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Paul C. Guest *Editor*

Identification of Biomarkers, New Treatments, and Vaccines for COVID-19

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Preface

Coronavirus 2019 (COVID-19) has caused a serious global pandemic in a span of just 11 months. Virtually every country and territory in the world has been affected with a total number of cases at more than 62 million and deaths exceeding 1.4 million as of November 28, 2020. The virulence and infection rate of this virus are profound and, at times, this has required extreme social distancing and lockdown measures throughout the world to stop the spread and reduce the burden on the healthcare services. The virus appears to have its greatest effects on elderly individuals and those who have co-morbid diseases such as diabetes and obesity. Although we have seen the number of cases rise and fall in many countries, the death rates are still high and there are justified fears of further waves of this virus, which could have even more devastating effects. In light of this, there have been a number of scientific and medical breakthroughs, and a worldwide mobilization effort has begun to identify potential treatments and develop vaccines.

There are many vaccine candidates worldwide in the effort to control COVID-19 disease. Many of these are being rapidly progressed, considering the global emergency. As of November 28, 2020, 10 of these vaccines are now in phase 3 clinical trials and several are already showing promise. This proposal aims to increase our understanding of the ongoing COVID-19 crisis with a series of reviews and clinical studies concerning the above issues. The authors in this series come from the six habitable continents from countries such as Australia, Brazil, Iran, Japan, Poland, South Africa, Switzerland, the United Kingdom and the United States of America.

The book will be of high interest to researchers in the areas of virology, metabolic diseases, respiratory disorders, as well as to clinical scientists, physicians, the major drug companies and the healthcare services. It is hoped that it will also be of interest to the general population as virtually everyone has been affected by this deadly pandemic in some way.

Debden, Essex, UK — Paul C. Guest

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Zari Naderi Ghale-Noie, Arash Salmaninejad, Robert Bergquist, Samaneh Mollazadeh, Benyamin Hoseini, and Amirhossein Sahebkar

Abstract

In the last two decades, the world has experienced outbreaks of three major coronaviruses with high morbidity and mortality rates. The most recent of these started in the form of an unusual viral pneumonia in Wuhan, China, and now the world is facing a serious pandemic. This new disease has been called COVID-19 and is caused by the SARS-CoV-2 virus. Understanding the specific genetic and phenotypic structure of SARS-CoV-2 in COVID-19 pathogenesis is vital in finding appropriate drugs and vaccines. With this in mind, this review sheds light on the virology, genetics, immune-responses, and mechanism of action of this virus.

Keywords

Covid-19 · 2019-nCoV · SARS-CoV-2 Genetics · Immune-responses · Respiratory

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1 Introduction

Coronaviruses are zoonotic RNA viruses vastly distributed among both mammals and birds, mostly causing respiratory or enteric diseases, but also producing hepatitis and neurological symptoms [1]. The human coronaviruses belong to the order nidovirales, subfamily coronavirinae, which has four genera: *alphacoronavirus* (α-CoV), *betacoronavirus* (β-CoV), *gammacoronavirus* (γ-CoV), and *deltacoronavirus* (δ-CoV) [2]. The former two are known to infect mammals, whilst γ-CoVs and δ-CoV infect birds [3]. Two outbreaks of human viral pneumonia called the severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) have been recorded in the last two decades, both caused by β-CoVs [2]. As noted by the World Health Organization (WHO), the SARS outbreak occurred in China in 2002 with the epidemic brought to an end within the next few years, while MERS was first diagnosed in Saudi Arabia in 2012, and is still continuing as rare reported cases [4, 5].

The current COVID-19 pandemic is due to infection by a new virus similar to that causing SARS called SARS-CoV-2 [3]. In natural populations, strategic evolutionary processes such as mutations, recombination, and reassortment are the roots of genetic diversity. With respect to CoV replication, the high incidence of homologous RNA recombination is prevalent [6]. Due to characteristics such as regular genome recombination, wide distribution, large genetic diversity, and high human–animal interface, CoVs might have emerged in humans owing to occasional spill-over and cross-species infection events [7, 8].

As shown by SARS, MERS, and COVID-19, the threat of the zoonotic movement of pathogens from animals to humans is a valid concern for human health. Most pandemics are viral diseases, which have emerged due to ecological intrusions forced by socioeconomic changes. In spite of their substantial effect on the global public health and world economy, it has not been possible to predict the cause of a potential human pandemic in sufficient time to establish effective countermeasures [9]. The hosts in which potential pathogens exist and emerge from, and the factors that drive the processes likely to result in human disease, can be studied by diagnostics and genetics followed up by mathematical modelling. In response to the "bird flu" (influenza A subtype H5N1) outbreak in 2005, the United States Agency for International Development (USAID) launched the PREDICT program in 2009 [10]. This program focused on biological threats in areas with high biodiversity and dense human–animal interaction, where the environmental conditions encourage the emergence of new viruses in human populations. In the next 10 years, this program collected more than a thousand viruses from many different animals, with a strong focus on bats that could potentially cause human disease, including more than 160 novel coronaviruses [10–12].

With regard to new coronaviruses, the level of viral diversity in infected patients is not only considerable, but these viruses can also quickly adapt to the human genome and physiology [13]. The signs of SARS-CoV-2 infection commonly appear 2–14 days after exposure but incubation can sometimes be longer. In addition to general symptoms such as fever, cough, and fatigue [14, 15], patients can develop anorexia and/or diarrhoea [16, 17], dyspnoea, chest pain, and cardiovascular involvement such as acute dysfunction of the left ventricle of the heart, arrhythmia, myocardial inflammation, microvascular injury, and thrombosis [18]. SARS-CoV-2 is preferentially transmitted through inhalation of infective droplets in the air coming from infected people, but can also be transferred by direct touch [19]. If symptoms develop, they generally involve the lungs first, which may lead to a severe respiratory infection and pneumonia that may require hospital care [20, 21]. In a minority of cases, symptoms may first appear in diverse other organs, but respiratory failure is still the main reason of death. In this review, we provide general information on the SARS-CoV-2 virus, shedding light on the virology, genetics, and infective mechanism of action, including involvement of different organs.

2 Epidemiology

In the city of Wuhan, China, a cluster of patients suffering from atypical pneumonia associated with visits to a seafood, wholesale market was reported in late 2019. Further research found the disease to be similar to SARS but caused by a new coronavirus, subsequently called SARS-CoV-2, which quickly led to an outbreak in China followed by a million confirmed cases worldwide only 10 weeks later [22]. In contrast to SARS and MERS, COVID-19 resulted in a global health emergency [22], and SARS-CoV-2 has proved to produce an unusual type of infection with many remaining unknowns such as origin, exact mechanism of action, and ways of transmission [23, 24]. This virus is highly contagious and despite control measures, such as isolations and lockdown of whole countries, it has not been possible to prevent further spread. Instead, the infection increased rapidly and was declared a pandemic by the WHO on 11 March 2020 leading to a global fear of infection, sometimes referred to as coronaphobia [25].

The first major COVID-19 outbreaks outside China occurred in South Korea, Iran, and Italy [26]. South Korea has been able to moderate the spread of the disease by massive testing and systematic updating through smartphone messaging, while Italy rapidly became the epicentre in Europe [27, 28]. In Iran, the infection spread rapidly around the whole country after two cases had been confirmed in Qom City on February 19th, 2020 [29]. According to the WHO, there were 230,211 confirmed cases and 16,343 deaths in Iran by 29 July 2020, translating into a case fatality rate (CFR) of 5.46% [30]. In South Korea and Italy, there were 14,251 and 246,488 confirmed cases, respectively, at this date [30]. The mortality was 300 and 35,123 resulting in CFRs of 2.1% and 14.24%, respectively, as reported by the WHO [30]. The global CFR has risen from 2% to 7% during the first 4 months of 2020 [30–32] and the United States has reported the highest number of cumulative confirmed cases and COVID-19-related deaths of any country (5,567,765 and 173,139, respectively) by August 17th, 2020 [Worldometer].

Although some groups of people, such as the elderly and those with underlying diseases and/or a weak immune system, are more at risk with respect to this virus, it does not mean that others are not susceptible [16, 33, 34]. To keep the transmission rate as low as possible, all people need to pay attention to personal health care and keep distance from each other [35]. Another way to slow transmission is isolation of suspected cases, which means that large numbers of close contacts should temporarily be isolated for medical observation to prevent the spread of the virus [36].

3 Main Coronavirus Groups

The known hosts of coronaviruses are all vertebrates, including humans [37]. According to traditional classification, there are three groups of coronaviruses, based on antigenic relationships [38]. The hosts of groups 1 and 2 are mammals with certain viruses from both groups having human hosts, while the Group 3 viruses have exclusively avian hosts (Fig. 1.1).

In 2003, after finding the agent of SARS, more efforts have been made to detect previously unknown viruses. Surveys have led to the discovery of two more human respiratory coronaviruses called HCoV-NL63 [39] and HCoVHKU1 [40]. Three different bat coronaviruses have also been isolated, with two of these from Group 1 and the third, considered as a Group 2 member, is believed to be the precursor of the SARS-CoV that infected humans [41]. Sequencing analysis of some SARS-CoV genomes has revealed that these viruses are only moderately related to other known coronaviruses such as HCoV-OC43 and HCoV-229E, both known to infect humans. The genome sequence was crucial in the discovery of the SARS virus but when analysed phylogenetically, the predicted viral proteins of SARS-CoV do not have close resemblance to any of the three known groups of coronaviruses [42]. It could therefore tentatively be considered as the first known member of a fourth group of coronaviruses [43]. On the other hand, unique and conserved parts of the genome and the proteome of SARS-CoV indicate that it may belong to the Coronavirus Group 2 Lineage [44].

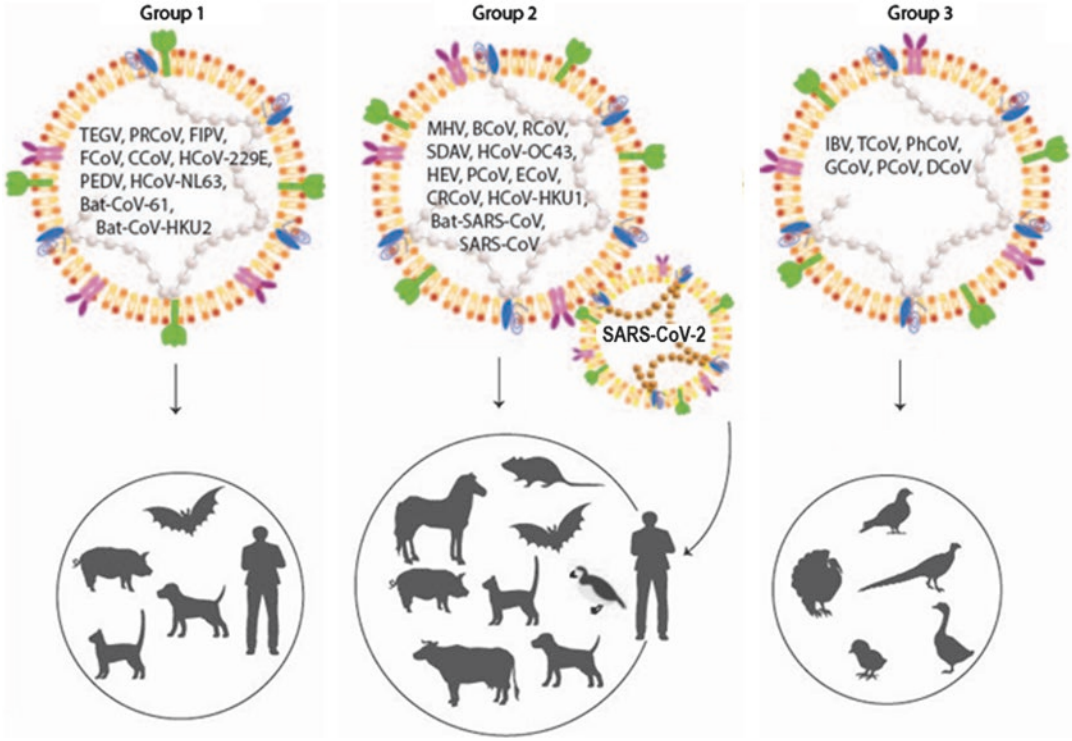

Fig. 1.1 Coronavirus taxonomy showing typical, specific hosts for each group

This finding is also supported by analyses of structural gene sequences [45] and secondary RNA structures in the 30 untranslated regions (UTR) of the genome [46]. Based on bioinformatics, however, it has been argued that the ancestor of SARS-CoV could be derived from multiple recombination processes among progenitors from all previous groups [47]. Although genetic recombination has been established in SARS-CoV [48], it has been shown only rarely in MERS-CoV [49, 50]. With respect to the latter virus, it has been proven that it is a lineage C β-CoV with a genotype very closely related to bat coronaviruses from the same lineage, such as BtCoV-HKU4 and BtCoV-HKU5 [51]. Although the three SARS-CoV, MERS-CoV and SARS-CoV-2 viruses are members of the β-CoV genus, the diversity [52] among them is apparent as seen in Table 1.1. Moreover, the new virus is closely associated (88% identity) with two bat-derived SARS-like coronaviruses, i.e. bat-SL-CoVZC45 and bat-SL-CoVZXC21 that were collected in 2018 in Zhoushan, Zhejiang Province in eastern China. It has been shown that the SARS-CoV-2 forms a distinct lineage within the subgenus of the sarbecovirus along with the SARS-like coronavirus from the bat [53]. Recently, an additional bat coronavirus, named RaTG13, has been identified and sequencing analysis suggests that it is almost identical (96.3% similarity) with SARS-CoV-2 [13]. Furthermore, in spite of its extensive similarity (>98·99%) among the virus sequences obtained from patients in China [54], RaTG13 has been found to be more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%) [55]. Although the bat is still a probable species of origin for SARS-CoV-2 due to the high level of genomic similarity, surveys have shown that other vertebrates such as Malayan pangolins, minks, snakes, and turtles might be potential intermediate hosts for the virus [52, 56–61]; however, this remains to be confirmed. On balance, the wide dissemination of SARS-CoV to various animals may reveal some novel intermediate hosts. Meanwhile, interspecies transmission blockage should be considered in advance [61].

Table 1.1 Significant differences between coronaviruses recently shown to infected humans

Aspect	SARC-COV-2	SARC-COV	MERS-COV	Ref.
Origin	Wuhan, China, 2019	Southern China, 2002	Saudi Arabia, 2012	[61]
Animal to human transmission	Possibly through touching slaughtered infected animals	Suspected spread from bats to humans via civets	By touching infected camels or consuming their milk or meat	[21]
Human to human transmission	Via air droplet dispersion or touch. Close contact needed	Via air droplet dispersion or touch. Close contact needed	Limited transmission between humans through close contact	[47]
Receptor-binding domain (RBD)	*Angiotensin-converting enzyme 2* (ACE2)	ACE2 (main receptor), CD209L lectin (alternative)	Dipeptidyl peptidase-4 (*DPP4*), also known as CD26	[61, 62]
Fatality rate	7%	9.6%	34.5%	[32]
Protein domain characteristics	Presence of ORF1a&b, S, 3a, E, M, 6,7 (a&b), 8, N, 10 Lacking 3b, 4(a&b), 5, 8a, 9(b&c)	Presence of ORF1a&b, S, 3 (a&b), E,M,6, 7 (a&b), 8 (a&b), 9 (b&c), N Lacking 4 (a&b), 5, 10	Presence of ORF1a&b , S, 3a, 4 (a&b), 5, E, M, 8b, N Lacking 3b, 6, 7 (a&b), 8a, 9 (b&c), 10	[63]

4 Genome, Genotype, and Protein Structure

The coronaviruses have non-segmented, positive-sense, single-stranded RNA genomes [64]. Their mRNAs structurally resemble eukaryotic mRNA and have 50 caps [65] and 30 poly (A) tails [66, 67]. The following genomic characteristics are specific for the coronavirus genomes:

(a) Extreme lengths ranging from 27.3 (HCoV229E) to 31.3 kb (MHV) – indeed, they are one of the largest mature RNA molecules known to biology.
(b) Multiple (from 6 to 12) open reading frames (ORFs) with small overlaps between adjacent ORFs.
(c) The first ORF represents around 67% of the entire genome – encoding 16 non-structural proteins [61].
(d) Encodes four structural proteins including: the spike glycoprotein (S), the envelope small membrane protein (E), the membrane protein (M), and the nucleoprotein (N).
(e) Both the 5′ and 3′ ends of coronavirus genomes contain short untranslated regions.
(f) One or a number of accessory genes are intercalated among the structural protein genes in the coding regions.
(g) The guanine-cytosine (GC) contents of the genomes vary from 32% to 43% [68].
(h) High recombination rate owing to the large genome size, discontinuous transcription, and sub- or fully transcriptionally active genomic RNA length [64].
(i) Thanks to the "copy-choice" mechanism which is critical to mediate the unique random template switching during RNA replication enabling the high frequency of homologous RNA recombination in these viruses [69, 70].

The SARS-CoV-2 genome consists of six major ORFs, the same in all coronaviruses, and a number of other accessory genes. The genome is arranged in the order of a 5′UTR -replicase complex (ORF 1ab)-structural proteins (Spike (S)-Envelope (E)-Membrane (M)-Nucleocapsid (N))−3′-UTR and non-structural ORFs [71]. Analysis has shown that some of the genes share less than 80% identity of nucleotide sequences with SARS-CoV [72] (Fig. 1.2). Amino acid sequences of the seven conserved replicase domains in ORF1ab, which have been used for CoV species classification, are 94.4% identical between SARS-CoV and SARS-CoV-2, which probably is the reason that these two viruses can infect the same species [72] (Table 1.1). Notwithstanding that the similarity between dif-

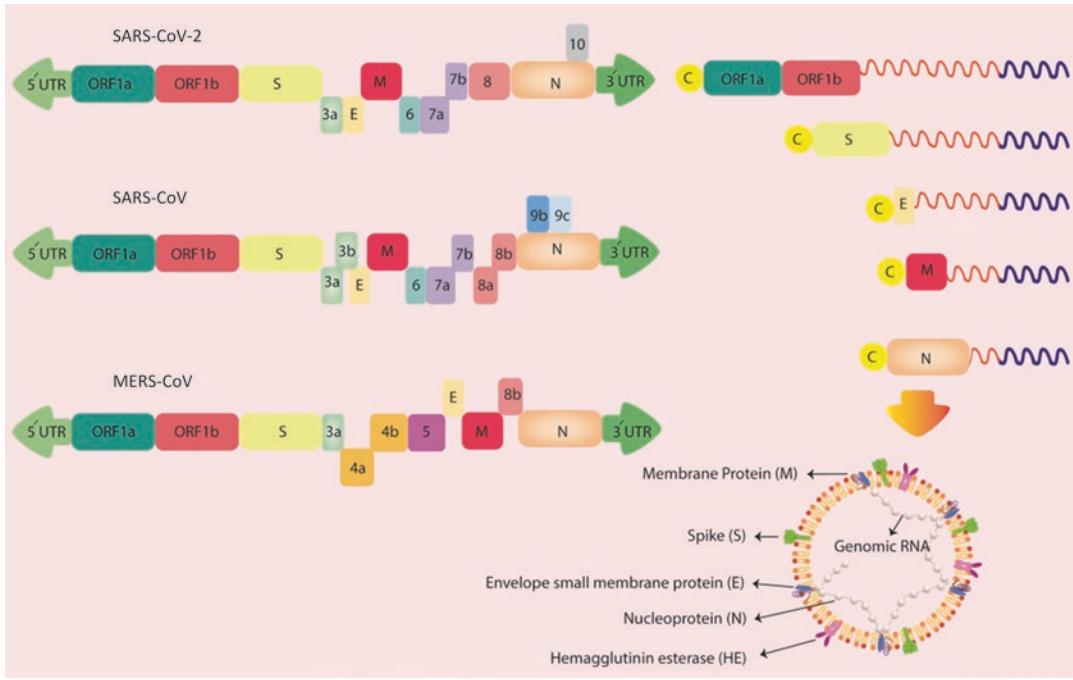

Fig. 1.2 The viral RNA is organized into 6-12 ORFs and includes around 30,000 nucleotides. The various elements and sub-genomic mRNAs are distinguished by the sequence used

ferent SARS-CoV-2 sequences investigated [13, 57, 73] is extremely high (around 99.9 %), mutations do exist [55]. Most of them occur in the S, N, ORF8, ORF3a, and ORF1ab regions of the genome with about 42% of the variations being non-synonymous [55]. The increased level of viral diversity in SARS-CoV-2 viruses shown in infected patients indicates that this virus might have begun to adapt to the human environment evolve in its new host. Tang et al. suggest that the development of novel variations in functional sites in the RBD of the spike seen in SARS-CoV-2 is likely caused by natural selection besides recombination [55]. It is therefore an urgent priority to study the precise detection, mutation tracking, evolution of the virus in the population, and investigation of cross-species transmission mechanisms [74].

5 Mechanism of Infection

All coronavirus ORF1 downstream regions contain specific genes which encode proteins for viral replication, nucleocapsid, and spike formation [75]. The glycoprotein spikes, located on the outer surface of the virus, are responsible for the attachment and entry of the virus to host cells. The virus may infect various hosts since complete attachment of the RBD is not required [76, 77]. The transmembrane protease serine 2 (TMPRSS2) might facilitate infection via proteolytic cleavage of the ACE2 receptor and coronavirus spike glycoprotein thereby promoting viral uptake [78]. Soon after the SARS-CoV-2 virus has gained entrance into the human host, the 394 glutamine residue in the RBD region of SARS-CoV-2 recognizes and binds the critical lysine 31 residue on the human angiotensin-*converting enzyme 2 (ACE2*) receptor [74]. These receptors are distributed throughout the body, not only in the lungs and the cardiovascular system but also in the pancreas, kidneys, intestines, and the central nervous system including the eyes. After binding, the conformation change in the spike protein leads to easing of the viral envelope and fusion with the cell membrane by the endosomal pathway, which allows release of the viral RNA into the host cell [79]. In this part of cycle, the RNA is translated into viral replicase polyprot-

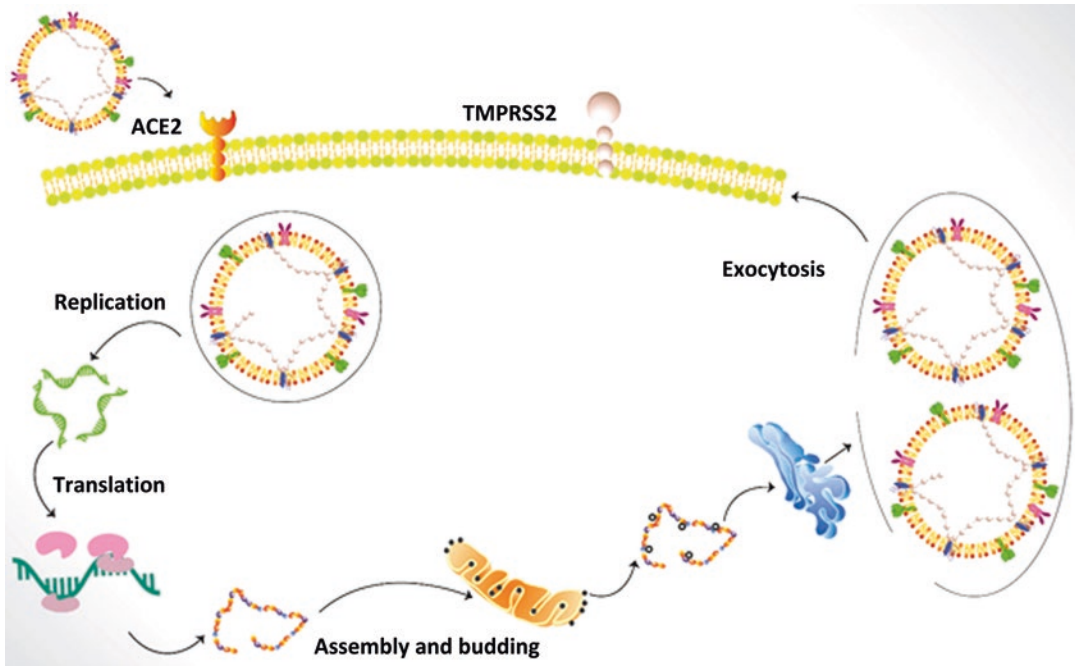

Fig. 1.3 TMPRSS2 activates SARS-CoV-2 virus S-protein binding to the ACE-2 host cell receptor, which enables viral fusion with the cell membrane and genome delivery into host cell. After viral RNA translation, proteins and RNA of daughter viruses assemble and move towards the cell membrane to be secreted

eins pp1a and 1ab, which are cleaved into subproducts by viral proteinases, while polymerases produce a series of subgenomic mRNAs by discontinuous transcription that results in translation into relevant viral proteins. Consequently, viral proteins and genome RNA are assembled into multiple virions in the endoplasmic reticulum (ER) and Golgi apparatus, then transported through vesicles and finally released from the cell [80] (Fig. 1.3).

6 COVID-19 Pathogenesis

The predominant symptoms of SARS-CoV-2 infection are related to the lungs. However, they are not only the now well-known pathology seen in older patients with comorbidities, such as hypertension diabetes and obesity, but signs are increasingly reported also in younger patients, including children. Indeed, the symptoms may come from almost any organ system or part of the body. Less common reported symptoms include production of sputum, headache, dizziness, haemoptysis, nausea, stomach pain, diarrhoea, and vomiting [74]. It was believed that dysgeusia and anaemia could be presented in COVID-19 infection [81]. Unknown aspects of SARS-CoV-2 infection not only make it difficult to prevent but also enable it to persist until large numbers of people have either died or recovered [31].

Patients may present with gastrointestinal symptoms such as abdominal pain, diarrhoea, and nausea [17, 34, 82]. Also, lymphopenia is commonly seen in cases with COVID-19 and may be a poor prognostic factor [83]. The incubation time is typically longer than that seen in influenza, generally 2 weeks and sometimes longer, which provides time for the beginning of antibody production, which is called the adaptive immune stage. This leads to a fall in virus titres and, in most patients, the elimination of symptoms. However, at his time, about 10% of patients enter a critical stage of the disease with increased risk of death. These patients suffer from viremia, acute respiratory distress, acute heart damage, failure of

several organs, and secondary bacterial infections [84]. Multiple organ dysfunction is thought to be due to an inflammatory immune response [85]. Furthermore, COVID-19 infection may lead to different dermatological presentations, which may help physician to diagnosis this infection [86]. Cytokine storms, a sign of immunological overreaction, are common in these cases, which often exacerbate the complications [87].

7 Respiratory Manifestations

Based on data on influenza virus infections and previous coronavirus epidemics (SARS and MERS), these kinds of viral infections mainly cause lung pathologies such as pneumonia and acute respiratory distress syndrome [88]. It seems that there are two clinical stages for COVID-19 disease. The first stage is the virus replication stage in which patients show relatively mild symptoms [83]. Most symptomatic patients with COVID-19 demonstrate fever, shortness of breath, tiredness, dry cough, and muscle pain. Findings of X-rays and CT scans of the chest are in favour of a pneumonia diagnosis [34, 84]. Infected subjects with mild respiratory symptoms may require supportive care such as supplemental oxygen.

8 Extra-pulmonary Presentations

8.1 Gastrointestinal Manifestations

Although gastrointestinal manifestations are uncommon [89, 90], some studies [16, 17, 91, 92] have noted that patients with SARS-CoV-2 infection may complain of gastrointestinal symptoms similar to the SARS outbreak [93]. Anorexia and diarrhoea are reported as the most prominent digestive system symptoms of SARS-CoV-2 [16, 17]; however, abdominal pain, vomiting, and nausea can also occur [16, 34, 72, 94]. The SARS-CoV-2 coronavirus has been detected in rectal swabs [84] and in patient's stools [16]. Thus, gastrointestinal manifestations should not only be thought of as a possibility, but this suspicion should immediately be raised in at-risk patients presenting with gastrointestinal symptoms. However, large-scale studies with emphasis on the gastrointestinal manifestations are needed to confirm these findings.

While some surgical aetiologies of bowel obstruction such as intussusception [95] are related to viruses, viral gastroenteritis such as sapovirus could be one of the nonsurgical aetiologies of intestinal obstruction and present with severe gastrointestinal symptoms [96]. Furthermore, different viruses, such as norovirus or rotavirus, can affect the enteric nervous system along with increased luminal contents from malabsorption and lead to severe intestinal distension [97] that may present as SARS-CoV-2 infection or just the common aetiology of adhesion band with asymptomatic SARS-CoV-2 respiratory infection.

High-resolution computed tomography (HRCT) plays an important role in the initial diagnosis of the SARS-CoV-2 infection as it shows a typical imagery. The demonstration of multiple, bilateral, ground-glass opacities in a patchy pattern with multiple lobular peripheral distribution are typical features of SARS-CoV-2 infection [90]. HRCT indicating lung infiltrations in patients with gastrointestinal symptoms accompanied with laboratory data such as lymphopenia and high CRP readings is a strong indication of SARS-CoV-2 infection [82, 98]. However, further studies are needed to assess the value and clinical utility of gastrointestinal-based testing for SARS-CoV-2 infection to improve diagnosis and reduce transmission.

8.2 Cardiovascular Complications

Importantly, primary heart injury has been developed even without respiratory failure [89]. Myocardial injury in SARS-COV-2-infected patients has been reported 8–14 days after the onset of symptoms [74]. Hypertension is commonly associated with COVID-19, and older patients with coronary problems and diabetes

often manifest cardiac injury associated with SARS-COV-2 infection [99]. The prevalence of cardiovascular complications in cases with SARS-COV-2 infection may be related to a more severe viral infection which accounts for increased risk of mortality rate [18]. Indeed, the most severe form of heart disorder is myocardial shock, which may occur in subjects with critical viral infections. Studies have shown that myocardial injury and myocarditis have been accompanied by increased serum troponin level and high viral loads [100].

SARS-COV-2 patients can be diagnosed with dyspnoea, chest pain, acute dysfunction of the left ventricular chamber, and heart beat arrhythmia [18]. Myocardial inflammation can result in electrocardiogram (ECG) abnormalities including T-wave inversion, non-specific ST-segment as well as PR-deviation. Moreover, focal wall motion defects of the heart can be demonstrated using echocardiographic assessment. Nevertheless, myocarditis can be associated with severe forms of SARS-COV-2 infections without abnormality or dysfunction of the motion of the cardiac wall [89]. Taken together, ECG and echocardiographic findings not only demonstrate the severity of this illness but also indicate an increased risk for severity of the infection and potential mortality [101].

Viral infection enhances the risk of coronary complications, which can be the essential cause of acute myocardial infarction (AMI) in SARS-COV-2 subjects, and extensive systemic inflammation in cases with SARS-COV-2 enhances the risk of AMI [102]. The mechanisms through which myocardium infected by viral particles can be explained by ACE2 receptors on myocardium and vascular endothelial cells, which account for myocarditis syndromes [89]. Moreover, vasculitis triggered by viral infection could hurt the myocardium [103]. The potential mechanism of myocardial injury possibly could be associated with oxygen supply and the demand mismatch can lead to type 2 AMI [104, 105].

The initial presentation of SARS-COV-2 infection can be acute heart failure with cardiomyopathy and enhanced risk of mortality [18]. Indeed, SARS-COV-2 infection can present with a range of heart dysrhythmias, mostly sinus tachycardia which is due to hypoxia or hypoperfusion, abnormal metabolism, fever, anxiety, etc. [101]. Studies suggest that systemic inflammation, multi-organ dysfunction, abnormal coagulation status, and critical complication are potential factors contributed to the elevated risk of venous thromboembolic event (VTEs) in SARS-COV-2 [94, 106]. Stress cardiomyopathy, especially cytokine storm, microvascular dysfunction, and sympathetic surge occur in SARS-COV-2 infection with unknown pathophysiology [107]. Microvascular injuries and thrombosis in various parts of the body have been highlighted by Magro et al. [108]. Furthermore, hospitalization because of pneumonia increases the risk for atherosclerotic problems [109].

Of note, current medication against SARS-COV-2 infections may cause cardiovascular dysfunctions, e.g., lopinavir/ritonavir therapy can result in PR and QT prolongation. Besides, these drugs can interact with cardiovascular medication such as antiplatelet and anticoagulant drugs and statins [18]. On the other hand, chloroquine and hydroxychloroquine not only account for cardiotoxicity, electrolyte abnormalities, and prolonged QT intervals but also affect antiarrhythmic medication [110]. Also, methylprednisolone can affect fluid retention, electrolyte derangements, and hypertension [18].

8.3 Haematological Disorders

The levels of serum ferritin, C-reactive protein (CRP), albumin, lactate dehydrogenase as well as the erythrocyte sedimentation rate (ESR) increase significantly during COVID-19, while the number of neutrophile leukocytes and haemoglobin levels fall [111]. The haem in haemoglobin is composed of four subunits (2-α and 2-β), each with an iron ion attached at the centre [112]. This constituent is imperative in haemoglobin and if the structure lacks the iron, it becomes porphyrin. In the SARS-COV-2-infected patients, the 1-β chain of haemoglobin is attacked by orf1ab, ORF3a, and ORF10 proteins resulting in iron dissociation and iron ion accumulation followed

by body inflammation together with CRP and albumin enhancement. Great amounts of serum ferritin are produced in response to stress and this decreases injury. The viral proteins (ORF8 and surface glycoproteins) attach to porphyrin. Parallel, the other coronavirus proteins (ORF1ab, ORF10, and ORF3a) attack the haem and release the iron which leads to less hemoglobin. Suppressing haem metabolism causes a wide range of complications and infection. It has been shown that chloroquine reduces the respiratory distress symptoms via inhibiting the binding of ORF8 as well as surface glycoproteins to porphyrins and preventing ORF1ab, ORF3a, and ORF10 to attack the haem [111].

A line of evidence shows that thrombocytopenia is diagnosed in critically severe SARS-COV-2 infection cases, which usually results in organ failure or physiologic decompensation and intravascular coagulation [113]. It has also been suggested that lymphopenia can be used as a reliable indicator of prognosis in infected patients. When data from severe SARS-COV-2 patients are stratified and compared with the composition of immune cells, an inverse correlation between disease severity and lymphocyte percentage is observed [114]. Lymphocytes play a substantial role in immune homeostasis and body inflammatory response. Therefore, finding an effective approach to improve SARS-COV-2 infection requires the elucidation of the mechanism of lymphocyte deficiency. Four mechanisms have been proposed: (1) The virus binds directly to the ACE2 receptors on the lymphocytes, which leads to demise of this type of cell [115]. (2) The lymphatic organs might be destroyed by viral particles resulting in lymphocyte depletion. (3) Derangement of inflammatory cytokines such as interleukin (IL)-6, tumour necrosis factor (TNF) α, and other pro-inflammatory cytokines leading to lymphocyte apoptosis [116]. (4) Metabolic disorders produce metabolic molecules such as hyperlactic acidemia, which suppress lymphocyte proliferation [117]. Also, leuko/erythroblastic reactions have been reported in cases with SARS-COV-2 infection [118]. Indeed, lymphopenia is a reliable predictor for the prognosis of SARS-COV-2 infection [83].

All the above bring forth the hypothesis that untypical gastrointestinal manifestations may be more common symptoms of SARS-CoV-2 infection than hitherto thought. It highlights the importance for diagnosis to be based on combined laboratory-based data and scanning imagery.

9 Immune Responses

The immune system operates through many different cells and we need to collect more information what is occurring in relation to COVID-19 infection. How the whole cell system, e.g., dendritic cells, helper T-cells, natural killer (NK) T-cells, B-cells, and macrophages, operates in both severely ill patients and those that show few signs of infection remains an open question. Although we have had only a few months to learn, it is known that, similar to other respiratory virus infections, a self-limiting respiratory disease occurs that in most cases ends with the development of neutralizing anti-viral T-cells and production of IgM, IgA, and IgG-specific antibody levels [119].

