Acta Biomed 2020; Vol. 91, N. 3: e2020038 DOI: 10.23750/abm.v91i3.10450

Therapeutic strategies against COVID-19

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Summary. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that mainly affects the upper and lower respiratory tract and is responsible for extremely different degrees of disease, ranging from flu-like symptoms to atypical pneumonia that may evolve to acute respiratory distress syndrome and, ultimately, death. No specific therapy for SARS-CoV-2 has yet been identified, but since the beginning of the outbreak, several pre-existing therapeutics have been reconsidered for the treatment of infected patients. The aim of this article is to discuss current therapeutics against SARS-CoV-2. A literature review was performed using PubMed, collecting data from English-language articles published until June 20th, 2020. Literature analysis showed that with the acquisition of more in-depth knowledge on the characteristics of SARS-CoV-2 and the pathogenesis of the different clinical manifestations, a more rationale use of available drugs has become possible. However, the road to defining which drugs are effective and which schedules of administration must be used to maximize efficacy and minimize adverse events is still very long. To date, it is only clear that no drug can alone cope with all the problems posed by SARS-CoV-2 infection and effective antivirals and inflammatory drugs must be given together to reduce COVID-19 clinical manifestations. Moreover, choice of therapy must always be tailored on clinical manifestations and, when they occur, drugs able to fight coagulopathy and venous thromboembolism that may contribute to respiratory deterioration must be prescribed. (www.actabiomedica.com)

Keywords: antiviral drug; COVID-19; drug repurposing; immunomodulatory drug; SARS-CoV-2.

1. Introduction

At the beginning of new coronavirus 2019 (COVID-19) pandemic, lacking specific preventive and therapeutic measures, prophylaxis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was limited to the use of quarantine, isolation, and infection-control measures, whereas therapy was based only on supportive procedures. However, starting from the evidence that COVID-19 had several similarities with two others recently emerged, coronavirus diseases, SARS and MERS [1], it was though that, at last for therapy of the most severe cases, compassionate use of the drugs previously used to fight these diseases should have been made [2]. Consequently, several already known drugs, alone or in combination, were administered to COVID-19 patients, even outside of properly authorized official protocols. A relevant number of data on the effectiveness, safety and tolerability of various drugs were collected. However, due to the low number of enrolled subjects and the methodological limitations of these reports, no definitive conclusion on the role of the different therapeutic measures could be drawn.

Some more information has been acquired when COVID-19 clinical characteristics, host immune response to infection, and SARS-CoV-2 structure and function were more precisely defined. Moreover, development of some potentially effective new drugs and new therapeutic measures was greatly favored [3]. Critical for the improvement of COVID-19 therapy was the evidence that in the first phase of the disease clinical manifestations were due to the direct viral damage, whereas in the last phase, the one with the most severe signs and symptoms, tissue damages were mainly due to an exaggerate host immune response resulting in multiorgan failure [4]. Of relevance was also the demonstration that SARS-CoV-2 could cause a complex coagulation disorder leading to both bleeding and thrombosis [5] and most of the infected subjects developed a significant humoral immune response that can be used for passive protection [6].

However, despite many studies, the best pharmacological approach to COVID-19 is not clearly established. None of the already licensed drug alone or in combination can be defined as the best solution to treat this disease (Table 1). In most of the cases, efficacy, safety and tolerability of the available drugs remains undefined and choice of the best pharmacological approach to a COVID-19 patient is a challenge. Unfortunately, development of drugs specifically tailored for fighting SARS-CoV-2 infection is far to be completed and the disease must be faced with what it is presently available. Aim of this narrative review is to discuss main current therapeutic approaches to COVID-19. A literature review was performed using PubMed, collecting data from English-language articles published until June 20th, 2020. Randomized controlled trials (RCTs), case reports, case series and review articles were included in our study from a search for "SARS-CoV-2", "SARS-CoV", "MERS-CoV", "coronavirus", and "COVID-19" in combination with "treatment", "pharmacology", "prophylaxis", and "pathogenesis". Information reported in this review was listed according to the main potential activity of the various therapeutic measures.

2. Antinfective Drugs

Agents able to reduce SARS-CoV-2 attachment, penetration and replication can be effective in any COVID-19 phase, from the contagion to the period with the most severe clinical manifestations. When present, the association of antiviral activity with immunomodulatory properties and effects on coagulation makes some of these drugs very attractive for COVID-19 therapy. Several compounds with different mechanism of action are included in this group. Among them, those that have been tentatively used in a substantial number of COVID-19 patients, alone or in combination are aminoquinolines (chloroquine or hydroxychloroquine), remdesivir, lopinavir/ritonavir, ribavirin, favipinavir, umifenovir, and azithromycin. All of them together with other drugs with previous poor use in humans for COVID-19 and some new drugs specifically prepared against SARS-CoV-2 treatment are presently in study with phase III clinical trials.

