Current treatments and scientific advancements to combat the epidemic novel coronavirus.

F.Ferrara 1, C.Pelliccia 2, A.Vitiello 1

1: Usl Umbria 1, Perugia, Italy
2: Usl Umbria 2, Terni, Italy

francesco.ferrara@uslumbria1.it

Abstract

An acute respiratory disease, caused by a novel coronavirus (SARS-CoV-2) has spread throughout China and other countries and received worldwide attention. On 30 January 2020, World Health Organization (WHO) officially declared the SARS-CoV-2 epidemic as a public health emergency of international concern. At the moment of writing this article (April 2020) a total of 2.240.191 cases confirmed worldwide since the outbreak and 153.822 deaths in 166 countries or regions confirmed cases globally had been reported. Meanwhile, several independent research groups have identified that SARS-CoV-2 has highly identical genome with SARS-COV-1. The novel coronavirus uses the same receptor, angiotensin-converting enzyme 2 (ACE2) as that for SARS-CoV, and mainly spreads through the respiratory tract. The clinical symptoms of patients include fever, cough, fatigue and a small population of patients appeared gastrointestinal infection symptoms. Currently, there are few specific therapeutic strategies, but several potent candidates of antivirals and repurposed drugs are under urgent investigation. In this review, we summarized the current treatment and scientific advancements to combat the epidemic novel coronavirus.
Keywords: SARS-CoV-2, treatment, coronavirus, infection, pneumonia

Introduction

In December 2019, a cluster of pneumonia cases, caused by a newly identified coronavirus (SARS-COV-2), occurred in Wuhan, China. This coronavirus, has rapidly spread throughout China and other countries, thus receiving worldwide attention. The SARS-CoV-2 is a β-coronavirus, which is an enveloped, non-segmented positive-sense RNA virus which can lead to severe and potentially fatal respiratory tract infections. It was found that the genome sequence of SARS-CoV-2 shares 79.5% identity to SARS-CoV. Based on virus genome sequencing results and evolutionary analysis, bats have been suspected as natural hosts of the original virus. SARSCoV-2 might be transmitted from bats via unknown intermediate hosts to infect humans. It is clear now that SARS-CoV-2 could use angiotensin-converting enzyme 2 (ACE2), the same receptor as SARS-CoV, to infect humans.

Epidemiology and Clinical characteristics of sars Cov 2

Based on current epidemiological investigation, the incubation period is 1–14days, mostly 3–7 days. The SARS-COV-2 is contagious during the latency period. It is highly transmissible in humans, especially in elder populations and in patients with previous disease. Most adults or children with SARS-CoV-2 infection present mild flu-like symptoms and only a few patients develop critical conditions due to the insurgence of acute respiratory distress syndrome, respiratory failure, multiple organ failure.
Common clinical manifestations include fever (88.7%), cough (67.8%), fatigue (38.1%), sputum production (33.4%), shortness of breath (18.6%), sore throat (13.9%), and headache (13.6%) [16]. In addition, a part of patients can manifest gastrointestinal symptoms, including diarrhea (3.8%) and vomiting (5.0%).

The elderly and those with underlying disorders (i.e., hypertension, chronic obstructive pulmonary disease, diabetes, cardiovascular disease), may rapidly develop acute respiratory distress syndrome, septic shock, hard to correct metabolic acidosis and coagulation dysfunction, even leading to death. In laboratory examination results, most patients have normal or decreased white blood cell counts, and lymphocytopenia, but in the severe forms, the neutrophil count, D-dimer, blood urea, and creatinine levels are more significant, and the lymphocyte counts shows a continued decrease. Additionally, inflammatory factors (interleukin (IL)-6, IL-10 and tumor necrosis factor-α (TNF-α) may increase, highlighting the state of the immune system. The data showed that patients mostly have higher plasma levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (GCSF), 10 kD interferon-gamma induced protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1-α (MIP-1α), and TNF-α .
Based on the current data, seem that most patients tend to have a good prognosis, while only a few patients may manifest very critical condition, especially the elderly and those with chronic underlying diseases. Complications include acute respiratory distress syndrome (ARDS), arrhythmia, shock, acute kidney injury, acute cardiac injury, liver dysfunction and secondary infections. The poor clinical outcome is related to disease severity. The disease tends to progress faster in elderly people, with the median number of days from the occurrence of the first symptoms to death shorter among people with 65 years of age or more. Neonates and elders need more attention and care due to their immature or weak immune system. (1-17)