Qin et al. [120] report that most severe cases they investigated demonstrated significantly decreased number of different classes of T-cells, particularly in the most severe cases. Both helper and suppressor T cells in patients with COVID-19 were below normal levels, T helper cells in particular [117]. Also, the percentage of naïve helper T cells increased; regulatory T cells and memory helper T cells decreased in severe cases [117]. That we are dealing with a dysfunctional immune response is further supported by Giamarellos--Bourboulis et al. [121] who found a unique pattern of immune dysregulation in severely affected COVID-19 patients. This was found to be characterized by IL-6-mediated low HLA-DR expression and lymphopenia resulting from depletion of CD4 lymphocytes, CD19 lymphocytes, and natural killer (NK) cells associated with sustained cytokine production and hyper-inflammation, a pattern distinct from bacterial sepsis or influenza [118].

High viral loads during the first infection and repeated exposure to virus especially in health-

care workers can be an important factor for the severity of disease. It should be noted that severe reactions, such as lymphopenia, eosinopenia, extensive pneumonia, and cytokine storm attacks leading to acute respiratory distress syndrome due to lung tissue damage and multi-organ failure, are unique to COVID-19 and are rarely observed in other respiratory viral infections. Lymphopenia is a sign of a defect in antiviral and immune regulatory immunity. At the same time, a cytokine storm starts with extensive activation of cytokine-secreting cells with innate and adaptive immune mechanisms both of with contribute to a poor prognosis. Elevated levels of acute phase reactants and lymphopenia are early predictors of high disease severity. Prevention of these developments should be main routes for future research areas. As we learn to live amidst the virus, understanding the immunology of the disease can assist in containing the pandemic and in developing vaccines and medicines to prevent and treat individual patients.

10 Therapy

There are not proven effective therapies for SARS-CoV-2, but current therapeutic method consists of supportive care, including invasive and non-invasive oxygen support and antibiotic treatments [122, 123]. Moreover, some patients with severe symptoms have received off-label or compassionate-use therapies such as: antiretrovirals, anti-parasitic agents, anti-inflammatory compounds, and convalescent plasma [124–127].

10.1 Drugs that Slow Viral Replication or Kill SARS-Cov-2

The use of protease inhibitors, such as lopinavir and ritonavir, is promising because these medications have been used to treat similar viral infections, e.g., HIV/AIDS. Lopinavir generally combined with ritonavir for inhibiting cytochrome P450 in order to raise the half-life of lopinavir [128]. These drugs are broadly reported to be able to deactivate Mpro and hence they can be a potential drug against SARS infection [129, 130]. After coding both for polyproteins ppla and pplb in SARS-CoV-2, these polyproteins are cleaved to be used as several functional proteins of spike, membrane, nucleoprotein, envelop, replicase, and polymerase in the virus [131–133]. This cleavage is performed by chymotrypsin-fold proteinase of 33-KD molecular mass or main protease (Mpro). This proteinase has been named 3C-like protease (3CLpro) as well [134–136].

Chloroquine/hydroxychloroquine has been recommended for treatment of SARS-CoV-2 [137]. Chloroquine is an immune-modulating drug which is traditionally used to prevent malaria infection but widespread malaria resistance against the drug limits its current use. The positive effect of chloroquine (with a standard dosing) has been revealed in reducing viral replication in some infections, including the SARS and MERS [137]. Its favourable penetration in tissues such as lung is noticeable [138]. The drug can block virus infection through increasing endosomial pH and interfering with the glycosylation of cellular receptor of SARS. Hence, there is a possibility that this drug enhances the antiviral effect *in vivo* [138]. It has been also discussed that the treatment with specific characteristics such as risk–benefit balance, the high safety, and the low expenditure, are in the context of the SARS-CoV-2 outbreak [139]. Although the usefulness of hydroxychloroquine has not been completely borne out and the U.S. Food and Drug Administration (FDA) cautions against its use outside hospital settings due to the risk of cardiac side-effects.

Nucleotide inhibitors constitute another line of promising chemical compounds that have been shown to be active against RNA viruses [140]. One of them, Remdesivir™, is currently in Phase 3 clinical studies (testing large numbers of people in the field) to evaluate the safety and efficacy in adults diagnosed with COVID-19. It is a nucleotide analogue that is intracellularly metabolized to an analogue of adenosine triphosphate that inhibits viral RNA polymerases. Remdesivir has been broadly used against members of several virus families such as filoviruses (e.g., Ebola)

and pervious coronaviruses (e.g., SARS-CoV and MERS-CoV) and has shown prophylactic and therapeutic efficacy in nonclinical models of these coronaviruses [141–143]. In addition, according to *in vitro* studies, this drug has shown the activity against SARS-CoV-2. Remdesivir provides humans with a clinical safety profile, as reported on the basis of experience in more than 500 persons, including healthy volunteers and patients treated for acute Ebola virus infection [144]. Some patients with severe COVID-19 have been treated with Remdesivir on a compassionate-use basis [145]. A study by Beigel et al. [146] showed that in adults hospitalized with COVID-19, Remdesivir can shorten the time to recovery and evidence of lower respiratory tract infection.

Type 1 interferons (IFN–I) have a broad antiviral activity. Some cytokines, including the ubiquitous α- and β-subtypes with different isoforms, as well as the ε, ω, and κ subtypes, have been designated IFN–I [147]. They are secreted by several cell types, particularly plasmacytoid dendritic cells, and lead to recognition of viral components through pattern recognition receptors (PRR) [148]. IFNs are thus amongst the first cytokines produced during viral infections. In most cells, they can be recognized by the IFNAR receptor present in the plasma membrane. With binding the interferon to IFNAR, some transcriptional factors such as STAT1 are phosphorylated and relocalized to the nucleus, where they activate interferon-stimulated genes (ISG). Most ISGs have a role in inflammation, signalling, and immuno-modulation. They interfere with viral replication; they also use several mechanisms like slowdown of cell metabolism and/or secretion of cytokines that activate adaptive immunity. ISGs include PRRs that promote sensitization of the cell to pathogens, proteins which decrease membrane fluidity, preventing viral egress or membrane fusion and antivirals that specially prevent one step of the viral cycle [149, 150]. The IFN-Is thus play a major role in antiviral immunity and it has been reported in the treatments against MERS-CoV and SARS-CoV [151]. IFN-I can be used in combination or not with lopinavir/ritonavir [143], ribavirin [152], remdesivir and corticosteroids [153], or IFNγ [154].

Favipiravir selectively contributes to inhibition of the RNA-dependant RNA polymerase (RdRP), an enzyme needed by RNA viruses for replication in human cells. It acts as a purine analogue and is incorporated into genome instead of guanine and adenine, which leads to termination of the RNA elongation among viruses; only a single molecule of favipiravir is needed for this to occur [4, 155]. The drug can be converted intracellularly into its active phosphorylated form and then is recognized as a substrate by viral RdRP. It has a wide spectrum of activity in RNA viruses (Influenza, Rhino, and Respiratory Syncytial Virus, etc.) but not against DNA viruses (such as Herpes) [5]. Several clinical trials of favipiravir in COVID-19 patients have been performed, whose results can clarify the role of favipiravir in the treatment of COVID-19 patients [156]. However, specific treatment schedules for COVID-19 have yet to be established.

The use of convalescent plasma had been suggested as an empirical treatment during previous outbreaks of various viruses like Ebola, MERS, SARS, H5N1 avian influenza, and H1N1 influenza [157–162]. In previous reports, the majority of the patients received the convalescent plasma by single transfusion [161–163]. Furthermore, the treatment of severe infection with convalescent plasma was associated with reduced respiratory tract viral load, serum cytokine response, and mortality [162]. It has been reported that in patients with SARS, administration of convalescent plasma was associated with more hospital discharge in comparison with patients who did not receive convalescent plasma [164]. Therefore, these findings raise the hypothesis that use of convalescent plasma transfusion could have influential effect on patients infected with SARS-CoV-2 [125].

10.2 Vaccination

Antiviral vaccines can be categorized into gene-based vaccines (live virus vaccines, nucleic acid vaccines, or recombinant vaccine vectors) or protein-based vaccines (whole-inactivated virus, viral proteins accumulated as particles, or individual viral sub-domains or proteins). Gene-

based delivery should stimulate CD8 T cells and trigger immune response based on CD4 T helper 1 cell-type [165]. Several DNA and RNA, as well as recombinant-subunit vaccine, platforms exist for COVID-19 vaccine development. An ideal vaccine should facilitate steady immune responses against pathogens. Although no RNA vaccine has been approved to date, various ones have progressed to clinical development [166].

DNA- and mRNA-based vaccines have enormous flexibility in regard to manipulation of antigen and quick synthesis. In fact, a mRNA-based vaccine mRNA-1273 has been moved to clinical investigation two months following sequence identification. Moreover, vaccines based on viral vectors not only express high levels of proteins that strongly stimulate immune responses, but also have long-standing stability. Besides, immunogenicity could be increased by adjuvants. Vaccines against SARS-CoV-2 strengthened by adjuvants are planned by about 10 developers. Unfortunately, knowledge about the exact SARS-CoV-2 antigen(s) to be applied in developing vaccine is inadequate. Indeed, this kind of information is needed to produce neutralizing antibodies against the viral S-protein, which would interfere attachment to the ACE2 receptor. Importantly, the most highly developed candidates such as Ad5-nCoV from CanSino Biologicals, mRNA-1273 from Moderna, INO-4800 from Inovio, and LV-SMENP-DC and pathogen-specific aAPC from Shenzhen Geno-Immune Medical Institute have entered clinical trials phase. The major platforms and examples of tentative SARS-CoV-2 vaccines are listed in Table 1.2. To efficiently evaluate the efficacy of a vaccine, specific animal models for COVID-19 have been developed, amongst them ACE2-transgenic mice, ferrets, hamsters, and non-human primates [30]. The challenges for SARS-CoV-2 vaccine are many; first, the viral S-protein is a primary immunogen for protection but optimizing the antigen design is essential to gain an acceptable immune response. Second, vaccine candidates can stimulate adverse effects such as exacerbating lung disorders. Third, the potential of immunity duration has not been established. Similarly, single-dose vaccines would be an advantage, but whether or not they would produce immunity is also unknown [166].

Furthermore, safety is an important point for vaccines and the vaccines' subsequent risk to make more severe infection of SARS-CoV-2 including antibody-dependent enhancement (ADE) and the enhanced respiratory disease (VAERD) is of great importance. Also, allergic inflammation can be produced by whole inactivated viral vaccines. So, parallel investigation of vaccines in healthy adults with vaccine evaluation in animal models as well as developing production capacity pave a way to minimize risk of vaccine in human cases and provide potential benefit via accelerating COVID-19 vaccine availability [165].

Table 1.2 Vaccine platforms and attributes

Technology	Vaccine candidate	Investigational level	Company
DNA	NCT04336410	Phase 1	Inovio Pharmaceuticals Plymouth Meeting, Pennsylvania, USA
Non-replicating vector	ChiCTR2000030906	Phase 1/2	CanSino Biologics
Non-replicating vector	ChiCTR2000031781	Phase 1/2	CanSino Biologics
Non-replicating vector	NCT04324606	Phase 1/2	University of Oxford/ AstraZeneca,
Non-replicating vector	NCT04276896	Phase 1/2	Shenzhen Geno-Immune Medical Institute,
Subunit vaccines	Preclinical work		
RNA	NCT04283461		Moderna/NIAID
RNA	NCT04368728	Phase 1/2	BioNTech/Pfizer

There are already more than 100 candidates [30] under development, but only a few have reached an advanced stage and only ten are at the first level of clinical trials (Phase I), which investigates safety. There is much hope for vaccine to be ready within the next 6 months, but that may represent wishful thinking rather than reality. Even if efficacy can be demonstrated, scaled-up production will become a bottleneck given the high number of doses needed to keep the world safe. Although access to the virus genome has reduced the laboratory work from decades to months, there is at least a year before a vaccine can become widely available as there are many steps required before a vaccine is released for public use. On the other hand, this will probably be cut short once safety has been shown. Vaccine evaluation can therefore be expected to move almost directly from Phase I to Phase IV, which represents continued studies after commercial release of a product.

11 Conclusion

COVID-19 is a serious infectious disease caused by the novel coronavirus SARS-CoV-2 that is highly contagious and spreads through droplets and close contact. Its major initial symptoms, like SARS and MERS, are fever, cough, and fatigue. The most probable origin of SARS-CoV-2 is a bat species, possibly transferred to humans via a mammal. It is an urgent need to control this pandemic reducing mortality and morbidity as soon as possible. Not only are the specific pathological mechanisms to a large extent unknown, but there is also a lack of specific antiviral drugs and vaccines. At present, it is vital to control the source of infection, reduce the route of transmission, and use existing drugs and means to control progress of the disease. We should focus on developing specific drugs, promote research and development of vaccines, and reduce morbidity and mortality of this virus in order to provide safety.

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Part II

Epidemiology

2 Prevalence of COVID-19 and the Continued Citizen-Based Control in Japan

Sumino Yanase and Hiroki Sugimori

Abstract

Since an outbreak of COVID-19 was detected among the crew and passengers of the Diamond Princess cruise ship in early 2020, the total number of cases of SARS-CoV-2 has surpassed 440,000 in Japan. However, that number remains small compared with other countries, such as the United States, a few European countries, and China. Despite the Japanese government not imposing a lockdown or implementing large-scale testing at the municipal level, the country has managed to contain the smaller outbreaks. To avoid infection, it is important to wear a face mask, wash one's hands, and observe social distancing. In addition, we focus on the clinical laboratory testing performed using the latest technology to detect SARS-CoV-2 RNA in a hospital in Yokohama, Japan. Large-scale testing of viral RNA would be useful for detecting asymptomatic virus carriers as is done in other countries, and could be carried out as a future measure for controlling COVID-19.

Keywords

COVID-19 · Outbreak · Face mask · Hand washing · Physical distancing · Influenza surveillance · Clinical laboratory testing

1 Introduction

After one year, coronavirus disease 2019 (COVID-19) has become prevalent in many regions worldwide. In Japan, since an outbreak of COVID-19 was detected among the crew and passengers of the Diamond Princess cruise ship in early 2020, the total number of COVID-19 cases leading to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has surpassed 440,000, including more than 8,000 deaths [1, 2]. Unfortunately, these numbers gradually increased since the emergency declaration was lifted at the end of May 2020 after just a month and a half. During Japan's state of emergency, most residents followed the advice of public officials to stay at home despite the lack of a compulsory lockdown or large-scale clinical test-

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ing as was the case in China and Korea. Meanwhile, the number of COVID-19 cases in Japan remains small compared with other countries, such as the United States and a few European countries before they entered their lockdown periods [2–4]. For instance, the number of COVID-19 cases and the number of COVID-19-related deaths in the United States are both approximately 70-fold greater compared with Japan [2, 5]. Although the reason why there are relatively fewer COVID-19 cases in Japan remains unclear, government measures aimed at eliciting the public's cooperation to prevent the spread of SARS-CoV-2 are obviously associated with the smaller number of cases, at least in part [2]. The approach taken by the Japanese government differs from the Swedish government's coronavirus strategy, which has attempted to deal with COVID-19 to save lives and slow down the outbreak [6–8].

SARS-CoV-2, which was identified in China as a novel coronavirus at the end of 2019, spreads mainly by human-to-human transmission via respiratory droplets containing the virus or by direct contact with the virus carrier [3, 9]. Notably, since the effects of social distancing and wearing face masks and eye protection to prevent the infection of SARS-CoV-2 were reported in June 2020, more and more people worldwide have begun to adopt these measures, not only in Asia but also in Europe and the Americas [10–12]. These preventive measures are similar to those used to protect against influenza. At present, these personal voluntary strategies are the main measures to protect against SARS-CoV-2 infection and are likely to remain so until a vaccine and efficient pharmaceuticals have been developed and distributed.

Here, we review the history to date of COVID-19 in Japan including the outbreak aboard the Diamond Princess cruise ship, the sequential meta-analysis of COVID-19 case demographics, and voluntary actions taken by the public to protect against SARS-CoV-2 infection. Moreover, we describe actual clinical laboratory testing for the detection of SARS-CoV-2 in Japan.

2 Cruise Ship Outbreak and the Environmental Assessment

2.1 COVID-19 Outbreak on the Diamond Princess Cruise Ship

In February 2020, the Diamond Princess cruise ship had docked at the Port of Yokohama in Japan. Of the 3711 crew and passengers from 57 countries, 712 people were infected by SARS-CoV-2. This was the first outbreak of COVID-19 in Japan [13, 14]. First, an octogenarian passenger who had disembarked in Hong Kong was diagnosed with COVID-19 on the first of February. The onset of cough was from January 19th and he was flagged by a medical quarantine inspection in Hong Kong [15]. Subsequently, the ship called in again at Yokohama after visiting other ports in Japan and was placed in quarantine on February 3rd. Of the 31 crew and passengers who developed fever and/or cough during the quarantine and were subjected to an oropharyngeal swab test, SARS-CoV-2 RNA was detected in 10 people [2, 13]. By April 15th, 14 of the 712 crew and passengers who were diagnosed with COVID-19 had died, and the initial case fatality rate (CFR) for COVID-19 was approximately 2.0%. In addition, 9 healthcare workers, including a quarantine officer and a medical doctor, became infected on the ship during the quarantine [13]. Japanese government officials performed more than 3000 reverse transcription polymerase chain reaction (RT-PCR) tests among the 3711 crew and passengers. There were 619 laboratory-confirmed cases of COVID-19 (approximately 17%), comprising the 318 asymptomatic cases and 301 symptomatic cases, as of February 20th [1, 13, 14, 16]. Ultimately, the asymptomatic COVID-19 cases were estimated to account for 17.9% of all diagnosed cases onboard the Diamond Princess [17]. According to a recent report, the corrected CFRs were estimated to be 2.6% across all age groups and 13% in those 70 years old and over based on data from large-scale PCR tests [1, 16]. Both the

initial CFR of 2.0% and the corrected CFR of 2.6% onboard the Diamond Princess were lower than the 3.7% estimated during that period in China based on data from the World Health Organization (WHO), but higher than the 1.2% calculated during the outbreak in Wuhan, China. Given that the mean age of those onboard the Diamond Princess was 58 years, the reported CFR seems to be high [16, 18].

2.2 Age-Related CFR Estimated from the Cruise Ship Outbreak

Regarding the age-related CFR of COVID-19, Russell and colleagues assumed that all deaths among COVID-19 cases were the result of COVID-19 even though passengers onboard the Diamond Princess may have had a different health status compared with the general population of their home countries [16]. Likewise, there were age-specific differences in the overall COVID-19 CFR calculated during the outbreak in China [16, 18, 19]. Furthermore, a large survey revealed that children 17 years old and under, who make up 22% of the United States population, accounted for less than 2% of confirmed COVID-19 infections across the United States [20]. However, there is also evidence which suggested that SARS-CoV-2 infection rates in children and teenagers are similar to those in older people [21]. Meanwhile, recent studies have uncovered the reason that older people generally show increased susceptibility to seasonal flu caused by viruses, such as influenza viruses [22–24]. Molony and colleagues revealed an age-dependent functional defect in monocytes, which are rapidly recruited to the respiratory tract and differentiate into macrophages and dendritic cells in response to influenza virus A infection [24]. Like influenza viruses, SARS-CoV-2 infects people through the respiratory tract, and the age-related difference in susceptibility may be associated with either dysfunction or abnormal response of the immune system as a result of aging [22, 25, 26].

2.3 Environmental Assessment of SARS-CoV-2 on the Cruise Ship

A report from the National Institute of Infectious Diseases in Japan, based on an environmental assessment of SARS-CoV-2 RNA on the Diamond Princess cruise ship, suggested the potential of the viral particles to spread through airborne means [27]. The results of environmental swab tests performed onboard the cruise ship revealed high concentrations of viral RNA in lavatories as well as on desks, telephones, and television remote controls, which were used or touched by each COVID-19 case in passenger cabins. Interestingly, there were no significant differences in the detectabilities of viral RNA between symptomatic and asymptomatic COVID-19 cases. Viral RNA was not detected in air samples from passenger cabins but was detected in swab samples from an exhaust slot in the ceiling of a passageway in the cruise ship [27]. Similar aerodynamic analyses of SARS-CoV-2 were performed at two hospitals in Wuhan, China after the outbreaks in February and March 2020. Those analyses concluded that transmission might occur not only through human respiration droplets and direct contact but also via a viral RNA aerosol [28]. These results suggest that room ventilation, open spaces, and sanitizing clothing and toilet areas might effectively reduce the concentration of SARS-CoV-2 RNA in aerosols, thereby enabling improved management of the COVID-19 outbreaks [27, 28].

3 Government-Recommended and Voluntary Measures to Protect Against COVID-19 Infection in Japan

After the outbreak on the Diamond Princess cruise ship, haploid genotypes (haplotype) analyzed using whole-genome sequencing of SARS-CoV-2 from clinical specimens of

RT-PCR-positive cases were compared with a single-nucleotide mutation isolated in December 2019 in China in which the guanine (G) nucleotide was substituted with a thymine (T) nucleotide at position 11,083 on the Wuhan-Hu-1 genome sequence (G11083T transversion) [14]. This single-nucleotide variation leads to a non-synonymous amino acid substitution (L37F) in the nonstructural protein 6 (NSP6), which affects viral autophagy in SARS-CoV-2 [14, 29]. The results showed that the haplotype isolated on the Diamond Princess cruise ship had a different genealogy compared with other genotypes detected in China, Europe, North America, as well as onboard a cruise ship docked in the United States [14]. This unique haplotype has not been detected in other COVID-19 outbreaks in Japan. Haplotype network analysis suggests that the more recent COVID-19 outbreaks in Japan (i.e., since March 2020) were derived from mainly Europe-related isolates, which originated in China or other countries [30–32]. In January and February 2020, SARS-CoV-2 was responsible for the initial COVID-19 outbreaks, such as those in Hokkaido (the second largest island of Japan), derived from the Wuhan-Hu-1 haplotype. Hokkaido is a frequent sightseeing destination for tourist groups from China throughout the year. Despite attempts to contain the early COVID-19 clusters, the number of cases has continued to increase since March 2020 (from approximately 2000 cases on March 31st). Eventually, the Japanese government declared a nationwide state of emergency on April 7th aimed at preventing the further spread of COVID-19. This state of emergency lasted for a month and a half and ended on May 25th.

During the state of emergency, Japan aimed to reduce the number of new daily COVID-19 cases to 0.5 per 100,000 people without a compulsory lockdown or large-scale clinical testing, which have been effective as non-pharmaceutical interventions in China, South Korea, Europe, and the United States [33–36]. Despite the compulsory quarantine and thorough testing of crew and passengers onboard the Diamond Princess, the Japanese government together with local governments, such as that of Tokyo, made efforts to identify new COVID-19 clusters and their underlying causes. Accordingly, they focused on spots where groups of people gathered, including fitness gyms, music halls, and nightclubs, and demanded that they close or restrict their business hours. Furthermore, the government urged the public to avoid what they called the 'three Cs' (i.e., closed spaces, crowds, and close-contact settings) and explained the efficacy of wearing face masks and washing one's hands to prevent (or reduce at least by more than 90%) human-to-human transmission of SARS-CoV-2. These messages were conveyed by an advisory committee consisting of specialists such as virologists and epidemiologists [2, 10–12, 33]. To make it easier for people to stay at home, many companies allowed their employees to telework and many schools switched to online classes. Likewise, many restaurants and bars closed or switched to offering food to go. In addition to wearing face masks, the public proactively washed their hands with soap or used ethanol-based sanitizers for disinfection. Many restaurants in Japan usually provide a wet towel to diners before they eat, and shops such as drug stores and convenience stores generally carry packets of disinfecting wipes. The use of wet towels and disinfecting wipes may also contribute to preventing oral infection of the microbes that cause influenza and COVID-19.

Indeed, the measures used to control COVID-19, including social distancing, wearing face masks, and washing hands, have been reported to dramatically reduce transmission of influenza in Japan and other countries, particularly in the Southern Hemisphere (Fig. 2.1) [37–39]. The results of these studies suggest that such voluntary non-pharmaceutical measures against the COVID-19 outbreak would be an effective strategy, although this seems to be simple at a glance [31].

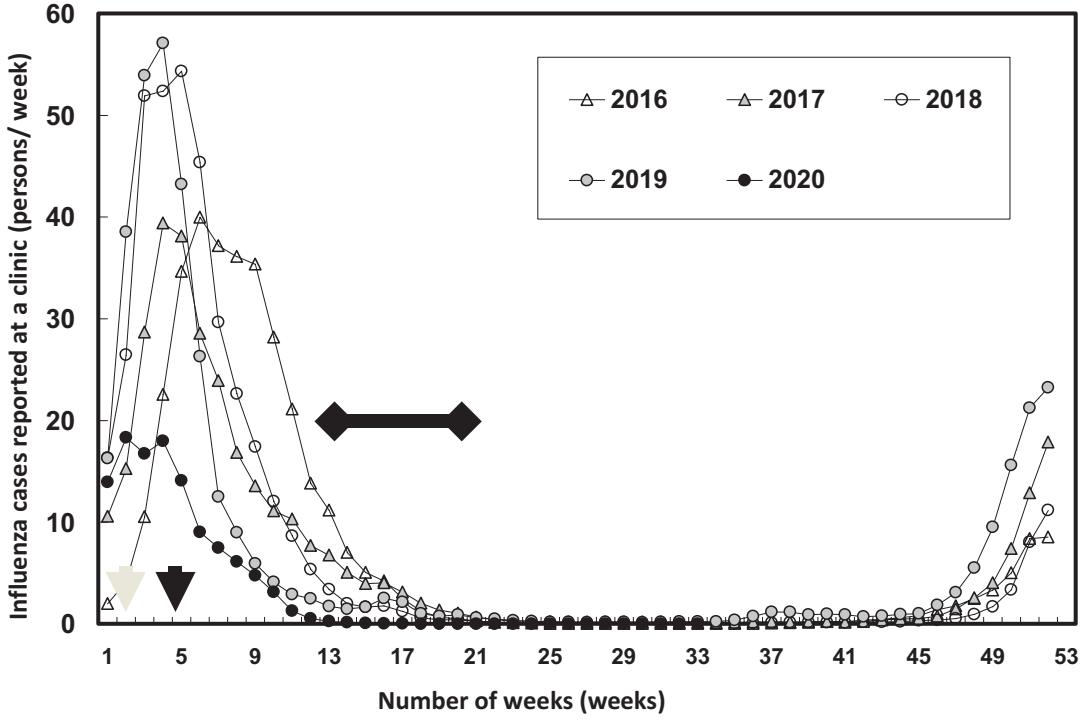

Fig. 2.1 Influenza cases reported per sentinel weekly in the last 5 years, published by the National Institute of Infectious Disease, Japan. This graph was modified from the published data [37]. The gray arrow indicates the day (January 15th, 2020) that the first COVID-19 case, who had visited Wuhan, China, was detected in Japan [40]. A few days later, the Japanese government issued travel bans to particular areas in China, which recommended prompt departures from Japan. The black arrow indicates the day (February 3rd, 2020) that the Diamond Princess cruise ship was quarantined in the Port of Yokohama. The double-headed arrow indicates the period of the state of emergency declared by the Japanese government, from April 7th to May 25th, 2020

4 Detection of the Coronavirus in Laboratories in Japan

Nearly all researchers consider asymptomatic cases to play a key role in the transmission of SARS-CoV-2 worldwide [17, 41–43]. However, few asymptomatic individuals voluntarily visit a clinic for diagnosis. Consequently, they act as a temporal carrier in the transmission of SARS-CoV-2 while the virus is shedding [44]. According to recent reviews, asymptomatic COVID-19 cases have been estimated to account for around 40% to 45% of confirmed SARS-CoV-2 infections [42, 43]. The lower rate of asymptomatic COVID-19 cases (approximately 17.9%) onboard the Diamond Princess cruise ship compared with other countries may be associated with the timing of non-pharmaceutical interventions (e.g., compulsory quarantine, lockdown, and large-scale clinical testing) and the age structure of the passengers [17, 42]. There were no significant differences in viral load between symptomatic and asymptomatic cases, the latter of which includes those that did not have symptoms at the time of swab testing as well as those that did not develop symptoms afterward. In addition, asymptomatic cases can transmit the SARS-CoV-2 virus to other people for at least 2 weeks after infection [45]. Therefore, identification of the asymptomatic individuals was suggested as an imperative task of the Japanese government to prevent the silent spread of COVID-19 through the country [45, 46]. Indeed, much progress has been made in the improvement of equipment used for diagnostic tests based on nucleic acid reagents and antibody-based serology, which reflects the cur-

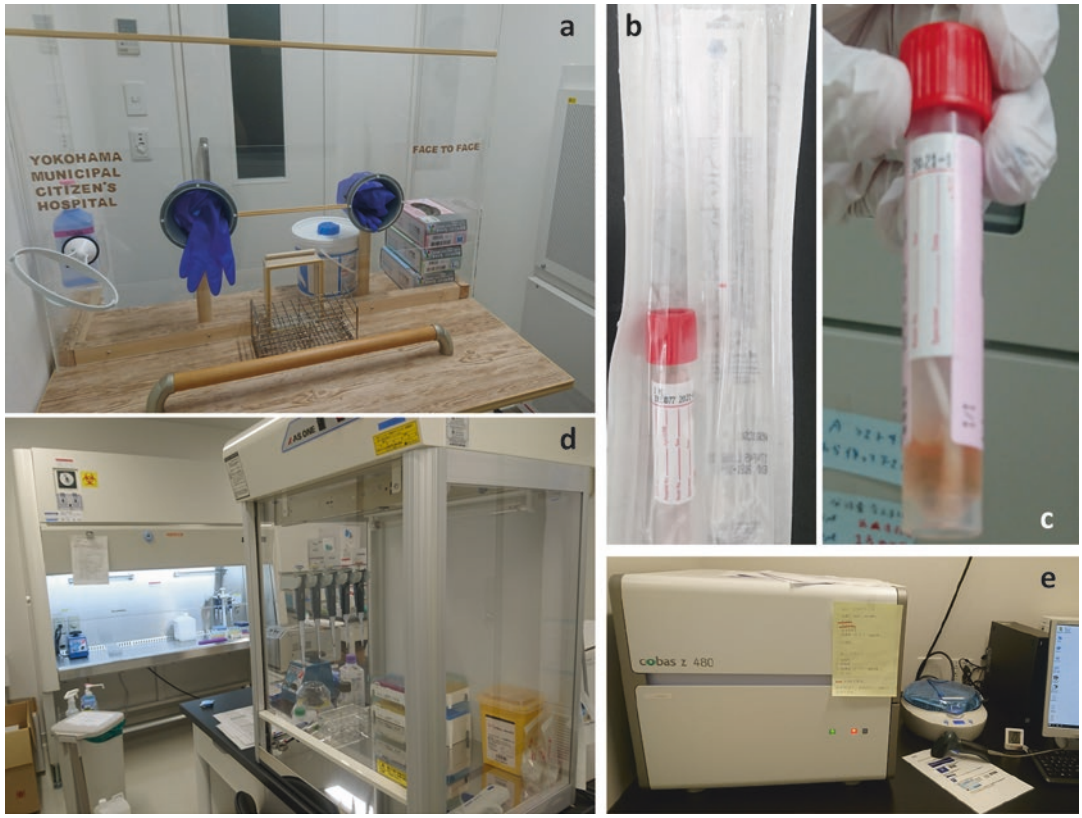

Fig. 2.2 Examples of equipment used to collect and test specimens from patients in the laboratory of a hospital in Yokohama, Japan. (**a**) A handmade partition used to protect staff while collecting nasal swabs from patients. (**b**) COVID-19 collection and transport kits in individually packaged peel-pouch transport media and a nasal/oral swab are made by Copan Manufacturer & Supplier, Italy. (**c**) Transport media containing a patient's nasal swab. (**d**) The inside and front biological safety cabinets are used for handling of specimens and reagents, respectively. (**e**) A COBAS Z 480 Analyzer Dialogue Diagnostics is the latest real-time PCR system (Roche Diagnostics, Switzerland)

rent thinking of limiting the transmission of SARS-CoV-2 as the main measure for controlling COVID-19 (Fig. 2.2) [46]. Based on the number of victims and COVID-19 research conducted around the world, to avoid the risk of infection while medical staff collect and test patients' specimens, it was shown that saliva can be used for easier SARS-CoV-2 detection [47]. Real-time PCR screening facilities at universities and institutes throughout Japan have assisted medical staff and patient testing at hospitals [48]. In the future, large-scale testing of viral RNA might be more effective in detecting asymptomatic carriers as in other countries, and could be carried out as a future measure for controlling COVID-19. However, limits of testing capacity using existing clinical laboratories and supply chains and difference in types of COVID-19 tests (to detect either viral RNA in the nasopharyngeal swab or serum antibodies against SARS-C0V-2) will lead to persistence of problems in prioritization of testing for either targeting of all citizen or contact tracing of virus clusters [49, 50].

5 Conclusions

Since the outbreak onboard the Diamond Princess cruise ship in the Port of Yokohama, the nation of Japan has experienced great

losses in terms of lives, health, and economic growth. However, much valuable information has been obtained from investigating outbreaks in such a closed population [1, 13–17]. This pandemic has provided a rare opportunity to understand the features of a new virus, including the age-related fatality rate, transmission through viral RNA aerosol, and coding the SARS-CoV-2 genome, which would otherwise be difficult to investigate [1]. In particular, the SARS-CoV-2 haplotype analysis of COVID-19 cases onboard the Diamond Princess revealed that the unique haplotype detected there was not the same as those detected in other outbreaks in Japan, providing hope in the fight against the novel coronavirus [14, 31]. Compared with the relatively easy act of quarantining a cruise ship, locking down an entire country is much more difficult. However, there are a few examples of such measures effectively suppressing the spread of SARS-CoV-2, including Wuhan, China and the Italian municipality of Vo'. The suppression of COVID-19 outbreaks in these cities and on the cruise ship was realized under political but not pharmaceutical interventions, including multipronged approaches such as compulsory lockdowns and political large-scale testing [13, 43, 45, 49]. These suggest the necessity of rethinking the restriction on capital and human resources that should prioritized to control the COVID-19 outbreaks in Japan without implementing a compulsory lockdown.

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COVID-19 Pandemic in Brazil: History, Characteristics, and Evolution

3

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Abstract

This chapter describes the eruption and spread of the SARS-COV-2 virus throughout Brazil. We also describe the governmental measures used to combat the virus, the regional influences impacting viral spreading, and the prevalence of the disease in different Brazilian subpopulations. It is hoped that such information will contribute to the control of the virus and help to prepare the region for future pandemics.

Keywords

COVID-19 · SARS-CoV-2 · Pandemic Brazil · Governmental measures · Regional characteristics

1 Brief Historical Facts of the COVID-19 Pandemic in Brazil

The coronavirus disease 2019 (COVID-19) is an infectious respiratory disease from a novel coronavirus named SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2). The first case of COVID-19 was reported on December 31, 2019, in Wuhan, China [1]. After a few weeks, the disease reached other countries in Asia, Europe, and North America. In South America, the disease

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was reported late. The first case occurred in Sao Paulo city, Brazil [2]. The World Health Organization (WHO) declared the outbreak a Public Health Emergency of International Concern (PHEIC) on January 30, 2020, and 2 months later, on March 11, 2020, it was declared a pandemic [1–3]. Two hundred fourteen countries worldwide have been affected by COVID-19, with more than 118 million confirmed cases and 2.6 million deaths as of March 11, 2021 [4]. In Brazil, one year into the COVID-19 epidemic, more than 11 million confirmed cases and 270 thousand deaths have been reported [5]. Brazil currently holds the record of the second highest COVID-19 cases and second highest number of deaths in the world. Since May 2020, the country has been considered the epicenter of the disease pandemic in Latin America [6]. The United States (US) occupies the first place in the total number of COVID-19 cases and deaths [4].

