COVID-19: The third wave of coronavirus infection outbreak

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Abstract

The novel coronavirus SARS-CoV-2 poses a great public health crisis. As of December 2019, it has spread all over the world with cases reported in more than 100 countries and the number of infected individuals surpassing 1,000,000. On March 11 World Health Organization officially characterized the COVID-19 as a pandemic.

Although genetic analysis revealed some similarities between the novel coronavirus and the causative agent of the 2002 SARS epidemic, both viruses have significant differences. Research done on SARS-CoV has greatly aided the understanding of SARS-CoV-2, as it served as a knowledge base and helped to identify the cell entry receptor and some other features of the disease, but not all of them. Several critical questions remain unanswered and specific therapeutics and vaccine candidates are lacking.

Introduction

Ever since the end of 2019, the novel coronavirus (SARS-CoV-2), causative agent of the coronavirus disease 19 (COVID-19) [1], has been the major concern of physicians, public health specialists and researchers. Major global public health initiatives are being implemented and rapid investigations of the biology of the virus and pathogenesis of COVID-19 are being conducted in research institutions all around the world.

Although COVID-19 originated in China, as of this March cases have been confirmed all around the world and in the majority of European countries [2] and the epicenter of COVID-19 seems to have moved from China to Western Europe. While only less than 100 cases have been confirmed in certain countries, COVID-19 should not be ignored as some of the possibly infected individuals may only be in the incubation period and the spread of the virus has been estimated to be higher in some countries due to aging populations [3].

Number of reported cases has surpassed 1,000,000 worldwide and many countries have declared state of emergency and implemented quarantine procedures. On March 11 World Health Organization officially characterized the COVID-19 as a pandemic [4].

Coronaviruses, their origin

Coronaviruses belonging to the Alphacoronavirus and Betacoronavirus genera represent a group of both human and animal viruses, causing respiratory or gastrointestinal infections, respectively. These viruses can cross species barriers by acquiring mutations in proteins responsible for host cell receptor binding as these regions are the most variable parts of the coronavirus genome [5]. The sporadic emergence and outbreaks of new types of coronaviruses, are thought to become more frequent as contact with wild animals increases due to habitat loss and with it the chance of new spillover events [6].

In humans' coronaviruses were associated with mild respiratory infections until the outbreak of severe acute respiratory syndrome

(SARS) in 2002 and Middle East respiratory syndrome (MERS) ten years later.

Sequence analysis has revealed that all human coronaviruses have animal origins, including the highly pathogenic ones – SARS and MERS causing coronaviruses, as early cases of these infections were traced to individuals being exposed to infected animals. Bats are likely the major natural reservoir of coronaviruses. [7]. COVID-19's causative agent seems to be no different, as the origin of the outbreak has been traced to wild animals possibly snakes, bats or more likely pangolins sold in Huanan market in Wuhan, China [8,9].

Misinformation and conspiracy theories spread across the internet have created doubt and misconception in the general public about the origins of this virus. Some misleading information suggested the novel virus could be manmade. Researchers have analyzed the genetic information of SARS-CoV-2, comparing it to other known coronaviruses, and their finding show that the virus is not an laboratory construct or purposefully manipulated [10].

Highly pathogenic coronaviruses

Highly pathogenic coronaviruses can cause these severe diseases due to the fact that they can infect the lower respiratory tract for instance the lung tissue, whereas the coronaviruses associated with the common cold and mild infections, infect the upper respiratory tract, like the nose and throat [8].

All three highly pathogenic coronaviruses share some similarities. They all infect the lower respiratory tract and the infection may present

itself in varying degrees of severity – from flu-like symptoms, to acute respiratory distress syndrome [8]. Phylogenic analysis has revealed that SARS-CoV-2 shares an ~80 % identity with SARS-CoV and only ~50 % identity with MERS-CoV, which explains certain characteristics that set MERS-CoV apart from the more related coronaviruses, for example MERS-CoV infection fatality rate is considerably higher as the virus mainly infects individuals with preexisting serious comorbidities, and human to human transmission is low [5]. In the case of SARS-CoV and SARS-CoV-2 human to human transmission has been proven and in the case of SARS-CoV-2 it is considered to be the main way the virus spreads. Accumulated information about SARS-CoV greatly aids the inquiries about the new virus.