Table 1. Main available therapeutic agents against COVID-19

Drug	Previous indication	Action against SARS-CoV-2	Possible adverse events
Chloroquine/ hydroxychloroquine	• Malaria (Plasmodium spp.) • Systemic lupus erythaematosus • Rheumatoid arthritis • Antiphospholipid syndrome • Sjögren syndrome	• Alkalization of endosomal pH • Impairment of ACE2 glycosylation • Inhibition of the S protein NTD domain link with sialic acids	• Retinopathy • QT prolongation
Azithromycin	• Intracellular bacterial infectious disease	• Interference between viral spike proteins and CD-147 • Immunomodulant action	• QT prolongation
Tocilizumab	• Rheumatoid arthritis • Juvenile idiopathic arthritis Crohn disease	• IL-6 receptor antagonist	• Increase in total and LDL cholesterol • Hepatotoxicity • Thrombocytopenia • Neutropenia • Infections
Corticosteroids	• Autoimmune diseases • Asthma • Septic shock	• Inhibition of pro-inflammatory cytokines transcription • Inhibition of NF-kB pathway	• Hypertension • Hypokalaemia • Hyperglycaemia
Remdesivir	• Ebola virus disease	• Adenosine analogue: inhibition of RNA polymerase	• Hepatotoxicity • Nausea, vomiting
Lopinavir/ ritonavir	• HIV • SARS • MERS	• Protease inhibitors (Ritonavir works as a booster by decreasing CYP450 metabolic activity, thus increasing the lopinavir half-life)	• Nausea • Diarrhoea • Asthenia • Hypertriglyceridaemia • Myalgia
Ribavirin	• SARS • MERS • HCV • RSV • Viral haemorrhagic fever (Lassa virus infection)	• Guanosine analogue: inhibition of RNA synthesis	• Nausea • Diarrhoea • Asthenia • Myalgia • Headache
Favipiravir/ Umifenovir	• Severe Influenza infection	• Guanosine analogue: inhibition of RNA synthesis	• Hepatotoxicity • Increase in cholestasis enzymes

2.1 Aminoquinolines

For years, aminoquinolines, mainly chloroquine (CQ) and hydroxychloroquine (HCQ), have been known as effective drugs for malaria prevention and treatment [7-9]. More recently, due to their immunomodulatory and anti-thrombotic properties, they have been included among drugs effective for treatment of some autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and anti-phospholipid syndrome [10]. Finally, *in vitro* and experimental studies have shown that CQ and HCQ could have a significant activity against several different viruses, including coronaviruses [9]. This explains why, when SARS and MERS epidemic occurred, both these drugs were indicated as possible measures for

treatment of these diseases [11] and at COVID-19 pandemic onset they were immediately considered for treatment of COVID-19.

A great number of studies have tested aminoquinolines, mainly HCQ, the less toxic of them, in this regard. However, whereas *in vitro* studies have totally confirmed theoretical assumptions, administration of these antimalarial drugs to humans for prevention and treatment of COVID-19 have left many experts convinced that they were poorly or nothing effective and, in a not marginal number of cases, very dangerous. *In vitro* studies have shown that CQ and HCQ inhibit viral attachment and entry in the host cell and block new viral particle maturation and spread [12-14]. Moreover, through multiple effects on the immune system cells and modulation of crucial pro-inflammatory cytokines they can reduce the cytokine storm that can follow SARS-CoV-2 infection and is the cause of the acute respiratory distress syndrome that can lead patients to death [15]. Finally, due to their ability to interfere with platelet aggregation, modify membrane binding of blood clotting proteins and improve biomarkers of endothelial dysfunction, these drugs are considered potentially capable to decrease the diffuse micro-vascular thrombosis that is generally found in patients with COVID-19 and that can strongly condition disease outcome [15].

From a clinical point of view, the first studies in patients with COVID-19 receiving CQ and HCQ reported favorable results. Compared to Lopinavir/Ritonavir, CQ was found more effective as patients treated with this drug had an earlier virus clearing, an earlier improvement of chest X-ray and a more rapid hospital discharge [16]. Similar results were obtained by Gautret et al. who reported that, given with alone or with azithromycin, HCQ induced a rapid fall of SARS-CoV-2 load with total clearing after few days of treatment and rapid discharge of COVID-19 patients from the hospital [17, 18]. Theoretically, these studies could be strongly criticized because they lacked internal validity. There was no blinding or randomization, only few patients were enrolled, criteria for enrolment were poorly defined, severity of disease was not taken into account for analysis and criteria for virus shedding evaluation were not uniform. Despite these limitations, results were considered important by several national institutions worldwide. Inclusion of the antimalarial medications among the drugs for emergency treatment of severe COVID-19 cases was suggested, a number of observational studies to evaluate the compassionate use of CQ and HCQ were initiated and the implementation of RCTs to verify their true role for COVID-19 treatment was encouraged [19, 20]. Further stimuli for CQ and HCQ prescription were the low cost compared to other potential anti SARS-CoV-2 drugs and the supposed relative low risk of severe adverse events following administration, as reported by studies in patients receiving the drugs for malaria or autoimmune diseases [21].

