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COVID-19 Outliers: Lessons to Better Understand SARS-CoV-2 Behavior

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Abstract

Purpose: COVID-19 has become a global epidemic with more than 6 million active cases worldwide. This is a huge number even when the active cases have started to decrease in most of the developed world and the threat is looming of a second wave. The question is where the second wave would come from? **Method:** Our hypothesis is that the answer lies in the COVID-19 outliers. In most epidemiological and clinical studies, focus is on the patients who represent the majority population however, there is every likelihood that re-emergence of the virus will be the from the cases that are unique and not following the norm. **Results and Conclusion:** This may allow us to understand the behavior of SARS-CoV-2 better and allow us to hypothesize better about its biology. This paper analyzes 6 such COVID-19 patients reported by different regional Centers for Disease Control in China, which should be considered with regards to further spread and developing effective control of SARS-CoV2 epidemic.

Keywords: COVID-19, clinical manifestations, epidemiology, special cases

1. Introduction

Since the onset of COVID-19 in Wuhan, China, in December 2019, SARS-CoV2 has expanded rapidly to all over the world infecting in excess of 25 million people and more than 840 thousand deaths [1]. Although more than seven months have passed since COVID-19 was declared a pandemic, our understanding of the disease is still somewhat limited particularly from the standpoint of pathogenesis [2] and how it will impact the spread of the virus [3]. We just carried out a large clinical study determining the efficacy of montelukast, hydroxychloroquine and invermectin in COVID-19 positive patients. The results of which are under review in another journal. During the data collection we found six very interesting COVID-19 patients, particulars of which should spark a debate in your readership and we would like to see what hypothesis they draw about SARS-CoV-2 biology and spread.

The SARS-CoV-2 is a novel coronavirus which is the infectious agent for COVID-19. It belongs to the β genus of coronaviruses where the diameter of the spherical, spiked coronovirus is 60 to 140 nm [4]. Structural analysis has revealed that SARS-CoV2 binds to human ACE-2 receptor through its spike protein [5]. Based on the current epidemiological survey, the incubation period is from 1-14 days, where 3 to 7 days is average for most of the population [6]. Clinical characteristics of most patients are mainly fever, fatigue and dry cough. However, a few patients are accompanied with symptoms such as nasal congestion, runny nose, sore throat and diarrhoea, and only a very small number of patients have transient anosmia. The more severe patients often have dyspnea and/or hypoxemia one or two weeks after the onset of the disease and critical patients end up with acute respiratory distress syndrome, septic shock, and even metabolic acidosis and coagulation dysfunction which are difficult to remedy.

Some special cases are different from the vast majority of patients, such as asymptomatic long incubation period, nucleic acid positive reexamination 10 days after discharge of the healer, confirmed diagnosis after the discharge of nucleic acid negative, sputum and pharynx negative stool positive.

2. Study Setting

A total of six COVID-19 outlier cases are reported here from various provincial hospitals across China, including Enshi, Hubei Province, Chengdu, Sichuang Province, Changde Hunan Province, Junan, Shandong Province, Zhou shan, Zhejiang Province, Zhou shan, Zhejiang Province and Jiujiang, Jiangxi Province. Only those patients are

selected in this report who were subjected to repetitive sputum and throat swabs to confirm SARS-CoV-2.

Case 1. Asymptomatic with an extra-long incubation period of 38 days
City: Enshi, Province: Hubei

A 41 years old female, who was taking care of her mother with a digestive tract tumour in the local hospital, was positive for nucleic acid detection sampling and negative for chest CT. The patient had no clinical manifestations of fever, cough, diarrhea, pneumonia and other related symptoms, with an incubation period of 38 days from coming in contact with an infected person. After which she stayed isolated at home, preventing contact with any other infected persons, to the final diagnosis of the positive nucleic acid test. Nevertheless, she surprisingly never developed any symptoms.

Case 2. COVID-19 Reinfection in a cured patient after 10 days of being discharged from Hospital
City: Chengdu, Province: Sichuan

A 55 years old male was cured and allowed to leave the hospital after two subsequent COVID-19 tests based on nucleic acid clearance according to COVID-19 Diagnosis and Treatment Plan (trial version sixth, China). The patient was advised to continue home isolation for further 14 days without contacting with any other infected persons and received a regular follow-up and consultation. After the reexamination of nucleic acid detection, he showed a positive test after 10 days of discharge. This has huge implications for the reinfection of the population and it points to two things, either there is a loss of infection immunity or the virus has started to mutate to the extent that it can re-infect the same host. This particular patient was admitted to the Chengdu Public Health Clinical Medical Center for further review. This situation has been reported many times in other regions of China as well.

