypical hemolytic uremic syndrome-related factor H mutation. Kidney Int 96:67-79.

9. Anticoagulants and other antithrombotic agents

9a. 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). Moreover, a pooled analysis of six original studies enrolling 1355 hospitalized patients with moderate to critical COVID-19 confirmed that D-dimer levels are significantly associated with the risk of mortality in these patients (6). 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 (7, 8). In addition, fibrin deposits are found in the lung parenchyma of patients with SARS-CoV-2 and acute respiratory distress syndrome (ARDS) (9). 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 (10). 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 (11). Moreover, the hypoxia that characterizes SARS-CoV-2 infection also contributes to thrombosis by enhancing blood viscosity (11).

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 (12). 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 (13). 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 (14). 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 (15).

Heparin appears to protect the vascular endothelium. Apart from pathogens, histones released from damaged cells can also cause endothelial injury (16). Heparin can antagonize histones thereby protecting endothelial cells (17). 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 (18).

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 (19). 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 (20). 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 (21). 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) (22). Moreover, a pre-print non-peer-reviewed evidence indicates that LMW heparin therapy in COVID-19 patients improved coagulation dysfunction (23).

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 x 10^9/L) (24). Recently ISTH has updated its recommendations for using LMW heparin or unfractionated heparin in special COVID-19 patient population, such as those with renal impairment or with obesity. Notably, obese individuals should be given a weight-adjusted appropriate prophylactic dose at admission with an increase to intermediate intensity or full therapeutic dose based on clinical parameters (25). 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 (26). On 11th April 2020 the Italian drug agency (AIFA) included LMW heparin among the drugs available for the treatment of COVID-19 patients (27). 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. Interestingly, a recent study in 324 non-critically ill patients with COVID-19 admitted to hospital has shown that the rate of relevant bleeding events and mortality was higher in patients given (sub)therapeutic doses of heparin (as unfractionated, LMW, or fondaparinux) than in those receiving prophylactic doses of the anticoagulant (28).

Several issues remain to be addressed to establish the optimal anticoagulant approach in patients with COVID-19, such as the proper time to initiate treatment, 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.

9b. Other antithrombotic agents

Pathophysiologic and pharmacologic rationale

Dipyridamole is an antiplatelet agent and acts as a phosphodiesterase inhibitor, eventually increasing intracellular cAMP and cGMP (29). 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 (30, 31), particularly against positive-stranded RNA viruses (32). Second, it suppresses inflammation and promotes mucosal healing, as demonstrated in pediatric patients with colitis or inflammatory bowel disease (33). Third, as a pan-phosphodiesterase inhibitor, dipyridamole may prevent acute injury and progressive fibrosis of the lung, heart, liver and kidney (34).

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 (35, 36). 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 (35). 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 (35, 36).

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).

References

1. Giannis D, Ziogas IA, Gianni P. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. J Clin Virol 2020;127:104362.
2. Klok FA, Kruip M, van der Meer NJM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res 2020;191:147-147.
3. Fogarty H, Townsend L, Ni Cheallaigh C, et al. COVID19 coagulopathy in Caucasian patients. Br J Haematol 2020;189:1044-1049.