Unfortunately, results of observational studies carried out in hospitalized patients with COVID-19 were generally disappointing. In most of them, including those enrolling several thousand patients and comparing different drugs, no benefit of the antimalarial medications on mortality or in speeding recovery was demonstrated [22]. Moreover, serious cardiac adverse events and other potential serious, life threatening side effects were evidenced in a greater number of treated subjects than in subjects receiving other treatments. QT interval prolongation on the electrocardiogram was the most common reported evidence of drug toxicity and the emergence of a specific ventricular arrhythmia called torsade de pointes, which could sometime degenerate into ventricular fibrillation, the most common reported cause of drug-related death. Despite all these studies had limitations high enough to make results debatable, uniformity of data arose the strong suspicion that CQ and HCQ could be not only ineffective but also dangerous for COVID-19 patients. Enrollment of new patients in these studies was stopped and team leaders of each study concluded that these drugs had no role for COVID-19 treatment. This was the case of the Solidarity Trial, launched by the World health Organization (WHO) and partners [23], the Recovery Trial (funded by the U.K. government) [24], and the REMAP-CAP trial [25]. Moreover, based on these findings, several national institutions, including the US Food and Drug Administration [26] and the Italian Medicinal Agency [27] suspended the authorization for compassionate use of CQ and HCQ, waiting the results of methodologically appropriate clinical

trials to establish whether aminoquinolines could be licensed for use in COVID-19 patients. This decision seemed further supported by the results of a multinational registry analysis of the use of CQ and HCQ with or without a macrolide carried out by Mehra et al. in hospitalized patients [28]. A total of 14,888 patients from 671 hospitals in six continents were enrolled excluding cases in mechanical ventilation and those receiving other anti-SARS-CoV-2 drugs. In-hospital mortality and *de-novo* occurrence of ventricular arrhythmias were considered to evaluate efficacy and safety, respectively. Analysis revealed that whereas patients receiving other therapies died in 9.3% of the cases, those given CQ or HCQ alone or in combination with azithromycin had a significantly higher mortality rate (HCQ 18.0%: hazard risk [HR] 1.335, 95% confidence interval [CI] 1.223-1.457; HCQ with a macrolide: 23.8%, HR 1.447, 95% CI 1.368-1.531; CQ: 16.4%, HR 1.365, 95% CI 1.218-1.531; CQ with a macrolide: 22.2%, HR 1.368, 95% CI 1.273-1.469). Similar negative findings were evidenced when the risk of *de-novo* ventricular arrhythmia was measured. In controls, this adverse event was evidenced in only 0.3% of the cases compared to significantly higher values in treated patients. HCQ was associated with arrhythmia in 6.1% of the cases (HR 2.369, 95% CI 1.935-2.900), HCQ with a macrolide in 8.1% (HR 5.106, 95% CI 4.106-5.983), CQ in 4.3% (HR 3.561, 95% CI 2.760-4.596), and CQ with a macrolide in 6.5% (HR 4.011, 95% CI 3.344-4.812). The study was published in a prestigious scientific journal but only few days later it was retracted after several clinicians, medical researchers, statisticians, and ethicists had pointed out that the study presented several substantial problems and some of the authors had admitted that they could no longer vouch for the veracity of the primary data sources [29].

In conclusion, data presently available raise quite a few doubts about the use of CQ and HCQ efficacy and safety for COVID-19 treatment but cannot be considerate adequate for their definitive exclusion from the list of drugs useful to fight COVID-19. The decision of suspending compassionate use and waiting results of RCTs that are presently ongoing seems the best solution to avoid wrong decisions.

The same watchful waiting strategy could be followed for the problem of the use of CQ and HCQ in prophylaxis. Presently, very few data in this regard are available. However, the only randomized, double-blind, placebo-controlled trial testing HCQ as post-exposure prophylaxis suggested no effect [30]. Adults who were at high- or moderate-risk were given, within 4 days after exposure, placebo or HCQ (800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 additional days). A new COVID-19 compatible disease was diagnosed in 11.8% of subjects with prophylaxis and in 14.3% of those receiving placebo. Side effects were more common with HCQ than with placebo (40.1% vs 16.8%), but no serious adverse reactions were evidenced. However, this study has several problems. Compliance with suggested therapy was not evaluated. Moreover, in a great number of cases, COVID-19 diagnosis was based only on signs and symptoms without laboratory confirmation. As some RCTs are presently ongoing [31], it seems appropriate to wait until they finish before drawing definitive conclusions.

Together with clinical efficacy, more attention should be paid to optimal dosing regimens, therapeutic and prophylactic serum and tissue levels, duration of treatment and variations of drug pharmacokinetic characteristics in patients with different severity of COVID-19 and different underlying disease. Mathematical models have shown that the antiviral effect of CQ and HCQ is strictly related to the dosage and those dosages that are considered safe in the clinic are in the lowest range of the therapeutic window, with significant, though yet partial, effects observable only at the highest doses administered. Virologic negativization should not be expected when HCQ is administered at dosages of 400 mg/day, but only at dosages of at least 600 mg/day [32]. Moreover, computer-aided simulations have shown that HCQ has effect on viral clearance only if the drug is administered early enough (i.e. when viral loads range from 1 to 1,000 copies/mL) [33]. If confirmed, these pharmacological evaluations could suggest well-defined schemes of therapy in some cases quite different from those used in clinical practice. Moreover, administration should be preferred to subjects that can be treated early and in pre-exposure prophylaxis.