The first case reported in Sao Paulo city, Brazil, occurred on February 26, 2020, when a 61-year-old man who arrived from Turin, Italy, presented with symptoms compatible with severe acute respiratory illness, such as fever, dry cough, sore throat, and runny nose [7, 8]. Brazil has 26 states and one Federative Unit located in five regions: North, Northeast, Midwest, Southeast, and South. Each Brazilian state displays specific characteristics, such as social, economic, and climatic features.

A couple of days later, 16 Brazilian states displayed around 182 suspected cases. In the Southeast region, the most populous one, Sao Paulo and Rio de Janeiro, Brazil's largest cities, were the most affected. After a few weeks, the pandemic rapidly spread into the rest of the country, reaching all Brazilian states. On March 9, 2020, the Brazilian Ministry of Health tested all patients in private and public hospitals with severe acute respiratory illness for COVID-19. A couple of months later, several states collapsed due to the Public Health System being overloaded after the first case. In April 2020, Sao Paulo, Rio de Janeiro, Pernambuco, Ceara, and Amazonas displayed the peak of deaths related to the COVID-19 [7].

Candido et al. reported that most of these infected people arrived from Italy, followed by China, France, Switzerland, South Korea, and Spain, between February and March [9]. This resulted in rapid virus dissemination around the country, differently to China and other countries [9]. The route from Italy to Sao Paulo was the main one for importing COVID-19 to Brazil, followed by Rio de Janeiro, Belo Horizonte, and other states. Sao Paulo becomes an epicenter of Brazil's disease, which would be associated with the destination for many people flying from Italy.

On March fifth, the Brazilian Ministry of Health reported the COVID-19 local transmission, with one person being infected by a man who arrived from Italy in Sao Paulo city [10]. Also, local transmission occurred in Rio de Janeiro and Bahia states. Two weeks later, it was declared the COVID-19 community transmission in the national territory by the Brazilian Ministry of Health, where health organizations could not identify the origin of the first patient infected. Several Brazilian institutes of research examined samples from patients who tested positive for COVID-19, evaluating the genetic sequencing. At the beginning of the pandemic, there were six different lineages of SARS-CoV-2. One specific lineage rapidly spread in the Brazilian territory, which would be associated with the COVID-19 community transmission. The virus lineage characterization might geographically elucidate the virus lineage circulation, identify the lineage in each state inside the country and outside, and understand the virus dissemination. After the community transmission, the contagion rapidly disseminated, making it hard to control the virus spread in Brazil, due to the difficulty of tracking the virus propagation.

Afterward, all Brazilian states followed the National Contingency Plan (NCP) to take action against COVID-19. The NCP measures follow scientific-based evidence and recommendations published by the WHO. By the end of March 2020, Brazilian authorities recommended physical distancing, although the lockdown was implemented only in some states, such as Amapa, Ceara, Maranhao, MatoGrosso, Para, Pernambuco, Parana, Piaui, and Tocantins. On July 29, Brazil reached 69,074 COVID-19 new cases in one day, peaking in daily new case numbers [11].

Classical drugs already used as an antiviral treatment, such as chloroquine, hydroxychloroquine, azithromycin, remdesivir, liponavir-ritonavir, and interferon-β-1b have been proposed as therapeutic strategies to treat the patients. However, several clinical research institutes have failed to show the drugs' efficacy and safety to treat COVID-19 patients. For example, Geleris et al. studied the hydroxychloroquine effect in severe patients with COVID-19 at a large medical center in New York City [12]. The authors did not find a significant effect on the risk of death in severe patients with COVID-19.

On June 11, the Butantan Institute in Sao Paulo signed an agreement to develop and produce a vaccine against SARS-CoV-2 (CoronaVAC), initially developed by the Chinese biopharmaceutical company, SinoVac Biotech. This vaccine already showed positive results in phase 1 and 2 preclinical studies. The phase 3 clinical test is in progress to certify the vaccine safety and efficacy [13]. Other vaccines also are in clinical tests in Brazil, including the Astra Zeneca/Oxford University and Johnson & Johnson vaccines. The most optimistic estimate for the conclusion of the COVID-19 vaccine studies is by early 2021 for global distribution [14].

2 Governmental Measures to Combat the Pandemic

The COVID-19 pandemic in Brazil started in February 2020, after it reached several countries in Asia, Europe, and North America [15]. Despite the scientific-based evidence and recommendations from the World Health Organization [16], the Federal Brazilian government has made conflicting and mismatching decisions that somehow influenced the disease epidemic in Brazil [17, 18]. The turbulence observed mainly in the first months of the pandemic directly impacted the disease dissemination in the country. Figure 3.1 highlights some important decisions and events related to the Federal Brazilian government during the COVID-19 pandemic progression in the country. An association has been between the decision-making of the Brazilian government and the subsequent disease-spread dynamics. The factors involved in this turbulence are complex involving several spheres and Brazilian authorities. The detailed description and discussion of each decision/event lie outside the scope of this chapter. The discordance between the Federal government and state/municipal governments and the discrepancy of the decision-

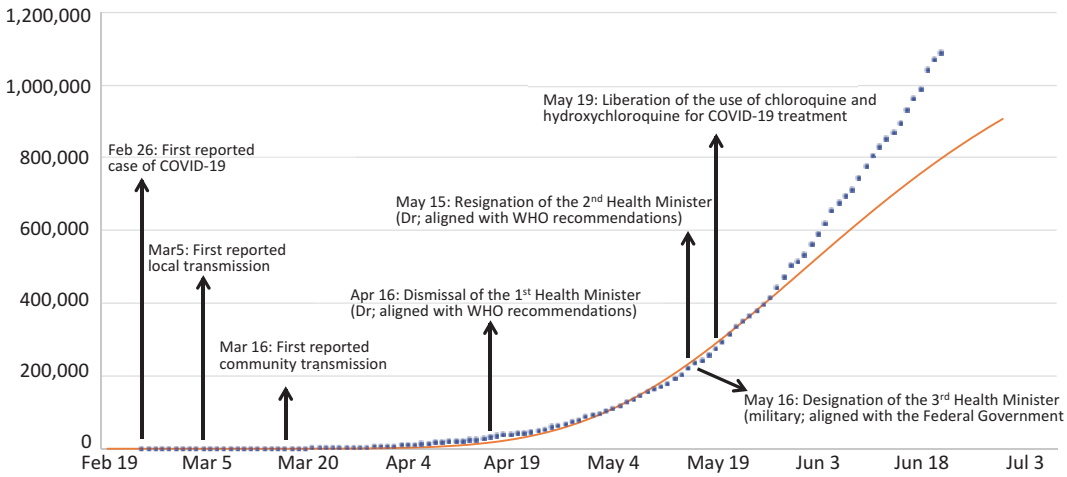

Fig. 3.1 Total number of COVID-19 cases in Brazil and Federal Brazilian government events related to the disease pandemic. Reported number was obtained from the Brazilian Ministry of Health (blue line) and the predicted number was calculated according to the mathematical model proposed by Tang et al. [6] (red line)

making among Brazilian states and municipalities also help to explain the specific response to the COVID-19 epidemic in each Brazilian state/city.

Apart the Federal Brazilian government decisions, distinct COVID-19 epidemic dynamics occurred among the Brazilian states and cities, probably due to particular characteristics and governmental measures to combat the spread of disease. Several interventional measures against COVID-19 dissemination were implemented, including quarantine, lockdown, and facemask use. These measures and implementations varied throughout the country due to intrinsic factors related to each Brazilian state and city with respect to specific, regional characteristics and extrinsic factors associated with safety. This included local and state government decisions concerning the disease management and the awareness of the population.

Using a simple mathematical model as previously proposed by our group [19, 20], we predicted the development of the COVID-19 epidemic in Brazil and in the five Brazilian states with the highest number of COVID-19 cases in each region: Para (North); Bahia (Northeast); Sao Paulo (Southeast); Minas Gerais (Midwest); and Santa Catarina (South). The total number of cases, daily new cases, and growth rate are shown in Figs. 3.2, 3.3, and 3.4, respectively.

Until June, the predicted number of total cases was close to the reported number according to this model in the whole country. However, after July, the reported number started to grow up in comparison with the predicted number, suggesting that interfering factors probably impaired the control of the disease pandemic (Fig. 3.2a). At the end of October, the reported number was about four times higher than the predicted number (Fig. 3.2a). Sao Paulo presented a similar response to the whole country with a reported number around two times higher than the predicted number (Fig. 3.2d). Bahia, Minas Gerais, and Para presented a less pronounced elevation in the reported number in comparison to the predicted number (Fig. 3.2b, c, e), and Santa Catarina had the best response (Fig. 3.2f). The daily new cases and the growth rate followed the results of the total number of cases (Figs. 3.3 and 3.4, respectively).

The interfering and interventional measures, including social distancing/isolating (quarantine and lockdown), the mandatory use of facemasks, and government decisions about the lives of the people and economic activities (reopening, flexibilization, and school returning) usually presented a direct correlation with the epidemic curve dynamics. The reported number of cumulative cases was expected to be higher than the predicted number in the Brazilian states, where these extrinsic factors are not completely efficacious to avoid the SARS-CoV-2 spread. On the other hand, when local authorities successfully implemented measures to combat the disease epidemic, the reported cumulative number of cases was expected to be similar or even lower than the predicted number [20].

The Sao Paulo state is the most populous in Brazil, with more than 46 million people (IBGE, 2019). Its capital is Sao Paulo city, with more than 12 million people. Sao Paulo state has been considered the epicenter of the COVID-19 pandemic in Brazil and Latin America since May 2020 [20]. The Sao Paulo state government established various preventive and protective measures and public health policies [21]. The government decreed a quarantine on March 24, and facemask use has been obligatory since May 7, 2020. On July 1, 2020, most of the Sao Paulo state reopened some trading sections, including shopping, commerce, and services (with restriction to 20% of the maximum capacity and 4 h per day), with more flexibility and reopening on August 7 (40% of the maximum capacity and 8 h per day). Another contributing factor for the increasing number of COVID-19 cases in the Sao Paulo state is the amount of testing for the disease. For example, in July 2020, the state performed around 54% more tests for COVID-19 than in June 2020. As shown in Fig. 3.1, the impact of reopening and flexibility procedures seemed to impair the COVID-19 epidemic curve. Similar effects, but less pronounced, were observed in the Bahia, Minas Gerais, and Para states, sug-

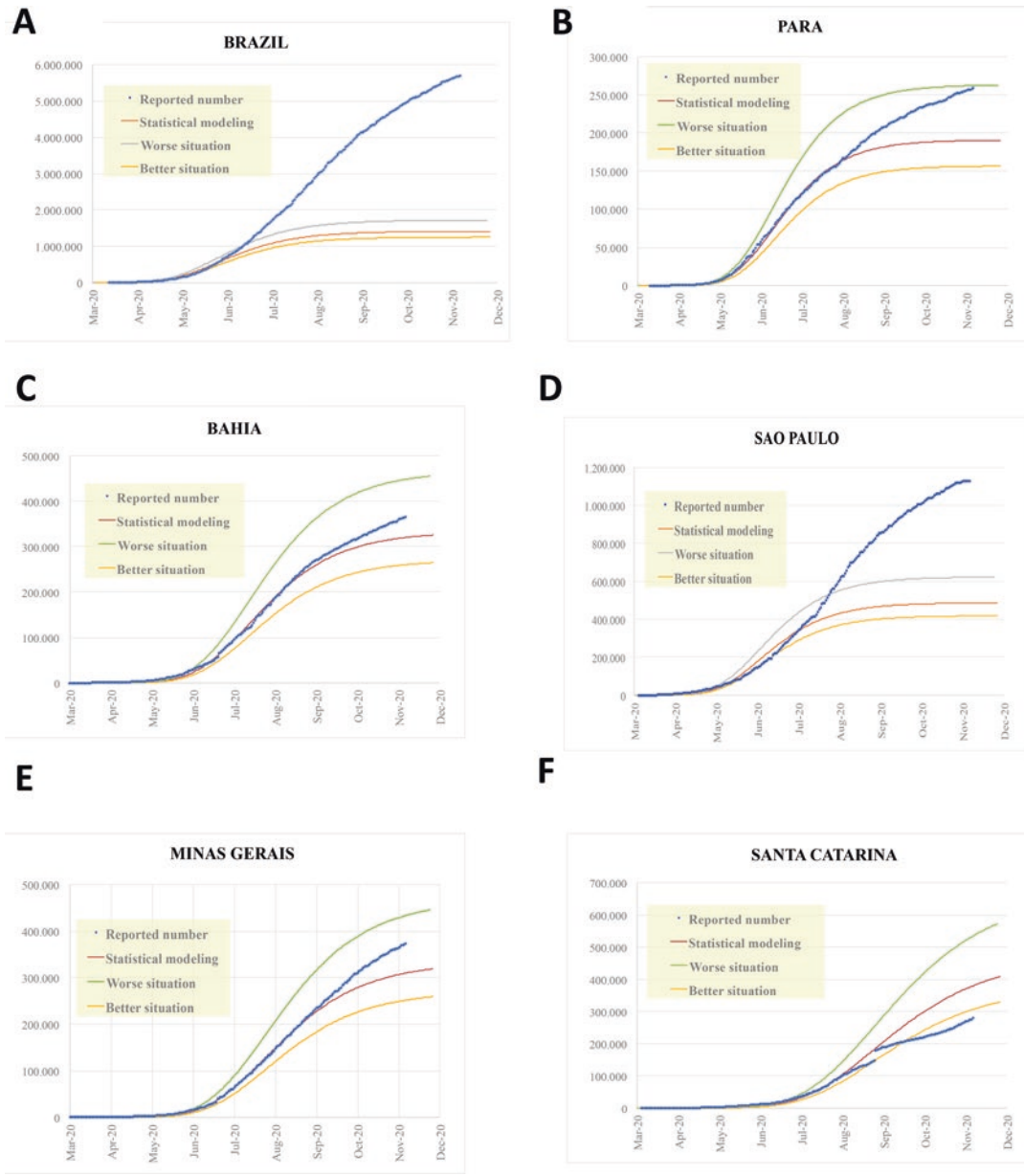

Fig. 3.2 Total number of COVID-19 cases in Brazil (**a**), Para (**b**), Bahia (**c**), Sao Paulo (**d**), Minas Gerais (**e**), and Santa Catarina (**f**)

gesting that the same factors may have contributed to the SARS-CoV-2 spread, including the reopening and increased flexibility. Santa Catarina state, on the other hand, presented the best response, indicating that appropriate measures were implemented there to combat the disease pandemic.

3 Regional Characteristics Influencing the COVID-19 Dissemination

Brazil is a vast country with continental dimensions. The total area consists of more than 8.5 million km^2, and the total population is almost

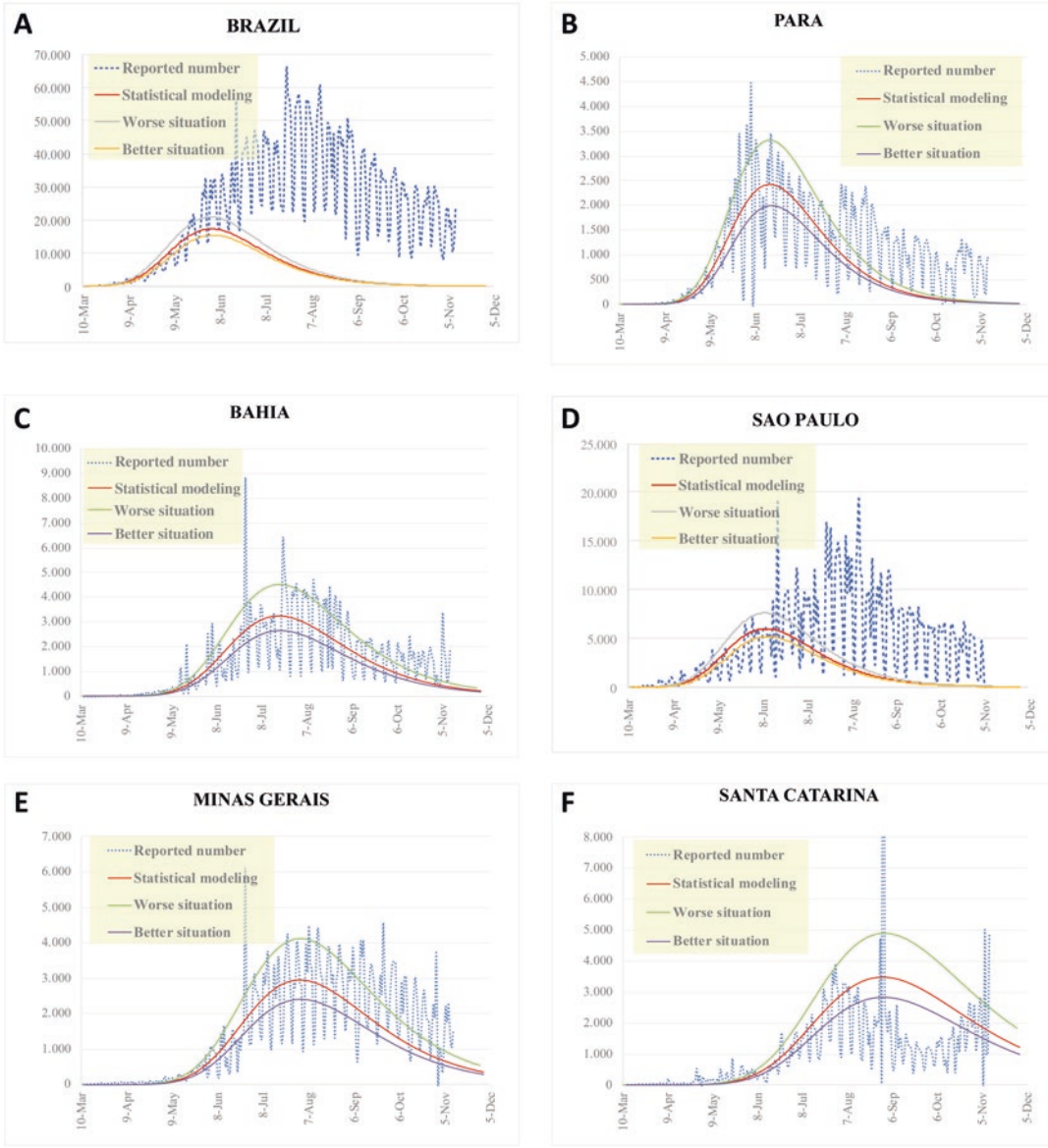

Fig. 3.3 Daily new cases of COVID-19 in Brazil (**a**), Para (**b**), Bahia (**c**), Sao Paulo (**d**), Minas Gerais (**e**), and Santa Catarina (**f**)

212 million people. The country is divided into five geographic regions, with a total of 26 Brazilian states and one Federative unit: North (7 states), Northeast (9 states), Midwest (3 states and one Federative unit), Southeast (4 states), and South (3 states). The high heterogeneity and complexity of the characteristics among the Brazilian regions and states and discrepancies among governmental authorities have directly influenced the dynamics of the COVID-19 pandemic within the country, resulting in specific and local disease epidemic curves. Mathematical modeling of the COVID-19 pandemic is affected by several interfering factors that have to be considered, including governmental decisions concerning social distancing and isolation (e.g., quarantine and lockdown), protective and preventive measures (e.g., mandatory use of face

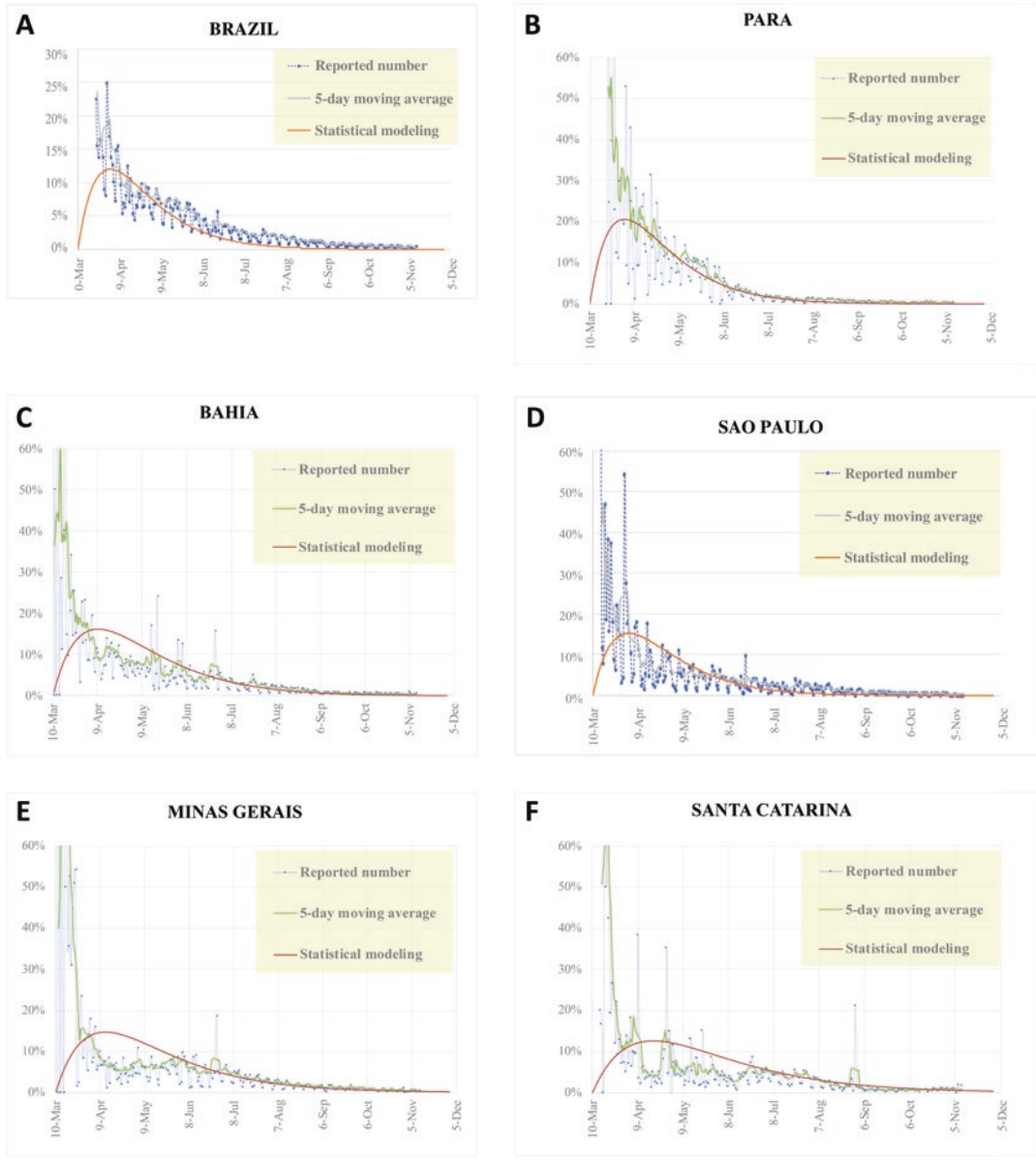

Fig. 3.4 Growth rate (%) of COVID-19 in Brazil (**a**), Para (**b**), Bahia (**c**), Sao Paulo (**d**), Minas Gerais (**e**), and Santa Catarina (**f**)

masks and frequent sanitation of the hands), and restriction of economic activities (e.g., closing/reopening of commercial trades and schools, measures of flexibility) [20, 22].

COVID-19 dissemination dynamics vary mainly due to two factors: a) intrinsic factors linked to the specific and regional/local characteristics, and b) extrinsic factors related to several external decisions to combat the pandemic growth. These latter include preventive measures, governmental decisions, and population awareness in combating the COVID-19 pandemic. The previous section discussed these factors. The intrinsic factors are associated with specific and local characteristics, including geographic localization, total area, population, demographic density, per capita income, and public health system.

Brazil is a developing country with high social differences. Fifty percent of Brazilian families earn about R$ 820 per month (~$200) and only 10% of the population take in 43.1% of the total income in Brazil (IBGE, 2019). The unemployment index was 11.2% in January 2020 (before the pandemic). The disease pandemic exacerbated this situation as millions of people lost their jobs, and the peak of the unemployment index in July 2020 was 13.8% (13.1 million people). Previous findings suggest that the most vulnerable people to the COVID-19 pandemic are those in low social classes, who are potentially more exposed to the virus.

Other important intrinsic factors account for the spatial occupation of each region or locality, presence of isolated communities (e.g., indigenous communities), accessibility to public health systems (e.g., disposal of intensive care units, doctors, and respirators per 100.000 people), and regional susceptibility to the disease (Table 3.1). Some studies suggest that previous immunization for other viruses, including MMR viral triplice vaccine (measles, mumps, and rubella), dengue vaccine, and poliovirus vaccine, could provide some immunity against SARS-CoV-2 [23]. However, additional studies are necessary to evaluate the real protective capacity of previous vaccines against SARS-CoV-2 infection.

4 Prevalence of the Disease in Different Subpopulations

About 80% of patients with COVID-19 infection have a mild form of the disease and are asymptomatic. Overall, among individuals with COVID-19, approximately 14% of the patients require hospital care because they present difficulty breathing, of which approximately 5% may need respiratory assistance in an intensive care unit [24]. This situation is exacerbated in the elderly and people with underlying comorbidities who are more likely to become seriously infected [25].

Patients with chronic diseases, such as diabetes, obesity, hypertension, asthma, chronic obstructive pulmonary disease, smokers, and pregnant women, are considered to be at risk of a worse COVID-19 outcome. Also noteworthy are hematological diseases, including sickle cell anemia and thalassemia, advanced chronic kidney disease (grades 3, 4, and 5), and chromosomal diseases, resulting from impaired immune function, as well as immunosuppression caused by treatment of autoimmune diseases such as lupus and cancer [26–30].

In Brazil, deaths associated with COVID-19 are strongly related to socioeconomic factors, demographics, and comorbidities. Wollenstein-Betech et al. evaluated data of 113,214 Brazilian patients with 50,387 deceased and reported that variables associated with the high prediction of mortality include the geographic location of the hospital, renal and liver chronic disease, immunosuppression, obesity, neurological, cardiovascular, and hematologic diseases, diabetes, chronic pneumopathy, immunosuppression, respiratory symptoms and hospitalization in a public hospital. The authors observed that patients with a low level of education showed a significant association with higher mortality and that mortality was inversely proportional to self-reported education level, suggesting that this may have also significantly impacted the COVID-19 scenario.

De Souza et al. analyzed data from Brazilian COVID-19 patients registered on the Influenza Epidemiological Surveillance Information System (SIVEP-Gripe) database, which includes suspected and confirmed COVID-19 cases as reported by public health and private services [31]. The authors reported that the most prevalent comorbidities were cardiovascular disease [23,085 out of 34,693 cases (66.5%)] and diabetes [17,271 out of 31,672 patients (54.5%)].

Diabetic patients have a dysfunction of the immune system and exhibit low-grade chronic inflammation [32, 33]. In diabetic patients, there is an elevation of pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor alpha [34–36]. This condition may favor cytokine storming in the late stage of the coronavirus infections through unknown mechanisms [37], leading to an increased risk of complications due to virus infection [38]. Patients with COVID-19 have raised blood total leukocytes and neutrophils, reduced lymphocyte number, and an

Table 3.1 Characteristics of the Brazilian states related to the COVID-19 pandemic

State/ Federative Unit	Total population	Population density (inhab./km^2)	Human development Index	Per capita income	Number of ICU per 100,000 inhab.	Number of doctors per 100,000 inhab.	Number of respirators per 100,000 inhab.	Total number of COVID-19 cases	Total of deaths related to COVID-19	Cases per 100,000 inhab.	Deaths per 100,000 inhab.	Mortality rate (%)
Acre	894470	5.4	0.719	890	5	108	16	30951	693	3917	77.5	2.24
Alagoas	3351543	120.4	0.683	731	9	132	15	91142	2254	2744	67.3	2.47
Amapá	861773	6.0	0.74	880	5	95	10	52832	751	7035	87.1	1.42
Amazonas	4207714	2.7	0.733	842	7	111	20	163399	4588	4218	109.0	2.81
Bahia	14930634	26.4	0.714	913	10	135	20	355753	7711	2352	51.6	2.17
Ceará	9187103	61.7	0.735	942	9	126	21	275671	9370	3118	102.0	3.40
Distrito Federal	3055149	530.3	0.85	2686	30	338	63	214655	3719	8352	121.7	1.73
Espírito Santo	4064052	88.2	0.772	1477	20	223	35	158768	3884	4087	95.6	2.45
Goiás	7113540	20.9	0.769	1306	15	169	24	258241	5797	3959	81.5	2.24
Maranhão	7114598	21.6	0.687	636	8	81	14	186572	4091	2723	57.5	2.19
Mato Grosso	3526220	3.9	0.774	1403	17	148	38	144258	3828	4474	108.6	2.65
Mato Grosso do Sul	2809394	7.9	0.766	1514	14	195	31	83439	1622	3185	57.7	1.94
Minas Gerais	21292666	36.3	0.787	1358	15	221	28	362340	9069	1736	42.6	2.50
Pará	8690745	7.0	0.698	807	8	85	16	254384	6767	3151	77.9	2.66
Paraíba	4039277	71.5	0.722	929	12	155	21	133958	3130	3397	77.5	2.34
Paraná	11516840	57.8	0.792	1621	18	209	31	216266	5269	1952	45.8	2.44
Pernambuco	9616621	98.1	0.727	970	16	157	29	163642	8667	1764	90.1	5.30
Piauí	3281480	13.0	0.697	827	7	126	14	114530	2441	3585	74.4	2.13
Rio de Janeiro	17366189	396.9	0.796	1882	25	248	42	313089	20759	1902	119.5	6.63
Rio Grande do Norte	3534165	66.9	0.731	1057	12	152	21	81876	2588	2402	73.2	3.16
Rio Grande do Sul	11422973	40.5	0.787	1843	14	244	28	253272	5903	2244	51.7	2.33
Rondônia	1796460	7.6	0.725	1136	13	142	25	72295	1464	4135	81.5	2.03
Roraima	631181	2.8	0.752	1044	4	136	17	57789	693	11629	109.8	1.20

(continued)

Table 3.1 (continued)

State/ Federative Unit	Total population	Population density (inhab./km^2)	Human development Index	Per capita income	Number of ICU per 100,000 inhab.	Number of doctors per 100,000 inhab.	Number of respirators per 100,000 inhab.	Total number of COVID-19 cases	Total of deaths related to COVID-19	Cases per 100,000 inhab.	Deaths per 100,000 inhab.	Mortality rate (%)
Santa Catarina	7252502	75.8	0.808	1769	12	221	28	266637	3163	3722	43.6	1.19
São Paulo	46289333	186.5	0.826	1946	19	260	39	1123299	39549	2551	85.4	3.52
Sergipe	2318822	105.8	0.702	980	10	162	21	84709	2228	3817	96.1	2.63
Tocantins	1590248	5.7	0.743	1056	8	146	19	75991	1105	5077	69.5	1.45

Data of the characteristics were obtained from IBGE (2019) and data of COVID-19 were obtained from the Brazilian Ministry of Health on November 4th, 2020. *Inhab* inhabitants, *ICU* intensive care units

increased neutrophil/lymphocyte ratio (NLR) [39–41]. These latter alterations positively correlate with the inflammatory state and COVID-19 severity [42–44].

In an analysis of 67,180 confirmed COVID-19 cases reported on the SIVEP-Gripe system, de Souza et al. observed that 65% (44,027 out of 67,180) of COVID-19 infections in individuals above 50 years of age and a lower proportion of 2.2% (1454 out of 67,180) in people less than 20 years old [31]. The authors reported 85% COVID-19 deaths in individuals above 50 years of age. In this same study, 16 newborns, 381 infants (1–12 months old), 518 children (1–12 years old), and 258 adolescents (12–17 years of age) were diagnosed with COVID-19.

Pachiega et al. extracted data from the bulletin about the epidemiological situation of COVID-19 available on the official website from the 26 Brazilian States and the Federal District [44]. They observed a high prevalence of comorbidities (83%) among patients who died from COVID-19 in Brazil, with 35% of these comorbidities being chronic heart diseases. The authors found that the group that presented chronic heart diseases was composed mainly of men aged over 60 years. This fact may indicate that this comorbidity may be predictive of a worse prognosis for COVID-19.

According to the Pan American Health Organization (PAHO), other groups also require the Public Health System's high attention due to their vulnerability. These groups include the indigenous population, prisoners, and employees of long-term institutions for the elderly, such as nursing homes.

The first COVID-19 case in Brazil's indigenous population was from the Amazonas and confirmed on April 1st [45]. This first case had previous contact with an infected doctor who had access to an Indigenous group. This infected woman spread the virus to her ethnic group. The first COVID-19-caused death was a 15-year-old Indigenous Yanomami teenager from Roraima State who did not have comorbidities. In data available by the Health Ministry on November 9th, 2020, 33,011 COVID-19 cases and 479 deaths among Indigenous individuals were reported. Brazil has 34 Indigenous Sanitary Districts (DSEIs), and the highest number of cases and deaths was reported in the Mato Grosso do Sul DSEI.

The overcrowding phenomenon in Brazilian prisons associated with structural aspects of these places, such as inadequate ventilation and poor health services, makes the occupants of these facilities more susceptible to COVID-19. On May 11, 2020, 531 confirmed cases and 22 deaths resulted from COVID-19 in the Brazilian prison system [46]. We evaluated actual data (November 9) that showed 36,132 cases and 121 deaths with the highest numbers in São Paulo State. As described before, the most affected people are those above 60 years of age and with comorbidities such as heart diseases and diabetes. However, the scientific data about this regarding the Brazilian prisoner population are still scarce.

5 Concluding Remarks

Because of the complexity of several interfering factors involved in Brazil's COVID-19 dissemination dynamics, the determining epidemiological features are not entirely understood yet. This work highlights intrinsic and extrinsic interfering factors on the COVID-19 epidemic in different Brazilian states and cities. Intrinsic factors include high heterogeneity and complexity of the regional/local characteristics. These include: total population and population density; the percentage of the elderly population; high-risk population, children, indigenous population, in jail population, and other vulnerable people; availability of public health systems; per capita income; and Human Development Index (HDI). Extrinsic factors also vary enormously among Brazilian states and cities and include governmental decisions to combat the COVID-19 epidemic and people's awareness. The governmental decisions involve social distancing/isolation (e.g., public distancing among people, quarantine, and lockdown), facemask use, frequent hand sanitation, and restriction of economic activities such as closing/reopening of commercial trades and schools, and measures of

flexibility. Mathematical models for predicting the COVID-19 pandemic for the understanding of the disease spread are still under investigation and validation.

Further studies are necessary to address several aspects of the COVID-19 pandemic in Brazil, especially in states and cities with persistent duration and those with risk of secondary waves of the epidemic, as well as to measure the effectiveness of the governmental measures and people awareness of the disease spread dynamics, helping to understand and to take other decisions against the disease dissemination.

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4 The Experiences of Recovered COVID-19 Patients in Baqiyatallah Hospital: A Qualitative Study

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Abstract

The emerging COVID-19 disease affects not only the physical health but also the emotional and psychological health of patients. This study aimed to explain the experiences of 22 recovered COVID-19 patients in Baqiyatallah hospital, Tehran, Iran. Data were collected through in-depth semi-structured interviews. All interviews were recorded and transcribed and then analyzed using the conventional content analysis method. This resulted in emergence of 3 themes "emotional-sensational paradox", "spiritual growth", and "experienced mental-psychological effects", with 11 main categories and 33 subcategories. The results of the study can be used to develop instructions and guidelines for the families of patients as well as healthcare teams to provide effective measures and interventions to minimize the suffering of patients and the damage to mental health.

Keywords

Experience · Patients · COVID-19 · Qualitative research

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1 Introduction

In December 2019, reports emerged of an infectious disease caused by a novel coronavirus in Wuhan, China, a virus that the World Health Organization (WHO) officially named COVID-19. One of the characteristics of

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COVID-19 is its high rate of transmission, which has caused it to quickly become a global health crisis. Contracting a disease like COVID-19 can have major impacts on a person's life, not only because of physical problems, but also due to exposure to emotional and psychological issues such as anxiety, fear, depression, labeling, avoidance behaviors, irritability, sleep disorders, and post-traumatic stress disorder (PTSD) [1].