Case 3. A woman with negative nucleic acid test was diagnosed after discharge
City: Chengde, Province: Hunan

A 35-year-old female COVID patient, was admitted to hospital with fever and no respiratory symptoms and were diagnosed with common acute upper respiratory tract infection with routine symptomatic treatment and Chinese patent medicine without any steroid hormone. She was discharged after 14 days with disappearance of clinical symptoms and two clear chest CT and negative nucleic acid tests and continued to have isolation rehabilitation. Two days later, the patient complained high fever and again admitted to the hospital. She was found COVID-19 positive on the basis of nucleic acid

detection and chest CT.

Case 4. Another COVID-19 Confirmed infection10 days after discharge
City: Junan, Province: Shandong

A 58 years old female COVID patient, was hospitalized for 2 days due to cold, fever and a slight cough. She was admitted in the isolation ward and discharged from the hospital after negative nucleic acid test repeated twice and a clear chest CT. After discharge, she remained in isolation at home. Ten days later, her sample returned a positive nucleic acid test. At present, she is isolated again in the isolation ward for treatment.

Case 5. A patient with negative sputum and pharynx test, and positive stool test
City: Zhoushan, Province: Zhejiang

A 47 years old male had spent two days with his COVID-19 positive parents. The patient with no clinical manifestation had a negative nucleic acid test of the sputum, throat swabs and chest CT twice. To reduce the risk of infection, he was sent to a designated hospital to stay in isolation. Two days later, the nucleic acid test was positive in stool samples twice, however, negative in Chest CT, negative for IgAs/IgGs) and surprisingly negative in sputum and throat swabs as well. Patient never developed any symptoms of the disease.

Case 6. A patient with delayed diagnosis and without fever symptoms
City: Jiujiang, Province: Jiangxi

A 36 years old female returned home from the COVID-19 endemic zone in Wuhan. After 15 days of isolation at home, she had a cough, slight chest distress and no fever. CT showed pulmonary inflammation and was admitted to the hospital with viral pneumonia. Three times the nucleic acid test was carried out and the results were found to be negative. However, the test results showed positive on the 21st day. This is a very long incubation period consistent with what is usually seen in immunocompromised patients in the case of influenza and west nile virus.

3. Discussion

SARS-CoV-2, has been shown to grow *in vitro* in human respiratory epithelial cells, where they can be seen to grow within 4 days and upto 6 days in other cell lines

including Vero E6 and Huh-7. It is an enigma then why such a varied response is being observed across the population. The patients where longer than 14 days incubation period observed are of great significance with regards to spread of the disease. Currently, all quarantine guidelines are based upon the premise that 14 day is enough to develop the symptoms and to become virus free. Perhaps this is one of the reasons for COVID-19 to be a much broader infection in comparison to SARS and MERS. A longer incubation time leads to a higher rate of asymptomatic and subclinical infections and allows the infection to spread broader in the communities [6]. The presence of virus in the fecal samples is a grave concern for the spread of virus particularly in the developing countries.

On one hand, China had a huge disadvantage to be the first country to deal with this epidemic while on the other hand, it might have been a blessing in disguise because they knew the geographical origin of the virus hence their COVID-19 Diagnosis and Treatment Plan (trial version sixth, China) divided people into two groups, based upon whether they were present in the Wuhan region within 14 days prior to the outbreak, had COVID-19 symptoms (fever and/or respiratory symptoms; imaging characteristics of pneumonia on X ray or Chest CT; the total number of WBCs and lymphocyte was either normal or decreased in the early stage of the disease) or exposed to people with symptoms. While for rest of the countries screening has become a huge challenge because all airports and seaports could potentially act as the source to bring the disease into a country. Screening is key particularly in context of the type of cases described in this paper where virus had a longer incubation time while patients remained asymptomatic. The challenge is nucleic acid-based RT PCR tests cannot be used at point of use and same is the case for a lot of enzyme link or chemiluminescent immunoassays; while the serological assays which may have the potential to be converted into point of care (POC) lateral flow tests have so far shown unreliable results. Until these tests are developed and become available, free movement of people will remain a huge challenge and life won't be able to go back to normality.