Treatment of COVID-19

There are currently no effective and direct drugs developed especially for treating SARS-CoV-2.
While waiting for new specific drugs to be developed, the off-label use of drugs already on the market, even if registered for different therapeutic indications, has proven to be a valid alternative. Based on the experience gained from previously fighting the SARSCoV and MERS-CoV epidemics, and given the high genomic homology between the two viruses, some treatment strategies could be similar. To date, ongoing treatments are mainly aimed at resolving the symptoms of pneumonia caused by the virus. Rescue treatments with convalescent plasma and immunoglobulin G are administered in some critical cases according to their clinical characteristics. Antiviral drugs and systemic treatment with corticosteroids commonly used in clinical practice, including neuraminidase inhibitors (oseltamivir, peramivir, zanamivir, etc.), ganciclovir, acyclovir and ribavirin, and methylprednisolone for influenza virus, showed no efficacy to date.

Remdesivir is an antiviral drug in the class of nucleotide analogues. It has been developed as a treatment for Ebola virus and Marburg virus infections. It has also shown antiviral activity against other single-stranded RNA viruses such as the human respiratory syncytial virus, the Junin virus (Argentine mamrenavirus), Lassa fever virus, Nipah virus, Hendra virus and coronavirus (including viruses causing MERS and SARS). It is undergoing studies for its possible use against SARS-CoV-2 infections. For the World Health Organization Remdesivir is one of the best candidate to fight the coronavirus SARS-CoV-2. The drug has been used as an experimental antiviral therapy at the National Institute for Infectious Diseases "Lazzaro Spallanzani" in Rome to successfully treat an increasing number of patients affected by Sars-Cov2. Following the example of the Spallanzani Institute various other Italian Hospital are currently utilizing this particular treatment protocol in SARS-CoV2 positive patients. A randomized, controlled clinical trial to evaluate the safety and efficacy of remdesivir in hospitalized adults diagnosed with SARS-CoV-2 has begun at the University of Nebraska Medical Center (UNMC) in Omaha and two Phase3 studies including a total of nearly 1500 SARS-CoV2 positive patients are going to published their results by the end of May 2020.

Lopinavir/Ritonavir is indicated, in combination with other antiretroviral drugs, for the treatment of children over 2 years of age, adolescents and adults with human immunodeficiency virus (HIV-1) infection. Lopinavir determines the antiviral action while Ritonavir increase the plasmatic volume of the latter. Lopinavir is an inhibitor of HIV protease 1 and HIV 2. The inhibition of HIV protease prevents the cleavage of the gag polyprotein, resulting in the production of immature, non-infectious, viruses. Few patients with SARS-CoV-2 were given antiviral treatment including