SARS-CoV-2

SARS-CoV-2 transmission

SARS-CoV-2 is transmitted to other people mainly by respiratory droplets during coughing or sneezing. Droplets containing viral particles are propelled several meters in the air and can be deposited in the mucous membranes of the mouth, nose or eyes of an individual. Transmission can also occur via contact with contaminated surfaces followed by self-inoculation of mucous membranes of the nose, eyes or mouth. Analysis of a number of coronaviruses showed that they can survive on inanimate surfaces for up to 9 days but can be efficiently inactivated by surface disinfection [11]. Recently published study reported that SARS-CoV-2 remained viable in aerosols for 3 hours, but with decrease in infectious titer. The same study reported that SARS-CoV-2 can remain viable for up to 72 hours on stainless steel and plastic, but with a 3-fold decrease of the virus titer [12].

As the pandemic progresses, new routes of transmission are being discovered. SARS-CoV-2 positive fecal samples indicate on the possibility of becoming infected via the fecal-oral route [13]. This is supported by the detection of SARS-CoV-2 in stool samples even after nasopharyngeal swab switched from positive to negative after treatment [8,14]. Fecal-oral route of transmission was also proposed during the SARS epidemic [15]. Proof that asymptomatic patients can take part in SARS-CoV-2 transmission has also been reported on multiple occasions [8,16]. The fact that asymptomatic individuals can act as infection vectors has urged countries to advise their citizens to self-isolate and practice social distancing.

Possibility of numerous transmission routes is strongly supported by the fact that the virus has been found in not only respiratory excretions and fecal matter, but also in blood and urine samples of COVID-19 patients [17,18].

3.1.2. Incubation period and basic reproductive number

Many parameters of the infection are still uncertain as the pandemic is still ongoing and they are being reported from many researchers. Incubation period on average seems to be about 6,4 days, but it can last as long as 24 [19-22].

The basic reproductive number (R0) is used as a measure to determine the potential and severity of an infectious disease. The larger R0 is, the stronger the transmission power will be, the smaller – the faster the infection will die out. It is defined as the number of new cases an existing case can create, respectively, how many individuals can an infected individual infect. A lot of R0 values have been reported. In the beginning of the outbreak WHO estimated the R0 to range between 1.4 – 2.5, but some researchers suggest that it may be as high as 3.8,

surpassing that of SARS-CoV and MERS-CoV. It is important to keep in mind that the estimation of R0 during an epidemic can be riddled with uncertainty and variability [23,24].

Symptoms and clinical manifestations

Main COVID-19 clinical manifestations include fever, cough, myalgia and shortness of breath but they are not limited to respiratory symptoms as gastrointestinal symptoms like diarrhea and vomiting and even neurological symptoms like headaches have been reported [14,21,25]. Some researchers even hypothesize that possible neurological infection with SARS-CoV-2 may be responsible for the acute respiratory failure in patient with severe infections as SARS-CoV viral particles were found in the brain, where the brain stem was heavily infected [26].

Disease severity could be associated with a phenomenon called "cytokine storm", as highly elevated levels of proinflammatory cytokines like IL-2, IL-6, IL-7, G-CSF, IP-10, MCP-1, MIP-1A, and TNFα were observed in the COVID-19 severe cases. "Cytokine storm" can initiate viral sepsis and inflammatory-induced lung injury which lead to other complications including pneumonitis, acute respiratory distress syndrome (ARDS), respiratory failure, shock, organ failure and potentially death. Reports have indicated that the main cause of death among COVID-19 cases is ARDS. Seeing as cytokines in some part are determinant of the severity of COVID-19 complications, some patients are being treated with anti-inflammatory drugs [27,28].

In severe cases significantly depleted lymphocyte numbers have been observed and could suggest that the infection may be associated with cellular immune deficiency, but the mechanisms of significant lymphocyte reduction remains unclear [29,30].

Some researchers suggest that individuals with severe COVID-19 symptoms may be experiencing the effects of antibody dependent enhancement (ADE) which may occur when an individual has been primed by prior coronavirus exposures, as coronaviruses causing mild infections are prevalent worldwide. These antibodies have low neutralizing activity against the novel coronavirus and could aggravate the infection. Similarly, ADE was characterized for the SARS coronavirus and even a priming coronavirus was suggested. Although this has not been confirmed for COVID-19, in vitro and mouse models studies have shown that Ade hinders the ability to manage inflammation in the lungs [31,32].

Age distribution and diversity of COVID-19 cases

Although pediatric cases have been reported, their symptoms are mild, most of the infected individuals are 30 years old and older suggesting that the elderly are especially at risk of developing more severe symptoms and even more at risk of death due to COVID-19. Also, a large percentage of the COVID-19 patients have some sort of underlying comorbidity, most commonly cardiovascular disease, diabetes, hypertension and others [6,20,22,33,34].