2.2 Remdesivir

Remdesivir was developed some years ago during the Western African Ebola virus outbreak [34]. It acts as an adenosine analogue by inhibiting RNA polymerase and interfering with viral synthesis. *In vitro* studies have shown that it could be effective not only against Ebola virus but also against many other RNA viruses, such as pneumoviruses, paramyxoviruses, Lassa virus, SARS-CoV and MERS-CoV, although with relevant difference in the half maximal inhibitory concentration (IC50) [35, 36]. *In vivo* studies in animal models have confirmed the potential use of this drug in clinical practice [37]. It was used in human Ebola patients, although with limited results. Safety and tolerability were generally acceptable [38, 39].

After the novel SARS-CoV-2 spread, several attempts to evaluate remdesivir against this virus have been made. *In vitro* activity was shown, although with half maximal IC50 5- to 25-fold greater than that reported for Ebola virus [12]. Despite this, in clinical studies the dose regimen for COVID-19 treatment was the same previously used to treat Ebola patient (an IV loading dose of 200 mg on day 1 followed by daily IV maintenance doses of 100 mg for 5–9 days). Unfortunately, presently available results of administration of remdesivir in severe COVID-19 cases do not definitively clarify the role of this drug for COVID-19 treatment. Data are, at least in part, conflicting, although in most of the cases some positive effects were evidenced. Administration of remdesivir on a compassionate-use basis to the first case of COVID-19 in the USA was followed by a rapid improvement in his chest imaging findings and clinical course, without any relevant adverse effects [40]. In a nonrandomized study, enrolling 53 patients with severe COVID-19 from the USA, Canada, Europe and Japan drug use was associated with significant clinical improvement in 68% of treated patients, including some patients under mechanical ventilation [41]. A double-blind, randomized, placebo-controlled trial of IV remdesivir in adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement showed that median recovery time was significantly shorter among the 538 receiving the drug than among the 521 subjects given placebo (11 days, 95% CI 9-12 vs 15 days, 95% CI 13 – 19; rate ratio for recovery, 1.32, 95% CI 1.12 - 1.55; $p<0.001$). Mortality by 14 days was lower in treated patients than in placebo recipients (7.1% vs 11.9%; hazard ratio 0.70, 95% CI 0.47-1.04) but difference did not reach statistical significance. Safety and tolerability of the studied drug were not statistically different from those of placebo as serious adverse events were reported in 21.1% and 27% of patients receiving remdesivir and placebo, respectively [42]. Despite some limitations, results of this study were considered very promising by US Food and Drug Administration that issued an emergency use authorization for the treatment with remdesivir of suspected or laboratory-confirmed COVID-19 in adults and children hospitalized with severe COVID-19. However, quite different results were reported in a randomised, double-blind, placebo-controlled, multicenter trial carried out in China enrolling a total of 236 evaluable patients (158 with remdesivir and 78 with placebo). Remdesivir was not associated with statistically significant clinical benefits although, among patients treated earlier, those receiving remdesivir showed a not significant faster time to clinical improvement (hazard ratio 1.52; 95% CI 0.5–2.43). Incidence of adverse events was similar in both groups (66% vs 64%), although a greater number of patients given remdesivir stopped early the treatment for adverse events (12% vs 5%) [43].

On the basis of these results, it could be concluded that the role of remdesivir for COVID-19 treatment is not precisely defined. Although remdesivir remains the most promising anti-SARS-CoV-2 drug, its efficacy must be confirmed. Moreover, dosage, route of administration and duration of treatment need a more careful evaluation. It has been calculated that, due to its physico-chemical and pharmacokinetic properties, when given intravenously at the presently recommended dosages, remdesevir could not reach adequate antiviral concentrations where the virus causes the most important lesions [44]. On the other hand, studies in monkeys infected by Ebola virus and receiving drug dosages substantially equivalent to those used in humans, showed that remdesivir and its active metabolite, nucleoside triphosphate, were not detectable or were in very low concentrations in the lung [45]. This explains why to improve remdesivir efficacy against lung SARS-CoV-2 damage pulmonary delivery of the

drug has been suggested [44]. Moreover, duration of treatment must be precisely defined as in a study in which 5 and 10 days of drug IV administration were compared, no advantage of a longer treatment was evidenced, although longer duration was associated with an increased incidence of severe adverse events leading to treatment discontinuation [46]. In addition, true safety and tolerability of remdesivir deserve attention. Generally, the drug was found safe and well tolerated but the potential role of remdesivir drug as cause of liver damage has not been clarified. It is not established whether increase of ALT reported in some patients receiving remdesivir can be ascribed to the drug itself or is simply the consequence of a virus-related liver damage [47]. Finally, it cannot be forgotten that remdesivir cannot be given orally and this is a significant limitation. If administered by mouth, it would be totally ineffective because immediately hydrolyzed to nucleoside monophosphate that is not absorbed. However, IV administration limits the use to hospitalized patients and excludes its use for prophylaxis. Modifications of remdesivir's structure and formulations in order to improve the poor tissue distribution/penetration in the target organs are possible solution to increase final efficacy of the drug [44]. It is desirable that the numerous ongoing studies can solve these problems.