Patients can be affected by physical, psychological, and financial effects of the disease as well as treatment, which can lead to depression [2]. Slow recovery, costly and tedious medical procedures, and doubts and frustration with illness can affect a person's family and social life [3]. These challenges are even more severe in the case of COVID-19 because of its ambiguous nature. Many have described this viral disease as a crisis, a term that in Eastern literature and religious mysticism refers to the presence of not only a threat but also an opportunity. Experience has shown that in the case of many difficult diseases such as cancer, people see the crisis that they go through as an opportunity to change [4]. Therefore, understanding how people experience a disease and cope with it and their new conditions and the impacts on their psychological concerns and social activities can greatly contribute to the quality of health services, especially rehabilitation support and nursing care [5].

Because of the nature and scale of the COVID-19 pandemic, it has resulted in the admission of large numbers of patients to hospitals. Research has shown that the experience of contracting an infectious disease with the possibility of death or unfortunate outcomes will have a more or less lasting effect on patients that is unpleasant to remember even after many years [1]. Considering the novel and emerging nature of COVID-19, there is still little scientific evidence regarding the experiences of COVID-19 patients. Although several studies have investigated the various physical and mental impacts of the disease, there is still much to discover about the views of the patients. Also, a review of the literature shows that there has been no qualitative study on the experiences of COVID-19 patients in Iran, which means there is indeed a large gap in our knowledge of experiences and concerns of recovered and recovering patients.

Quantitative and questionnaire studies are not suited for documenting and studying individual concerns and experiences. Dealing with crises such as difficult diseases is a personal, multidimensional experience affected by many factors as well as social context. Therefore, research in this area should be done in the form of qualitative and in-depth analysis of the experiences of people who have faced or are facing the challenges of this disease. This study was designed as a qualitative research based on semi-structured interviews with open-ended questions with the goal of capturing the views and opinions of COVID-19 patients and understanding their thoughts, feelings, and emotions, so that the knowledge of their actual experiences can be used in the development of better solutions for people under similar conditions. Indeed, documentation of patients' experiences from their own point of view can give therapists and families a better understanding of strengths and shortcomings of treatment and care services. This qualitative study was focused on the experiences of COVID-19 patients in Baqiyatallah hospital, Iran.

2 Methods

The present study was a qualitative research using conventional content analysis approach. The study was conducted in April 2020 in the recovery unit of Baqiyatallah hospital. Qualitative content analysis is a research method commonly used to study people's experiences and understanding of phenomena through the interpretation of the content of subjective data [6]. This method involves extensive examination of the participants' descriptions of the subject of interest to identify explicit and implicit concepts and then coding, summarizing, and categorizing them into themes and subthemes [7].

In this method, codes are formed based on meaning units found in participants' comments and then classified based on their differences or similarities [8, 9]. Content analysis is a good way

to obtain valid and reliable results from textual data in order to create knowledge, reach new insights, present a set of facts, or provide practical guidance for action. In conventional content analysis, categories can be extracted directly from the text [9–11].

The research population of this study composed of COVID-19 recovered patients at Baqiyatallah hospital. All patients with COVID-19 enrolled in this study were diagnosed according to the World Health Organization interim guidance [12]. Upon admission, patients underwent chest computed tomographic (CT) scan plus swab test. Since the scan results were available in real time compared to the swab test, which takes at least 24 h, diagnosis was made based on the CT results. However, positive results on a reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of the nasopharyngeal swab provided confirmation of COVID-19. Thus, all participants in the study were positive based on the two methods. The interested patients who were able to express their experiences were sampled for maximum variance sampling in terms of occupation, education, economic status, and social and cultural variables. It should be noted that these patients had overcome the disease and were placed in the recovery unit of the hospital for 2 weeks to prevent transmission to others. Data were collected using in-depth semi-structured face-to-face interviews.

2.1 Data Collection

The interviews were conducted at the recovery unit of the hospital. Before each interview, the researcher introduced themselves and attempted to establish a good relationship with the participant and gain their trust. The researcher then explained the objectives of the research, the reason for recording the interview, the voluntary nature of participation, and confidentiality of identity and information of the interviewees. Participants were then asked to provide verbal and written informed consent. The interview sessions included a centralized group interview with 10 people and one-to-one semi-structured interviews with 12 people. In the one-to-one interviews, eight people allowed the researcher to record the interview and the remaining four subjects underwent the interview without recording. The group session was not recorded due to the reservations of some participants. The length of interviews was determined based on the amount of information provided and the conditions of the participant, but typically ranged from 35 to 70 min.

All interviews were conducted by the lead researcher. The interview began with a general open-ended question, followed by more specific questions based on the results of present and past interviews and the main themes detected in comments in line with research objectives. The exploration of participants' views and experiences from the onset of the disease up to the present continued until the researcher concluded that the concepts were sufficiently captured. The main questions of the interview were followed by exploratory and follow-up questions such as "Can you explain more?" Or "What did you mean by that?" to clarify the concept and eliminate ambiguities.

During the interview, the researcher provided feedback when needed, gained the trust of the interviewee, avoided communicating personal opinions to the interviewee, and did not correct the interviewee's statements.

Some examples of the main questions used in the interviews are as follows:

What is your opinion about coronavirus?
What is your experience with this disease?
How did you find out about your disease?
How did you feel when you were diagnosed with the disease?
Explain your feelings at the time of illness and also your present feelings?

The interviews were recorded by a voice recorder. Sampling continued until data saturation. Complete data saturation was achieved after 22 interviews.

Data analysis was performed by an inductive approach for qualitative content determination. For this purpose, the records of the interviews were listened to several times and transcribed verbatim. The analysis units in this part of the study were sec-

tions of transcriptions that were related to the research question. After selecting the analysis units, they were reviewed repeatedly to achieve immersion and gain a general sense of data. The initial coding was then performed based on participants' own words and also what they suggested (researcher's perceptions of their comments). Then, more interviews were conducted to further explore the identified concepts. More meaning units were then extracted from the new analysis units. After removing the excess meaning units, the remaining units were compressed into codes and reviewed. This analysis was carried out continuously and simultaneously with the process of data collection. The codes derived from the initial codes were grouped based on their differences or similarities, labeled based on their contents, and then classified into subcategories. Finally, the resulting subcategories were merged into several main categories and the themes in collected data were identified accordingly.

The interviews and analyses were performed according to the instructions given by Speziale et al. to increase the credibility, dependability, transferability, and conformability of the study [13]. Conformability was ensured by conducting in-depth interviews, combining several data collection methods such as interviews and field notes, submitting the coding process for review by experts to ensure the consistency of categories with participants' statements, remaining constantly engaged with data, audit trail by independent researchers to determine whether they have a similar understanding of data and to find contradictions between the initial codes obtained from the interpretation of participants' experiences. To ensure transferability, sampling was performed using the purposive method with the goal of maximizing diversity in interviewees in terms of age, education, and economic and sociocultural status. Examples of direct statements were included attempts made to provide detailed accounts of the information to allow independent judgment about the transferability of findings. Also, the results were presented to a number of researchers who did not participate in this study for comparison with their own results and experiences. Further, the researcher's ideas and assumptions were outlined in advance to prevent them from affecting data analysis. To ensure rigor, colleagues with expertise in qualitative research were also asked to review the coding process.

2.2 Data Analysis

In qualitative research, data analysis is performed simultaneously with data collection, and therefore there is a mutual effect between data collection and analysis. In this study, the data were analyzed using the conventional content analysis. The researcher listened to the interviews repeatedly until gaining a general sense of the data, transcribed them verbatim, reviewed the transcripts to extract codes, placed the identified concepts and codes in groups and discussed them further with interviewees, continued this process until reaching a consensus on the categorizations, and finally categorized the extracted concepts into themes accordingly [14].

3 Results

In-depth interviews were conducted with a total of 22 COVID-19 patients. The patients had an age range of 19–87 years. In terms of education level, the patients ranged from illiterate to master's degree. Most of the patients were married and had 0–5 children. From the analysis of the interviews, the researchers extracted 3 main themes with 11 categories and 33 subcategories, which are described below (Table 4.1).

3.1 Theme I: Emotional-Sensational-Paradox

One of the main themes identified in the interviews was the paradox in patients' emotional-sensual experiences. The paradoxical experiences that many patients expressed in their statements included being satisfied and also dissatisfied, describing experiences as positive and also negative, being relaxed and also stressed, and feeling support but also rejection.

Table 4.1 Themes and subthemes experiences of recovered COVID-19 patients

Themes	Categories	Subcategories
Emotional-sensational-paradox	Satisfaction–dissatisfaction	Good performance of medical staff Appropriate equipment and facilities Lack of rapid diagnosis Lack of timely information Insufficient training
	Positive experiences–negative experiences	Acceptance of the disease Hope for the future Overcoming the disease Disappointment Wandering
	Relaxation–stress	Recovery statistics Empathy with the patient by relatives Talk to a doctor Infection statistics Death statistics Disease side effects
	Support–rejection	Sympathy Compassion Accompanying Social exclusion Getting away from others
Spiritual growth	Accepting and submitting to divine providence	Belief in divine destiny Belief in divine providence
	Rethinking material possessions	Understand the meaning of life No fear resulting from heartfelt faith
	Seeking stronger divine connection	Prayer Appeal Mention Patience Trust
	Tendency to spirituality	Religious beliefs Disease is from God Cure God willing Observance of health as observance of people's rights (Haqqonnas) Altruism Close to God
Experienced mental–psychological effects	Unpleasant thoughts	Death Despair Hopelessness
	Perceived mental stress	Fear Anxiety and stress Deprivation Obsession Hopelessness
	Positive thoughts	Feeling empowered Positive attitude toward the disease

Satisfaction–Dissatisfaction

In this regard, patients expressed satisfaction with the good performance of the medical personnel and the quality of hospital equipment and facilities, but complained about the media doing a poor job in informing people at the onset of the pandemic, insufficient public education, and the sluggishness of the diagnosis process. Some of the statements of the participants in this regard are provided below. Commenting on the satisfaction with the hospital staff and facilities, one participant said:

We were in a hospital that had good facilities and also good expert staff who were really committed and sincere in doing their job and were treating us good and helping us not worry (Participant No. 2).

Why they didn't say don't come out right from the start at the 30th? (referring to the onset of pandemic in Iran). Because of this, people were still going everywhere at that time. I can claim that it was poor and late announcements that caused me to become sick (Participant No. 4).

If they had announced it in early February, this wave of disease would not have occurred; if they were good in educating people, the disease would not have occurred ... (Participant No. 5).

It took a long time for them to diagnose my coronavirus. First they said it's common cold, then they made other diagnoses ... After changing a few hospitals and a few new doctors ..., they realized it's coronavirus (Participant No. 1).

Positive Experiences–Negative Experiences

While having negative experiences such as frustration and confusion, patients also expressed that the acceptance of the disease and overcoming it were positive experiences. Referring to this issue, one of the participants stated:

After realizing that I had coronavirus, I didn't know what to do. I had a feeling of helplessness because I thought I might not be treated, but then I submitted to God's will and accepted that this is my illness and that I must be treated. After starting the treatment and once I got better, I began feeling a sense of overcoming the disease and that whatever God wants will happen. I felt that God has given me the ability to overcome the disease ... (Participant No. 3).

Relaxation–Stress

Many patients had experienced anxiety and stress because of high rate of infection and relatively high mortality rates, and also complications of the disease. But at the same time, they were also heartened by the counseling of physicians, how people around them had understood their condition, and the recovery statistics. In this regard, one participant stated:

I was feeling anxious, but the doctor's words that I am ok were calming. Television reports giving the death statistics makes people stressful and anxious... People will be more relaxed if they show the recovered statistics, but reporting the death statistics increases the stress ... it was heartening enough for me to see my family understand my condition (Participant No. 9).

Support–Rejection

Patients had received support, sympathy, and compassion from their families, healthcare workers, and people around them, but they had contradictory feelings about how people had kept their distance from them to avoid contracting the disease. Examples of these contradictory feelings can be observed in the following statements:

A friend was telling me that I don't pray, but I told people to pray for you. Hospital personnel come ask how I'm doing. So there is some sympathy. At the same time, people are afraid to come close, even my family, despite their sympathy, they keep their distances. Well it's natural because the virus is infectious (Participant No. 6).

It feels bad that everyone is keeping their distancing from you. Since I knew how people would react, when I found out that my test was positive, my wife called my brother to give me a lift to the hospital. But he said call an ambulance. But we didn't do it, because if the neighbors had seen the ambulance and found out about me having coronavirus, they wouldn't let me back in our apartment. So we went with Snap (car rental service) and I didn't even tell the driver because I was afraid he would drop me off...

This reminds me of the judgment day, as everyone is running away from others (Participant No. 2).

3.2 Theme II: Spiritual Growth

While accepting the disease and its negative consequences as a fact, some patients stated that some things that they experienced during the disease led to their spiritual growth. These factors are listed below.

Accepting and Submitting to Divine Providence

Belief in religion and the consequent view toward the suffering and hardship due to a disease that

has no definitive treatment plays a significant role in people's tolerance of hardships and effects of the disease and can also affect recovery. In religious people, who believe in divine providence and mercy, the experience of the disease may lead to spiritual growth. In this regard, some of the participants shared their experiences as follows:

> *This reminds me of the judgment day, as everyone is running away from others, but in the end our destiny is in God's hand...* (Participant No. 2).

> *God has sent this disease to test humans and to tell them that the cure is in my hand. I believe that this is God's will. He has sent the disease to me and he also has sent the cure. If he didn't want to, it wouldn't happen. I submit to God's will. I am not afraid of the disease because I believe that life is in the hands of God and I believe in him ...* (Participant No. 1).

Rethinking Material Possessions

Another participant stated:

> *After contracting the disease, I just realized what life is, the life is not these material possessions and things, I realized that I'll be judged by my actions and nothing else ... money, wealth, cars, houses, they are nothing ...* (Participant No. 11).

Seeking Stronger Divine Connection

Many participants cited prayer for patience and trust in God as a way to relax. An example of such statements is given below:

> *Showing patience and perseverance, praying, and reading Quran have an impact and make people more peaceful; believers can keep their spirits up by praying and supplication*"

Tendency to Spirituality

The examples of tendency to spirituality include not only the religious belief that the disease is from God and the cure will also be at his will but also altruism. Interestingly, one of the participants likened hygienic measures to respect for Haqqonnas (Islamic public rights):

> *We are responsible for the lives of the people and we have a commitment, whether religious or cultural, to respect hygiene .... it is Haqqonnas.... the religion makes it my responsibility .... If someone gets the disease from me, I have done wrong to him, I am obligated to not transmit the disease, we just have to take care of each other* (Participant No. 10).

3.3 Theme III: Experienced Mental–Psychological Effects

The categories of this theme include unpleasant thoughts, perceived psychological stress, and positive thoughts. The participating patients made the statements below about this theme.

Unpleasant Thoughts

(a) Death – Uncertainty about the lethality of the disease had caused a sense and experience of fear in people. One participant stated:

> *The disease has no clear symptoms and no clear cure. They just say it's fatal, so you start feeling that you're dying...* (Participant No. 5).

(b) Despair – Many patients stated that at one point they had no hope of treatment and recovery:

> *Since there's no definitive cure, I had no hope in the outcome of the treatment and did not expect to recover ...* (Participant No. 3).

(c) Hopelessness – Many patients were feeling unable to deal with the illness. In this regard, one participant said:

> *I was very weary. I felt like I was fainting. I couldn't fight with the disease neither mentally nor physically....* (Participant No. 2).

Perceived Mental Stress

(a) Fear – Fear of death, fear of transmitting the disease to other people, especially family members, and fear of exacerbation of one's conditions were the factors observed in this subcategory. The remarks of one participant, which were also expressed in other ways by several other participants, were as follows:

> *My biggest concern was that my children not get infected. Also since the disease is new and has no clear cure, some were saying it kills instantly. Also,*

I was afraid that it would become a large outbreak... (Participant No. 2).

(b) Stress and anxiety – The anxiety due to the concern of getting others infected, the ambiguity in the nature of the disease, the uncertainty of treatment, and finally the concern of getting judged by others were the subcategories that constituted this category.

It's a new disease, it's not clear what it is, it doesn't have a definite treatment, and that if you get sick, people start looking at you differently and run away from you. This gives me stress ... (Participant No. 4).

(c) Deprivation – Quarantined patients have reduced social communication, are distanced from their family and community. These experiences were common among the statements.

I was thinking when can I start working again? When can I return with my family? When can I go out with my friends? Will I be able to go back to society? Won't people run away from me (Participant No. 7).

(d) Hopelessness – Hopelessness was the prevailing experience of people with the disease. This hopelessness was mostly about the effectiveness of treatments and recovery.

...honestly I didn't expect to be cured... I thought I was going to die ... (Participant No. 8).

(e) Obsession – This subcategory refers to the tendency of some patients to completely avoid social interactions and show obsession about health and hygiene issues. In this regard, one participant stated:

Since I was discharged from the ward, I've been doing a lot of things like washing my hands, putting on a mask, keeping distance from people ... so much so that I might wash my hands ten times in an hour....

Positive Thoughts

(a) Feeling empowered – Recovered patients described their ability to overcome the disease and regain their physical and mental strength as an empowering experience:

I feel good. I think, God willing, my body strong and spirit was so strong that I managed to defeat the disease, and that's very good for me ... (Participant No. 9).

(b) Positive attitude toward the disease – In addition to their negative experiences, some participants spoke about their positive views regarding the disease, and specifically hope for the future, seeing the disease as a divine test, and positive thinking:

After recovering and putting those difficult days behind, I started hoping for the future, and I think the disease was a divine test. The disease made me another person and I consider this to be the positive side of the disease... (Participant No. 8).

4 Discussion

This study aimed to examine the statements of patients recovered from COVID-19 in Baqiyatallah hospital in Tehran to determine how they expressed their experience of contracting this viral infection. Based on the findings, the experiences of these patients were classified into 3 themes with 11 main categories and 33 subcategories. The themes identified in this study were emotional–sensual paradox, spiritual growth, and experienced mental–psychological effects.

Examining the experience of the patients showed that they had conflicting feelings about their condition. For example, while they were satisfied with the services provided, they complained about some factors that led to them contracting the virus. Also, while they felt the support, compassion, and sympathy of family and community members, they also sensed that people distanced themselves from them. In this regard, the findings also showed the mutual presence of positive and negative thoughts in the patients and the feeling of relaxation as well as stress. These results are consistent with the findings of a study by Ashing-Giwa et al., which reported conflicting emotions in cancer patients and attributed these to their different reactions and behavioral responses to social conditions and personal mental conditions [15].

A study by Khansari et al. reported the common emergence of such feelings in patients who received bad news such as a diagnosis of cancer [16]. In the present study, one factor that may have helped the patients to accept their disease and cope with it was spiritual growth. Similarly, two other studies reported that many patients considered their illness to be the will of god and that they continued living by trusting in god. Thus, they viewed the disease as a reason to strengthen their spirituality and their connection to God, a phenomenon referred to as spiritual relief [17, 18].

Most of the participants in the present study believed that religious beliefs, seeking divine help, and trust in divine power were important factors in them overcoming the disease and regaining their health. Similar studies on other serious diseases in different cultures also confirmed the impact of religious factors [15, 19–22]. In these patients, appealing to spirituality and trust in God served as an important source of support and hope for overcoming the illness, similar to the findings of Ashing-Giwa et al. [15]. A study by Stanton also reported the impact of returning to religious beliefs for overcoming illness in women with breast cancer [23].

Trust in a divine power helps the patient to remain peaceful and reduces the fears of the disease. A similar conclusion was drawn in a study by Sajadian and Montazeri [22], with the difference that patients in that study were even more inclined to pray and seek divine connection. Similar to the study of Sajadian, our study found that receiving support, especially from the family, played an important role in the recovery and relaxation of patients.

An important source of anxiety for patients was their poor knowledge about the disease, which can be attributed to the absence of good education about the issue, as well as the novelty of such a crisis. Considering the statements made by some patients, it is necessary to create an education program for the patients being discharged as well. In this regard, a study by McPhail et al. reported that informing patients about follow-ups, necessary care in the post-treatment period, and recurrence reduced the anxiety of patients [24].

The psychological effects of the disease on the interviewed patients included the sense of anxiety, stress, fear, despair, and deprivation. In a study by Imanzadeh, it was stated that several factors can help reduce disease-induced anxiety and there are different ways for patients to control this anxiety themselves. Being hopeful, seeking meaning in life, quality of life, optimism, receiving support, access to facilities, counseling services, and happiness are some of these factors and strategies. The results of the present study about understanding the meaning of life and rethinking material possessions are also similar to the findings of the findings of another study of cancer patients, in which it was reported that religious people find life more meaningful and consequently have higher hopes for living and lower anxiety and fear of death [25].

Another source of anxiety for patients in the present study was the fear of being infected or infecting others, especially family members. Other unpleasant thoughts and feelings that the interviewed patients experienced included the sense of loneliness, deprivation, hopelessness, stigma, stress, anxiety, and fear. Consistent with these findings, other studies have reported that such unpleasant thoughts may reduce the patient's commitment to treatment and even make people think of avoiding quarantine [26, 27]. This may even cause the patients to lose the psychological support of their family and friends, which could exacerbate their stress and lead to even greater psychological damage.

The limitations of this study included gender bias (all subjects were male) and also non-generalizability, because of the place of study and the nature of qualitative research. In addition, the seriousness of the illnesses was not accounted for in the findings. This aspect could have been achieved through the use of biomarkers such as measurements of the levels of inflammatory serum proteins and imaging approaches included computed tomography chest scans [27]. The strengths of the study included the leading researcher conducting all interviews and also the short interval between the treatment and the interview (interviews were conducted within 2 weeks of discharge from wards), when participants could more likely to express their experiences more accurately.

5 Conclusions

The results of this study showed that conflicting emotions, spiritual growth, and experienced mental–psychological states were among the important factors that affected the mental health of COVID-19 patients. In addition, it had been explained to the patients about the results of their laboratory and clinical tests, such as CT scans and PCR results, and they were also kept informed about the course of the disease and treatment. This may have led to them experiencing a milder form of the disease, resulting in their having greater peace of mind and stronger hopes for the future, compared to individuals who experience more severe disease forms. Therefore, there should be a policy to educate COVID-19 patients upon admission in order to teach them how to overcome the problems that may emerge during their illness. This could help them to cope with the stressful situations and avoid negative experiences. Taking necessary measures to give patients or susceptible people access to free care, treatment, social and psychological counseling can be helpful in this regard. The results of this study can be used to develop instructions and guidelines for the families of patients as well as healthcare teams to help them understand the patients' typical condition and experiences and provide effective measures and interventions to minimize the suffering of patients and the damage to their mental health. The main goal is to restore balance and stability to the lives of individuals affected by this global pandemic.

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Part III

Risk Factors and Outcomes

5

Age-Specific Differences in the Severity of COVID-19 Between Children and Adults: Reality and Reasons

Amir Tajbakhsh, Khojaste Rahimi Jaberi, Seyed Mohammad Gheibi Hayat, Mehrdad Sharifi, Thomas P. Johnston, Paul C. Guest, Mohammad Jafari, and Amirhossein Sahebkar

Abstract

In severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, children experience mild symptoms compared to adults. However, the precise explanations for this disparity are not clear. Thus, we attempted to identify rational explanations about age-related differences as reported in different studies. Given the incomplete data on SARS-CoV-2, some information has been gathered from other studies of earlier coronavirus or influenza outbreaks. Age-related differences in disease severity are important with regard to diagnosis, prognosis, and treatment of SARS-CoV-2 infections. In addition, these differences impact social distancing needs, since pediatric patients with mild or asymptomatic are likely to play a significant role in disease transmission.

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1 Introduction

The family of coronaviruses has different genera and each of them causes a variety of illnesses. In the last two decades, coronaviruses have included three major types. These are severe acute respiratory syndrome-coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and the novel coronavirus 2019 (2019-nCoV or SARS-CoV-2) which causes COVID-19 disease [1]. SARS-CoV began in Guangdong, China, and spread to other countries such as Europe, Southeast Asia, South Africa, and North America [1]. Over 8000 persons were infected with SARS-CoV and it led to the deaths of 775 people with a 9.5% mortality rate. MERS-CoV emerged 9 years later with fewer cases (2509 as of January 2020) but a fatality rate of almost 35% [1]. MERS-CoV was successfully controlled, since the contagion rate of this virus was low with a basic reproduction number (R_0) of approximately 1. This meant that each patient infected about one other person. In contrast, the R_0 for SARS was around 4.

On December 30, 2019, a group of patients who suffered from pneumonia with an unusual etiology were detected in Wuhan, China. On January 7, 2020, a new type of coronavirus (SARS-CoV-2) was identified as the source [2]. The World Health Organization (WHO) named the disease associated with this virus as COVID-19. Many people were quickly infected and the R_0 for this new virus was somewhere between 2 and 3.5 [2, 3]. Generally, COVID-19 is an acute disorder and severe cases may result in death due to major alveolar injury, the development of respiratory damage, circulatory failure, and organ failure [4, 5]. The mortality rate of COVID-19 is lower than SARS and MERS with current rate of approximately 4.2% (as of July 20, 2020) [6]. However, in the case of SARS-CoV-2, there have already been more than 14 million cases (almost 10,000 times greater than SARS and MERS combined) in more than 200 countries worldwide and the disease has not run its full course.

Based on the previous studies, one of the important questions that have arisen concerning this disease is the difference in the severity of symptoms, its progression, and the death rate between adults and children. Another important issue was whether or not this difference could be leveraged in the development of a treatment strategy [7–9]. In this review, we attempted to address the issue.

2 Age-Related Effects of Different Infections

It is known that the severity of many infectious diseases is considerably lower in children compared with adults [9]. For example, paralytic polio appeared in about 1 in 1000 infections of infants, and in approximately 1 in 100 infections among adolescents [10]. Similarly, the tendency of adults and teenagers toward exhibiting symptomatic rubella with systemic manifestations is considerably higher than in young children. In general, the case-fatality rate of the earlier SARS-CoV was in the range of 7–17%. Moreover, patients above 65 years old and those with underlying medical conditions showed mortality rates as high as 50%, although the mortality rate in children was approximately 0%. In addition, the prevalence of human coronavirus-NL63 (HCoV-NL63) infection was higher in adults compared to children [11]. An age-related difference of infection is also seen for SARS-CoV-2, which may be related to epidemiological and biological factors [12, 13]. It has been reported that the mortality rate increases as the patient age increases, with highest mortality rates among the elderly [14, 15]. Table 5.1 lists factors which have been implicated in the lower rate of SARS-CoV-2 infections in children.

Table 5.1 Age-specific differences in COVID-10 among children and adults

Categories	Reasons	Children vs. adults
Epidemiology	Epidemiology (infection rate)	▽
Clinical features	Symptoms	▽
	Severity of COVID-19 (worse outcomes)	▽
	Prolonged hospital stays	▽
	Life-threatening complications	▽
Underlying condition	Underlying disease	▽
	Chronic conditions	▽
Immunity	Innate immunity	▲
	Innate immune receptors	▲
	Adaptive immunity	▽
	Effector T cells/ regulatory T cell ratio	▽
	Chronic, low-grade inflammation (inflamm-aging)	▽
	Vigorous immune response	▽
	Immune dysfunction	▽
	Lymphocytes	▲
	Vaccination (high-titer antibodies)	▲
	Bacterial adhesion ligands in the lungs	▽
Hormone and/or receptors	Sex hormone receptor	▽
Saturation with O_2	Fetal hemoglobin (HbF)	▲
	Number and content of hemoglobin	▽
	Higher glycated hemoglobin	▽
Environment	Outdoor activities	▽
	Cigarette/smoking	▽
	Air pollution	▽
Nutrition	Alcohol consumption	▽
	Healthy nutrition	▲
Diagnosis	Number of tests	▽
	Early recognition	▽
Transmission	Infected by family transmission	▲

3 Age Effects of COVID-19 Disease

Epidemiological risk factors of infected persons reported in Wuhan, China, identified traveling and close contact with other persons as significant risk factors [16]. In the early stage of the pandemic, a report from the Chinese Centre of Disease Control and Prevention indicated that there were 416 pediatric cases with an age between 0 and 9 years (0.9%) with no deaths and 549 cases in patients with an age between 10 and 19 years (1.2%) with only one death (0.2%) among 44,672 confirmed cases [14]. A number of studies have now revealed that the number of COVID-19-related deaths is higher in adults compared to children (Fig. 5.1) [8, 17–19]. Importantly, children display different symptoms and some exhibit no obvious clinical symptoms [20, 21]. In this respect, a report was released describing 34 children admitted to a hospital in China. It was reported that infection had been linked to a family member in the case of 28 children, and 26 of those children had either traveled to or resided in Hubei province in China [22]. However, data

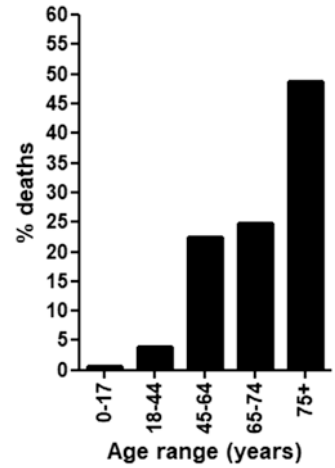

Fig. 5.1 Percentage of COVID-19-related deaths by age

on the susceptibility to SARS-CoV-2 infection based on the ages of children have also been contradictory. For example, a report from Venice, Italy, showed few positive cases for children under the age of 14 [23], while other studies reported that the probability of getting infected with SARS-CoV-2 was similar in children and adults [24–26]. A recent study carried out in Mexico of 236,439 individuals found that laboratory-confirmed diagnosis of COVID-19, hospitalization, and poor outcome were disproportionately associated with male gender, older age, and having one or more comorbidities [27].

4 Clinical Features in Children

The common symptoms of SARS-CoV-2 infection include fever, cough, sore throat, stuffy nose, sneezing, and rhinorrhea [17]. Among nine children with COVID-19 in China, 5 had no symptoms, 4 had a fever, 2 had cough, and 1 had rhinorrhea [28]. In a study of children infected with SARS-CoV-2 in the USA, 73% of children exhibited characteristic COVID-19 signs and symptoms including shortness of breath, cough, or fever [9]. In a study of infected children in China, all of the patients were either asymptomatic, or had mild disease. In this respect, fever (50%) and cough (38%) were the most prevalent symptoms and no deaths were reported. However, a single case of multi-organ dysfunction syndrome (MODS) and severe pneumonia was reported in a child in China [22]. Another study of discharged patients in China found that age was negatively associated with physical function during a 1-month follow-up examination [29].

More recent studies have described an inflammatory condition due to SARS-CoV-2 infection in children, which has been named pediatric inflammatory multisystem syndrome, with similarities to Kawasaki disease or toxic shock syndrome [30]. A systematic review of 114 pediatric cases with COVID-19 infections showed that the symptoms were mainly mild and included fever (64%), cough (35%), and rhinorrhea (16%), or no symptoms (15%) [31]. However, the study also found the presence of ground-like opacities in 54% of the cases with laboratory findings of lymphopenia, elevated D-dimer, and C-reactive protein in 33%, 52%, and 40% of the cases. Notably, 15% of the patients were diagnosed with pediatric inflammatory multisystem syndrome, with symptoms overlapping with Kawasaki disease and more than half required intensive care. No deaths were reported.

5 The Different Etiology and Severity of COVID-19 Based on Age

There are various degrees of sensitivity to COVID-19 for all groups, such as elderly, people with underlying health issues (hypertension, diabetes, etc.) [32]. According to the WHO report for children with COVID-19 (2.4% of all reported cases), the rate of severe cases is 2.5%, with 0.2% of patients progressing to clinical illness. This information suggests that different factors play an important role in COVID-19 illness [33]. Another study revealed that the age range among children infected with COVID-19 was 1 month to 17 years in 28 confirmed pediatric patients [20]. Moreover, in 507 patients, it was shown that most patients were elderly and few were children [34].

In a systematic review by Ludvigsson et al. it was reported that COVID-19 appears to have a milder disease course and better prognosis in children than in adults [19]. Moreover, in the case of children, basic medical treatment was sufficient for all of the children to recover and no intensive care was required [35]. Similarly, neonatal cases that have been reported had a mild form of the disease [36–38]. Furthermore, data from the USA indicated that most hospitalizations occurred with pediatric patients less than 1 year old and in patients with severe conditions [9]. In a patient population of 95 children under 1 year of age with known hospitalization, 59

(62%) had been hospitalized and 5 had been admitted to an ICU [9]. It was reported that among patients in the age range of 1–17 years, a lower percentage of patients were hospitalized (4.1–14%), with little variation among different age groups [9].

Family transmission has been reported to be the main cause of COVID-19 in children, although symptoms were generally milder and the prognosis better compared to adults [39]. Although several studies found no cases of infants acquiring the infection when born to mothers with the disease, two reports identified SARS-CoV-2 in 4 out of 34 tested newborns [40, 41]. In a study by Zhou et al. 9 children in China showed an epidemiological history, and family clustering was observed for all 9 infected children [26]. According to another study, there can be differences in symptoms even among family members infected with SARS-CoV-2 [5]. For example, confirmation of infection by SARS-CoV-2 using polymerase chain reaction (PCR) and samples of stool from children showed that confirmation of infection took a longer period of time, relative to the time of initial infection, when compared to adult family members [39]. However, it should be noted that early recognition of infection is challenging in children due to the mild or asymptomatic clinical course and 5 children in the above study were readmitted into the hospital due to later positive results for SARS-CoV-2 infection.

6 Underlying Conditions and Severity of COVID-19 in Children

Since the severity of COVID-19 is mostly related to underlying chronic conditions common in the elderly population, infection is more prevalent in older patients [34, 42, 43]. However, the most frequent underlying conditions in children were chronic lung disease (including lung disease mediated by immunosuppression; 10%), cardiovascular disease (25%), and asthma (40%). In a study of 295 pediatric cases, it was reported that 77% of hospitalized patients suffered from one or more underlying medical conditions, and that 6 of these pediatric patients were admitted to an ICU [9]. On the other hand, among patients with no hospitalization, 12% suffered from underlying conditions. Moreover, the number of deaths among the pediatric cases described above was reported to be 3 [9].

7 Outdoor Activities and the Potential to Contract the Virus

Compared to adults, children undergo less international travel and, in general, do not engage in more outdoor activities (e.g., employment outside of the home). Therefore, the probability of contracting the virus is lower in children. This issue might be one possible reason for the difference in the infection rate between adults and children [44]. Another reason for lower infection rates in children may be associated with their lung epithelial cells and their healthier and more "alert" immune systems as it relates to the respiratory system. Children typically have healthier respiratory tracts, because they are exposed to less pollution than adults, as well as the fact that smoking and alcohol consumption is more common in adults [33]. A greater probability of developing pneumonia, acute respiratory distress syndrome (ARDS), and respiratory syncytial virus infection has been reported in adults with alcohol use disorder (AUD) [45]. The reason for elevated susceptibility to these and other pulmonary infections is that people with AUD have impaired immune responses. In these conditions, alveolar macrophages, lymphocytes, neutrophils, and the cells responsible for innate immune responses are considered "critically important" immune cells [45].

8 Immune System and Age-Related Differences

It has also been reported that the innate and adaptive immune response change as a function of age, and that these changes lead to some significant clinical consequences, such as increased risk of infection, malignancy, autoimmune disease, and a reduced response to vaccination [33]. There are marked changes that occur in the immune system due to aging, which include epithelial cells, neutrophils, monocytes, macrophages, mast cells, and eosinophils associated with the innate immune response. Furthermore, there are age-specific changes in immune function for components of the adaptive immune response, including dendritic cells (DCs), T cells, regulatory T cells, and B cells [33]. Holcar et al. observed that the ratio of effector T cells/regulatory T cells is higher in adults compared to children [46].