Lopinavir/Ritonavir. After treatment, three out of four patients showed significant improvement in pneumonia associated symptoms, two of whom were then confirmed to be SARS-CoV-2 negative and discharged, and one of whom was negative for the virus at the first test. Two reviews, including a Chinese one, showed that Lopinavir/Ritonavir may be a valid drug treatment option for SARS-CoV-2. However, a retrospective study enrolling 134 NCP (Novel coronavirus pneumonia) patients revealed that there is no significant difference between the Lopinavir/Ritonavir treated group (n=52), and control group (n=48) in improving symptoms or in reducing viral loads. The efficacy of Lopinavir/Ritonavir antiviral treatment warrants further verification in future studies. Nine randomized controlled trials of Lopinavir/Ritonavir in patients with COVID-19 have been registered in China up to February 22. Currently, the combination of Lopinavir/Ritonavir is a recommended antiviral regimen in the latest version of the Diagnosis and Treatment of Pneumonia Caused by SARS-CoV-2 issued by the National Health Commission of the People's Republic of China, but to date, there are no FDA-approved treatments for any human CoV infection. Most in vitro studies have shown that SARS-CoV2 could be inhibited by Lopinavir/Ritonavir. Furthermore, two retrospective matched cohort studies of SARS patients revealed that Lopinavir/Ritonavir plays an essential role in the clinical outcome, especially in the early stage. Lopinavir/Ritonavir treatment alone or in combination with interferon had improved clinical outcomes in experiments involving common marmosets and in some MERS patient. Although some data indicate the potential efficacy of Lopinavir/Ritonavir, adverse reactions should be kept in mind. Diarrhea, nausea and asthenia are the most frequently reported reactions in patients receiving LPV therapy. Elevated total bilirubin, triglyceride and hepatic enzyme levels have also been reported. A retrospective study of MERS showed that the most common symptoms and laboratory tests of Lopinavir/Ritonavir were diarrhea (40.9%), nausea (40.9%), stomatitis (18.2%), fever (13.6%), anemia (45.0%), leukopenia (40.0%) and hyperbilirubinemia (100%). However, the symptoms and laboratory tests returned to normal after Lopinavir/Ritonavir therapy ceased. The protease inhibitor Lopinavir/Ritonavir could be an effective treatment based on experience accumulated from the SARS and MERS outbreaks but, to date, scientific results in the current emergency have not been convincing. An open-label, randomized clinical trial, which will compare the efficacy of lopinavir/ritonavir vs. hydroxychloroquine in 150 patients with mild COVID-19, is currently ongoing in the Republic of Korea and the treatment in question is already used in Italy. As for other antiviral treatments at the end of March 2020 the Italian Medicine Agency (AIFA) started the evaluation of available scientific evidences with the aim to understand if a clinical program to assess the efficacy and safety of favipiravir is appropriate.

Chloroquine has been used to treat malaria for many years and presents an antiviral activity against many other viruses but with an almost unknown action’s mechanism. It presents anti rheumatic properties with a slow onset. The therapeutic action in this case is based on various pharmacological effects such as: interaction with sulfhydryl groups, modulation of enzymatic activity (in particular phospholipase, NADH-cytochrome C reductase, cholinesterase, protease and hydrolase), DNA fixation, stabilization of lysosomal membranes, inhibition of prostaglandin synthesis, polymorphonucleate chemotaxis and phagocytosis, possible interference with interleukin 1 production by monocytes and inhibition of superoxide release by neutrophils.
Both the anti-rheumatic effect and the antimalarial effect can be explained in relation to the concentration reached in the intracellular acid vesicles and through the increase of their pH. Several possible mechanisms have been duly investigated: Chloroquine can inhibit the PH dependent phases of viral replication with a powerful effect on SARS-CoV infection and diffusion. In addition, chloroquine has an immune-modulating effect, suppressing the production/release of TNF-α and IL-6. It also functions as a class of autophagy inhibitor, which may interfere with virus infection and replication. Several studies have found that chloroquine interfered with SARS-CoV cellular glycosylation receptors and worked on both entry and post-entry stages of SARS-CoV-2 infection in Vero E6 cells. A combination of remdesivir and chloroquine has shown effective inhibition on the recently emerged SARS-CoV-2 in vitro.