2.3 Lopinavir/ritonavir

Lopinavir and ritonavir were developed in 2000 to fight HIV, although subsequent studies have demonstrated their *in vitro* and likely *in vivo* action against other RNA viruses, such as SARS-CoV-2 and MERS-CoV [48, 49]. Ritonavir works as a booster of lopinavir by lowering CYP450 metabolic activity, thus increasing the lopinavir half-life. This oral combination drug belongs to the class of protease inhibitors and supposedly works by inhibiting the 3-chymotrypsin-like protease of SARS-CoV-2. In 2004, during the SARS epidemic, Chu et al. demonstrated, through an open nonrandomized clinical trial, that the administration of a combination of ribavirin and lopinavir/ritonavir (LR) was associated with a milder clinical course of the disease, less chance of serious complications and reduction in the viral load in comparison to another historical group treated with ribavirin alone [50]. This led several health authorities to include LR among the therapies potentially effective and the combination was frequently used on a compassionate basis and later included in several trials.

Unfortunately, despite some case reports have suggested a potential positive effect of LR also in COVID-19 patients [51, 52], results of the only randomized clinical trial evaluating LR efficacy till now published were disappointing [53]. A total of 199 subjects with severe COVID-19 were studied and it was shown that LR administration did not reduce mortality, duration of illness and virus shedding compared to standard of care. Poorly satisfactory were also the results of a study that examined the benefit-risk profile of LR in COVID-19 patients compared to standard of care, placebo or other treatments [54]. Although some data seem to indicate that patients given LR had a lower risk of adverse events than controls, authors concluded that the benefit-risk profile for LR in severe COVID-19 cannot be considered positive. However, drawing definitive conclusion on the true role of LR for COVID-19 seems presently not possible. Efficacy data available regard only patients with severe disease. It is not known if treatment of less severe cases may be effective. Moreover, treated patients were given the combination after several days of disease and it is not precisely defined whether an early administration may lead to more satisfactory results. In addition, dose of LR used for COVID-19 treatment is derived from that given to patients with HIV. Taking into account the IC50 for HIV and those for SARS-CoV-2, it was calculated that the doses prescribed to COVID-19 patients could not be adequate to inhibit viral replication [55]. In addition, some experts are using LR against COVIS-19 in association with other drugs. It seems clear that also for LR a definitive evaluation about its role in COVID-19 treatment will be available only after the conclusion of the clinical trials presently ongoing.

2.4 Ribavirin, favipinavir and umifenovir

Ribavirin, a guanine analogue, has shown activity against several RNA viruses, as it has been approved for the treatment of HCV, respiratory syncytial virus

(RSV) and Lassa virus infections and was supposed to be effective against SARS-CoV-2, although no clear advantages were observed after its administration to patients affected by SARS and MERS [56]. Additionally, severe side effects, such as hemolytic anemia and hepatotoxicity, related both to the mechanism of action of the drug and to the high dosage requested, have been reported [57, 58]. For these reasons, IV use of ribavirin in COVID-19 patients is generally not recommended. On the contrary, its compassionate use for inhalation has been considered by several health authorities worldwide and a number of open-label clinical trials have been authorized and are presently ongoing [59, 60].

Favipiravir is a guanine analogue that is licensed in Japan and China for oral treatment of selected influenza cases, those due to a novel or re-emerging influenza virus resistant to all other available influenza drugs [61]. It is generally safe and well tolerated, although concerns regarding a potential teratogenic activity have been raised. Favipinavir is *in vitro* effective against several RNA viruses, including SARS-CoV and MERS-CoV [62], and was found able to induce a rapid viral load decrease and clinical improvement in experimental animals infected by RNA viruses [63]. For these reasons, favipiravir has been tested in Vero E6 cells against SARS-CoV-2, showing that it could be effective in inhibiting viral replication [64]. Results of studies carried out in humans with COVID-19 are scanty, although several clinical trials in which this drug was administered together with other anti SARS-CoV-2 drugs have been authorized and are ongoing. However, in a nonrandomized trial comparing favipiravir and LR in addition to standard therapy in patients with COVID-19 it was shown that patients given favipiravir had a faster viral clearance and a greater improvement of disease course and chest X-ray than those receiving LR [65].

Umifenovir is an oral antiviral drug licensed for prophylaxis and treatment of influenza in Russia and China. As favipinavir, it is effective against several RNA viruses, including SARS-CoV-2 and has been used in prophylaxis and therapy of COVID-19 [66]. However, results are conflicting and real effectiveness of this drug is unknown. A retrospective study carried out in a group of 81 patients with mild to moderate disease did not show any advantage of umifenovir administration as the prevalence of negative swabs after 7 days of therapy and the duration of clinical manifestations were quite similar between treated and untreated patients. Moreover, treated patients had a longer hospital stay than patients in the control group (13 days vs 11 days; p=0.04), although adverse reactions were mild and with similar frequency in both groups [67]. Results showing no effect of umifenovir are reported also by Zhou et al., who carried out a study in which patients with COVID-19 were treated with either nebulized interferon (IFN)-α2b, umifenovir or a combination of IFN-α2b plus umifenovir [68]. Only treatment with IFN-α2b was effective as patients receiving this medication with or without umifenovir significantly reduced the duration of virus shedding from the upper respiratory tract and showed a decreased serum interleukin (IL)-6 and C-reactive protein concentration. A little better were the results of a study in which umifenovir has been used for prophylaxis of health professionals on the front line of the COVID-19 outbreak. Results showed that subjects given the drug were at a significant low risk of SARS-CoV-2 infection than those without prophylaxis (log-rank test, $\chi 2 = 98.74$; $p<0.001$). However, risk of hospitalization was not different between treated subjects and controls (p=0.091) [69].