It has also been reported that the symptoms of SARS-CoV-2 infection are milder in children than in adults due to differences in the innate immune response, which is primarily mediated by monocytes and macrophages [33]. Macrophages and monocytes express Toll-like receptors (TLRs) and recognize pathogen-associated molecular patterns (PAMPs). However, with aging, their cell surface expression is reduced, and consequently, TLR-mediated signaling is altered, which leads to an increase in the risk of respiratory tract infections [33, 47]. T cells are critical for clearing coronavirus in-vivo [48]. Both antibody- and T cell-responses are needed to overcome COVID-19 infection. It has been suggested that a "young" immune system, with its efficient T cells, is more effective in responding to SARS-CoV-2 [48].

9 Innate Immune Cells and Age-Related Differences

In older adults, the innate immune system exhibits a reduced capacity to respond to specific pathogenic threats. This reduction results in a greater incidence of specific infectious disorders in elderly persons. Along these lines, the prevalence and mortality of lung infections is known to rise with age. Moreover, specific pathogens often contribute to worse outcomes, as well as prolonged hospital stays, for adults [49]. The innate immune cells recognize PAMPs via pathogen recognition receptors (PRRs), which include TLRs. Activation of the downstream signaling cascade is triggered by PPRs, which assists in the generation of type I and III interferons (IFNs), as well as other pro-inflammatory mediators. The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, which make an important contribution to the regulation of the immune response, are activated by type I IFNs. Moreover, immunity can be directly activated via IFNs through the stimulation of DCs. Additionally, the activity of natural killer (NK) and cytotoxic T cells is increased by IFNs [50]. In this scenario, IFN-γ is produced in response to viral infection, boosting the adaptive immune response, and killing virus-infected cells via NK cells migrating to infected sites [50]. Importantly, IFNs and cytokines make a significant contribution in lung injury during an acute viral infection. For example, high levels of type I IFNs and innate pro-inflammatory cytokines have been documented in serious cases of SARS-CoV-2. It has also been reported that lymphocytes are reduced and neutrophils are increased in SARS-CoV-2 patients at the onset of the cytokine storm [2, 51]. However, no reductions in leukocyte or lymphocyte counts were reported in children in a study carried out in China [28]. Out of the 9 children infected with SARS-CoV-2, 6 actually had an increase in their lymphocyte count and showed increased leukocyte counts.

"Trained immunity" is often suggested as a reason for the less severe form of COVID-19 in infants [52, 53]. Trained immunity is a way to enhance the innate immune defense [54]. This term refers to the ability of developing an exacerbated immunological response for the purpose of protecting against a second infection, which is not related to adaptive immunity [55]. This immunological "memory" can endure from 1 week to several months, and is only described

for innate immune cells, including macrophages, monocytes, and NK cells [55]. An innate immune memory is representative of trained immunity in which innate immune cells are converted to memory cells after antigen exposure. This occurs via a mechanism distinct from "classical" immunological memory, which is carried out via T and B lymphocytes [52, 56]. An investigation by Mitroulis et al. showed that functional, metabolomic, and transcriptomic alterations in hematopoietic progenitor cells were determined by systemic antigens, which assist in the development of myeloid cells with a quicker responsiveness to infections [57].

In hematopoietic progenitor cells and the innate immunity system, trained immune memory is achieved via epigenetic modifications to cells, which results in cross-protection against various pathogens. Importantly, vaccines have the capacity to activate trained immune memory [52]. After pathogen exposure, activation is increased by antigen-presenting cells (APCs) which offer cross-protection for other pathogens. Cross-reactivity can be induced by vaccines, which leads to training of the innate immune system. Along these lines, the mortality rate has been reported to be reduced in measles-vaccinated children [58].

The epidemiological manifestations of the additional serious clinical presentation between SARS-CoV-2-infected children and older infected children might potentially be due to trained immunity [26]. The maturation of the immune response takes place with age and occurs from frequent stimuli, which leads to improved innate immune function and protection for older children [53]. In this respect, several modifications occur in group 2 innate lymphoid (ILC2), bone marrow, and NK cells. Lung ILC2 has been shown to have the capacity to "remember" or recognize their activation status following stimulation by inhaled allergens [59, 60]. ILCs are enriched at barrier surfaces of the mammalian body. These barrier surfaces are places for the quick response to environmental, host, or microbial stimuli to improve tissue homeostasis or immunity [60]. Moreover, ILCs have a complicated contribution in directly affecting the adaptive immune response in terms of infection, inflammation, development, or homeostasis [60].

10 IFN-γ and SARS-CoV in Children

A growing body of evidence suggests that coronavirus targets the most important proteins in the IFN signaling pathway to evade the immune system. Considering the immunological differences between adults and children, IFN-γ induction via NK cells is higher in adults but lower in children [61, 62]. It has been suggested that children respond faster to the virus in the incubation period [63]. Therefore, their immune system inhibits viral replication and prevents high titers. In contrast, the immunological response in adults is delayed as the virus impairs the innate immune response. In a study on the dynamics of the innate immune response of human cells to SARS-CoV infection, it was shown that the activation of the IFN regulatory factor (IRF)-3/7 pathway did not occur until 48 h post-infection. The delayed IFN-related antiviral response is a possible strategy implemented by coronaviruses to evade the immune response [64]. Moreover, the virus circumvents the immune system by hiding its double-stranded RNA in vesicles, causing less IFN induction [65]. A delayed IFN-related antiviral response can impair the immunity of the host, thereby impairing the host defense against rapid viral replication during the early stages of infection. Furthermore, patients with COVID-19 infection suffer from many abnormalities in the late stages of the disease, which are the result of immune system imbalance and malfunction and lack of effective IFN-specific immune responses. These events can lead to pro-inflammatory reactions and immunopathological responses, which present clinically as lethal inflammation in the lungs and multi-organ failure [66].

11 Adaptive Immune Response and Age-Related Difference

In addition to the innate immune response, the adaptive immune response is mobilized in SARS-CoV-2 infection. Specifically, B cells, $CD4^+$, and $CD8^+$ T cells are activated by pro-inflammatory mediators leading to a potent virus-specific antibody response [67]. It has been reported that adults infected with SARS-CoV-2 can have lower numbers of peripheral blood lymphocytes (lymphocytopenia) [48, 51, 68, 69] although this appears to be normal in children with SARS-CoV-2, indicating a lower degree of immune dysfunction [49, 52, 70]. This lower immune dysfunction in children is due to the higher numbers of lymphocytes, and especially NK cells, in children compared to healthy adults [71]. Furthermore, lymphocyte counts are most likely higher in children due to frequent immune system activation, which arises from repeated exposure to viral infections during childhood [53].

12 Cross-Protection Against SARS-CoV-2 and Age-Related Differences

Activation of the innate immune system can be induced by frequent viral infections in children, and has the potential for better defense against various pathogens [58]. Respiratory viral infections are normally more prevalent in children under the age of 5 years in comparison with adults [8]. Moreover, the pathogenic mechanism of the SARS-CoV-2 infection may be associated with immune system maturity. Thus, a strong response to several viral infections might be prevented by the immature immune system in children. For example, primary Epstein–Barr virus infection in young children typically causes a mild form of the disease, although it can cause infectious mononucleosis in older children, adolescents, and young adults [72, 73]. In addition, the susceptibility of children to infection with a respiratory virus is higher compared to adults [74–76]. Moreover, children are usually inoculated with various antiviral vaccines according to immunization schedules and guidelines [21]. In fact, a summary of vaccinations for routine immunization has been suggested by the WHO for children worldwide [77]. Importantly, there are several high-titer antibodies in the blood of children that might provide "cross-protection" against coronavirus infection [21]. It should be noted that previous immunity from infection with a related coronavirus might be the cause of potential protection for children from SARS-COV [78, 79], as well as from SARS-CoV-2 [34].

13 Improved Efferocytosis by Bacillus Calmette-Guerin (BCG) Vaccine to Protect Against COVID-19 Pneumonia

Efferocytosis is a process that leads to clearance of apoptotic cells and maintenance of homeostasis [80–83]. It has been reported that increased efferocytosis by alveolar phagocytes resulting from BCG vaccination leads to effective efferocytosis, lung homeostasis, decreased inflammation, and protection of the host against lethal influenza A virus (IAV) pneumonia [84]. BCG improves the expression of T-cell immunoglobulin mucin protein 4 (TIM4) on alveolar phagocytes and decreases the expression of Rab5 and Rab7, which results in enhanced efferocytosis [84]. Gursel et al. speculated that the smaller than predicted number of cases observed in countries in Asia and Africa with substantial travel and business transactions with China might be a result of BCG immunization [85]. Since BCG vaccination has been shown to reduce acute respiratory tract infections even in the elderly, it has been suggested that susceptible populations of SARS-CoV-2-infected patients should be immunized with BCG vaccination until a specific vaccine is developed [85]. Such a plan might be advantageous for frontline health workers [86]. In this regard, there are two documented clinical trials evaluating the effects

of BCG vaccination on health-care workers involved in the treatment of COVID-19 patients (NCT04328441; NCT04327206) [87].

14 Age-Related Difference and Higher Hemoglobin in COVID-19 Pneumonia

Fetal hemoglobin binds with greater affinity to oxygen than adult hemoglobin, which facilitates the transition of oxygen from the mother to the prenatal fetus [88–90] (Table 5.1). Hemoglobin levels are also age- and gender-dependent. The majority of patients with COVID-19 pneumonia are older with chronic illnesses, such as diabetes [91]. Importantly, patients with diabetes exhibit higher glycated hemoglobin, which may be another reason for the high risk of infection in elderly people [91]. Notably, it is has been reported that several SARS-CoV-2 proteins, such as ORF1ab, ORF10, and ORF3a, can mount an attack on the hemoglobin chain to detach the iron and form porphyrin, leading to lower oxygen-carrying capacity [92].

15 The Role of Sex Hormones and Age-Related Differences in COVID-19

Due to the significant effect of age on mortality and morbidity seen with COVID-19 illness, separating the effects of sex and age is challenging as levels of sex hormones change with age and throughout the lifetime [93, 94]. Gender differences in the severity and incidence of respiratory viral infections, such as IAV, SARS-CoV, and MERS, are related to sex hormones and their interaction with innate immune cells during a lung infection [93, 95–97]. In a similar way, alterations in sex hormone levels through menopause/aging, pregnancy, and puberty are related to quantitative and qualitative differences in innate immunity. Hormone receptors are expressed by immune cells including progesterone (PR), androgens (AR), and estrogens (ERα and ERβ). Additionally, it has been demonstrated that the activity of sex hormone receptors usually underlie gender differences in immune cell numbers and/or responses in the respiratory system [98–100]. It has been previously shown in several investigations that NK cells exist in greater numbers and have greater cytotoxic activity in males compared to females [101–103] although this trend has been shown to be reversed with aging [104].

The angiotensin converting enzyme 2 (ACE2) and cellular serine protease transmembrane serine protease 2 (TMPRSS2) are used by the SARS-CoV-2 virus to gain entry into host cells [105]. TMPRSS2 is primarily found in spermatids and spermatogonia, whereas ACE2 is extensively expressed in Sertoli, Leydig, and spermatogonia cells. Moreover, genes related to viral reproduction and transmission have been found to be enriched in ACE2-positive spermatogonia [106]. It has also been documented that SARS infection leads to orchitis [107].

Patients over 60 years of age or with chronic diseases, including hypertension and Type II diabetes, are more prone to exhibit a cytokine storm, or suffer from systemic inflammation [2, 5, 108–111]. On the other hand, it has been shown that gender-specific hormonal changes may occur during COVID-19 illness [112]. It has been determined that serum luteinizing hormone (LH) are significantly increased and the ratio of testosterone to LH reduced in males with COVID-19, which may suggest that gonadal function in males is negatively impacted by COVID-19 [112].

16 Interferon as a Target Therapy in Coronavirus Disease 2019 (COVID-19)

Based on the above information, one route for reducing COVID-19 fatality might be the stimulation of the innate immune responses to trigger IFN production at the early stages of the disease. This could be accomplished by agents capable of inducing production of IFNs, such as poly-L-lysine and carboxymethylcellulose (poly ICLC).

Despite proof of the effectiveness of IFNs in the treatment of coronavirus-induced infections, the proper dosing and scheduling of such treatments must be evaluated in clinical trials. Jian-ya et al. reported that treatment with IFN, lopinavir, ritonavir, and corticosteroids was successful and resulted in recovery and discharge of 50 out of 51 patients [113]. Along these lines, the National Health Committee of the People's Republic of China recommended oral lopinavir/ritonavir and inhaled IFN-α as antiviral treatments. Since IFN-α is a broad-spectrum antiviral drug, it may have potential advantages for patients with SARS-CoV-2 infections. It has also been previously reported that treatment with lopinavir/ritonavir is not an appropriate treatment option for children with mild symptoms [21]. Qin et al. demonstrated that the administration of moxifloxacin, IFN, and lopinavir to non-ICU patients showed favorable results, and 16 of 89 patients were discharged after being admitted to the hospital. Moreover, administration of IFN, lopinavir, moxifloxacin, and methylprednisolone to SARS-CoV-2-infected patients resulted in 26 of 35 patients being discharged from the ICU [114].

17 The Role of Lactoferrin in Immune System Age-Related Differences

Human and cow's milk is an excellent source of enzymes, hormones, growth factors, and nutrients for infants and children, including lactoferrin, which plays an important role in protection against an extensive range of microorganisms [115]. It is known that breast feeding, as well as the extensive use of lactoferrin-containing infant formulas, might account for the milder symptoms shown in children with SARS-CoV-2 infection [116]. Lactoferrin is known to bind to specific receptors and play a critical role in efferocytosis, as a "Keep-Out" signal [117, 118]. From a pharmacological perspective, lactoferrin is a multifunctional glycoprotein with an extensive spectrum of antiviral activity against herpes simplex virus (HSV), hepatitis B virus (HBV), respiratory syncytial virus (RSV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), hepatitis C virus (HCV), poliovirus, IAV, rotavirus, human papillomavirus, parainfluenza virus, Hantavirus, and SARS-CoV-2 [119–121]. Lactoferrin inhibits neutrophil apoptosis via proximal apoptotic signaling events [117]. Inhibition of the "unnecessary recruitment" of activated neutrophils is involved in several inflammatory and autoimmune disorders, such as diabetes, atherosclerosis, rheumatoid arthritis, psoriasis, as well as acute lung injury that can result from influenza pneumonitis and COVID-19 infection [122–125]. From a mechanistic standpoint, it appears that lactoferrin potentially confers a protective role in host defense against SARS-CoV-2 by binding to heparan sulfate proteoglycans (HSPGs) and blocking the preliminary interaction between host cells and the virus [121]. However, it should be noted that there have been no reports for the interaction of SARS-CoV-2 with this receptor.

18 The Recommendations for Selecting Treatment in Patients Based on Age and Neutrophil-to-Lymphocyte Ratio (NLR)

It has been demonstrated by Liu et al. that the incidence of severe disease with a neutrophil-to-lymphocyte ratio (NLR) $\geq$ 3.13 and age $\geq$ 50 years old was 50%, whereas it was only 9.1% in patients with an age $\geq$ 50 years old and a NLR < 3.13 [126]. It follows form this that patients <50 years old and a NLR < 3.13 should be treated in either a community hospital (general isolation ward), or via home isolation. In contrast, patients with $\geq$ 50 years old and with a NLR < 3.13, who are at moderate risk, should be admitted to an isolation ward with respiratory monitoring and supportive care. Patients with an age $\geq$ 50 years old and a NLR $\geq$ 3.13 who are at high risk should be transferred to an ICU with invasive respiratory support equipment. Thus, early determination of the NLR metric, combined with the patient's

age, can be helpful with risk-stratification so that medical resources, as well as patient care can be appropriately managed [126].

19 Conclusions

Age-related differences are evident in the transmission, presentation of symptoms, diagnosis, treatment, and outcome of patients infected with the SARS-CoV-2 virus [34]. A strong response to some viral infections may be prevented by an immature immune system in children. Since the exposure of children to air pollution, cigarette smoke, and underlying chronic disorders are considerably lower than for adults, children tend to have healthier respiratory tracts and a more active innate immune response. Additionally, increased activation of the innate immune system can be achieved by vaccines, as well as frequent viral infections, which can lead to a more robust defense against different pathogens. In contrast, adults may experience a more detrimental immune response associated with ARDS. Thus, because children have a healthier immune system overall, they tend to have a healthier respiratory tract and exhibit milder symptoms to SARS-CoV-2 infection. Consequently, children have a lower rate of hospitalization and mortality than adults infected with the SARS-CoV-2 virus. We suggest that family transmission is the primary method by which pediatric patients (particularly for infants less than 1 year of age and children with underlying medical conditions) become infected with the SARS-CoV-2 virus. Therefore, it is highly recommended that children undergo testing if there is a confirmed SARS-CoV-19 case in the household.

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Sex Differences and COVID-19

6

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Abstract

Biological sex and psychosocial gender both play a role in many disease outcomes, and the novel coronavirus disease (COVID-19) is no different. Clinical observations in COVID-19 patient data delineate clear disparities between males and females, indicating males are at a higher risk for poorer disease outcomes. Although we are yet to understand the sex and gender-based disparities specific to COVID-19, there is evidence for sex-based differences in the endocrine, immune and renin–angiotensin system, all systems implicated in COVID-19 outcomes. Such disparities are largely thought to be driven by sex chromosomes and modulating sex hormones, which are known to vary between sex, and across the reproductive lifespan. Understanding and exploiting these driving factors are critical to understanding the pathobiology of SARS-CoV-2 virus and may lead to the development of novel therapies and increase the efficacy of preventative vaccine strategies currently under development. This chapter focuses on the endocrine, immune and renin–angiotensin system and genetic sex-based differences that could account for the meaningful differences observed in the outcomes of the SARS-CoV-2 infection.

Keywords

COVID-19 · Sex differences · Sex hormones · Renin–angiotensin system · Immune system · ACE inhibitors · Pregnancy

1 Introduction

1.1 COVID-19

Biological sex and psychosocial gender factors both play a role in many disease outcomes, and coronavirus disease 2019 (COVID-19), a novel coronavirus caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is no different. At the time of writing, COVID-19 has affected 118,760,083 people and claimed more than 2.6 million lives worldwide [1]. Virtually all biological systems and organs can

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be affected from this coronavirus infection, either by the virus directly targeting specific tissues or due to secondary, indirect effects [2]. Consequently, highly heterogeneous clinical presentations have been reported from asymptomatic cases to severe respiratory disease and multisystem organ failure. Symptoms of SARS-CoV-2 infection can also include fever, cough, dyspnoea, myalgia, headache, and diarrhoea, and unexplained anosmia and dysgeusia [3], while laboratory tests often show lymphopenia and elevated lactate dehydrogenase levels [4]. While a majority of infected patients remain asymptomatic or present with mild symptoms, in approximately 20% of cases the disease evolves into a severe manifestation of acute respiratory distress syndrome (ARDS) that can require hospitalisation and intubation [5]. Current data estimate approximately 5.7% of those cases die as a result of SARS-CoV-2 infection [6].

The SARS-CoV-2 virus is an enveloped positive-sensitive, single-stranded RNA virus which encompasses RNA genetic material and structural proteins required for infection and invasion of host cells [7]. After activation of the spike protein of SARS-CoV-2 by protease transmembrane protease serine 2 (TMPRSS2) [8], the angiotensin-converting enzyme 2 (ACE2) vector facilitates binding and entry into host cells. ACE2 has a broad expression pattern in the human body with strong expression levels shown in the heart, oesophagus, kidney, testis, bladder and ileum [9] and in specific cell types including type II alveolar cells in the lungs [10]. Accordingly, diverse systems can be affected including the immune, endocrine, angiotensin, cardiovascular and neurological systems with diverse clinical symptoms. The density of ACE2 in each tissue appears to correlate with the severity of organ-specific pathology [11]. With such a highly heterogeneous clinical course, establishing the underlying biological mechanisms and optimal therapeutic approach to SARS-CoV-2 infection poses a significant challenge for both researchers and medical professionals.

1.2 Sex and the COVID-19 Response

Known risk factors for severe clinical outcomes of COVID-19 include older age (65 years and older) and underlying comorbidities including cardiovascular and endocrine conditions, such as hypertension, diabetes mellitus and obesity [12, 13]. Clinical observations in COVID-19 patient data also delineate disparities between males and females, indicating a male vulnerability to the disease [14]. Although sex-disaggregated data are still not provided by all countries, the Global Health 50/50 research initiative have comprehensively collated and reported on the gender differences of COVID-19 infection and death rates from 170 countries. The current worldwide data report similar numbers of diagnosed cases in women and men (1: 1.1 ratio), but an increased case fatality in men (1: 1.4 ratio, females and males, respectively) [15]. In contrast, one study investigating patient data from South Korea did not show significant differences in mortality rates between the sexes, although did demonstrate that males were more likely to receive oxygen therapy, be admitted to intensive care unit (ICU), and have longer lengths of stay after admission to ICU [16]. A recent meta-analysis collating data from 8 countries did, however, demonstrate that once infected males had more severe adverse clinical outcomes, leading to higher death rates [17].

Sex differences in COVID-19 outcomes are likely to be driven by both psychosocial moderators and biological sex differences. Psychosocial moderators including patterns and prevalence of smoking and alcohol intake have been hypothesised to drive, in part, the more severe outcomes to COVID19 in males [18]. Clinically, males also have higher rates of underlying health conditions including hypertension, type II diabetes, cardiovascular disease and chronic lung diseases. At the biological level, analysis of viral RNA in COVID19 patients indicates that males show delayed viral clearance as SARS-CoV-2 RNA was detected for a longer time in males compared with females (preprint) [19]. Additionally, in preclinical rodent models that were infected with

severe acute respiratory syndrome (SARS), a virus with similar homology to SARS-CoV-2 [20], male mice had a 90% mortality rate in contrast with females who had a 20% mortality rate. Further, when the infection load was doubled, all male mice died, while 40% of female mice survived [21].

It also appears that a sex X age interaction exists, with a study showing pre-menopausal females demonstrated milder symptom severity and better outcomes to COVID-19 compared to age-matched males. These differences disappeared when comparing post-menopausal women with age-matched males, suggesting that protective factors associated with female reproductive phase exist [22].

Although we are yet to understand these gender-based disparities, there is evidence for sex-based differences in the immune, endocrine, and renin–angiotensin system, all systems implicated in COVID-19 outcomes. Such disparities are largely thought to be driven by sex chromosomes and modulating sex hormones, which are known to vary between sex, and across the reproductive lifespan. Understanding these driving factors is critical to understanding the pathobiology of SARS-CoV-2 virus and may lead to the development of novel therapies and preventative vaccine strategies which can exploit these dimorphic differences. This chapter focuses on the endocrine, immune and renin–angiotensin system and genetic sex-based differences that could be attributed to the meaningful differences observed in the outcomes of the SARS-CoV-2 infection.

2 Physiological Sex Differences as Potential Moderators of COVID-19 Outcomes

Physiological differences between males and females have critical implications for differential susceptibility and response to a variety of diseases, treatment efficacy and the differences in the way medications are metabolised. Gender determination and expression of a person is the result of the complex interaction between psychosocial entities and sex chromosomes and chromosomal gene regulation of sex hormones. Sex is defined as the biological and physiological difference between women and men; sex chromosomes and gonadal hormones primarily contribute to these differences at the cellular, organ, and systems levels and are referred to throughout this chapter. Sex chromosomes encode sexual differentiation through the presence of Y genes and via an increased dose/inactivation of X genes.

The endocrine system controls the synthesis, release and feedback of hormones released from internal glands directly into the circulatory system, subsequently regulating distant target organs. The ‘sex hormone’ endocrine hypothalamic–pituitary–gonadal axis (HPG axis) is a hormone-regulating mechanism containing three different component structures that operate in a coordinated fashion and is involved in the regulation of several reproductive, developmental and regulatory processes. Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus, and GnRH in turn stimulates the pituitary gland to release luteinising hormone (LH) and follicle-stimulating hormone (FSH), stimulating the gonads to produce sex steroid hormones, including oestrogen, progesterone and testosterone. The increase in LH and FSH during puberty induces the maturation of the gonads, leading to marked increases in estradiol and testosterone in females and males, respectively. Sex steroids, particularly in females, fluctuate greatly across the reproductive lifespan.

In addition to reproductive function, sex hormones including oestrogens, progesterone, and testosterone modulate a diverse range of biological systems and consequently affect broad disease processes including respiratory, cardiovascular, renal and immunological disease. While the exact molecular mechanisms underlying these processes are not yet completely elucidated, sex hormones appear to interact with genetic and environmental factors to influence multiple aspects of the immune system, including the contribution to cell differentiation, inflammatory cytokine profiles and epigenetic alterations [23].

2.1 The Immune System and COVID-19

The SARS-CoV-2 virus infects host cells and begins the process of viral replication which induces proinflammatory cytokines that recruit components of the innate immune system. The wide range of presentations from asymptomatic to respiratory distress is thought largely due to the variable control of this positive inflammatory feedback [24]. An uncontrolled innate inflammatory response paired with an impaired adaptive immune response has been associated with the clinical severity in patients with COVID-19 [25]. Additionally, a subgroup of patients with severe COVID-19 can experience the so-called cytokine storm syndrome, characterised by hypercytokinemia leading to inflammatory infiltration of the lungs and acute respiratory distress syndrome, an uncontrolled and generalised inflammatory response. Increased production and elevated local and systemic proinflammatory cytokines and chemokines precipitate and sustain the resulting aberrant systemic inflammatory response. Subsequently, this response can be followed by the immune system 'attacking' the body, causing ARDS and multiple organ failure, which can lead to death from SARS-CoV-2 infection [26]. Sex differences are reported in immune response during the disease course of COVID-19.

2.2 Sex Differences in the Immune System

The nature and strength of the immune response differ between males and females across the lifespan due to the unique challenges known to each sex. On average, females across species tend to develop a stronger immune response to contagions and improved pathogen clearance and vaccine efficiency. From an evolutionary perspective, this increases reproductive fitness of a species, supported by evidence that in cases where the father is responsible for carrying the offspring to term (e.g. seahorse), there is an observed upregulation in immune function [27]. Additionally, the immune system must be flexible enough to allow for pregnancy without attacking the foetus or the sperm required to make offspring [28].

The immune response of the innate arm is a rapid and first line of defence against contagions and viral infections. Innate immune cells with specialised functions include neutrophils, eosinophils, basophils, mast cells, monocytes, dendritic cells and macrophages, whose main ability is to respond quickly and broadly when a pathogen is introduced, typically leading to inflammation. Innate immune cells express Toll-like receptors that can detect pathogen-associated patterns of potentially harmful pathogens, including viral proteins and nucleotides. This induces interferons (IFNs) and other proinflammatory cytokines including interleukin 6 (IL-6) and TNFα. These low-molecular-weight soluble proteins function as chemical messengers for regulating the innate and adaptive immune systems. It has been demonstrated that immune cells in females exhibit a 10x-fold higher expression of Toll-like receptors [29].

The adaptive immune response expands over time, storing information about previous infections and activating a pathogen-specific and long-lasting response. The cells of the adaptive arm are highly specialised, with adaptive lymphocytes (B and T cells) bearing unique receptors that recognise specific patterns, rather than general patterns. Antigens are processed by innate immune cells and presented to the adaptive cells in the lymph nodes. Regardless of age, females demonstrate higher proliferation of regulatory T cells, immunoglobulins and higher B cell numbers compared to age-matched males. Global analysis of B cell gene expression signatures reveals that the majority of genes differentially expressed between the sexes are significantly upregulated in females compared to males [30].

T lymphocytes expressing CD4+ cell surface molecules are known as helper T cells (Th) and regarded as the most prolific cytokine producers. Of these CD4+ expressing cells, they are further subdivided into helper T cells 1 (Th1) and helper T cells 2 (Th2) with the cytokines they produce known as Th1-type cytokines and Th2-type cytokines [31]. Th1 type cytokines tend to have a pro-

inflammatory response and include interferon gamma, IL-2 and TNFβ, evoking cell-mediated immunity and phagocyte-dependent inflammation. Like all homeostatic systems, this proinflammatory state must be regulated to prevent tissue damage, and the Th2/regulatory T (Treg) cell-related cytokines such as cytokines IL-4, IL-5, IL-6, IL-10 and IL-13 largely promote an anti-inflammatory response and a strong antibody response [32]. While both Th1 and Th2 responses are required at different time points to effectively eradicate an infectious agent [33], an optimal homeostatic balance needs to be maintained. In general, Th1 and the cellular immune response predominate in males, while the Th2 controlled antibody-mediated immune response predominates in females [34, 35]. Sex hormones are thought to be largely responsible for this difference.

2.3 Sex Hormones and the Immune Response

Sex hormones including oestrogen, progesterone and testosterone modulate the activity of immune cells, resulting in differential immune responses between the sexes. Oestrogen receptors are expressed in all immune cells, serving as key transcriptional regulators of cellular activity by binding to oestrogen response elements on multiple target genes.

In general, high estradiol concentrations, the most potent form of oestrogens in humans, favour a Th2 anti-inflammatory response over the Th1 cell response. Estradiol has been shown to suppress the production of Th1 proinflammatory cytokines, including IL-6, IL-1β and TNF-α by monocytes and macrophages (a major factor in the COVID-19 cytokine storm) while stimulating Th2cell production of anti-inflammatory cytokines IL-4, IL-10 and interferon γ, promoting immune tolerance. It has also been shown that immunoglobulins G (IgG) and M (IgM) production in human peripheral blood mononuclear cells, of both males and females, are increased by estradiol treatment in a dose-dependent manner, without altering cell viability or proliferation [36].

Progesterone also has important immunomodulatory and anti-inflammatory functions. Progesterone receptors are expressed in most immune cells, including epithelial cells, macrophages, dendritic cells, lymphocytes, mast cells and eosinophils. Progesterone inhibits proinflammatory cytokines IL-1β and interleukin 12 production by macrophages and dendritic cells. Additionally, it has been shown that progesterone favours the skewing of T helper cell response from Th1-type towards Th2-type and the production of anti-inflammatory IL-4, IL-5 and IL-10 cytokines [37]. Treatment of human natural killer cells with progesterone has also been shown to reduce the activation and production of IFNy via caspase-dependent apoptosis [38].

In contrast to the largely immunoenhancing role of estradiol and progesterone, higher serum total testosterone levels are associated with an immunosuppressive effects on various components of the immune cell-mediated response, including inhibiting T helper cell differentiation [39]. Worse outcomes for males are reported in response to the seasonal influenza infection [40], and higher levels of influenza A-specific memory B cells in females relative to males after an influenza vaccination have been demonstrated [41].

2.4 Sex Hormones and COVID-19

Broadly speaking, oestrogen and progesterone favour a state of decreased innate immune inflammatory response, responsible for acute inflammatory cytokine storms, while enhancing immune tolerance and antibody production as part of the adaptive response, while male sex hormones could lead to susceptibility and severity towards pathogenic infection. Although evidence is currently sparse, it has been hypothesised that testosterone plays a role in the clinical course of SARS-CoV-2 leading to ARDS and multiorgan failure [42]. It has therefore been proposed that estradiol and progesterone treatment may provide beneficial effect in COVID-19 response, with estradiol and progesterone treatment hypothesised to blunt the innate immune inflammatory responses and at the same time stimulate

B-cell responses and antibody production without noticeable side effects (33, 51). Currently, two separate clinical trials are testing estradiol (ClinicalTrials.gov identifier NCT04359329) and progesterone (ClinicalTrials.gov identifier NCT04365127) in COVID-19 patients [43], which will hopefully yield promising results as a novel treatment.

2.5 Sex Chromosomes and the Immune System

Sex chromosomes contribute to genetic differences and can play an important role in system function and disease outcomes. Females have two copies of the X chromosome while males have one. The X chromosome expresses several genes implicated in the immune response, including toll-like receptors 7 and 8, genes that regulate B and T cell activity including FOXP3 and multiple cytokines [44, 45]. Although the majority of alleles on one X chromosome are silenced, there is the possibility of incomplete X-chromosome inactivation occurring in females leading to an over-expression in females of these immune-related genes. Furthermore, polymorphism of X-linked genes and cellular mosaicism may offer a female advantage during the host response, by providing more adaptive and balanced cellular machinery during innate immune responses [46].

2.6 Ageing, Sex and the Immune System

Collectively, the major changes of the innate and adaptive immune system with ageing result in vulnerability to certain infections and decreased efficacy of many vaccines. Although sex differences in the ageing immune system have not been studied extensively, addressing such sex differences may aid in greater effectiveness of vaccines and immunotherapies. In general, adaptive antibody responses are lower in older individuals than in their younger adult counterparts [47].

Females who are in their menopausal phase of their reproductive lives exhibit increased concentrations of peripheral pro-inflammatory markers, higher numbers but reduced cytotoxicity of NK cells and reduced numbers of B cells and T (CD4+) cells relative to women in their reproductive phase [23, 48–50]. Proliferation of B and T cells decreases with age, although this rate of decline is significantly lower in females than in males [51]. Data also suggest that elevated inflammatory cytokines (including CRP, a marker for generalised systemic inflammation) in females compared to males persist among aged individuals [52]. The efficacy of vaccines, particularly in older individuals, is consistently higher in females than in males. In a study profiling the immune cells of 172 healthy adults aged 22–93 years, matched for sex, age and body mass index (BMI), the largest sex-based immune differences were observed after the age of 65. Males displayed clear genomic differences compared to females, with males having higher innate and proinflammatory activity, but lower adaptive (B and T cell) activity [39]. The authors hypothesised this may explain why males are more prone to infectious diseases.

These sex differences in sex steroid levels, in addition to differential X chromosome dosage, are likely to influence both the innate and adaptive immune response to SARS-CoV-2 and the inflammatory outcomes and vaccine efficiency when and if a successful vaccine is developed. As the largest sex-based immune differences have been observed after the age of 65 [39], this may be the reason for the stark sex-based fatality rate differences reported in the elderly.

3 Sex Differences in the Renin–Angiotensin–Aldosterone System

The renin–angiotensin–aldosterone system is a pathway that is critically implicated in SARS-CoV-2 infection. Sex differences in renin–angiotensin system (RAS) system have been hypothesised as one contributor to the gender disparity in morbidity and mortality from the COVID-19 disease [53]. The RAS is an endocrine system essential for the regulation of blood

pressure and fluid balance and involves multiple organs including the liver, lung, adrenal gland, kidney and vasculature. Briefly, renin is a peptide hormone secreted from afferent arterioles in response to renal hypoperfusion, decreased distal chloride delivery to the macula densa and increase sympathetic activity. Angiotensinogen, synthesised and released from the liver, is cleaved by renin in the systemic circulation to form angiotensin 1 which can then be converted to angiotensin 11. The RAAS then divides into a classical 'activator' system by converting angiotensin I to angiotensin II by angiotensin-converting enzyme (ACE1) which binds to the AT1 receptor and an 'inhibitor' system converting angiotensin I to angiotensin (1-7) via angiotensin-converting enzyme 2 (ACE2), promoting theA2 receptor/Mas receptor pathway. This two-arm, counter-regulatory system works in opposing and regulatory actions with ACE1and AT1 receptor activity stimulating SNS tone, vasoconstriction, inflammation, and a decrease of baroreflex sensitivity and NO release, while the ACE2 and AT2 axes promote a decrease of SNS tone, loss of blood pressure, and an increase of baroreflex sensitivity and NO release and vasodilation.

Excess activation of the 'activator' system leads to renal and cardiovascular disorders, such as hypertension and chronic kidney disease, and is a major risk factor for stroke, myocardial infarction, congestive heart failure, atherosclerosis and renal failure. Studies have demonstrated reduced angiotensin-converting enzyme 2 (ACE2) activity in pre-hypertensive subjects and similarly reduced ACE2 activity in diabetic and renal disease patients [54–56]. ACE inhibitors that prevent Angiotensin I converting to angiotensin II by inhibiting ACE1and drugs that prevent angiotensin II binding to AT1 receptors called angiotensin receptor blockers (ARB) are widely prescribed for these conditions with improved clinical outcomes.