Infection during pregnancy has also been reported but initial reports suggest that intrauterine transmission of SARS-CoV-2 is not likely even if the infection happens late in the pregnancy as SARS-CoV-2 was not found in the amniotic fluid, cord blood, neonatal throat swab, and breastmilk samples from pregnant women with COVID-19 pneumonia. But since a newborn developed COVID-19 symptom just 30 h after the birth, more inquiries should be made concerning SARS-CoV-2 and pregnancy [22,35].

Diagnostic techniques

Diagnosis of COVID-19 mainly includes molecular assays, but imaging using CT scans is also used, as COVID-19 patients show ground glass opacities in the lungs – a characteristic of viral pneumonias. Molecular assays are based on viral nucleic acid detection and quantification using RT-PCR. Genome sequencing techniques have also been used [25]. A number of assay protocols have been developed by leading research institutes. Most of the RT-PCR assays have primers designed to be specific for the spike protein or nucleocapsid genes of SARS-CoV-2 [36]. Wide variety of sample types can be used for viral nucleic acid detection, such as throat or nasal swabs, lower respiratory secretions, sputum, stool or blood. Lower respiratory tract samples like bronchoalveolar lavage fluid aspirate are considered to be the most representative and they show higher rates of positive nucleic acid tests. Although upper respiratory tract specimens can be used too, they subject healthcare workers to greater risks due to the closer contact with patients. As mentioned before, stool samples have been found positive for viral nucleic acids even after no detectable virus can be found in upper respiratory swabs, so clinicians have to be cautious when discharging patients based on negative upper respiratory swabs [19,22].

While most of the available assays are developed for the RT-PCR method, some researchers are searching for different nucleic acid detection approaches. Researchers from USA have developed an COVID-19 detection protocol using the CRISPR technology SHERLOCK (Specific High Sensitivity Enzymatic Reporter Unlocking). This protocol is only about one hour long and is highly specific. It includes extracted RNA amplification and detection using the Cas-13a protein, which cleaves reporters if COVID-19 RNS is detected, followed by visual read out of the results using a commercially available paper dipstick. Although this protocol is not yet fully implicated in the clinical practice, its developers offer starter kits to any researchers working with COVID-19 patient samples [37-39].

Viral cell entry

The diversity of both clinical manifestations and transmission routes, can be by part explained by the cell entry receptor of SARS-CoV-2. Coronaviruses are enveloped viruses. All coronaviruses code a spike protein which is integrated in the envelope and binds to the host receptor and facilitates viral entry via the receptor binding domain [5,8]. Computer modeling of the spike protein of the novel coronavirus revealed that its receptor binding domain's 3D structure is practically identical to that of SARS-CoV's. Causative agent of COVID-19 and SARS-CoV are thought to share the same cell entry receptor – angiotensin-converting enzyme 2 (ACE2) and interestingly SARS-CoV-2 seems to have a higher affinity for it, which could facilitate a faster human to human spread [40].

Expression of the ACE2 receptor is found in many extrapulmonary tissues including heart, kidney, endothelium and the intestine. The lungs seem to be the most vulnerable ACE2-expressing tissues due to the fact they represent a large surface area, which an inhaled virus can infect and the ACE2-expressing lung cells have high levels of viral process-related genes, which facilitate coronaviral replication. ACE2 is highly expressed on the luminal surface of intestinal epithelial cells and functions as a co-receptor for nutrient uptake.

ACE2 is an carboxypeptidase which converts angiotensin I and angiotensin II into several molecules with important roles in vasodilatation, heart function regulation, protection during acute lung injury [40,41]. ACE2 levels can be increased by the use of renin–angiotensin–aldosterone system inhibitors, which are used for the treatment of hypertension, so patients with cardiovascular disease have more severe respiratory symptoms [42].

Although multiple studies have shown the importance of ACE2 in viral entry and pathogenesis, SARS-CoV has two alternative cell entry receptors – L-SIGN (CD209L) and DC-SIGN (CD209), which should be further explored as SARS-CoV and SARS-CoV-2 share a certain level of homology [43].

Therapeutics

Even though this is the third global coronavirus outbreak, there is no single specific antiviral therapy for coronaviruses and the main treatments are supportive. Drug development and implementation in active clinical practice can take for up to ten years, but taking into account the rapid spread of the virus, potential therapeutics are being searched for among drugs/immunotherapeutic agents that have proven efficacy against viral agents that are similar to COVID-19 or among broadly acting anti-viral drugs that have already been used for other viral infections. Some potential drug candidates are being used with varying degrees of efficiency.