3. Anti-inflammatory Drugs

To fight the over-reactive immune response that characterizes the third stage of the COVID-19 course before it results in life-threatening multi-organ dysfunction, the use of immunomodulatory agents capable of reducing the systemic inflammation has been repeatedly suggested [70]. They should be added to antivirals already administered in the first phases of disease. Theoretically, macrolides, corticosteroids, cytokine inhibitors such as tocilizumab or other monoclonal antibodies against different cytokines, and intravenous immune globulin (IVIG) could be effective in this regard. To elucidate their role, several phase III clinical trials are presently ongoing.

3.1 Macrolides

Macrolides are antibiotics that work by binding 50s bacterial ribosomal units, thus inhibiting protein synthesis. They are mainly active on Gram-positive and atypical bacteria [71]. Moreover, they have anti-inflammatory and immunomodulatory activity. *In vitro* and *in vivo* studies have shown that macrolides downregulate the inflammatory cascade, attenuate excessive cytokine production in viral infections, and may reduce virus-related exacerbations of chronic respiratory diseases. Finally, some macrolides and, in particular, azithromycin might have a direct antiviral activity through the blockage of virus internalization into host cells during the early phase of infection [72]. Efficacy in rhinovirus, RSV, and influenza virus infection has been reported [73]. Potentially effective to treat COVID-19, macrolides were initially included in some protocols for empirical treatment of this disease. However, contrary to expectations, use of macrolides in COVID-19 patients was disappointing. Most of the data have been collected in subjects with mild to moderate disease that received azithromycin together with a second drug, mainly an aminoquinoline. Results were conflicting, as previously reported for CQ and HCQ [17, 18, 28, 29]. Moreover, as macrolides can cause the same heart problem of aminoquinolines [74], patients included in protocols in which macrolides were associated with CQ or HCQ frequently suffered from severe heart problems.

3.2 Corticosteroids

At the beginning of the pandemic period, corticosteroids were not included in the list of drugs recommended for COVID-19 therapy [75]. This because all the studies that had tested corticosteroids for treatment of respiratory infections, including those as SARS and MERS with similarities with the new pandemic, had given negative results. Children with RSV infection had no benefit from corticosteroids administration [76]. Administration of corticosteroids to patients with influenza has been associated with increased mortality, increased length of stay in an intensive care unit and higher risk of secondary bacterial or fungal infection [77]. Finally, patients with SARS and MERS had no appreciable advantages from corticosteroid use and, in some cases, these drugs were found dangerous as they delayed virus clearance or caused relevant adverse events such as psychosis and diabetes [58, 78]. On the other hand, when corticosteroids were added to standard therapy in COVID-19 no effective outcome was observed [79, 80].

However, a recent report seems to subvert any previous negative judgment. Although the assumptions did not favor their use, corticosteroids were included in the Recovery Trial. In an arm of this study, COVID-19 patients receiving dexamethasone 6 mg once daily (oral or IV) for up to 10 days together with standard treatment were enrolled and compared to subjects given standard treatment only [81]. Results showed significant dexamethasone efficacy as 28-day mortality and duration of hospitalization. Both these markers of drug efficacy were significantly lower in subjects given dexamethasone than in controls. Mortality was 21.6% and 24.6% (rate ratio [RR] 0.83; 95% CI 0.74 - 0.92; p<0.001] and median duration of hospitalization was 12 and 13 days (RR 1.11; 95% CI 1.04 - 1.19; p=0.002) in treated patients and in controls, respectively. Subgroup analysis revealed that benefit was strictly related to the severity of lung impairment as mortality was reduced by 35% in patients needing invasive mechanical ventilation (RR 0.65; 95% CI 0.51 - 0.82; p<0.001) and by 20% in patients receiving only oxygen (RR 0.80; 95% CI 0.70 to 0.92; p=0.002). On the contrary, no advantage of dexamethasone was evidenced in patients with mild disease who did not need respiratory support (RR 1.22; 95% CI 0.93-1.61; p=0.14). Similarly, the greatest effect on duration of hospitalization was seen among patients receiving invasive mechanical ventilation at baseline (test for trend, p=0.002). Finally, progression to severe damage requiring mechanical ventilation was reduced among patients given dexamethasone compared to those with usual care (RR 0.76; 95% CI 0.61-0.96; p=0.021). Unfortunately, this study has been published before certification by peer review and some doubts on the validity of the results might be arisen. However, it is very interesting to encourage further studies regarding steroid use for COVID-19 treatment. As indicated by the authors themselves, results suggest the steroids can be effective when proper dosages of the drugs in accurately

selected patients are given. Corticosteroids should be given to patients in phase III of disease, when hyperinflammation begins to cause tissue damage. An earlier administration can be totally ineffective and, on the contrary, can cause adverse events. If these results are confirmed, current recommendations for the therapy of COVID-19 should include corticosteroids, as already done in UK [82].