Every government is trying to develop some sort of track and trace system, the quality of which is essentially dependent upon the level of engagement by general population. When a healthcare worker contacts a suspected patient for the first time, reliable and rapid pathogen detection and feasible differential diagnosis based on the clinical description are essential. It is very important to monitor SARS-CoV-2 future host adaptation, evolution, infectivity, transmissibility and pathogenicity. The COVID-19 is a new pathogen, and our understanding is somewhat limited. We should give serious concentrations to the drug which have potential to block the viruses cycle at various stages (Figure 1)[7]. While the vaccine development is ongoing and it very likely that we

may be waiting till the third quarter of 2021 to have something ready for distribution, it will be great to check the prophylactic capability of some of these drugs particularly montelukast which has proven track record of low toxicity and long-term use. Further areas which require immediate improvement is the poor performance of diagnostic reagents, and training of medical and laboratory staff. In the initial days of the epidemic, it was great to see the positive contribution UK academia made to NHS by donating RNA extraction robots, Thermocyclers, qPCR reagents and sharing of appropriate personal protective equipment (PPE). To beat this epidemic, we would need to analyse every single data point, therefore, these COVID-19 outlier patients may provide some useful clues for prevention and treatment and give your readers an opportunity to take these examples into consideration while making hypothesis.

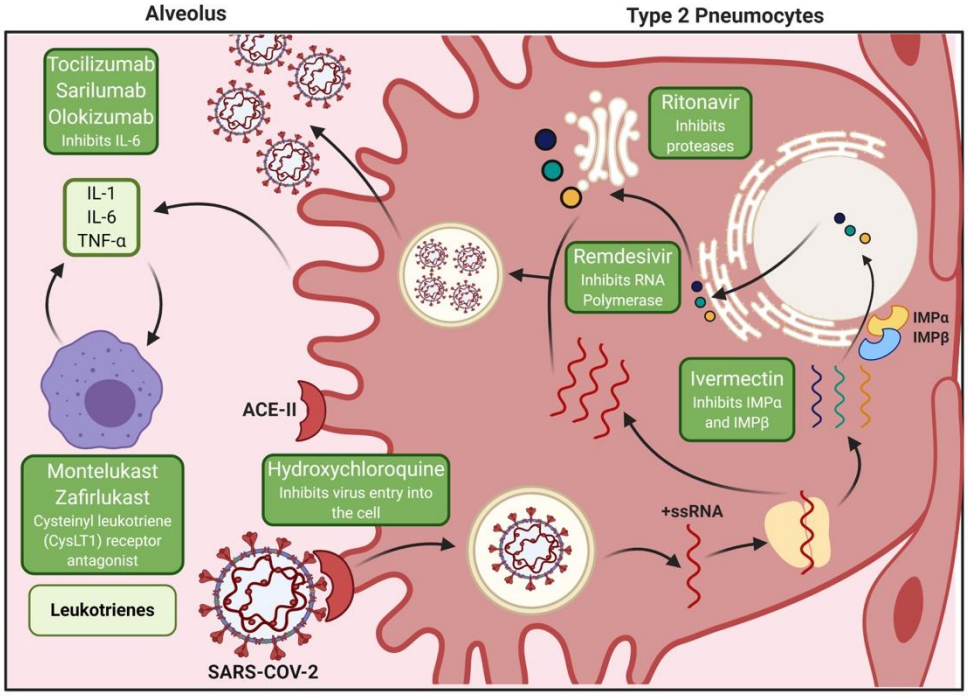

Figure 1: Therapeutic targets of potential drugs for COVID-19

The pathophysiology of SARS-CoV-2 begins with inhalation of the virus, its invasion & replication in type 2 pneumocytes in lungs, which induces an inflammatory response with the release of IL-1, IL-2, IL-6, TNF-α, NF-κB alongside leukotrienes & prostaglandins. This increases vascular permeability resulting in pulmonary & interstitial oedemas. Increased vasodilation recruit immune cells like neutrophils, macrophages, & monocytes. Degradation of these immune cell's damages pneumocytes further, decreasing surfactant production & increasing likelihood of alveolar collapse. Spike glycoproteins bind to the cellular receptors angiotensin-converting enzyme 2 (ACEII) & the virus is endocytosed into the cell. The viral +ssRNA is uncoated in the cytoplasm and is imported into the nucleus by IMPα & IMPβ. It is then transported to the golgi to be packaged as viral structural proteins. Alongwith the viral +ssRNA, this forms a new virus which is exocytosed into the surrounding alveoli. The drugs which may prevent are Hydroxychloroquine that inhibits viral entry into host cell; Remdesivir, disrupts viral replication; Ivermectin/Ritonavir prevents viral protein production; Tocilizumab / Sarilumab / Olokizumab / Zafirlukast / Montelukast reduces the inflammatory response and binds to various viral proteins (Figure created with Biorender.com) (Adapted from Rehman et al. 2020)

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