WHO has released a landscape analysis of therapeutics, which contains numerous therapeutic products ranging from protease and kinase inhibitors and other antivirals to interferons and other anti-viral immunity signaling components? These therapeutics are in various stages of clinical development and have reported effect on COVID-19 or other coronavirus or other viral diseases ("Landscape analysis of therapeutics as 21st March 2020" n.d.).

One of the listed therapeutics is baricitinib. Researchers using BenevolentAI, which is a large repository of structured medical information, identified this therapeutic as potentially capable of blocking viral infection. Baricitinib is a kinase inhibitor acting on AP2-associated protein kinase 1, janus kinase and other regulators of endocytosis, showing that it may reduce both the viral entry and the inflammation in patients, as it is used for such indications as rheumatoid arthritis [44-46].

Interferons are involved in the body's natural antiviral response. Some viruses, including SARS-CoV-2 have evolved mechanisms to suppress the synthesis of interferons. A respiratory drug discovery and development company has received expedited approval for clinical trials in COVID-19 patients for an Inhaled formulation of interferon-beta-1a, to aid lung function in patients with severe symptoms [47,48].

Multiple research teams have recently reported on the anti-viral action of the anti-malarial agent chloroquine against SARS-CoV-2, which is in line with the agents with previously reported activity against SARS-CoV and MERS-CoV and the fact that the in vitro anti-viral activity of chloroquine has been identified since the late 1960's. In vitro studies showed that chloroquine blocked SARS-CoV-2 infection at low micro-molar concentrations and had effects at both entry and post-entry stages of the infection [49]. At least 16 different trials for SARS-CoV-2 already registered in the Chinese Clinical Trial Registry and results demonstrate that chloroquine phosphate is superior to the control treatment in inhibiting the exacerbation of pneumonia, improving lung imaging findings, promoting a virus negative conversion, and shortening the disease course. Recommendations to include the drug in the next version of the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 issued by the National Health Commission of the People's Republic of China were made in

February 15 [50]. The use of chloroquine (or hydroxychloroquine, or sulfate and phosphate salts of chloroquine) is especially attractive due to the fact that it is cheap, widely available and the drug has been used in humans for many decades and is considered to be safe, with only mild and transitory side-effects.

Also neutralizing antibodies are being considered as possible treatment options [20,51]. Monoclonal antibody isolated from supernatants from SARS-CoV spike protein specific hybridomas showed cross-reactivity to SARS-CoV-2 spike protein and neutralized SARS-CoV-2 infection in vitro [52].

Vaccine development

Vaccine development is never easy and fast, a lot has to be done to ensure that an efficacious and safe vaccine reaches the public. With this new coronavirus it is especially challenging since there are no existing vaccines or production processes for coronavirus vaccines, but the rapid response of researchers has aided the process.

The causative agent of COVID-19 was identified in record time and the genomic sequence of the virus was swiftly made available aiding the development of potential therapeutics and vaccines. Also, data from studies on SARS-CoV and MERS-CoV vaccines showed that the spike protein is an ideal target for a vaccine, as antibodies targeting the spike protein interfered with virus binding and neutralized the virus and the structure of SARS-CoV-2 has already been solved [53].

The SARS-CoV-2 vaccine will have to overcome numerous issues, like the waning of the antibody response, as antibody titers of SARS-CoV waned after 2-3 years, which could be dangerous if the virus becomes endemic with recurrent outbreaks. Also, higher titers of neutralizing antibodies will be necessary since older individuals are more affected and they are more immunosenescent [53].

As SARS-CoV neutralizing antibodies can cross-react with SARS-COV-2, SARS-CoV-1 vaccines might cross-protect against SARS-CoV-2, but these vaccines were not developed further than phase I clinical trials and are not available for use [53].

The Coalition for Epidemic Preparedness Innovations has given funds to several prospective vaccine developers one of whom has started phase I clinical trials. This is an mRNA-based vaccine which expresses the stabilized spike protein of the virus in vivo. First participant has already been dosed with the vaccine and a total of 45 healthy adults will be enrolled in this trial [54]. Several vaccine candidates are still in the preclinical phase, such as recombinant protein based, viral vector based, DNA and live attenuated vaccines [53].

Conclusion

COVID-19 is surrounded with numerous uncertainties as the pandemic is still ongoing. Although the progress made by scientists is undeniable, new properties of the virus are emerging and certain problems remain unanswered, for example the possibility of recurrent infection, the possible persistency and the capability of the virus to infect a multitude of cell types and cause complications outside of the respiratory tract and the lack of SARS-CoV-2 specific therapeutics and vaccine.

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