3.3 Monoclonal antibodies

With the aim of reducing cytokine storm and systemic hyperinflammation that characterize the phase III of COVID-19, the use of monoclonal antibodies effective against those cytokines that have been systematically found increased in serum of patients with very severe disease has been suggested [70]. Reduction of serum concentrations and activity of IL-2, IL-6, IL-7, IL-10, and 17, GM-CSF, interferon-inducible protein 10, MCP-1 macrophage inflammatory protein-1 α, and TNF-α have been considered a possible measure to reduce signs and symptoms of COVID-19 and improve final outcome [83, 84].

Tocilizumab (TCZ), that acts by binding to both soluble and transmembrane IL-6 receptors, so inhibiting their activation, is the most largely studied monoclonal antibody for COVID-19 therapy. Before pandemic development, this monoclonal antibody had been licensed for treatment of a number of severe autoimmune diseases, such as rheumatoid arthritis, juvenile idiopathic arthritis and Crohn's disease. In patients with these diseases results of therapy was considered satisfactory as, with the reduction of serum IL-6 levels, disease course was controlled without relevant adverse events, although the risk of superimposed infections could not be excluded [85]. Starting from these evidences, already during the first weeks of the pandemic TCZ was included in the therapy of the most severe COVID-19 cases and, after the evidence that some patients had had benefits, several randomized controlled trials to establish the relevance of TCZ therapy were planned and authorized.

With some exceptions [86], results were positive as TCZ administration was associated with a relevant reduction of clinical and laboratory manifestations of COVID-19 in most of the treated patients. Need for oxygen support was curtailed, chest X-ray was rapidly improved, lymphopenia was reduced as they were the serum level of C-reactive protein and IL-6 [87-93]. The most striking results were evidenced in the most severe patients who less frequently needed mechanical ventilation and went to death. Klopfenstein et al. studied frequency of intensive care unit (ICU) admission and death rates in a group of 45 patients among whom 25 were given TCZ [94]. They reported that, despite patients given TCZ had a more severe clinical picture (higher level of oxygen therapy at 13 L/min vs 6 L/min, $p<0.001$), and poorer biological findings (severe lymphopenia: 676/mm^3 vs 914/mm^3, $p=0.037$; higher CRP level: 158 mg/L vs 105 mg/L, $p=0.017$), they were less frequently admitted to the ICU or went to death (25% vs 72%, $p=0.002$). Similar findings were evidenced by a greater prospective study in which a total of 544 patient were enrolled [88]. TCZ was given by the IV or subcutaneous route on the base of the availability of specific formulation. After adjustment for several confounding factors such age, sex, duration of illness and sequential organ failure assessment score, it was shown that, compared to patients given only standard treatment, those receiving TCZ together standard care had a significant lower risk of invasive mechanical ventilation and death (adjusted HR 0.61, 95% CI 0.40–0.92; $p=0.020$). Further results, suggesting the importance of TCZ in limiting risk of COVID-19 negative evolution, are those derived from the CORIMMUNE-19 study, a French study in which 129 patients (64 treated with standard of care and 65 with standard of care plus TCZ) were enrolled [95]. However, despite these encouraging findings, definitive conclusions about when and how use TCZ in COVD-19 patients and what can be expected from its use cannot be drawn. Analysis of the methods used to collect data regarding TCZ indicates that in some cases studies lack a clear analytical approach and show poor methodological quality. Moreover, criteria used to define COVID-19 severity, characteristics of the patients that could have benefit, choice of drug dosage and schedule of administration and number and type of markers for evaluation of disease improvement were frequently very different from study to study, making pooling of data and final evaluation very difficult. Two examples can clearly highlight these problems.

Regarding dosage and schedule of administration, it is not definitively established whether TCZ must be given once or twice daily. Moreover, it is not known whether the 8 mg/kg/dose that is commonly administered in rheumatologic diseases is the right dosage against to obtain the greatest efficacy with the lower risk of adverse events. A recent small study has shown that a single dose of 400 mg can be enough to obtain satisfactory results [96]. Regarding choice of the patients to treat, in a recent comprehensive review it was concluded that most of the available information leads to consider TCZ use in patients with extensive lung involvement, and severe or critical patients with high IL-6 levels. However, cut-off levels are not established, and severity is not defined [97]. Considering overall the data, we can conclude that even in the case of TCZ only further randomized clinical trials can offer solutions to these problems.

4. Convalescent Plasma

Several studies have reported that administration of convalescent plasma containing high titers of neutralizing antibodies to patients with severe viral respiratory disease can be effective in improving clinical course and survival rates against SARS-CoV-2 without any relevant adverse event. A recent meta-analysis of studies published between 1918 and 1925 regarding Spanish influenza pandemic has shown that administration of convalescent human blood products to treat severe influenza cases was already in use with satisfactory results about a century ago [98]. This practice has been later followed for respiratory diseases due to different viruses including SARS-CoV. In this regard, plasma infusion has been associated with a 23% reduction of case-fatality rate (95% CI 6%-42%; p=0.049) [99]. A positive effect was, finally, evidenced in some, even if not all, patients with MERS [100].