Tocilizumab is indicated for the treatment of severe, active and progressive rheumatoid arthritis (RA) in adults not previously treated with MTX, treatment of moderate to severe active RA in adults who have not responded adequately or are intolerant to previous therapy with one or more disease-modifying anti-rheumatic drug(s)(DMARD) or tumor necrosis factor (TNF) antagonists. Tocilizumab binds specifically to both soluble and membrane IL-6 receptors (sIL-6R and mIL-6R). Tocilizumab has been shown to inhibit signals mediated by sIL-6R and mIL-6R. IL-6 is a pleiotropic proinflammatory cytokine produced by several cell types, including T and B cells, monocytes and fibroblasts. IL-6 is involved in various physiological processes, such as activation of T cells, induction of immunoglobulin secretion, induction of liver synthesis of acute phase proteins and stimulation of hematopoiesis. IL-6 is involved in the pathogenesis of diseases, including inflammatory diseases, osteoporosis and neoplasms. The Chinese National Health Commission in its new treatment guidelines for SARS-CoV-2 established that Tociluzumab, may also be used for the treatment of coronavirus patients with severe lung damage. Some coronavirus-infected patients may develop an uncontrolled immune response, leading to life-threatening lung tissue damage. After verifying the promising results in clinical practice, the Chinese authorities have made the decision to recommend the use of the medication. The scientists initiated a randomized clinical trial to evaluate the application of the drug. According to the Chinese Clinical Trial Registry, the investigators aim to enroll 188 patients, half of whom will be treated with Tocilizumab. The drug does not affect the new coronavirus, nicknamed SARS-CoV-2. It is an interleukin 6 receptor inhibitor (IL-6), a proinflammatory cytokine. In SARS-COV 2 disease the body can respond to the pathogen by overproducing immune cells and their signaling molecules in a dangerous phenomenon called cytokine storm. Similar lung inflammation was observed in SARS patients during the 2003 outbreak, especially in China. Research groups have recently identified IL-6 as the main culprit for this immune system overreaction among SARS COV2 patients, hence the Tocilizumab clinical study. In its previous treatment guidelines, the Chinese authorities have already included a high level of IL-6 as an indicator of worsening the disease and in its current update, the use of Tocilizumab is limited to patients with that marker. Lastly a non-randomized phase II clinical trial recently started aiming to evaluate the efficacy and safety of varius drugs related to an anti-inflammatory effect like baricitinib, lopinavir/ritonavir, hydroxychloroquine and sarilumab in the treatment of 1,000 hospitalized patients with COVID-19. With the aim of reducing inflammation symptoms, sunitinib, fedratinib and ruxolitinib, which are all selective JAK inhibitors, may be potentially effective against SARS-CoV-2.

In vitro studies conducted by a group of Chinese researchers have revealed that SARS-CoV-2 virus seems to disappear in contact with high concentrations of enoxaparin sodium, an anticoagulant among the most used for the prevention of venous thromboembolism. The interesting discovery has led Chinese scientists to start clinical studies, administering a high dosage of the active ingredient to patients affected by SARS COV2, and the preliminary results seem very promising. Hepalink Group, world leader in the production of heparins, has in fact started two clinical trials in Shenzhen No. 3 at People's Hospital and Concord Hospital linked to Tongji Medical School, within the Middle China Science & Technology University complex, aimed at confirming and deepening the data. Sodium enoxaparin is a low molecular weight heparin with a strong antithrombotic activity. Due to its anticoagulant efficacy, it is authorized and normally used in the prophylaxis of venous thromboembolism (VTE), particularly in patients undergoing surgery or at risk of developing clots caused by being bedridden. In recent years, numerous scientific evidence has confirmed the role of heparin even in cases of Covid-19 infection, in reducing death caused by to thromboembolic phenomena, which are far from infrequent in patients affected by the virus. During the inflammatory processes, typical of viral infections, the development of widespread thrombosis in the lungs and, in serious cases, also in other vital organs, is often seen and presents a serious risk to life. It is no coincidence that the WHO (World Health Organization) in its recent Recommendations to Improve Clinical Management of Severe Acute Respiratory Infections (SARS), urges to prevent a complication such as venous thromboembolism in adults and adolescents in hospital by resorting, in the absence of contraindications, to the subcutaneous administration of heparin, preferably low molecular weight. Now the usefulness of the known anticoagulant seems to go further, since data from the Far East are coming in that suggest its role on the very mechanism of action of the virus. By putting enoxaparin sodium in vitro in high concentration with the new coronavirus, the active

ingredient seems to determine a significant reduction of the pathogen, which would bind to heparin instead of attacking the cells of the body. Such encouraging data prompted Chinese researchers to start some clinical trials directly on infected patients, using the drug to fight the virus, at dosages higher than those normally used for prophylaxis, to prompt the reduction of inflammatory markers and the negation of tests.