4. Zhou F, Yu T, Du R*, et al.* Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054-62.
5. Tang N, Li D, Wang X*, et al.* Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020;18:844-7.
6. Sakka M, Connors JM, Hekimian G*, et al.* Association between D-Dimer levels and mortality in patients with coronavirus disease 2019 (COVID-19): a systematic review and pooled analysis. *J Med Vasc* 2020;45:268-74.
7. Yao XH, Li TY, He ZC*, et al.* [A pathological report of three COVID-19 cases by minimally invasive autopsies]. *Zhonghua Bing Li Xue Za Zhi* 2020;49:411-417.
8. Dolhnikoff M, Duarte-Neto AN, de Almeida Monteiro RA*, et al.* Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19. *J Thromb Haemost* 2020;18:1517-1519.
9. Whyte CS, Morrow GB, Mitchell JL*, et al.* Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. *J Thromb Haemost* 2020;18:1548-1555.
10. Wright FL, Vogler TO, Moore EE*, et al.* Fibrinolysis Shutdown Correlates to Thromboembolic Events in Severe COVID-19 Infection. *J Am Coll Surg* 2020;231:193-203.
11. Subramaniam S, Scharrer I. Procoagulant activity during viral infections. *Front Biosci (Landmark Ed)* 2018;23:1060-81.
12. Gray E, Hogwood J, Mulloy B. The anticoagulant and antithrombotic mechanisms of heparin. *Handb Exp Pharmacol* 2012:43-61.
13. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* 2013;13:34-45.
14. Poterucha TJ, Libby P, Goldhaber SZ. More than an anticoagulant: Do heparins have direct anti-inflammatory effects? *Thromb Haemost* 2017;117:437-44.
15. Camprubi-Rimblas M, Tantinya N, Guillamat-Prats R*, et al.* Effects of nebulized antithrombin and heparin on inflammatory and coagulation alterations in an acute lung injury model in rats. *J Thromb Haemost* 2020;18:571-83.
16. Xu J, Zhang X, Pelayo R*, et al.* Extracellular histones are major mediators of death in sepsis. *Nat Med* 2009;15:1318-21.
17. Zhu C, Liang Y, Li X*, et al.* Unfractionated heparin attenuates histone-mediated cytotoxicity in vitro and prevents intestinal microcirculatory dysfunction in histone-infused rats. *J Trauma Acute Care Surg* 2019;87:614-22.
18. Liu Y, Mu S, Li X*, et al.* Unfractionated Heparin Alleviates Sepsis-Induced Acute Lung Injury by Protecting Tight Junctions. *J Surg Res* 2019;238:175-85.
19. Milewska A, Zarebski M, Nowak P*, et al.* Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. *J Virol* 2014;88:13221-30.
20. Mycroft-West C, Su D, Elli S, et al. The 2019 coronavirus (SARS-CoV-2) surface protein (Spike) S1 Receptor Binding Domain undergoes conformational change upon heparin binding. BioRxiv Posted: 2 March 2020 preprint doi 10.1101/2020.02.29.971093.
21. Tang N, Bai H, Chen X*, et al.* Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. *J Thromb Haemost* 2020; 18: 1094-1099.
22. Paranjpe I, Fuster V, Lala A*, et al.* Association of Treatment Dose Anticoagulation with In-Hospital Survival Among Hospitalized Patients with COVID-19. *J Am Coll Cardiol* 2020; 76: 122-124.