Experience in COVID-19 patients is limited for the small number of treated patients, difference in dose, schedule of plasma administration, severity of disease and time of administration. First reports seemed to indicate that the treatment could be effective, as it was associated with a reduction of clinical manifestations, duration of virus shedding and reduced mortality. In an uncontrolled case series of 5 critically ill patients with COVID-19 and acute respiratory distress syndrome, treatment was followed by a rapid improvement of clinical conditions, with significant decrease of sequential organ failure assessment score and reduction of oxygen administration, mechanical ventilatory support and viral shedding within few days from the beginning of therapy [101]. Another study enrolling 10 severely affected patients showed that in most of the cases transfusion was followed within 3-7 days by improvement of pre-therapy clinical symptoms, radiological manifestations and laboratory tests, although with differences among patients. In general, respiratory support was significantly reduced, lung lesions on computed tomography (CT) examination were partially or totally absorbed, oxyhemoglobin saturation and lymphocyte increased, whereas C-reactive protein decreased [102]. Starting from these and other anecdotical positive results, several national institutions planned and approved clinical trials with the aim to establish the real role of convalescent plasma in COVD-19 treatment and decide the best dose and schedule of administration. Unfortunately, results of one trial enrolling a substantial number of patients with severe or life-threatening COVID-19 (52 with convalescent plasma in addition to standard treatment and 51 with standard treatment alone) did not show any significant effect, arising doubts about the importance of convalescent plasma transfusion. Clinical improvement within 28 days was evidenced in 51.9% of the convalescent plasma group vs 43.1% in the control group (HR 1.40; 95% CI 0.79-2.49; p=0.26) without differences according to the severity of disease. Moreover, no significant difference was found in 28-day mortality (15.7% vs 24.0%; OR 0.65; 95% CI 0.29-1.46; p=0.30) [103].

However, starting from the available data it seems highly likely that to be effective convalescent plasma transfusion must take place as soon as possible after the infection. Cheng et al. studying patients with SARS had already shown that the prevalence of those who had had a good clinical outcome was significantly higher among those who were given convalescent plasma before day 14 after symptom onset than among those treated later (58.3% vs 15.6%; p<0.001) [104]. In COVID-19 patients, data supporting the need for an early convalescent plasma

transfusion are reported in the study by Duan et al., in which it is clearly evidenced that those patients with the poorest outcome were just those who received convalescent plasma later [102]. This is not surprising. SARS-CoV-2 load peaks after few days from infection, while antibody production begins no earlier than a week and became significant may days later. This seems to indicate that, if the intent of the convalescent plasma transfusion is to reduce viral replication and have a substantial effect on tissue damage reduction, antibodies must become available in the first phase of disease. However, other problems that are essential to assure maximal efficacy of convalescent plasma transfusion must be solved. Characteristics of the donors, time of donation after COVID-19 resolution, anti-SARS-CoV-2 antibody titer required for plasma effectiveness are not precisely defined and must be known before the convalescent plasma use can be definitively included among measure to treat COVID-19.

5. Drugs Against Coagulopathy and Thromboembolism

Among the clinical manifestations that characterize COVID-19, coagulopathy and venous thromboembolism are common. Two different coagulation abnormalities have been identified. The first is due to the direct pathological activity of the virus on vasal endothelial that causes microvascular clot formation in the lung and in other organs. The second is part of the consequences of the cytokine storm as the enhanced cytokine production stimulates hypercoagulability that may be associated with vessel thrombosis and major thromboembolic complications [105]. As coagulopathy may contribute to rapid deterioration of clinical picture, monitoring of coagulation variables is mandatory, particularly in patients with symptoms severe enough to need hospitalization. To avoid risks of venous thromboembolism, administration of low molecular weight prophylaxis is recommended [106]. However, the best dosage is not precisely defined, and clinical trials are presently ongoing to define this problem.

6. Conclusions

Waiting the development of vaccines and drugs specifically addressed to the prevention and treatment of SARS-CoV-2 infection, health systems around the world have tried to cope with the worst pandemic of the last century with a number of drugs with undocumented efficacy. The search for verification of which of them could play an important role in the management of COVID-19 was, at least in part, hindered by the enormous number of infected people and the urgency of trying to reduce as much as possible the number of severe cases who needed invasive mechanical ventilation and were a high risk of death. Choice of drugs, possible associations, dosages and duration of treatment were based on opinion of physicians and previous experience about the efficacy of the same therapeutic measures in other similar conditions. Only later, with the acquisition of more in-depth knowledge on the characteristics of SARS-CoV-2 and the pathogenesis of the different clinical manifestations of COVID-19, a more rationale use of available drugs has become possible. It was possible to identify which drugs could be used in the different phases of COVID-19 and implement many randomized clinical trials specifically devoted to elucidating the best drug use. However, the road to defining which drugs are effective and which schedules of administration must be used to maximize efficacy and minimize adverse events is still very long. To date, it is only clear that no drug can alone cope with all the problems posed by SARS-CoV-2 infection and effective antivirals and inflammatory drugs must be given together to reduce COVID-19 clinical manifestations. Moreover, choice of therapy must always be tailored on clinical manifestations and, when they occur, drugs able to fight coagulopathy and venous thromboembolism that may contribute to respiratory deterioration must be prescribed.

Author Contributions

SE planned the review and co-wrote the manuscript giving a substantal scientific contribution; MGn and MGa wrote the first draft of the manuscript; PA,

LV and MEC gave a substantial scientific contribution; CN and AA performed the literature review; NP critically revised the paper and gave her scientific contributions. All of the authors read and approved the final version of the manuscript.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Received: 15 August 2020
Accepted: 17 August 2020
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