A treatment can be developed from competitive interceptor of SARS- CoV and other coronaviruses by preventing binding of the viral particle to the surface-bound, full- length ACE2. Indeed, *in vitro* studies showed that SARS-CoV replication was blocked by a soluble form of ACE2 in the monkey kidney cell line, Vero-E6. Moreover, ACE2 fused to the Fc portion of immunoglobulin has just been reported to neutralize SARS-CoV-2 *in vitro* and the SARS-CoV-2 binds ACE2 with higher affinity than SARS-CoV. In this context, provision of soluble recombinant human ACE2 protein could actually be beneficial as a novel biologic therapeutic to combat or limit infection progression caused by coronaviruses that utilize ACE2 as a receptor. Soluble recombinant ACE2 protein has therapeutic potential for a vast array of therapeutic indications and novel shorter ACE2 variants are being tested in mouse studies for treatment of kidney diseases. If given in its soluble form as an appropriate recombinant ACE2 protein, a new tool may be at hand to fight the spread of coronavirus in susceptible individuals by limiting coronavirus attachment to the cell membranes, cell entry, and replication. To our knowledge, studies in animals or humans testing the therapeutic potential of soluble recombinant ACE2 proteins are not yet available. The potentially beneficial effect (or not) of soluble recombinant ACE2 proteins to attenuate coronavirus infection should be urgently tested. Mouse models that carry the human version of ACE2 with the mouse version deleted should greatly facilitate research in this rapidly emerging field. To date the more promising molecules with an effect of this kind are camostat mesylate and nafamostat mesylate, both approved in Japan for the treatment of the remission of acute symptoms of chronic pancreatitis and currently undergoing trials testing their efficacy in the potential treatment of SARS-COV2. A randomized, placebo-controlled clinical trial is evaluating the efficacy and safety of camostat mesilate in 180 patients with COVID-19 and both drugs will be evaluated in clinical trials launched by the University of Tokyo.

Many are the possible drugs being tested at the moment in which there are several monoclonal antibody such as Bevacizumab and Camrelizumab or different drugs like Thalidomide, but the results to date are not yet clear and mostly inconsistent.

Talking of the possibility of using vaccine, a Phase 1 clinical trial evaluating an investigational vaccine designed to protect against coronavirus has begun at Kaiser Permanente Washington Health Research Institute (KPWHRI) in Seattle. The National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health, is funding the trial. KPWHRI is part of NIAID's Infectious Diseases Clinical Research Consortium. The open-label trial will enroll 45 healthy adult volunteers with age 18 to 55 over approximately 6 weeks. The first participant already received the investigational vaccine. The study is evaluating different doses of the experimental vaccine for safety and its ability to induce an immune response in participants. This is the first of multiple steps in the clinical trial process for evaluating the potential benefit of the vaccine. Finally, Umifenovir (trade name Arbidol) is an antiviral marketed in Russia for the prevention and treatment of Influenza A and B virus infections. Umifenovir is not licensed in Europe nor in the United States. Although in China, during the SARS COV 2 epidemic, umifenovir has been used in some patients in combination with other pharmacological treatments, the available data are scarce, not of high scientific value and obtained on a very small number of patients. (18-55)

Conclusion

SARS-CoV-2 is spreading around the world at an alarming rate. Elders and immunocompromised patients are more vulnerable to the deadly repercussions of the virus. While some treatment protocols have shown some promise, including lopinavir/ritonavir, remdesivir, chloroquine and tocilizumab for

which scientific evidence and everyday clinical observation support their favorable efficacy and safety profile, there is no globally approved cure for the virus and no vaccine has been developed yet. It is opinion of the writers that at the moment, missing any widespread and specifically made treatment, the best course of action should be to pay as much attention as possible during the monitoring phase especially for possible drug-drug interaction seen the massive number of drugs used to date. Also is our opinion that the focus on prevention should be maintained as high as possible to prevent the further spread of the infection

Main statements

I, the undersigned, Francesco Ferrara and any other author, declare that:

- I have no conflict of interest;
- We have not received funding;
- There are no sensitive data and no patients were recruited for this study;
- The document does not conflict with ethical legislation.

Regards

The authors

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