23. Shi C, Wang C, Wang H, et al. The potential of low molecular weight heparin to mitigate cytokine storm in severe COVID-19 patients: a retrospective clinical study. MedRxiv Posted: 15 April 2020. preprint doi: 10.1101/2020.03.28.20046144.
24. Thachil J, Tang N, Gando S*, et al.* ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost* 2020;18:1023-1026.
25. Thachil J, Juffermans NP, Ranucci M*, et al.* ISTH DIC subcommittee communication on anticoagulation in COVID-19. *J Thromb Haemost* 2020;18:2138-2144.
26. Baumann Kreuziger L, Lee A, Garcia D, et AL. (2020) COVID-19 and VTE/Anticoagulation: Frequently Asked Questions. www.hematology.org/covid-19/covid-19-and-vte-anticoagulation.
27. https://aifa.gov.it/-/covid-19-scheda-informativa-aifa-su-eparine-a-basso-peso-molecolare.
28. Pesavento R, Ceccato D, Pasquetto G*, et al.* The hazard of (sub)therapeutic doses of anticoagulants in non-critically ill patients with Covid-19: The Padua province experience. *J Thromb Haemost* 2020. doi: 10.1111/jth.15022.
29. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. *Br J Clin Pharmacol* 2011;72:634-46.
30. Kuzmov K, Galabov AS, Radeva K*, et al.* [Epidemiological trial of the prophylactic effectiveness of the interferon inducer dipyridamole with respect to influenza and acute respiratory diseases]. *Zh Mikrobiol Epidemiol Immunobiol* 1985;6:26-30.
31. Kozhukharova MS, Slepushkin AN, Radeva Kh T*, et al.* [Evaluation of dipyridamole efficacy as an agent for preventing acute respiratory viral diseases]. *Vopr Virusol* 1987;32:294-297.
32. Fata-Hartley CL, Palmenberg AC. Dipyridamole reversibly inhibits mengovirus RNA replication. *J Virol* 2005;79:11062-11070.
33. Huang B, Chen Z, Geng L*, et al.* Mucosal Profiling of Pediatric-Onset Colitis and IBD Reveals Common Pathogenics and Therapeutic Pathways. *Cell* 2019;179:1160-76 e24.
34. Insel PA, Murray F, Yokoyama U*, et al.* cAMP and Epac in the regulation of tissue fibrosis. *Br J Pharmacol* 2012;166:447-456.
35. Liu X, Li Z, Liu S*, et al.* Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19. *Acta Pharm Sin B* 2020. doi: 10.1016/j.apsb.2020.04.008.
36. Verma DK, Kapoor S, Das S, *et al.* Potential inhibitors of SARS-CoV-2 Main protease (Mpro) identified from the library of FDA approved drugs using molecular docking studies. *Preprints* (www.preprints.org) Posted: 9 April 2020 doi:10.20944/preprints202004.0149.