Since the ACE2 enzyme is the receptor that allows SARS-COV-2 entry into cells, it has been speculated that the use of ACE inhibitors or ARB may increase the development of severe acute respiratory syndrome in response to COVID-19 infection [57]. Conversely, it has also been suggested that ACE inhibitors and ARBs could benefit infected patients, as ACE2 converts angiotensin II (with known vasoconstrictive, pro-inflammatory and fibrotic effects) into angiotensin, which may protect lungs from acute injury, and upregulating ACE2 through therapy may enhance this process [57]. However, to date there is no evidence that ACE inhibitors nor ARB medications facilitate SARS-CoV-2 cell entry by increasing ACE2 tissue expression in either animal or human studies [58] and a recent meta-analysis demonstrated that compared with untreated subjects, those using either ACE inhibitors or ARBs showed a similar risk of severe or lethal COVID-19 (summary OR: 0.90; 95% CI 0.65 to 1.26 for ACE inhibitors; 0.92; 95% CI 0.75 to 1.12 for ARBs) [59].

Sex differences have been described with regard to the RAS system, with sex hormones thought to be the main regulators [60–62]. Adult women exhibit higher circulating concentration of Ang (1-7) compared with men, presumably with increased activity of the inhibitory, protective Ang (1-7)/mas receptor arm of the RAS system. Angiotensin II levels, however, have been shown to be similar between the sexes [63]. In a study performed in atrial tissue of elderly males, it was shown that estradiol substantially modulated the local RAS system via downregulation of ACE and simultaneous upregulation of ACE2, AT2R and MAS expression levels, resulting in a shift from the classical stimulatory RAS axis to the inhibitory RAS/ACE2 axis [64]. Progesterone also works in an 'inhibitory' manner by competing with aldosterone for the mineralocorticoid receptor [60]. Additionally, the ACE2 gene is located on the X chromosomes [65], and it has therefore been hypothesised that females may display higher ACE2 activity [60].

Age appears to moderate these sex differences observed in the RAS system, with increasing ACE activity and Ang II levels during the menopause transition. Menopause has been shown to increase ACE activity, thus increasing Ang II levels [66]. In healthy adults, ACE activity is higher in males; however, during perimenopause (the time preceding menopause), ACE activity in

plasma has been shown to be similar to males [60]. Hypertensive rat models also show that ACE activity is higher in male rats compared to female rats [67], and oestrogen administration decreases ACE activity in the plasma, kidney and aorta of ovariectomised females [68]. Children are also susceptible to SARS-CoV-2 infection, although they are seemingly mostly resistant to the effects of the SARS-CoV-2 virus, particularly in regards to mortality [69]. As prepubertal children have low levels of sex hormones, under the premise that sex hormones modulate the sex differences observed in ACE2 expression in lung tissue, it may be hypothesised that the low levels of sex hormones may be one contributing factor explaining the low morbidity in children [53].

3.1 Sex Differences in the Renin–Angiotensin System and COVID-19

ACE2 has been established as the most relevant protein in COVID-19 pathobiology as a binding site for SARS-CoV-2 spike glycoprotein, mediating entry into the host cell. The transmembrane protease serine type II (TMPRSS2) also plays a critical role, priming the viral spoke protein to allow viral entry into host cells. In-vitro studies have demonstrated a positive correlation between membrane expression and/or tissue activity of ACE2 and risk of viral infection of COVID-19 [70]. Furthermore, it has also been suggested that by binding to ACE2, the SARS-CoV-2 induces a decrease in the ACE2 tissue activity, with consequential increases activity of the stimulatory RAS pathway, aggravating COVID-19-induced inflammation in organs such as the lung [58, 71].

Single-cell RNA-seq sequencing data suggest that males have a higher amount of ACE2-expressing pulmonary alveolar type II cells than females [72], hypothesised as implicated in the male vulnerability to the virus. Of note, although ACE2 is only moderately expressed in healthy lung tissue compared to the heart, kidneys and testes, staining of lung tissue sections from adults with pulmonary hypertension has revealed increased ACE2 protein in the endothelium of pulmonary arteries compared to healthy controls [73], for which males have higher rates. Further, the ACE2 gene maps to chromosomal location of Xp22.2 on the X chromosome [74]. This gene diversity between sexes may be one of the factors for modulating sex differences in immune responses to pathogens such as SARS-CoV-2.

Additionally, as previously noted, in order for SARS-CoV-2 to infect host cells, priming of the spike proteins of the virus is first required by TMPRSS2. It has been reported that the androgen (testosterone) receptor activity may be a necessary requirement for the transcription of the TMPRSS2 gene, as presently there are no other currently known TMPRSS2 gene promotors [75]. TMPRSS2 mRNA levels have been shown not to be affected by estradiol treatment [64].

4 Reproduction, Pregnancy and COVID-19

Immunological adaptations in pregnancy allow for maternal tolerance of a growing foetus; however, this also leads to an increased risk of susceptibility to acute viral infections due to this immunosuppressed status, especially when the infection is acquired in the third-quarter of pregnancy [76], and this would likely be the same for COVID-19 [77]. The sex steroid estradiol and progesterone concentrations are considerably higher during pregnancy than during other times in the female reproductive cycle and increase over the course of pregnancy, with the highest levels reached during the third trimester. Pregnant women are at a greater risk of a wide spectrum of adverse consequences for both mother and foetus, with vertical transmission from mother to foetus having the ability to effect development, growth, and preterm birth and stillbirth outcomes, while the mother is predisposed to heightened morbidity and maternal death [76]. The mortality rate for SARS among pregnant woman was 25%, as compared to 10% for all other adults [78].

A clear picture of the clinical characteristics and maternal, foetal or neonatal outcomes associated with COVID-19 infection remains elusive. Although early, preliminary data suggested that the prognosis of SARS-CoV-2 infection could be

more severe in pregnant women [79], accumulating data appear reassuring in terms of maternal health and birth outcomes.

One observational study assessed the impact of SARS-CoV-2 on the delivery and postpartum services in a single health system in New York City during the period of peak COVID-19 – 25 March 2020–15 May 2020. The rate of preterm birth among SARS-CoV-2-positive women was lower than both the national preterm birth rate of 10.02% and in among SARS-CoV-2 negative women, and no increase in the number of stillbirths during the period of peak pandemic was observed. Further, the number of preterm births before 35 weeks (those at highest risk for child morbidity related to prematurity) was exceedingly low. A strength of this study was the availability of testing in the entire population of interest, rather than testing only symptomatic women. Another study carried out in the USA reported a significant decrease (25%) in the odds of a preterm delivery during the pandemic compared to a similar pre-pandemic period in the available peer-reviewed literature, with both early preterm delivery (49% decrease for <34 and 63% decrease for <28 weeks), known to have the highest risk of neonatal morbidity and mortality [80]. Further, it has been demonstrated that there are no significant differences in key pathological markers between pregnant women with COVID-19 and pregnant women without COVID-19 with regard to white blood cells, lymphocytes, neutrophils, NLR, immunological markers or coagulation fibrinolysis markers [81].

In contrast, a population-based cohort study in Wuhan, China, demonstrated that a SARS-CoV-2 infection in late pregnancy was associated with an increased risk of preterm birth (OR 3.34, 95% CI 1.60–7.00) and caesarean section (OR 3.63, 95% CI 1.95–6.76) although there was no statistical difference in low birth weight, neonatal asphyxia, nor premature rupture of membranes (a rupturing of the amniotic sac before labour commences), between the mothers with and without COVID-19. Of those infants born to mothers with confirmed infection, none tested positive to infection or had abnormal CT results [82].

In terms of maternal health, a systematic review of 2567 pregnancies affected by the SARS-CoV-2 virus demonstrated that there were a 7% ICU admission rate and a mortality rate of 1%, levels not significantly different than normal [83]. A national population-based cohort study of 427 pregnancies hospitalised with COVID-19 (UK) also showed no evidence of increased risk of severe outcomes compared to the general hospital population [84].

It also remains unclear the ways in which the virus or immunity to the virus may be passed from the pregnant mother or breast-feeding mother to her infant. Available evidence suggests that in contrast to SARS-CoV and MERS-CoV, vertical transmission appears to occur in only a small proportion of cases of SARS-CoV-2 [85]. Although a low level of potential infection has been cited, due to evidence of low ACE2 and TMPRSS2 gene expression levels in human placenta and foetal tissue compared to adult tissues [86], the presence of the SARS-CoV-2 genome has been shown in umbilical cord blood and in at-term placentas, in vaginal mucosa of pregnant women and in milk specimen [85]. Further investigations are needed to address this question.

High levels of ACE2 expression have also been shown in other organs including in the testis, cardiovascular and gastrointestinal system. Such high levels in the testis suggest SARS-CoV-2 this may affect sexual development for younger boys and cause infertility in adult males if they had been infected by the SARS-CoV-2. Taken together, even though lung cells may be the main target cells of SARS-CoV-2, other organs may also be vulnerable, particularly in the testis which may lead to male infertility if previously infected with COVID-19 [87].

5 Long-Term Outcomes of COVID-19 and Sex Differences

A subset of individuals with COVID-19 disease has been observed to develop a chronic, post-viral condition that persists well after the initial presentation the so-called long COVID. Symptoms that include fatigue, cognitive dysfunction, dizziness, anxiety, headache, rashes, joint pain, shortness of breath and fevers,

with difficulty with memory, thinking, sleep and vision are also reported. These symptoms largely mimic that of post-viral fatigue syndrome and myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), for which the most common triggering factor is infection and for which there is a 3:1 higher prevalence in females [88].

The increased immune function observed among females largely being advantageous and protective against pathogen infection is accompanied by an increased risk of autoimmune diseases, with females being 9 times more likely to develop an autoimmune disorder [89, 90]. This has been hypothesised as a risk factor for post-viral fatigue syndrome and ME/CFS onset and a reason for the sex bias observed. In SARS-related conditions, fortunately, increased immune function pertains to enhanced anti-inflammatory regulation and antiviral defence in female and appears to be protective. However, the long-term fallout of COVID-19 may be worse for females than for males, due to psychosocial, economic and biological reasons, and remains to be explored and revealed.

6 Conclusion and Perspectives

Since COVID-19 was declared a pandemic by the World Health Organisation in March 2020, reports consistently describe males as having more severe outcomes and higher mortality rates, despite similar infection rates. These differences are likely due to gender-specific psychosocial behaviours and biological disparities particularly at the level of genetics and hormonal factors. The natural biological sex differences observed in systems implicated in the COVID-19 response, including the immune and RAS systems, suggest a female advantage. Such differences in these systems can be studied and ultimately exploited to develop pharmacological innovations that help mount a more effective response to the virus and increase the efficacy of vaccines under development. Moreover, vulnerable populations including the aged and immunosuppressed and reproductive life events including pregnancy must be considered within the context of sex differences and sex hormones to comprehensively understand, exploit and facilitate a personalised treatment approach to protect those most vulnerable. Large-scale studies with sex-specific reporting and suitable analyses are required to elucidate how sex moderates the response to COVID-19. This will improve clinical management and treatment in response to both the acute phase of infection and the long-term, chronic effects of SARS-CoV-2.

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Intestinal Microbiota in the SARS-CoV-2 Infection: What Is Known?

7

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Abstract

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiological agent of COVID-19, emerged last year in China and quickly spread to millions of people around the world. This virus infects cells in different tissues and causes pulmonary (e.g., pneumonia and acute respiratory distress syndrome), neurological, cardiovascular, and intestinal manifestations, which can be the result of a direct viral effect or secondary to endothelial, thrombotic, or immunological alterations. In this chapter, we discuss recent studies which highlighted the relevance of the intestinal microbiota for other infectious respiratory diseases. We present the "altered microbiota" (dysbiotic) as a point of connection between conditions that are risk factors for the development of severe forms of COVID-19. In addition, we describe the findings of recent studies reporting alterations of microbiota composition in COVID-19 patients and speculate on how this may impact in development of the disease.

Keywords

Coronavirus · Microbiota · SARS-CoV-2 · Short-chain fatty acids

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1 Introduction

1.1 Epidemiological Characteristics of Coronavirus Diseases

Coronaviruses (CoVs) are a group of single-stranded RNA viruses (+ssRNA) that present spike projections, resembling a crown aspect [1, 2]. Belonging to the order *Nidovirales* and the family *Coronaviridae*, these viruses can be divided into the subfamilies *Orthocoronaviridae* and *Torovirinae*, the first being subdivided into four genera: α and β, which are capable of infecting mammals, and γ and δ that are predominantly infectious in birds [3, 4]. The CoVs have been described as zoonotic viruses since 1936. The first human infection by CoVs was reported in 1966 [5]. Varieties of HCoVs, which preferentially infect the upper respiratory tract, are common causes of colds [6–9]. In addition to this mild respiratory disease, viruses of this family can also infect the lower respiratory tract, thus causing infection and inflammation in the alveoli and lung parenchyma, as described below.

The first epidemic caused by HCoV began in 2002 at the province of Guangdong, China. The clinical manifestation started with high fever and mild respiratory symptoms but progressed rapidly to pneumonia in a few days [10]. At the end of February 2003, the disease spread to neighboring regions and countries. The transmission had an interpersonal characteristic, affecting not only the community but also health workers [11, 12]. The etiological agent of this syndrome was identified as a coronavirus, and it was named severe acute respiratory syndrome coronavirus (SARS-CoV). In July 2003, the World Health Organization (WHO) registered more than 8400 cases of SARS-CoV worldwide and 813 deaths associated with complications and more severe forms of the disease [13–16]. A second epidemic caused by HCoV virus emerged in 2012 at Saudi Arabia. The etiological agent was called Middle East respiratory syndrome coronavirus (MERS-CoV) and the most common symptoms of MERS infection included fever, cough, and shortness of breath. Since 2012, the WHO reported 2494 cases and 858 deaths associated to MERS-CoV infection [17].

More recently (December 2019), a third epidemic caused by HCoV emerged. This began in the city of Wuhan, China, and quickly spread to the world [18]. Initially, on January 12, 2020, the WHO named it 2019-nCoV and the pneumonia caused by it as COVID-19. On February 11, 2020, the International Committee of the Coronavirus Study Group (CSG) proposed the nomenclature SARS-CoV-2, which became the official name [19]. As of March 1, 2020, 79,968 cases of COVID-19 had been recorded in China and 2873 deaths [20]. In the subsequent months, the epidemic spread to all the continents leading to more than 30 million cases and 958,703 deaths [21]. At the time of this report on March 11, 2021, there were over 118 million cases and 2.6 million deaths.

1.2 Coronavirus Disease 2019 (COVID-19)

One of the main challenges of treating COVID-19 patients is the diversity and variability of clinical manifestations. While some individual are asymptomatic or present only a few and mild symptoms, others may have severe symptoms, as described below [4]. In general, the symptoms begin gradually, with an incubation period of 5–14 days, and the occurrence of symptoms varies according to the evolution of the disease [19]. The most common clinical manifestations of COVID-19 are fever, dry cough, dyspnea, and tiredness, while other less common symptoms include body pain, nasal congestion, headache, conjunctivitis, sore throat, diarrhea, loss of taste and smell, rashes, and discoloration of fingers and toes. The most severe symptoms include breathing difficulty, which causes reduction in blood oxygen saturation, and it is associated with the alveolar damage, edema, and inflammation. Some patients can present derived complications of the disease itself as acute respiratory distress syndrome, septic shock, or failure of nonrespiratory organs [22].

In addition to the respiratory manifestations, pathological alterations in various organs and tis-

sues including the digestive tract, central nervous system, spleen, and kidney have been reported in COVID-19 patients [23]. These extra pulmonary alterations may be related to direct effects of the SARS-CoV-2 that uses the protein angiotensin-converting enzyme 2 (ACE2), which is expressed in multiples tissues and organs, as the main receptor for invading the cells, or they may be indirect effects due to respiratory, endothelial, thrombotic, or immunological alterations [23, 24].

An important extrarespiratory manifestation occurs in the gastrointestinal tract with patients often presenting diarrhea, nausea/vomit, and abdominal pain. Initially, these symptoms were found in a small number of cases, but as the virus spread all over the world, they were found to be relatively frequent [25]. Jin et al. performed a retrospective study in which 8.1% of the SARS-CoV-2 patients evaluated had diarrhea for a median of 4 days [26]. The first case reported in the USA presented diarrhea for two consecutive days and tested positive for the presence of the virus in stool samples [27]. A few studies analyzed the correlation between the severity of the disease and the gastrointestinal manifestations. The presence of diarrhea as a symptom was most seen in patients with the severe form of COVID-19 when compared to the mild form of the disease [26, 28]. The findings of the latter study indicated that patients with diarrhea were most likely to require mechanical ventilation and present respiratory distress [28].

Lymphopenia, increased levels of C-reactive protein (CRP), lactate dehydrogenase (LDH), and clotting factors are parameters commonly seen in other types of respiratory viral infections and are also observed in COVID-19 patients [29, 30]. Meta-analysis studies showed that 35–75% of adult individuals infected with SARS-CoV-2 presented lymphopenia, being more frequent in patients who died and also being a predictor for possible admissions to Intensive Care Units (ICUs) [31]. CRP, an indicator of acute inflammatory conditions [32], was found in a meta-analysis to be increased by 75–93% in patients with COVID-19, especially in those whose disease severity was severe [31]. LDH is an enzyme responsible for catalyzing the production of pyruvate for lactate in several cell types and is released after cell damage [32]. This enzyme was found to be elevated in individuals with COVID-19 who required ICU admission and was commonly related to unfavorable outcomes [31, 33, 34]. Coagulation factors related to COVID-19 infection include Prothrombin Time (PT) and D-dimer. In studies that measured both factors among individuals who survived and those who died, it was demonstrated that the values of both PT and D-dimer were higher in non-surviving patients, which suggested the activation of the coagulation cascade in these individuals [35].

Considering the aspects mentioned, it is easy to understand why COVID-19 is a complex disease and represents a huge challenge for development of effective forms of treatment.

2 Microbiota and Respiratory Infection

The microbiota consists of a large number of microorganisms including bacteria, viruses, protozoa, and fungi, which colonize the human body and establish important interactions with human cells. Sites in the interface between the inner and external environments including the skin, the respiratory, genitourinary, and gastrointestinal epithelium are home to a vast number of microorganisms which we only started to identify and understand in the last 15–20 years with the advent of new technologies, such as next-generation sequencing.

Understanding the complex interactions between host and microbiota is of great interest because they are relevant for the proper functioning of several tissues including the epithelial barriers and the immune system. Reflecting the importance of the signals provided by the microbiota to the host, several studies have indicated that changes in its composition and/or diversity induce or participate in a vast number of diseases including inflammatory bowel diseases, irritable bowel syndrome, obesity and their comorbidities, cancer, diabetes, hypertension, and neurodegenerative diseases [36].

Recent studies indicate that the intestinal microbiota is relevant for respiratory health, playing a role in the development of diseases that affect this system including asthma, chronic obstructive pulmonary disease (COPD), lung cancer, cystic fibrosis, and respiratory infection [37–40]. This crosstalk between the lungs and the gut contributes to the maintenance of the pulmonary homeostasis and immunity and is known as the gut–lung axis [41, 42]. However, the mechanisms involved in these interactions are not completely clear.

Respiratory infections are the most common infectious disease in the world. Lower respiratory tract infections are a significant cause of morbidity and mortality, accounting for more than 4 million fatalities each year [43]. Pneumonia and bronchiolitis are the most frequent comorbidities. The common causes of pneumonia are *Streptococcus pneumoniae* and *Haemophilus influenza* type b, responsible for 15.4 million total illnesses in children [44]. Pulmonary tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), is also an important cause of deaths worldwide [45]. In regard to viral respiratory infections, influenza leads to severe illness in 3–4 million people every year and respiratory syncytial virus (RSV) infection causes almost 34 million episodes annually [46]. Other viruses are also responsible for acute respiratory tract infections such as parainfluenza, metapneumovirus, adenovirus, rhinovirus, and human coronavirus [47].

Recent studies have demonstrated the existence of a multifaceted relationship between respiratory infections and the intestinal microbiota. Gray et al. demonstrated that exposure of neonatal mice to intestinal commensal bacteria induced a robust defense against bacterial pneumonia [48]. This response is characterized by dendritic cell activation and a quick influx of interleukin-22-producing group 3 innate lymphoid cells (IL22+ILC3+) into the lungs of the mice [48]. Gut microbiota has been shown to be relevant for the control of *S. pnemoniae* respiratory infection, an effect that was associated with a molecule called peptidoglycan recognition protein 4 [49]. The importance of the intestinal microbiota in the protection against pneumonia was highlighted in microbiota-depleted mice. These mice presented increased bacterial dissemination, inflammation, and organ damage after intranasal infection with *S. pneumoniae*, and fecal microbiota transplantation restored the protection against infection [50]. Similarly, the use of probiotics such as *Bifidobacterium longum 5(1A)* protects against *Klebsiella pneumoniae* infection in mice [51]. Moreover, a recent study described that the commensal Bacteroidetes protect against *Klebsiella pneumoniae* colonization and contagion, an effect that is dependent on IL-36 signaling [52].

Metabolites such as the short-chain fatty acids (SCFAs), which are derived from the metabolism of dietary fibers, play an important role in this context. These molecules protect against *K. pneumoniae* lung infection in mice [53]. The intestinal microbiota can also protect against lung colonization by Mtb [41, 54, 55]. However, in this latter context, the production of SCFAs was associated with an increased susceptibility to tuberculosis through their inhibitory effects on the immune response to Mtb [56, 57].

The gut–lung axis is also relevant for respiratory viral infections. Influenza infection is often accompanied by gastrointestinal symptoms [58]. In animal models, respiratory infection with Influenza [59] or RSV [60] has been shown to modify the microbiota. Intestinal dysbiosis caused by Influenza infection can contribute to secondary pulmonary pneumococcal superinfection [61]. Alterations in gut microbiota seen in RSV-infected children are associated with severe disease [62]. Oral administration of commensal probiotics improved the response of mice to influenza infection [63–66]. The use of probiotics also has protective effects on RSV infection [67]. However, the massive prophylactic use of probiotics to prevent respiratory infection is still controversial, and the mechanisms of protection are not clear [68].

Intact microbiota is essential for the establishment of type 1 $CD4^+$ T cells, $CD8^+$ cytotoxic T cells, and IgA responses to influenza infection [69]. The desaminotyrosine (DAT), a microbiota associated metabolite, protected from influenza

by attenuating lung immunopathology through amplification of the innate antiviral response mediated by type I interferon signaling [70]. The bacteria *Clostridium orbiscindens* is responsible for DAT production and its administration rescued the influenza response of antibiotic-treated mice [70]. Other microbiota metabolites rather than DAT can contribute to protection against respiratory infection, as it is the case of the SCFAs. The role of the SCFA propionate in protection against RSV infection was demonstrated in an experimental model [71]. Acetate, which is one of the SCFAs, protected against RSV infection by increasing the antiviral response mediated by type I interferon response [72]. Together, these data suggest a broad effect of microbiota in the protection of respiratory infection and potential for future interventions.

3 Potential Connections Between Intestinal Microbiota and COVID-19

As described in the previous section, the gut microbiota modulates the responses during respiratory infections. Recent evidence indicates that this may also be the case for COVID-19. There are several points of connection between gut microbiota and COVID-19, as discussed in more detail in the next sections.

Older age and conditions such as hypertension, cardiac conditions, diabetes, and obesity are risk factors for severe forms of SARS-CoV-2 infection and are associated with higher mortality in COVID-19 patients [73, 74]. All of these conditions, as described below and exemplified by findings of a few recent studies, are associated with significant alterations in intestinal microbiota that seem to contribute to their development (Fig.7.1).

3.1 Age Effects

The elderly population has a poor prognosis for SARS-CoV-2 infection [75]. Different factors contribute to the increased severity of SARS-CoV-2 infection in these individuals including the higher incidence of diseases such as cardiovascular disease and type 2 diabetes mellitus (T2DM) and age-related changes in mechanisms involved in the protection of the organism such as removal of virus particles and immune response to the virus [76]. Smits and collaborators demonstrated in an experimental study that aged nonhuman primates inoculated with SARS-CoV-2 had stronger innate responses to viral infection than younger adults and a decreased type I interferon beta expression [77]. Consistent with these findings, recent studies have shown that older age is associated with defects in T-cell and B-cell function, deficiency in control of viral replication and more prolonged pro-inflammatory responses, potentially leading to severe forms of COVID-19 [75, 78–80]. Furthermore, elderly individuals show alterations in composition of intestinal microbiota with a reduction of short-chain fatty acids (SCFA) that may cause loss in control of intestinal permeability and reduction in motility [81, 82]. The dysbiotic state can also alter immune responses and contribute to the poorer prognosis in COVID-19 cases [83].

3.2 Hypertension

Hypertension is a medical condition that increases the risk of many other diseases such as heart diseases, stroke, heart attack, and chronic kidney disease and is a major cause of early death worldwide [84]. High blood pressure is characterized by a systolic pressure $\geq$ 130 mm Hg and a diastolic pressure $\geq$ 80 mmHg. Different studies all over the world have demonstrated higher rates of hypertension in patients with the severe form of COVID-19, at 56% in New York, USA [79], 50% in Wuhan, China [75, 85], and 49% in Lombardy, Italy [86, 87]. Despite being a highly prevalent medical condition in COVID-19 patients, it is not yet clear how much hypertension alone influences the development of a severe disease course and death by COVID-19 [75]. This is because, among the factors that predict the severe form of SARS-CoV-2 infection such as sex, age, and obesity, there is already a relationship with the pres-

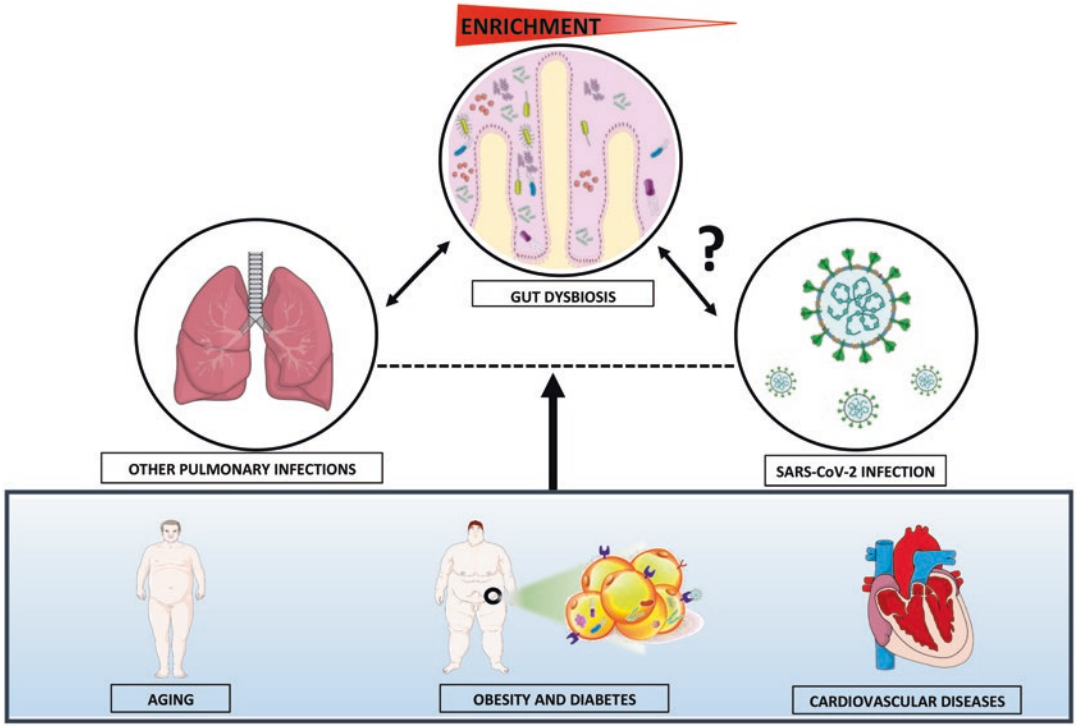

Fig. 7.1 Intestinal dysbiosis is associated with the development of pathologies including cardiovascular diseases, respiratory infections, and obesity and its comorbidities. These conditions are risk factors for the development of severe forms of SARS-CoV-2 infection. The direct or indirect involvement of intestinal dysbiosis for the development of COVID-19 is an aspect that needs to be addressed in future studies

ence of high blood pressure levels and hypertension, forming a network of interactions that needs to be better explored and clarified [88].

Changes in intestinal microbiota richness, diversity, and composition were reported in patients with hypertension compared to healthy controls [89]. Interestingly, the dysbiotic microbiota identified in patients with hypertension were also observed in pre-hypertensive individuals, and when transferred to germ-free mice, it caused a significant increase of blood pressure compared with mice colonized with a "healthy microbiota" [89]. Another recent study, which involved 529 participants, found a relationship between the diversity and composition of gut microbiota and blood pressure [90]. Several other studies have also corroborated the idea that the gut microbiota contribute through different mechanisms to the regulation of blood pressure [91] and that alterations in microbiota diversity or composition are associated with the development of hypertension and other cardiovascular diseases (i.e., heart failure and coronary artery disease), as reviewed by Troseid et al. and shown by other studies [92–94].

3.3 Diabetes

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia (fasting plasma glucose $\geq$ 126 mg/dL), which is a result of reduction in insulin production and action or both. The increased glycemia and the other metabolic alterations present in these patients chronically lead to damage in several organs and tissues such as the heart, blood vessels, kidneys, nerves, and eyes [95]. For both SARS [96, 97] and MERS [98], DM was described as a risk factor. DM is also associated with an increased risk of developing severe forms of COVID-19 [74]. A study involving 605 patients hospitalized for

COVID-19 in Wuhan described higher rates of complications (58.5%) and mortality (33%) in individuals presenting high fasting blood glucose on the day of hospitalization [99]. Increased circulating levels of glucose may boost virus replication and inflammatory activation of immune cells such as monocytes, thus contributing to the pathophysiology of COVID-19 [100].

DM patients present important alterations in the microbiota, which may play a role in COVID-19 development. In this regard, a recent systematic review that recapitulated the evidence from 42 human studies found consistent associations between specific taxa and type 2 diabetes mellitus (T2DM). A negative association was reported for some genera (*Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Akkermansia,* and *Roseburia*), which may have a protective role in this disease and a positive association was observed for others (*Ruminococcus*, *Fusobacterium*, and *Blautia*) [101]. In this same study, the authors also pointed out mechanisms involved in these associations including the effect of some microorganisms on regulation of inflammation and insulin signaling [101]. Additionally, they also highlighted the use of microbiome knowledge and of strategies that affect it as tools for prevention and treatment of T2DM patients in a personalized/individual manner [101].

3.4 Obesity

Obesity is a chronic disease characterized by an excessive accumulation of fat in the adipose tissue and other tissues, insulin resistance, and a low-grade systemic inflammatory state [102]. These alterations are associated the development of secondary pathologies such as T2DM and cardiovascular diseases, which are themselves risk factors for severe COVID-19, as mentioned above [103]. Obesity is associated with respiratory disorders such as asthma, COPD, pulmonary embolic disease, and obstructive sleep apnea [104]. Studies indicate that obesity is a risk factor for severe virus respiratory infections caused by H1N1 influenza A [105] and by SARS-CoV-2 [106].

Obese individuals present intestinal dysbiosis characterized by a reduction in microbiota diversity and alteration in the abundance of some components [107]. Several studies involve experimental models and humans found significant changes in the microbiota composition when comparing obese and lean individuals (as reviewed by Fan and Pedersen [108]). These alterations may be relevant for different physiological processes that are impaired in obese individuals including, for example, the adiposity and inflammation of adipose tissue [109, 110]. The intestinal dysbiosis associated with obesity may also have a role, directly or indirectly, in the development of severe forms of SARS-CoV-2 infection, which will need to be addressed in the future.

In summary, intestinal dysbiosis is frequently present and seems to contribute to the development of conditions associated with an increased risk of illness by COVID-19. Addressing the relevance of this relationship and its contribution to COVID-19 will be important because it will potentially impact on both preventive and therapeutic strategies used in this condition, such as the use of probiotics, antibiotics, and changes in diet composition.

4 COVID-19 and Microbiota

Recent studies have indicated that COVID-19 patients present significant differences in gut microbiota composition and, potentially, on the mechanisms of interaction between microbiota and host cells. A study conducted in Hong Kong involving 15 patients with COVID-19, 6 patients hospitalized with community-acquired pneumonia, and 15 healthy individuals found significant differences in the fecal microbiome. COVID-19 patients presented an enrichment in opportunistic bacteria associated with bacteremia and a depletion of bacterial species known for their beneficial effects on the host including two important sources of butyrate, the *Faecalibacterium prausnitzii* and *Eubacterium rectale*. The study also indicated a correlation between components of the microbiota and disease severity. The researchers found a positive correlation for *Coprobacillus*,

C. ramosum, and *C. hathewayi,* while a negative correlation was found for *Alistipes ondedonkii* and *F. prausnitzii*. Interestingly, the authors observed that some of the microbiome alterations were present even after SARS-CoV-2 was not detected anymore and the respiratory symptoms were resolved [111]. A subsequent study by the same group showed that the fungal microbiome (mycobiota) was also altered in COVID-19 patients. They found that opportunistic fungi such as *Candida albicans*, *Candida auris,* and *Aspergillus flavus* were present at higher proportions in samples from COVID-19 patients compared with healthy controls. As described for bacteria, some changes observed for fungal components were present even after resolution of respiratory symptoms [112].

Alterations in gut microbiota of COVID-19 patients were also observed in another study [113]. In this study, the authors compared the microbiota of COVID-19 with the microbiota of healthy controls and H1N1 patients. Interestingly, they found in both diseases a reduction of community richness and microbial diversity. Several significant changes in microbiota composition were observed when comparing COVID-19 samples with control. At the genera level, while the healthy microbiota had higher abundance of *Romboutsia*, *Faecalibacterium*, *Fusicatenibacter,* and *Eubacterium hallii group*, COVID-19 patients had more *Streptococcus*, *Rothia*, *Veillonella*, *Erysipelatoclostridium,* and *Actinomyces*. These taxa enriched in COVID-19 were positively associated with CRP, a key inflammatory biomarker [113], which may indicate leakage of microbial molecules or bacteremia. A recent study conducted on a primate model (the macaque), which recapitulates COVID-19 symptoms, also found significant changes in microbiome composition, some persisting for a long period after infection

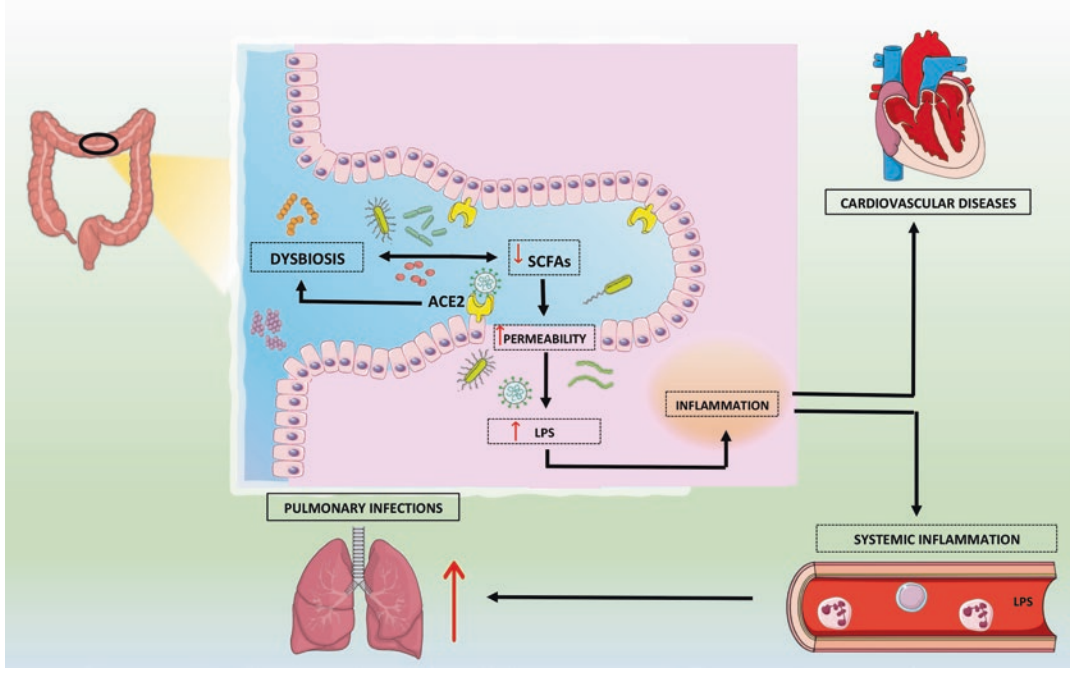

Fig. 7.2 Hypothesis linking the alterations in intestinal microbiota composition and functions and the development of pulmonary and extrapulmonary (cardiovascular) clinical manifestation associated with SARS-CoV-2 infection. COVID-19-infected individual may have intestinal dysbiosis characterized by reduction in the production of short-chain fatty acids. These metabolites have an important contribution for the intestinal barrier integrity and changes in their production may impair the intestinal permeability, thus permitting the entry of microbial components, e.g., lipopolysaccharide (LPS) that can be correlated to the severity of COVID-19

(26 days post infection), and reduction of bile acids, tryptophan metabolites and SCFAs intestinal production SARS-Cov-2 infection [114]. Considering these studies indicating reduction of SCFAs concentrations during SARS-CoV-2 infection, we tested if these metabolites had a direct impact on intestinal epithelial cells infection. Using Caco-2 cells and human intestinal biopsies, we did not observe any effect of SCFAs on SARS-CoV-2 entry or replication in intestinal cells and only minor effects on the production of anti-viral and inflammatory mediators. These findings indicate that SCFAs do not interfere with the SARS-CoV-2 infection of intestinal cells, an aspect that will need further analysis in vivo [115].