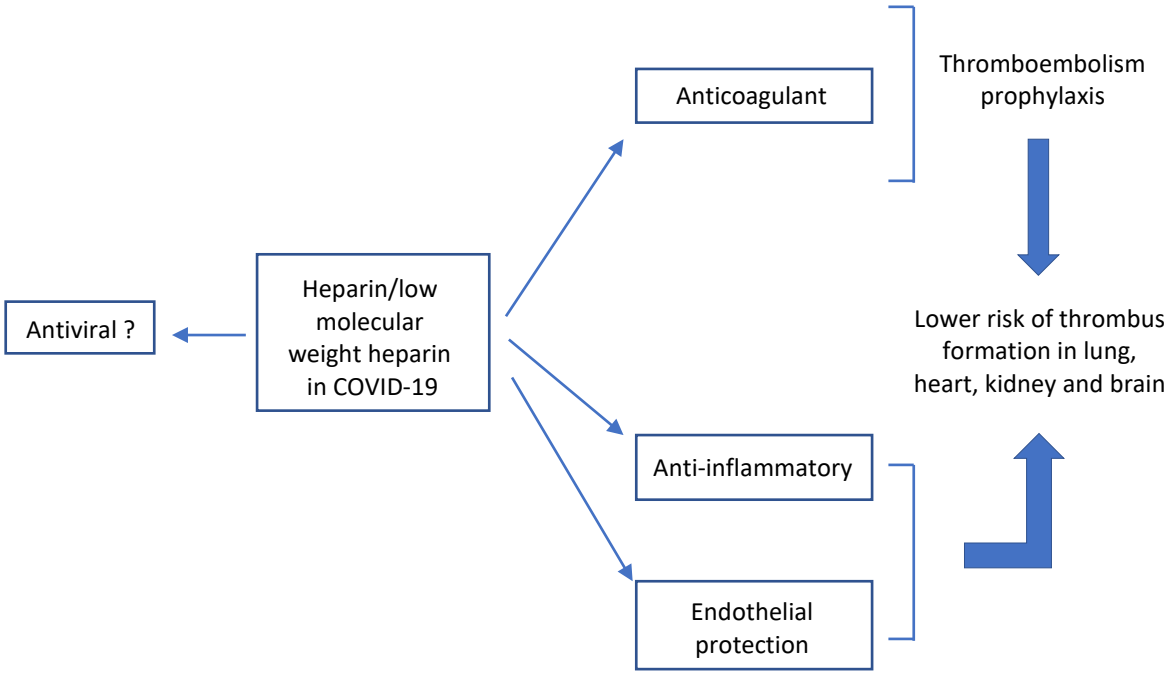


Figure. Possible effects of heparin and low molecular weight heparin in COVID-19

10. Corticosteroids

The place of corticosteroids in the treatment of COVID-19 has been first established by the outcome of the RECOVERY trial in the UK (1). This was a randomized, controlled, open-label, adaptive, platform trial comparing a range of possible treatments with usual care in patients hospitalized with COVID-19. Around 15% of all UK hospitalized patients with COVID-19 were enrolled in the trial and the control arm fatality rate is consistent with the overall hospitalized case fatality rate in the UK.

Prior to this trial there was considerable confusion about the place of steroids in the treatment of severe viral infections. On the one hand, slower clearance of viral RNA has been observed in patients with SARS, MERS and influenza treated with systemic corticosteroids. On the other, steroids offered a theoretical benefit after the phase of viral replication when immunopathology is dominant. However, prior to the RECOVERY trial, clinical trials of sufficient size and rigor had not been performed in such settings.

In this trial, the comparison of dexamethasone 6 mg given once daily for up to ten days vs. usual care alone was assessed (1). The primary outcome was 28-day mortality. In contrast to SARS and MERS, the phase of viral replication in COVID-19 is early after infection, declining thereafter.

As reported (1), 2104 patients randomly allocated to receive dexamethasone were compared with 4321 patients concurrently allocated to usual care. Overall, 454 (21.6%) patients allocated dexamethasone and 1065 (24.6%) patients allocated usual care died within 28 days (age adjusted rate ratio [RR] 0.83; 95% confidence interval [CI] 0.74 to 0.92; P<0.001). The proportional and absolute mortality rate reductions varied significantly depending on the level of respiratory support

at randomization (test for trend p<0.001): Dexamethasone reduced deaths by one-third in patients receiving invasive mechanical ventilation (29.0% vs. 40.7%, RR 0.65 [95% CI 0.51 to 0.82]; p<0.001), by one-fifth in patients receiving oxygen without invasive mechanical ventilation (21.5% vs. 25.0%, RR 0.80 [95% CI 0.70 to 0.92]; p=0.002).

Thus, this trial provides clear evidence that treatment with dexamethasone 6 mg once daily for up to 10 days reduces 28-day mortality in patients with COVID-19 who are receiving respiratory support. Based on these results, 1 death would be prevented by treatment of around 8 patients requiring invasive mechanical ventilation or around 25 patients requiring oxygen (which, in the UK, is recommended when oxygen saturations on room air are 92-94%) without invasive mechanical ventilation.

A cautionary note is the possibility of harm amongst those patients who did not require oxygen at the time of randomization. In these patients mortality was higher in those receiving dexamethasone (17.0% vs 13.2%) although the difference did not attain statistical significance (RR 1.22 [95% CI 0.93 to 1.61]; p=0.14).

The RECOVERY trial also reported that hydroxychloroquine and the HIV protease inhibitors lopinavir and ritonavir do not improve outcomes in patients hospitalized with COVID-19.

The usefulness of dexamethasone in patients with severe pulmonary complications of COVID-19 infection has been supported by further investigations (2,3).

References

1. Horby P, Lim WS, Emberson JR, et al. RECOVERY Collaborative Group. Dexamethasone in Hospitalized Patients with COVID-19 – Preliminary Report. NEJM doi: 10.1056/NEJMoa2021436.
2. Tomazini BM, Maia IS, Cavalcanti AB, et al. Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients With Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19: The CoDEX Randomized Clinical Trial. JAMA 2020: 324 (13):1307-1316. doi: 10.1001/jama.2020.17021.
3. WHO REACT Working Group. Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. JAMA 2020: 324(13): 1330-1241. doi: 10.1001/jama.2020.17023.

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