Hoel et al. found increased levels of LPS-binding protein (LBP), a marker that suggest impairment in the intestinal barrier function of COVID-19 patients [116]. Increased levels of CRP were also observed in these patients. In addition, the authors found increased concentrations of IL-18 and IL-18 binding protein, which suggested inflammasome activation. These changes correlated with biomarkers of myocardial damage and cardiac involvement in COVID-19 patients. These findings indicate that cardiac alterations observed in COVID-19 patients may be secondary to changes in the intestinal barrier and consequent activation of inflammatory mechanisms, an interesting hypothesis that needs further validation (Fig. 7.2).

5 Conclusions and Future Perspectives

This chapter described how changes in microbiota composition and function might be associated with the development of SARS-CoV-2 infection. A dysbiotic microbiota is present in several conditions that are risk factors for severe forms of COVID-19. In addition, other factors that interfere with the gut microbiota make the scenario more complex and need to be considered. First, interventions used in the treatment of COVID-19 patients have a dramatic impact on the intestinal microbiota (e.g., oxygen therapy and antibacterial agents). Second, SARS-CoV-2 can directly or indirectly affect specific tissues and lead to systemic alterations that can impact on the microbiota composition, including the gastrointestinal alterations (e.g., diarrhea and emesis) and hyperactivation of the immune system. Understanding the impact of these factors in the microbiota and the relevance of the dysbiosis to the development of acute and long-term changes associated with COVID19 disease are important. We propose that this knowledge will lead to the development of measures for preventing the impairment of the "health microbiota-host interactions" and may point the way to additional strategies for treatment of infected individuals.

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8 SARS-CoV-2 Infection and the Kidneys: An Evolving Picture

Jaya A. George and Siyabonga Khoza

Abstract

Since December 2019, a novel coronavirus known as Severe Acute Respiratory Virus 2 (SARS-CoV-2) has caused an outbreak of a respiratory illness worldwide. Even though SARS-CoV-2 primarily affects the respiratory system, other organs such as the heart and kidneys are implicated. The pathophysiology of Acute Kidney Injury (AKI) in coronavirus 2019 (COVID-19) patients is not clearly defined. Direct kidney injury results from virus entry through angiotensin-converting enzyme-2 (ACE2) receptors which are highly expressed by the podocytes and proximal convoluted tubules, as suggested by "viral-like" particles on electron microscopy. However, the link between the presence of viral particles in kidney tissue and kidney injury has not been fully explained. Furthermore, it is also hypothesized that collapsing focal segmental glomerulosclerosis (FSGS), myoglobin toxicity, sepsis-linked, and glomeruli fibrin thrombi is part of the mechanism for AKI. Reported cases link FSGS and high-risk apolipoprotein 1 (*APOL1*) alleles in patients of African ancestry. Typically, these patients present with AKI and nephrotic-range proteinuria. The rate of AKI in hospitalized patients is high and associated with a higher mortality rate in older patients with comorbidities. Even higher mortality is now being reported in patients with chronic kidney disease and kidney transplant recipients due to immune system dysfunction. Herein, we review the current literature on kidney disease and pathogenesis in COVID-19 patients.

Keywords

COVID-19 · CKD · AKI · Transplantation · Dialysis · APOL1

1 Introduction

The novel coronavirus, Severe Acute Corona Virus (SARS-CoV-2 or COVID-19) was initially described in Wuhan, Hubei province of China, in December 2019. It has since spread throughout the world with over 126 million cases reported worldwide and 2.7 million deaths as of March, 2021[1]. It manifests primarily as an acute respiratory infection but also affects multiple organs such as the heart, gut, blood, nervous system, and

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the kidneys [2]. Coronaviruses are a large family of single-stranded RNA viruses that cause illness in humans and other animals. Diseases range from the common cold to more severe diseases like Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). COVID-19 disease is more contagious than these illnesses. It is spread by droplets, contact, airborne transmission, and possible fecal spread [3, 4]. Manifestations of disease range from asymptomatic infections to fulminant disease with sepsis and respiratory failure. A study of 44,672 patients from China reported that 81% of patients had mild manifestations, 14% had severe manifestations, and 5% had critical manifestations (defined by respiratory failure, septic shock, and/or multiple organ dysfunction) [5].

The SARS-CoV-2 virus enters cells by binding of the spike protein (S protein) to the angiotensin-converting enzyme-2 (ACE2). The S protein is then activated and cleaved by cellular transmembrane serine proteases (TMPRSSs), after which membrane fusion occurs [6]. ACE2 is expressed in the lungs, heart, liver, GIT, and kidneys, which suggests that these organs may be infected by the SARS-CoV-2 virus [7]. The disease has been shown to cause acute kidney injury (AKI) and other renal abnormalities. Patients with chronic kidney disease (CKD), end-stage renal disease (ESRD), and transplant recipients may be at increased risk of severe disease.

In addition, data from electronic health records of patients in California suggest patients with renal disease may be predisposed to acquiring COVID-19 infection [8]. The increased risk may be the consequence of treatment modalities such as dialysis, immune deficiency as seen in transplant recipients, and genetic predisposition. What is more certain is that patients with preexisting morbidities including renal disease are at risk for more severe disease and for increased mortality [9–14]. In this review, we briefly discuss the epidemiology of renal risk factors and COVID-19 disease as well as possible pathogenic mechanisms.

2 Dialysis

Rates of renal replacement therapy vary widely from 2 to 73% in the critical care setting [1, 15, 16]. Haemodialysis poses an increased risk to COVID-19 because of the clustering of patients and staff in close proximity [17–19]. For example, Wang et al reported a 16.09% incidence in haemodialysis patients and a 12.12% incidence in staff at a dialysis center in Wuhan [18]. These patients are often asymptomatic or mildly symptomatic, and this has been ascribed to reduced circulating inflammatory cells and pro-inflammatory cytokines in patients on haemodialysis compared to those not on haemodialysis [20]. The reduced immune response may increase susceptibility to COVID-19. In spite of the mild symptoms seen, the mortality rate is reportedly higher. For example, the mortality rate from haemodialysis patients in Spain was 30% which was similar to that reported in haemodialysis in Italy at 29% [21, 22]. Data from these studies suggest that death in dialysis patients was due to cardiovascular disease or electrolyte abnormalities and not pulmonary failure.

3 Transplant

Another group at risk of a high mortality from COVID-19 is transplant recipients [23]. The reported mortality for transplanted patients in New York was 28% compared to 1–5% mortality seen in the general population [24]. A study in France reported an incidence of 5% in kidney transplant recipients compared to 0.3% in the general population [25]. Fever and cough are the predominant symptoms, with more males affected. Reports indicate that transplant recipients were on immunosuppressant drugs such as tacrolimus, mycophenolate, and prednisone [24–30]. The immunosuppressants cause lymphopaenia or impaired lymphocyte function, and cellular immunity is key in determining the course and outcome of COVID-19 infection. The presence of comorbidities also contributes to increased mortality [27].

4 Chronic Kidney Disease

Epidemiological evidence shows that CKD is a risk factor for AKI with COVID-19 infection [31]. A systematic review of 22 observational studies comprising 17,391 patients gave a pooled prevalence of preexisting CKD of 5.2% (2.8–8.1). CKD increases the risk for death in patients with COVID-19 [14, 32, 33]. Elevated serum creatinine on admission has also been associated with disease severity and increased incidence of AKI and hospital death [34, 35].

CKD is accompanied by systemic inflammation and an acquired immunosuppression, which may predispose to infection or contribute to the worse outcomes seen. These include accumulation of pro-inflammatory cytokines, altered T and B cell function, increased oxidative stress with attendant damage, and loss of proteins such as immunoglobulins, complement, and transferrin [36, 37]. In addition, systemic inflammation in CKD contributes to defective adaptive and innate cells, which increases the risk to infections [38].

5 Apo L1

The apolipoprotein–L1 (*APOL1*) risk genotypes were originally identified in African Americans with primary glomerular, focal, and segmental glomerulosclerosis (FSGS). Subsequently, *APOL1* has been associated with several nondiabetic chronic kidney diseases such as HIV-associated nephropathy (HIVAN) and "hypertension-attributed" ESRD [39–41].

APOL1 is trypanolytic for T. *brucei rhodesiense* which causes acute African sleeping sickness [42]. Similar to the hemoglobin variant that causes sickle cell disease, but also confers protection against malaria, two copies of *APOL1* increase risk for renal disease. About half of all African Americans inherit at least one *APOL1* risk allele, while 12–15% are homozygous [43]. However, most individuals with the *APOL1* risk genotype do not develop kidney disease, and it is thought that a second hit is needed to cause disease.

Infection with SARS-Co-V2 may be a newly identified risk factor for renal disease in those who carry *APOL1* risk alleles. Support for this hypothesis is twofold. Biopsies on SARS-CoV-2 infected patients show that they experience both glomerular and tubular disease [44]. In addition, a number of recent case reports have shown that many of those that present with proteinuria and FSGS carry risk alleles for *APOL1* (Table 8.1). Currently, kidney biopsy data are limited and mostly come from postmortem studies which show a wide range of histopathological findings (Table 8.1).

6 The Clinical Picture of Kidney Disease in COVID-19

While the renal manifestations of COVID-19 are not clearly defined, emerging data suggest that it leads to adverse outcomes like AKI, albuminuria, proteinuria, haematuria, elevated blood urea nitrogen and serum creatinine, as well as electrolyte and acid–base abnormalities [54, 55](Table 8.2). These factors are associated with high mortality [34].

6.1 Acute Kidney Injury Pathophysiology

The incidence of AKI varies with severity of COVID-19 infection [54, 56]. It appears to be more common in the elderly, in males and in the obese [15, 56–58]. It develops early after hospitalization [56, 58]. The highest incidences of AKI in COVID-19 patients are reported in those that are critically ill [59]. Several studies show that mortality in COVID-19 patients with AKI is higher than in those without AKI. The most common comorbidities seen are cardiovascular disease, respiratory disease, diabetes, and hypertension (Table 8.3).

AKI may be due to acute tubular necrosis (ATN), intrarenal injury or both. AKI may result from multiple mechanisms such as sepsis, bleeds, or other causes of volume depletion while the

Table 8.1 APOL1 and COVID-19 kidney disease

References	N	Biopsy finding	Antemortem or postmortem	Clinical findings	Presence or absence of viral particles and APOL1
Santoriello et al. [45]	42	ATI, Diffuse thrombotic microangiopathy, Acute GN, Collapsing FSGS	Postmortem	31/34 developed AKI. 23/29 has proteinuria (dipstick)	Absence viral particle
Su et al. [46]	26	Diffuse proximal tubular injury, Microvascular lumen occlusions, Peritubular erythrocyte aggregation, Interstitial inflammation around interstitial fibrosis, and glomeruli fibrin thrombi, Pigmented cast	Postmortem	7/26 exhibited proteinuria	Viral like particle with distinct spikes in tubular epithelium and podocytes.
Kudose et al. [44]	17	Collapsing glomerulopathy (5) Isolated acute tubular injury (4) Crescentic transformation of lupus nephritis (1) Anti-GBM nephritis (1) Membranous glomerulopathy (2) MCD (1)	Antemortem	15/17 developed AKI. Nephrotic-range proteinuria (9/17) 7/17 were dialysis-dependent	Absent viral particle *APOL1*(3/17)—two patients had G1/G1 and 1 with G1/G2)
Golmai et al. [47]	12	ATN (12), Early diffuse and nodular glomerulosclerosis (2)	Postmortem	N/A	Absent viral particle
Sharma et al. [48]	10	Acute tubular necrosis (ATN), FSGS with healed/collapse glomeruli, Cortical necrosis with ATN, ATN with myoglobin cast nephropathy, Crescentic GN with ATN	Antemortem	10/10 had varying degree of proteinuria (5/10 with nephrotic-range proteinuria) Haematuria (6/10)	N/A
Wu et al. [49]	6 (all black)	Collapsing glomerulopathy, ATN (6)	Antemortem	6/6 had proteinuria (dipstick)	*APOL1* (6/6)—G1/G1 in four patients, G1/G2 in one, and G2/G2 in one. Absent viral particle
Peleng et al. [50]	1 (black)	Collapsing glomerulopathy	Antemortem	Nephrotic-range proteinuria	*APOL1* (G1/G1) Absent viral particle
Larsen et al. [51]	1 (black)	Collapsing glomerulopathy	Antemortem	Nephrotic-range proteinuria	*APOL1* (G1/G1) Absent viral particle
Sharma et al. [52]	2 (black)	Collapsing glomerulopathy	Antemortem	Proteinuria	*APOL1*(G2/G2) Absent viral particle
Gautier-Vargas et al. [53]	1	Thrombotic microangiopathy	Antemortem	Haematuria, Proteinuria	N/A

ATI acute tubular injury, *AKI* acute kidney injury, *GN* glomerulonephritis, *FSGS* focal segmental glomerulosclerosis, *GBM* glomerular basement membrane, *ATN* acute tubular necrosis, *MCD* minimal change disease, and *APOL1* apolipoprotein L-1. (N) denotes the numbers patients with biopsy findings

Table 8.2 Clinical features of renal involvement in COVID-19 infection

Complication
Acute kidney injury
Urine abnormalities: proteinuria, haematuria
Electrolyte abnormalities
Acid–base disturbances
Cardiorenal syndrome

cause of intrarenal injury is uncertain. It could also result from direct viral injury or inflammation accompanying the immune response. The use of nephrotoxic drugs or high levels of positive end-expiratory pressures is also possible contributing factors (Fig. 8.1). Reports suggest that a significant number of patients with AKI may have persistently elevated serum creatinine; however, there are no long-term studies yet that have reported on CKD following AKI in COVID-19 patients [66].

Both ACE2 and TMPRSS protease activity are expressed on podocytes and kidney tubular cells. This suggests that direct infection can occur [67, 68]. Direct tubular or podocyte infection or injury would explain the proteinuria seen in these patients. In a study looking at viral particles in different body fluids, Ling et al. showed their presence in the urine of a few infected patients[69]. Biopsies on postmortem patients showed tubular necrosis and nucleocapsid protein in the renal tubules, lending further support to a viral cytopathic effect. [70]. These results are in contrast to other studies which have not shown viral particles in kidney biopsies or in the urine of infected patients [44, 45, 47].

COVID-19-associated collapsing glomerulopathy may result from an immune dysregulation-mediated "second hit" to podocytes by the virus, especially in individuals of African ancestry with *APOL1* risk alleles [52].

ACE is involved in the regulation of the renin–angiotensin–aldosterone system (RAAS) for the maintenance of blood pressure, fluid volume, and sodium and potassium balance. Angiotensinogen is cleaved by ACE to produce angiotensin II. Angiotensin II binds to the angiotensin type I receptor (ATIR) and causes vasoconstriction, inflammation, fibrosis, and oxidative damage [71, 72]. Angiotensin II can be further cleaved by ACE2 to form angiotensin-(1-7) which, on binding to its receptor (Mas), exerts vasodilatory and anti-inflammatory effects [73]. When SARS-CoV-2 binds to ACE2, it is internalized and downregulated. ACE2 counteracts the physiological role of ACE, and the eventual effects of RAAS activation depend on the tissue ACE/ACE2 balance, which also determines the balance between pro-inflammatory and pro-fibrotic, and anti-inflammatory and anti-fibrotic pathways [74].

This imbalance of ACE2/ACE levels in COVID-19 and the dysregulated angiotensin-II / AT1R axis of RAAS may be partially responsible for the cytokine storm. The cytokine storm has been well described since the early reports of this disease [75, 76]. It is characterized by an increase in pro-inflammatory cytokines, with ensuing recruitment of pro-inflammatory cells, endothelial dysfunction, and arterial and venous thrombosis [77–79]. In addition, there are changes in the cellular immune system such as monocytes and macrophages show activation. They are responsible for secretion of pro-inflammatory cytokines [79]. Immune dysfunction is also evidenced by lymphopaenia in severe disease and reduced natural killer cells (NK), T cells, and B cells [79].

The complement system, which is one of the host immune system's first response to pathogens, contributes to acute and chronic inflammation, intravascular coagulation, and cell injury. This ultimately leads to multiple organ failure and death. Postmortem analysis of kidney biopsies has shown glomerular and tubular deposits of the membrane attack complex, C5b-9[80]. In addition, complement and neutrophil extracellular traps work together to activate and drive the coagulation pathway with the generation of microthrombi and subsequent organ injury [81].

COVID-19 patients often show signs of increased coagulation activity. These include a prolongation of the prothrombin time (PT) and international normalized ratio (INR) and partial thromboplastin time (PTT), elevation of D-dimers, decreased fibrinogen levels, and thrombocytopenia. Elevated D-dimers on admission and increasing D dimers are associated with

Table 8.3 Mortality and comorbidities in acute kidney injury in COVID-19 infections

References	N	Age (Years, Median) AKI vs Non-AKI	Mortality (%) AKI vs Non-AKI	Comorbidities
Ng et al. [60]	9657	71 vs. 62	31.1 vs. 10.8	HTN, HF, PVD, CAD Diabetes Chronic liver disease COPD, Asthma Malignancy CKD
Hirsch et al. [56]	5449	69.0 vs. 61	24.4 vs. 5.6	HTN, HF, PVD, CAD Diabetes HIV Chronic liver disease COPD, Asthma, OSA Malignancy Obesity
Fischer et al. [57]	4610	67.1 vs. 60.7	33.7 vs. 9.3	HF, Diabetes Obesity CKD
Chan et al. [15]	3993	71 vs. 63	50 vs. 8	HTN, HF, PVD Diabetes Liver disease CKD
Chen et al. [61]	1392	N/A	72 vs. 10	HTN Diabetes Chronic lung disease CKD Malignancy
Russo et al. [62]	854	76 vs 68	63 vs. 27	HPM, HF, CAD, arrhythmia Diabetes COPD CKD Malignancy
Cheng et al. [34]	701	73 vs. 61	33.7 vs 13.2	HTN, COPD Diabetes CKD Malignancy
Nimkar et al. [63]	370	75 vs. 67	58.1 vs 19.6	HTN, cardiac diseases Hyperlipidaemia COPD Malignancy CKD Stroke and Dementia
Pelayo et al. [64]	223	70 vs. 61.6	31 vs. 9	HTN, HF, atrial fibrillation, CAD COPD, Asthma Diabetes CKD Obesity

(continued)

Table 8.3 (continued)

References	N	Age (Years, Median) AKI vs Non-AKI	Mortality (%) AKI vs Non-AKI	Comorbidities
Cui et al.[65]	116	61.05 vs. 58.5	57.1 vs. 12.6	Cardiovascular diseases HTN HF, CAD, and pericardial effusion Arrhythmia Respiratory disease COPD, Asthma, TB, lung cancer Diabetes CKD
Pei et al. [66]	333	N/A	57.1 vs. 3	HTN Diabetes

HTN hypertension, *HF* heart failure, *PVD* peripheral vascular disease, *CAD* coronary artery disease, *HIV* human immunodeficiency virus, *COPD* chronic obstructive pulmonary disease, *OSA* obstructive sleep apnea, *CKD* chronic kidney disease, and *TB* tuberculosis

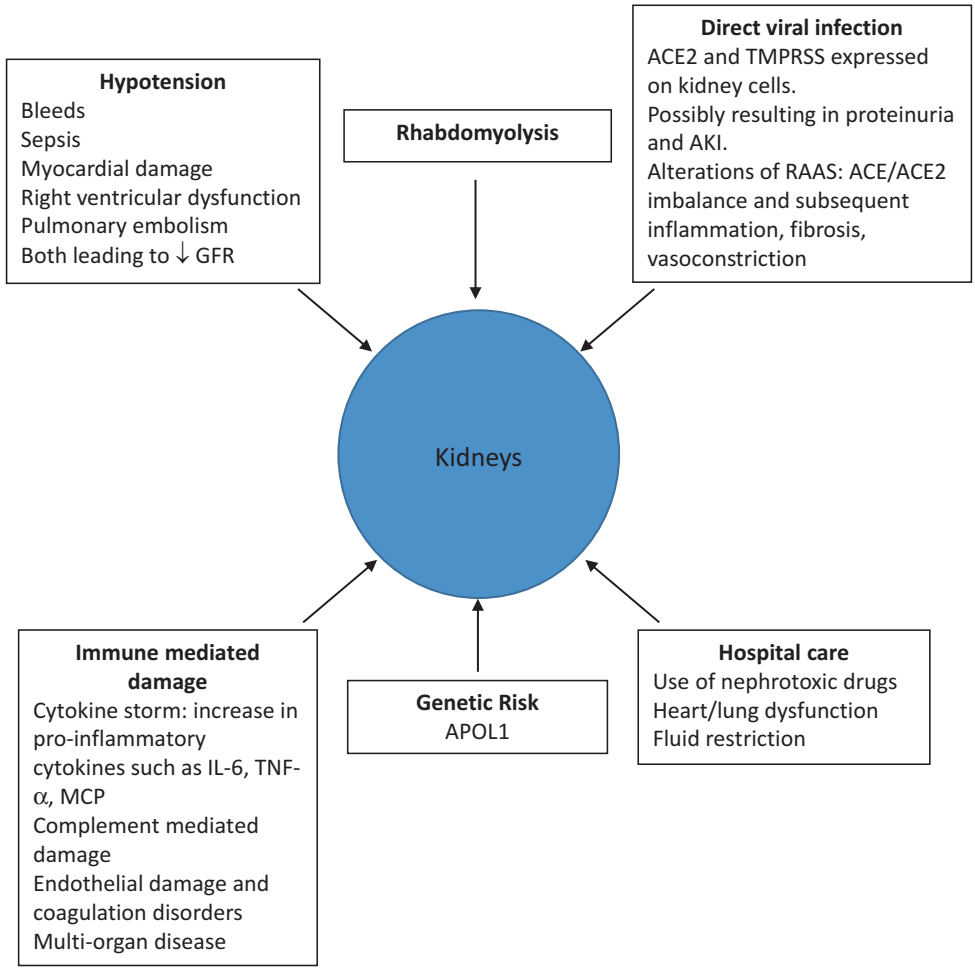

Fig. 8.1 Mechanisms for kidney damage in COVID-19 infection

increased mortality [82]. The cause of altered coagulation is multifactorial.

Hypoxia associated with COVID-19 pneumonia promotes thrombosis by increasing blood viscosity directly and through the hypoxia-inducible transcription factor-dependent signaling pathway [82]. Anti-phospholipid antibodies are another cause of thrombosis in COVID-19 infection [83]. Direct viral entry of endothelial cells with damage has been reported in renal biopsies and may be another cause AKI [46]. ACE2 and TMPRSS-2 expressions are present on the surface of arterial and venous endothelial cells, making viral entry possible [84–86]. Furthermore, the IL-2 receptor, IL-6, and TNF are increased in COVID-19 disease, resulting in the loss of the antithrombotic and anti-inflammatory functions of endothelial cells. The outcome is activation of the coagulation system, complement and platelets and leukocyte influx into the microvasculature.

COVID-19 patients on mechanical support, especially those who require prolonged support, often have severe sepsis. This predisposes to severe hypotension. The use of inotropic agents in these patients may result in vasoconstriction and a subsequent drop in glomerular filtration and, if untreated, may cause acute tubular necrosis [27]. Inadequate fluid replacement, myocardial injury, and pulmonary embolism can all contribute to hypotension and a reduction in glomerular filtration rate.

There is some evidence that the virus causes myositis which is the first sign of rhabdomyolysis [87]. In addition, severe tissue hypoxias due hyperventilation or potentially drug-induced tissue damage have been suggested as possible cause of AKI in some COVID-19 patients. The presence of myoglobin casts in kidney tissue suggests that myoglobin toxicity may be implicated in AKI [46, 48].

Electrolyte abnormalities are associated with severe COVID-19 infection including lower sodium, potassium, and calcium levels [88]. Hyponatraemia is thought to BE extrarenal in origin and occurs as a result of the syndrome of inappropriate antidiuretic hormone (SIADH) secondary to pneumonia [89]. Hypokalemia may be due renal loss and to a lesser degree from diarrhea [55].

Proteinuria and haematuria are also common in patients admitted with COVID-19 pneumonia [66]. Proteinuria may be from fever or systemic inflammation. It could also be due to viral cytopathic effect. SARS-CoV-2 has been shown to cause proximal tubular dysfunction with low molecular weight proteinuria, phosphaturia, and uricosuria with a neutral aminoaciduria. The uricosuria and associated hypouricaemia are associated with increased disease severity [90]. A recent prospective study showed that patients with mild or moderate proteinuria and patients with mild or moderate haematuria were at increased risk of in-hospital death after adjusting for age, sex, comorbidities, and leukocyte counts [34].

7 Conclusions

In summary, kidney injury is common in hospitalized COVID-19 patients. The cause is multifactorial and includes direct viral injury, cytokine storm, and volume depletion amongst others. Urine abnormalities are common. Acute kidney injury is associated with increased disease severity and increased mortality. Further research is needed to determine the pathogenesis and to develop appropriate management strategies. Currently, there is a lack of data on the extent of renal disease in patients from Africa. While Africa appears to have been spared major mortality from COVID-19, there are currently no data on associations of COVID-19 and kidney disease from this vast continent. What we do know is that there is a substantial burden of CKD in Africa and that a large proportion of those with CKD has risk factors such as hypertension, diabetes, and HIV [91]. We also know that early detection of AKI is limited by resource constraints and sometimes by limited awareness [92]. It is important to use the COVID pandemic to increase awareness of and testing for renal disease in COVID-19 affected African patients and to study the long-term renal effects of COVID-19.

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9 Cutaneous Manifestations of COVID-19: Early Diagnosis and Prognostic Information

Beatriz Burger and Hosana Gomes Rodrigues

Abstract

Coronavirus disease 2019 (COVID-19) is a multiple organ disease caused by SARS-CoV-2 virus infection. Among the organs and tissues affected by the disease, the skin has received less attention. Skin is the largest tissue in the body and is responsible for temperature maintenance, protection against external dangers and dehydration, and other roles. Although the skin manifestations of COVID-19 are common, the lack of standardization in the description of its signs makes it difficult to group them together. Considering the literature available so far, the skin manifestations can be divided into 4 patterns: exanthem, urticarial lesions, vascular and acro-papular eruptions. The localization, age, onset, symptoms and severity vary among them. The treatment, when necessary, is usually focused on the inflammatory response control. The pathophysiological mechanisms seem to involve the apoptosis of keratinocytes as well as endothelial cell dysfunction, favouring the establishment of skin inflammation. The better characterization of the skin manifestations is essential to understand the possible effects of COVID-19 on skin as well as for the development of appropriate treatments.

Keywords

Dermatology · SARS-CoV-2 · Urticarial lesions · Exanthem · Vascular and acro-papular eruptions

1 Introduction

The skin is the largest and most visible organ in the human body and separates the body from the outside environment [1]. The skin consists of three main parts: the epidermis, dermis and subcutaneous tissues. For being constantly exposed to potential hazards (physical, chemical and microbial insults), various types of immune cells reside in or are recruited into the skin to maintain homeostasis critical for health upon inflammation, wounding and viral infection [1].

Viral infection caused by the pathogen SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) causes coronavirus disease 2019 (COVID-19), which initiates an immune response in many organs of the body, including the skin [2]. Patients with COVID-19 often present with fever, dry cough, fatigue, sputum production,

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headache, myalgia, asthenia, anosmia, shortness of breath and loss of smell and taste [3–5]. Severe disease is characterized by dyspnoea, blood oxygen desaturation, respiratory failure and venous thromboembolism [5].

The skin manifestations of COVID-19 were first reported by Recalcati et al. in Lombardy, Italy [6]. In this study, 18 out of 88 patients in the study developed dermatologic manifestations such as erythematous rash, widespread urticaria and chickenpox like vesicles. Eight patients (9%) developed cutaneous involvement at the onset of the disease and 10 (11%) after the hospitalization. The reported prevalence of cutaneous manifestations of COVID-19 has been variable. In studies that examined patients with and without skin findings related to COVID-19, skin disease prevalence varied from 0.19% to 20.45% [6, 7]. Disparities among reported cutaneous manifestation prevalence suggest a potential underreporting of skin symptoms associated with COVID-19 disease [4].

Reports of skin lesions are increasing, but clinical images and/or histopathological findings of these manifestations are often not shown, and the similarities of the clinical presentations have not yet been summarized and/or standardized [2, 8]. This lack of standardization makes it difficult to have a clear conception of the COVID-19 skin manifestations and, as a consequence, the establishment of a proper treatment.

It is emerging that there are 4 cutaneous clinical manifestations and several characteristics associated with COVID-19 that could be classified into the following patterns: exanthem (vesicular eruptions, maculopapular eruptions), urticarial lesions, vascular (pseudo-chilblain, purpuric/petechial and livedoid lesions) and acropapular eruptions [5, 9]. These patterns appear at different time points during the disease development and are correlated with infectivity, duration and severity, providing potential utility in epidemiological control [4, 5, 9, 10].

The aim of this chapter is to provide information on cutaneous manifestations of COVID-19 disease, focussing on early symptoms and on prognostic information.

2 Exanthem Pattern

An exanthem is a widespread nonspecific rash characterized by a generalized eruption of erythematous papules and macules that may be associated with fever or other symptoms [11, 12]. Exanthem may be the manifestation of an infectious disease or reactions to drugs [11]. Exanthem is not uncommon in viral infections and is a dermatological manifestation in COVID-19 infection [5].

2.1 Vesicular Eruptions or Varicella-Like Exanthem

The vesicular eruptions were first described by Marzano et al. and Galván et al. as a rare but specific COVID-19-associated skin manifestation [9, 13]. In the study conducted in Spain, which included 375 patients diagnosed with COVID-19, 9% of the patients presented the exanthema manifestation, characterized by small monomorphic vesicles, fluid-filled blisters, haemorrhagic content, with possible evolution to larger or diffuse vesicles [9]. The main regions affected have been reported as trunk and upper limbs [9]. The highest occurrence is in middle-aged patients, and it is associated with moderate disease severity. This manifestation appeared before the classical symptoms or up to 3 days after symptoms and lasted for 8 or 10 days. Itching was reported in some patients [5, 9].

In a case report article, Genovese et al. described an 8-year-old girl with a 3-day history of an asymptomatic papulovesicular skin eruption [14]. There were about 40 eruptions and few vesicles scattered bilaterally and symmetrically on the trunk. These eruptions appeared 6 days before the patient was diagnosed with COVID-19, and the skin manifestations were resolved within 7 days without any therapy.

Gioanotti et al. described two cases with a histopathological picture consistent with that of a viral exanthema [15]. One case was a 59-year-old woman admitted to the intensive care unit (ICU) at the University of Milan with bilateral interstitial pneumonia. Three days after admission, she

developed widespread erythematous macules on her arms, trunk and lower limbs that spontaneously improved within 5 days. In a second case, an 89-year-old woman was suffering from fever and cough for a 1-week duration. An exanthem on her trunk and arms was observed on admission at the ICU, but it improved spontaneously 8 days later [15]. Histological analysis showed vacuolar degeneration with disorganized keratinocytes and enlarged and multinucleate keratinocytes with dyskeratotic (apoptotic) cells. A dense inflammatory infiltrate may also be present [5].

2.2 Maculopapular Eruptions or Maculopapular Rash

Català et al. described seven major maculopapular patterns: morbilliform (45.5%), purpuric (14.2%), erythema multiforme-like (9.7%), pityriasis rosea-like (5.7%), erythema elevatum diutinum-like (2.3%), perifollicular (2.3%) and other maculopapular (20%) [16]. These manifestations appear frequently on the antecubital fossa and axillary folds [5]. A few cases showed infiltrated papules on the extremities, mostly the dorsum of the hands, that looked pseudovesicular or resembled erythema elevatum diutinum or erythema multiforme [9].

Galván et al. reported a maculopapular eruption prevalence of 47% among 375 patients [9]. These appeared at the same time as the other symptoms of infection and lasted for 3–10 days, and itching was reported in most of the patients. This was associated with more severe disease in older patients [5, 9].

A 3-year-old female patient diagnosed with COVID-19, presented with high fever but, after the fever resolved, she experienced a maculopapular rash which worsened by day 6 of the disease [17]. The identification of rashes in children with COVID-19 is an unusual and important condition that must be recognized to minimize or avoid viral transmission.

In another case, a 34-year-old man was admitted into the emergency room with a skin eruption that was not itchy but had started 2 days prior [18]. Seven days prior to his admission, he tested positive for COVID-19 and the main symptom was a slight fever of 37.5–38 °C. At consultation, a generalized maculopapular eruption was observed on his trunk, upper limbs, legs and face without involvement of the palmoplantar areas. He was administered an intravenous steroid bolus and antihistamines. The patient was discharged from the hospital 3 days after admission, following a complete regression of the cutaneous symptoms [18]. Skin biopsy showed some nonspecific features of viral-related exanthema, such as slight spongiosis and mild perivascular lymphocytic infiltrate [5].

3 Urticarial Lesions

The urticarial-like pattern has been described as acute, swollen, red wheals or plaques typically associated with pruritus disseminated in skin rash that healed in few days [5, 19]. These are mostly distributed on the trunk or dispersed. A few cases were palmar and itching, which were common for urticariform lesions [9]. Urticarial lesions lasted for a shorter period (mean 6.8 days), usually appeared at the same time as the other symptoms and were associated with more severe COVID-19 disease in adults [5, 9]. In the study of Galván et al., urticarial lesions had a prevalence of 19% [9].

Henry et al. observed one case of urticarial eruption before the onset of any fever or respiratory symptoms [20]. A 27-year-old woman presented with an odynophagia followed by diffuse arthralgia and pruritic disseminated erythematous plaques eruption with particular face and acral involvement, without cough or fever. The diagnosis of urticaria was confirmed by a dermatologist, and she was diagnosed for COVID-19 48 h later.

Fernandez-Nieto et al. demonstrated an example of an urticariform rash in a 32-year-old woman with COVID-19 [21]. The skin manifestation appeared 6 days after the onset of symptoms. Histological examination revealed a perivascular infiltrate of lymphocytes, with some eosinophils and upper dermal oedema. Oral antihistamines were added to her treatment, with clinical and symptomatic improvement within 5 days.

4 Vascular Pattern

Several ischemic/coagulopathy lesions have been described in SARS-CoV-2 infection, including chilblain-like lesions (the "COVID toe"), especially common in children, along with livedoid, purpuric and acral necrotic lesions [5]. Most of these clinical manifestations may have a thrombotic or microthrombotic pathological counterpart. In addition, cases of immune thrombocytopaenic purpura and of the antiphospholipid antibody syndrome have been described with their well-known cutaneous manifestations [5].

4.1 Acral Areas of Erythema with Vesicles or Pustules (Pseudo-Chilblain)

Patients can present with erythematous or violaceous papules and macules, bullae or digital swelling [22]. Acral areas of erythema and/or oedema with some vesicles or pustules (pseudo-chilblain) are usually asymmetrical. These lesions have purpuric areas, acral sites, mostly toe, soles and hands, with possible bullous evolution. In these manifestations, distal arteries and veins constrict, which can lead to pruritic and tender wounds on the extremities [9, 19].

A 19% prevalence of chilblain-like lesions was documented in the Spanish series [9]. There are nearly 100 cases of chilblains associated with COVID-19 already described in the literature because some studies focused on this finding or anatomic region specifically [3, 4, 9, 22, 23].

Pseudo-chilblain lesions typically affect young patients (mean age 32 years), take place later in the course of COVID-19 disease and are associated with less severe disease (in terms of hospital admission, pneumonia, intensive care unit admission or mortality). These lesions could cause pain (32%) or itch (30%) [9]. Galván Casas et al. described 71 cases which had a mean duration of 12.7 days [9]. This was also evidenced by Recalcati who described 14 cases occurring in mostly children and young adults [22]. In this latter case series, 10 patients had foot involvement, while only 6 had hand involvement [22]. The pseudo-chilblain is a specific COVID-19-associated skin manifestation as vesicular eruptions.

4.2 Purpuric/Petechial Lesions

A wide spectrum of purpuric and petechial lesions has been described as possible manifestations associated with SARS-CoV-2 infection. These appear at any time during the COVID-19 course, are localized on the trunk, buttock and limbs, typically in adult patients and could be associated with symptoms such as a burning sensation [5]. These lesions may cause difficulties with differential diagnosis. In Thailand, a COVID-19-related skin rash with petechiae was initially misdiagnosed as a Dengue virus infection [24].

Magro et al. examined cutaneous tissues from 5 patients with SARS-CoV-2 infection and severe respiratory failure in the USA and described two patients with widespread purpuric skin manifestation. The first developed a retiform purpura with extensive surrounding inflammation on the buttocks, while the second had mildly purpuric reticulated eruptions on the chest, legs and arms [5, 25]. These dermatologic signs are a generalized microvascular thrombotic disorder [25].

In a French study, Bouaziz et al. found that 2 out of 14 patients with purpuric rash evolved in necrotic lesions [23] and Zulfiqar et al reported a case of a lower extremity purpura as a skin sign of immune thrombocytopenic purpura related to SARS-CoV-2 infection [26]. At the same time, Diaz-Guimaraens et al. observed a patient with erythematous macules, papules and petechiae affecting the popliteal fossae, buttocks and anterior thighs but sparing acral and mucosal regions [27].

4.3 Livedo or Necrosis

Galván et al. considered livedo and necrosis as belonging to the same clinical pattern, suggesting a major systemic occlusive vascular disease. In their series, these lesions had a prevalence of 6% and were present in older patients and in patients with more severe disease and a 10% mortality rate [9]. These manifestations appear at any time

during the disease course and are localized on the limbs [5].

Manalo et al. reported two cases of transient livedoid lesions [28]. A patient developed a transient nonpruritic blanching unilateral livedoid patch on the right anterior thigh resembling livedo reticularis. Another patient showed a transient unilateral asymptomatic rash on her right leg resembling livedo reticularis that appeared after occasional sun exposure. This lasted for almost 20 min and did not recur. According to the authors, these might be primary lesions of COVID-19 or simply indicate complications leading to vascular occlusion, as COVID-19 has been linked to alterations in coagulation and vascular damage [28, 29]. Livedoid or necrotic lesions occur late in the evolution and are probably unhelpful for diagnosis. However, they are consistent with the idea of vascular damage due to COVID-19 [9].

5 Acro-Papular Eruption

An acral papular eruption can be the expression of several viral diseases. Recently, papular eruptions with acral distribution have been associated with SARS-CoV-2 infection. Estébanez et al. reported one case of COVID-19 associated with the development of confluent erythematous-yellowish pruritic papules 13 days after being tested for SARS-CoV-2 [30]. Despite topical steroid treatment, the lesions became confluent erythematous plaques with a pruritic component 3 days later.

Proposed immunopathological mechanisms for the viral associated papular eruptions include immune complexes or delayed hypersensitivity reactions, and it is plausible that similar immunomediated mechanisms may be also operative in COVID-19-associated papular eruptions [5].

6 Treatment

Oral antihistamines, local corticosteroids and triamcinolone cream are some treatments for cutaneous manifestations of COVID-19 disease [20, 21, 30, 31]. These agents are used in combination with systemic drugs for infectious complications and symptoms. Oral antihistamines contributed to clinical and symptomatic improvement in two patients with urticaria [20, 21]. Local corticosteroids were given to a patient with erythematous-yellowish papules, although these papules progressed into hardened and pruritic erythematous plaque 3 days later despite the treatment [30]. Another patient with confluent erythematous patches and worsening dermatitis demonstrated a good response to triamcinolone cream [31].

Considering the lack of standardization in the description of the COVID-19 skin manifestations, it is difficult to establish effective treatments. On the other hand, some of the skin lesions reported so far resolved with no treatment. This evolution probably depends of some variants such as age, pattern of the manifestation, immunological and nutritional status of the patient among others.

7 Pathophysiological Mechanisms

To understand the pathophysiological mechanisms for COVID-19 disease, it is important to identify targets for specific interventions, with consideration that some aspects of SARS-CoV-2 infections have possible correlations with the skin [2]. The vascular lesions have been described during COVID-19 infections and may be due to different possibly overlapping mechanisms, including a direct action of the virus on endothelial cells, an indirect effect involving the triggering of immune or autoimmune reactions or to an exaggerated and uncontrolled host response accompanying the well-known “cytokine storm” [5, 23].

Angiotensin-converting enzyme 2 (ACE2) is a cellular receptor for SARS-CoV-2, which is expressed in several tissues, including endothelial cells and keratinocytes in skin [5, 32–34]. The entry of virus in human cells induces angiotensin II accumulation, and this excess may contribute to vessel dysfunctions such as vasoconstriction, vascular permeability and abnormal tissue remodelling [32].

The microvascular dysfunction can lead to increased vasoconstriction and organ ischaemia, inflammation and a further pro-coagulation state [5, 23]. Coronavirus primo-infection-induced chilblains (perniosis) may represent microangiopathic changes induced by an increased type I interferon reaction. The reaction may protect the host from viral replication, thereby explaining why young patients run a short and indolent course of the disease in contrast to older patients with a delayed or insufficient type I interferon reaction, resulting in increased morbidity and mortality [35].

Soluble biomarkers of complement activation have been observed in patients with COVID-19 disease, and diffuse microvascular vasculitis could be the result of complement system activation [36]. Colocalization of SARS-CoV-2-specific spike glycoproteins with complement components in the skin was also documented [25]. One study found significant complement protein deposition (C5b-9, C4d, and MASP2) in the dermal capillaries and an interstitial and perivascular neutrophilia with prominent leukocytoclasia, suggesting a vasculitic phenomenon. A similar pattern has been demonstrated in the skin of patients with retiform and purpuric lesions [25]. This has been based on high concentrations of lymphocytes without eosinophils, papillary dermal oedema, epidermal spongiosis and lymphohistiocytic infiltrates [8].

Dastoli et al. have speculated that urticaria in the setting of COVID-19 is likely to be associated with systemic eosinophilia, which in turn leads to better outcomes of COVID-19 infection because eosinopenia is frequently observed in patients with COVID-19, and may have a prognostic value in more severe cases [37]. Figure 9.1 shows a schematic of the pathophysiology of COVID-19 in skin.

8 Conclusions

Patients with COVID-19 are most commonly with respiratory symptoms. However, multiorgan involvement can occur, with multiple skin manifestations. Some case reports have described that the dermatological findings may appear prior to respiratory symptoms, although most studies suggest skin manifestations are present several days after the onset of other symptoms. These signs and symptoms may assist clinicians in identification of the disease before the development of respiratory symptoms and may also be used to identify complications requiring treatment [19]. Dermatological findings may include a maculopapular rash, urticaria, vesicular rash, petechiae, purpura, chilblains, livedo racemosa, and distal ischemia. These rashes should trigger consideration of COVID-19, and understanding these manifestations is important to help identify potential COVID-19 patients and properly treat complications. The patterns of cutaneous manifestations associated with COVID-19 infection are summarized in Table 9.1.

The signs and symptoms of skin manifestations precede the classical COVID-19 symptoms, as evidenced in some patients with urticarial rashes. Therefore, clinicians should be familiar with urticarial rashes and other cutaneous manifestations of COVID-19 to enable early diagnosis of infected individuals and limit viral spread [38]. The severity of the associated disease appears to follow a gradient, from less severe disease in pseudo-chilblain to most severe cases in patients with livedoid presentations, as shown by the increasing percentages of pneumonia, hospital admission and intensive care requirements [9].

Future studies should include race/ethnicity information because some skin findings may be more common in some races [4].

Careful documentation and robust reporting of cutaneous manifestations associated with COVID-19 are needed to augment our understanding of disease presentation and epidemiology. Improved understanding of the cutaneous manifestations, comorbidities and treatments will enhance our ability to provide better clinical care and support our colleagues on the front lines of this rapidly evolving pandemic [4]. However, the overall similarities in the clinical presentation of these skin manifestations have not yet been summarized and require further study.

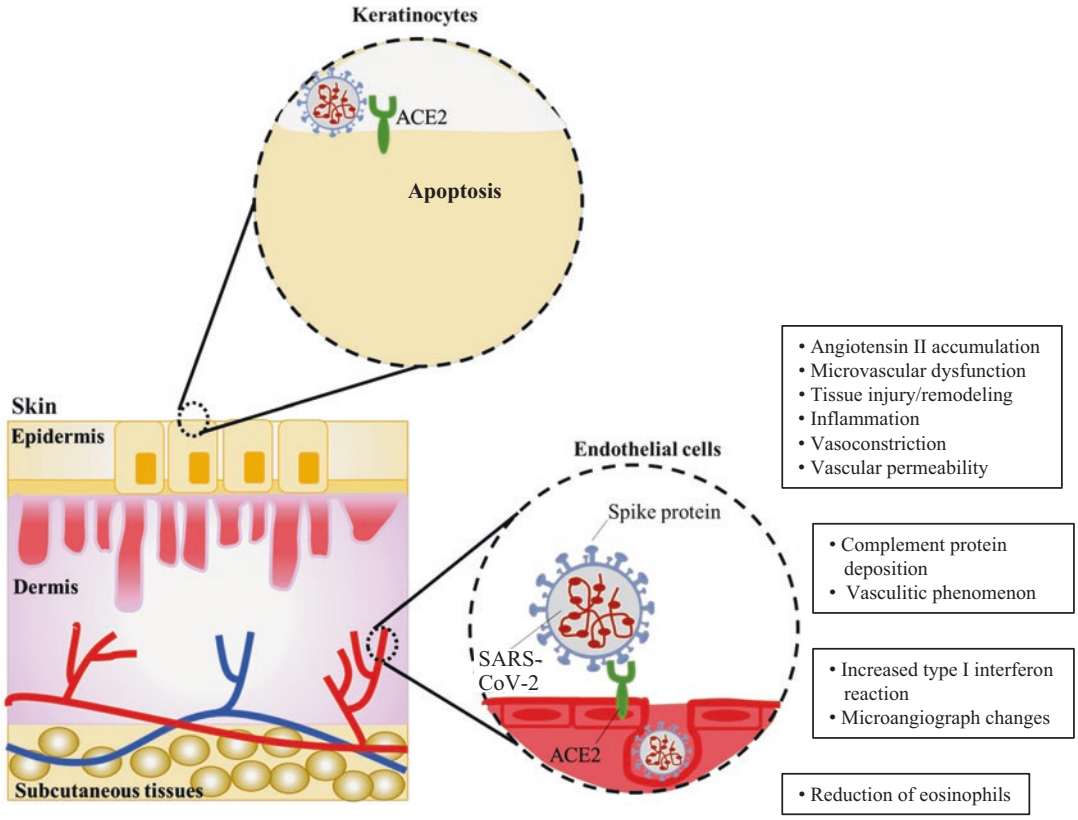

Fig. 9.1 Pathophysiology of COVID-19 in skin

Table 9.1 Pattern of Cutaneous Manifestations of COVID-19

Pattern	Subtype	Localization	Onset	Age	Symptoms	Severity
Exanthem	Vesicular eruptions	Trunk, upper limbs	Early	Middle-aged patients	Itching and fever	Moderate
	Maculopapular eruptions	Antecubital fossa and axillary folds	Early	Older patients	Itching and fever	Severe
Urticarial lesions	Urticarial lesions	Trunk or dispersed	Early	Adult patients	Pruritus, fever	Severe
Vascular	Acral areas of erythema with vesicles or pustules (pseudo-chilblain)	Acral sites, mostly toe, soles and hands	Late	Young patients	Pruritus, burning and pain	Less
	Purpuric/petechial lesions	Trunk, buttock and limbs	Any time during the COVID-19 course	Adult patients	Burning	Severe
	Livedo or necrosis	Limbs	Any time during the COVID-19 course	Older patients	Pruritus, burning and pain	Severe
Acro-papular eruption	Acro-papular eruption	Limbs	Late	Adult	Pruritus	Moderate

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How Do We Manage Breastfeeding During the COVID-19 Pandemic?

10

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Abstract

The COVID-19 pandemic is causing global disturbances and creating many questions in every aspect of life. Since it influences health in multiple ways, including sexual and reproductive health, publishing in all of these areas has increased lately. One aspect that requires basing on scientific evidence is breastfeeding. There are some controversies in the literature on the breastfeeding management in confirmed COVID-19 mothers. Breast milk is excellent for the infant's nutritional needs and growth, because it includes all of the nutrients an infant requires. It promotes the immature immune system of the infant and reinforces defense mechanisms against infectious and other agents during the breastfeeding period. While limited clinical research is available, we can build on what we know about breastfeeding and previous similar outbreaks to plan and manage this crisis. The aim of this chapter is to provide pediatricians with further guidance on breastfeeding and associated safety measures during the COVID-19 crisis, particularly in instances where a mother has or may have COVID-19. This will also be a benefit to future epidemics and pandemics.

Keywords

Coronavirus · SARS-CoV-2 · COVID-19 · Breastfeeding · Breast milk

1 Introduction

The novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) erupted in Wuhan, China, in December of 2019 and spread rapidly to all parts of China [1]. Since this time, the entire world has been affected [1] and the World Health Organization (WHO) announced the outbreak of the disease as an international public health emergency [2]. While most of the infections occur in adults older than 60 years [1], the health impact of SARS-CoV-2 spans over to all age groups including pregnant mothers. For mothers and their newborns, breast-

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feeding during this pandemic needs special attention because of both the potential short- and long-term health consequences. A few studies have evaluated the infection of neonates with SARS-CoV-2, and, thus far, none of these demonstrated breastfeeding as a means of transmitting the virus [3–6]. Reports and case series from China have proposed that, unlike other infections caused by coronaviruses and the H1N1 virus, the impact of COVID-19 in pregnant women is similar to that of adults of the same age group [4, 7, 8]. There is no evidence of greater clinical severity throughout pregnancy or of a higher prevalence of obstetric problems in patients infected by COVID-19 [4, 9, 10]. For example, a study that included 9 pregnant patients with pneumonia caused by COVID-19 did not find the presence of the virus in samples of 6 patients collected from amniotic liquid, blood from the umbilical cord, breast milk, or oropharynx swab of the newborn [4]. Thus, the vertical mother–fetus transmission appears to be possible but has not been reported. Zeng et al. [5] reported on 33 newborns of mothers infected by SARS-CoV-2 who presented mild to moderate signs and symptoms of early neonatal infection. Three of the newborns had positive molecular tests for COVID-19, and the authors questioned the existence of possible vertical transmission of the virus since all measures recommended for infection control were adopted. However, the samples of amniotic fluid, umbilical cord blood, and breast milk did not demonstrate the presence of the virus, raising the possibility that the infants may have been infected separately.

There are some controversies in the literature on breastfeeding management in confirmed COVID-19 patients. Wang et al. did not recommend breastfeeding in suspected, uncured cases and in those taking lopinavir or ritonavir medications [11]. Moreover, neonatal isolation was recommended in newborns suspected or confirmed to have SARS-CoV2 infections [12]. On the other hand, the Union of European Neonatal and Perinatal Societies advise direct breastfeeding under precise measures of infection control in asymptomatic mothers with COVID-19. However, when the mothers had severe infections, the neonates should be fed apart from the mothers with freshly expressed breast milk [13].

Other international health organizations and medical societies such as the WHO [14], Royal College of Obstetricians and Gynaecologists (RCOG) [15], and the Italian Society of Neonatology (SIN) [13] also advise breastfeeding with hygiene precautions and recommend mother and newborn separation only in instances of poor maternal health conditions or in cases where treatments need to be provided to the neonate. In contrast, USA agencies, including the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics (AAP), considered a more conservative approach with regard to potential maternal–child transmission. Modified guidelines published by the AAP suggested both direct breastfeeding and use of extracted breast milk. As another factor, the CDC recognized the importance of mother–baby contact and for shared final decision making between family and physician, but this agency does not give clear guidance on breastfeeding [16]. The AAP recommends temporary separation of mother–infant and administration of extracted breast milk rather than direct breastfeeding in cases where the mother has COVID-19 to reduce the risk of postnatal infant infection from maternal respiratory droplets [17].

It should be stated that separations of mother–infant risk a potentially detrimental impact to the infant. This can lead to a vicious cycle of separation-induced stress, decreased breast milk production, poor breastfeeding success, and loss of skin-to-skin contact, all of which increase the risk of infant instability and the potential requirement for support in a neonatal intensive care unit [18]. Also, breast milk contains different antimicrobial substances, anti-inflammatory agents, and factors that enhance the development of the immune system and decrease the occurrence of respiratory tract infections [19–21]. Furthermore, breastfeeding provides health advantages for both the mother and infant and is thought to be the ideal food for children in the first 6 months of life for healthy growth [22, 23]. It promotes the immature immune system of the infant and reinforces defense mechanisms against infectious and other

agents during the breastfeeding period [23]. Numerous studies indicate that continued, frequent breastfeeding is related to greater linear growth and greater protection of child health by delaying maternal fertility postpartum and reducing the child's risk of morbidity and mortality [24].

While limited clinical research is available, we can build on what we know about breastfeeding and previous similar infection outbreaks to plan and manage this crisis. This review is aimed at healthcare professionals involved with maternal care and breastfeeding, in both an acute care and community health setting, by providing up-to-date evidence to promote health in infants who may or may not have been exposed to COVID-19. As our knowledge about COVID-19 is rapidly evolving, all recommendations may be subject to change. Furthermore, long-term studies that may take place years after this virus has passed may provide different information and lead to different recommendations. Accordingly, the final aim of this chapter is to provide pediatricians with the most up to date guidance on breastfeeding and associated safety measures during the COVID-19 pandemic, particularly in instances where a mother has or may have COVID-19 disease, and considering the caveat that these recommendations could change as new information becomes available or a new virus emerges.

2 Benefits of Breastfeeding

Breast milk protects neonates, infants, and children against morbidity and mortality [25, 26]. The protective impact is particularly strong against infectious diseases, because of the direct transfer of antibodies, anti-infective agents, and long-lasting immunological capacity and memory [27]. Breastfeeding has both short- and long-term advantages for the mother and her infant. To achieve the greatest benefit from the protective agents in breast milk, every effort should be made to support early initiation of the process. Early initiation of breastfeeding stimulates hormones and facilitates mother–baby bonding [28–30].

In 2016, the Lancet Breastfeeding Series reported that breastfeeding could prevent about 823,000 child deaths annually [25]. Breastfeeding decreases 64% of morbidity and mortality due to diarrhea and more than 70% in the severity of respiratory syncytial virus (RSV) [26]. These findings indicate protective advantages of breastfeeding, which could be seen in cases of COVID-19 infections. Although the neonate has an immature immune system, the colostrum which is the first form of milk produced by a mother acts as a powerful immune enhancer, protecting infants against infections via bioactive factors and immunoglobulin A (IgA) antibodies. Breast milk has an abundance of immunoglobulins, lactoferrin, lysozyme, and cytokines, which play an important role in defending against detrimental microorganisms and by regulation of the immune responses [31]. In addition, human milk contains large quantities of oligosaccharides made by the mother's microbiome, which provides probiotics for the infant's developing immune system [32–34].

The best way to enhance successful breastfeeding is to ensure that mother–infant skin-to-skin contact occurs [35]. By ensuring this occurs immediately after birth, the infant's microbiome can develop from the mother's microflora [36]. Skin-to-skin contact in this way also enhances blood glucose levels 75–90 min after birth, as well as cardiorespiratory stability [30], and it significantly decreases stress levels in the infant and mother [37]. Thus, keeping the mother and infant together can decrease birth stress and neurodevelopmental disorders in the infant. Furthermore, the smell, touch, and voice of the mother normally soothe an infant [38].

3 COVID-19 and Breastfeeding: Current Evidence

A recent paper published in the Lancet discussed the point that all of the information available on pneumonia caused by COVID-19 disease is based on data from the general population [39]. This suggests that the data may not be relevant to breastfeeding. However, data from previous studies regarding the SARS-CoV epidemic in 2002–2004

could have relevance to the current SARS-CoV-2 pandemic, potentially enabling us to draw on the experience gained from management of this and other epidemics to inform present practice guidelines [40].

Vertical transmission is the term used for passage of a pathogen from mother to infant during the period before and after birth. The potential routes include placental blood during pregnancy, the birth canal during delivery, and during postpartum feeding [3]. However, Zhu et al. reported no evidence of vertical transmission in COVID-19 cases [3], and a retrospective review of clinical records of 9 pregnant women by Chen et al. did not find any data to support transmission of SARS-CoV-2 from mother to child in amniotic fluid, cord blood, neonatal throat swab samples, and breast milk samples [4]. While some case studies of infants infected with COVID-19 have been reported, none of these tested for the presence of the virus in amniotic fluid, cord blood, neonatal throat swabs, or breast milk [6, 41–43]. In addition, none of the case studies published by researchers in Spain [44], Vietnam [41], China [43], and the USA [45] demonstrated transmission of the SARS-CoV-2 virus through breast milk. In support of this, a systematic review by Duran et al. and a study by Lu and Shi noted that breast milk does not seem to be a route of transmission of the virus [10, 46]. Samples of breast milk from 18 women infected with SARS-CoV-2 were investigated, and even though the viral RNA was detected in one sample of milk, follow-up culture of the same sample was negative [47].

4 Guidelines on Breastfeeding for Mothers with Suspected or Confirmed COVID-19

Reviews by the North-American Centers for Disease Control and Prevention [48] and the Royal College of Obstetricians and Gynaecologists, London [49], suggested that if the mother is willing and in clinical conditions to breastfeed her child, she must be well-informed and agree with the preventive measures necessary. In agreement, the chief editor of Breastfeeding Medicine stated: “Given the reality that mothers infected with coronavirus have probably already colonized their nursing infant, continued breastfeeding has the potential of transmitting protective maternal antibodies to the infant via the breast milk. Thus, breastfeeding should be continued with the mother carefully practicing handwashing and wearing a mask while nursing, to minimize additional viral exposure to the infant [50].” Similarly, the Brazilian Federation of the Gynecology and Obstetrics Associations published a note emphasizing that the advantages of breastfeeding outweigh any potential risks of virus transmission via breast milk [51]. Hence, they suggested that women infected by COVID-19 who wish to breastfeed should be encouraged to do so [51].

The WHO, the Ministry of Health, the Secretary of Health of the State of São Paulo, the Brazilian and Global Network of Human Milk Banks, and the American Academy of Pediatrics all support the point that breastfeeding is maintained with the necessary precautions in case of infection by COVID-19 for the health of the child and mother [48, 52–55]. This guidance is valid only if the mother wishes to breastfeed her child and is in a clinical condition to do so. If the mother does not feel safe to breastfeed while infected, it is suggested that their milk is extracted and given to the child that way. In cases of COVID-19 infections, it is recommended that the precautions below are followed since an infected mother could pass the virus via respiratory droplets when in contact with the child, such as during breastfeeding [48, 53–56]:

- Wash hands for at least 20 s before touching the baby or extracting breast milk.
- Wear a face mask completely covering the nose and mouth and avoid talking or coughing through breastfeeding.
- Immediately change masks if coughing or sneezing occurs or at every feeding.
- In the case of manual or mechanical extraction of human milk, strictly observe guidelines.
- Follow guidance for cleaning the pumps for milk extraction after each use.

- Consider the probability of asking for help from a healthy individual to feed the newborn with the breast milk using a cup or spoon.
- It is important that the person who will feed the newborn with breast milk learns how to do this with the help of healthcare professionals.

5 Conditions Recommended for Starting and Maintaining Breastfeeding

The mother should be encouraged to breastfeed her child, always complying with the protection measures mentioned above. It is important she receives all the support and guidance necessary from a multiprofessional team, including mental health objectives. This should also be considered due to the long-term health benefits linked to breastfeeding. Breast milk not only protects against morbidity and mortality in the neonatal and postneonatal periods, but also can extend throughout childhood and even to adolescence and adulthood. This protection includes prevention of dental malocclusion, overweight and obesity, allergies and other chronic diseases. It also favors the bond between mother and child and has been linked with higher intellectual coefficients and greater professional success [52, 54, 55].

There is also the protection resulting from breastfeeding to the maternal health, with observations of less postpartum bleeding, aiding in the decrease of weight gained during the pregnancy, decreased risk of breast and ovarian cancers, as well as lower incidence of type 2 diabetes and postpartum depression. Exclusive breastfeeding also provides a contraceptive effect which assists in the spacing between pregnancies, even when no other method is used for this aim [55].

In the case of COVID-19 and other pandemics, the contact between mother and child during breastfeeding is likely to benefit the mother from an emotional aspect, providing a means of coping with difficult periods of quarantine and social isolation. However, given reports of cases in which the newborn contracted the disease (usually a mild form) via respiratory droplets from the mother or health professionals, it is important to comply at all times with the protection protocols during the infectious period [4, 5, 9, 10].

6 Conditions in Which It Is Recommended to Extract Breast Milk

Mothers who are not in good clinical condition or who do not feel safe to maintain direct contact with their child should choose for extraction of breast milk and get help from a trained professional on how to feed the newborn. Milk extraction should also be an option when the newborn is premature or when they are not in a clinical condition that allows sharing of the same room. The raw milk must be extracted in an isolated room, ideally with the assistance of a trained professional, and can be stored for up to 12 h in a refrigerator. Milk extracted from mothers with suspected or confirmed COVID-19 infection should not be kept inside a milk bank. Instead, it should be stored in a locked refrigerator used specifically for that purpose. It is recommended that the mother be informed about the frequency of extraction, which should be 4–6 times per day, to maintain sufficient milk production until the end of isolation [53–55].

7 Conditions in Which Recommended for Not Breastfeeding or Extracting Breast Milk

Mothers may fear transmitting the virus to the newborn, either by contact or through breast milk, or they may not be in a clinical condition to breastfeed or extract their milk. Under these conditions, they should be separated from their baby, and the baby should receive milk from another source throughout the period of isolation. Even in these situations, it is recommended that the mother still extracts milk in order to maintain production levels, although this milk will be discarded [55].

In the case of the baby, a bottle should not be used for the administration of milk from the other

source so that there is the probability of resuming breastfeeding after the isolation period. The use of a cup or spoon is recommended. Also, the donor milk can only be used for the newborn after pasteurization. Although this process maintains the nutritional value of the milk, it decreases its immunological effects by about 30% to 40%. Regarding the nutritional components, only lipase and some thermolabile vitamins are destroyed in pasteurized milk [54, 55].

8 Breastfeeding by Mothers Undergoing Medications for COVID-19

For mothers who are receiving medications for SARS-CoV-2 infection and wish to breastfeed, the infant's exposure to the antiviral medication is a potential concern. Remdesivir [57], hydroxychloroquine, and sarilumab [58] are the most common current treatment options under investigation for confirmed COVID-19 with remdesivir receiving emergency use authorization on May 2020 from the US Food and Drug Administration (FDA) for adults and children with confirmed COVID-19. While transition of remdesivir to infant through breast milk is unknown, no adverse effects were reported in a newborn whose mother received this therapy for infection with Ebola [59]. Moreover, remdesivir has a large molecular weight (602.6 g/mol), significantly limiting its transition into breast milk. A study on 33 mothers undergoing long-term hydroxychloroquine medication found low levels of the medication in the milk between 1.9 and 3.2% of the maternal dose [60]. As mentioned in the National Library of Medicine LactMed database, infants exposed to hydroxychloroquine during breastfeeding receive only small volumes of this drug in breast milk. In infants up to at least 1 year of age, careful follow-up reported no adverse events on growth, vision, or hearing [61]. Sarilumab is also a large protein molecule probably with low levels in human milk. If sarilumab is needed by the mother, the European League Rheumatism (EULAR) task force does not suggest discontinuation of breastfeeding, while the NLM database suggests exercising caution until more evidence is available [62].

9 Conclusions and Future Perspectives

Breastfeeding is the safest and best protective measure available for healthy and at-risk infants and their mothers and should therefore be continued during the COVID-19 pandemic. In addition, mothers and infants should not be separated and their skin-to-skin contact is encouraged. In exposed or infected mothers, additional droplet protection should be taken by the mother by her wearing a face mask when handling and feeding her infant. When mothers are too sick to breastfeed, they should still be supported to extract their milk, and the infant should be fed by a healthy individual. Breastfed infants have an advantage in receiving additional protection against SARS-CoV-2, and the current evidence suggests that the virus is not transmitted via this means. Breast milk includes antibodies and other factors, which help to protect children against many common childhood diseases, and breastfeeding confers many short- and long-term health advantages for both the mother and child. Efforts should be made to advise and support mothers with breastfeeding, even in states when the mother has suspected or confirmed COVID-19 infection. Therefore, the advantages of continued breastfeeding outweigh the risk of breastfeeding cessation and potential COVID-19 transmission [63].

The current WHO recommendations suggest that mothers should breastfeed their babies, exclusively during the first 6 months of life [64, 65]. After the addition of complementary food, it is suggested that mothers continue breastfeeding for at least 6 additional months and, if possible, carry on with this practice up to the age of 2 years and beyond. Hence, COVID-19-affected or COVID-19-suspected mothers should be informed about the importance to continue providing their infants breast milk and that this goal can be achieved by adopting suitable hygiene and

safety protocols. However, it should be stated that further research is required including studies aimed at determining any long-term effects of breastfeeding from mothers with different severities of COVID-19 infection. This will also provide important insights into future epidemics and pandemics.

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11 Deep Learning Analysis in Prediction of COVID-19 Infection Status Using Chest CT Scan Features

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Abstract

Background and aims

Non-contrast chest computed tomography (CT) scanning is one of the important tools for evaluating of lung lesions. The aim of this study was to use a deep learning approach for predicting the outcome of patients with COVID-19 into two groups of critical and non-critical according to their CT features.

Methods

This was carried out as a retrospective study from March to April 2020 in Baqiyatallah Hospital, Tehran, Iran. From total of 1078 patients with

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COVID-19 pneumonia who underwent chest CT, 169 were critical cases and 909 were non-critical. Deep learning neural networks were used to classify samples into critical or non-critical ones according to the chest CT results.

Results

The best accuracy of prediction was seen by the presence of diffuse opacities and lesion distribution (both=0.91, 95% CI: 0.83-0.99). The largest sensitivity was achieved using lesion distribution (0.74, 95% CI: 0.55-0.93), and the largest specificity was for presence of diffuse opacities (0.95, 95% CI: 0.9-1). The total model showed an accuracy of 0.89 (95% CI: 0.79-0.99), and the corresponding sensitivity and specificity were 0.71 (95% CI: 0.51-0.91) and 0.93 (95% CI: 0.87-0.96), respectively.

Conclusions

The results showed that CT scan can accurately classify and predict critical and non-critical COVID-19 cases.

Keywords

COVID-2019 · Chest CT scan · Computed tomography · Deep learning · Prediction

1 Introduction

The new coronavirus disease 2019 (COVID-19) was first encountered in November 2019 in Wuhan, Hubei Province, China [1, 2]. It has now affected people in over 200 countries around the globe, and the World Health Organization (WHO) declared it as a pandemic on the March 11, 2020 [3]. The Regular daily reports from countries around the worlds have shown a total of 118,760,083 confirmed COVID-19 cases, 94,337,038 recovered cases, and 2,634,301 deaths as of March 11, 2021. The first report of the COVID-19 outbreak in Iran was reported from Qom city on February 19, 2020 [3]. The infection rapidly disseminated to all provinces of Iran, consequently putting it in the list of the top 10 affected countries in the world with respect to COVID-19-related deaths with 61,016 out of 1,723,470 cases at present (March 11th, 2021) [4].

One of the most important consequences of COVID-19 besides liver disease and gastrointestinal disorders [5, 6] is severe acute respiratory syndrome, including pneumonia, pulmonary edema, and acute respiratory distress syndrome (ARDS) [7]. For this reason, precise diagnostic tests are needed for fast diagnosis, isolation, and treatment of infected patients. One method of diagnosis is the use of laboratory testing kits. However, according to the Center for Disease Control and Prevention (CDC) in the first wave of the virus, these kits may have led to falsely negative results in some cases, potentially due to laboratory error or indefinable viral material in the specimen [8, 9]. In addition, most of these kits are time-consuming and expensive [10]. Therefore, chest computed tomography (CT) scanning was employed as a potentially helpful instrument for diagnosing pneumonia in all patients, as well as in suspected positive cases for COVID-19, as this provides a visual indication of viral effects in lungs [11, 12]. Previous studies have shown an important visual specification in lung of patients with COVID-19 symptoms such as ground-glass opacities (GGOs) and areas of increased lung density called consolidation [13, 14]. If a patient has longer time of infection, these specifications become more frequent and more likely to spread across both lungs [15]. Studies from China have demonstrated that increasing time from onset of symptoms leads to greater severity of disease and other findings as specific signs, such as linear opacities, crazy-paving pattern, reverse halo sign, pleural effusion, intraregional traction bronchiectasis, and lymphadenopathy [16, 17].

Deep learning neural networks are statistical methods and algorithms for predicting future events and have shown good predictive accuracy in different fields of research including public health [18–21]. This method has high flexibility as a mathematical tool and has also been widely

used for classification and forecasting [22, 23]. Here, we have used deep learning to predict the outcome of COVID-19 patients as either critical or non-critical, according to chest CT findings. This model can also facilitate prediction of outcome in new cases.

2 Methods

2.1 Study Design and Participants

This study was designed as a retrospective analysis and carried out in the Baqiyatallah Hospital, Tehran, Iran from March to April 2020. Patients with COVID-19 who underwent chest CT scans participated in the study. A positive result on a reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay of a specimen collected via nasopharyngeal swab was considered for diagnosis of COVID-19 infection [24]. This led to a final number of 1,078 confirmed COVID-19 patients being enrolled in this study. Demographic characteristics including sex and age and radiological chest CT data were gathered and evaluated. Inpatient clinical follow-ups led to classification of participants into two groups of critical and non-critical cases. Of these, 169 patients were recognized as critical by either being admitted to the intensive care unit (ICU) or having died, and 909 patients were recognized non-critical, with 55 patients admitted to the routine ward, and the rest discharged as outpatients (n=854).

The Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran, approved this retrospective study with the code: IR.BMSU.REC.1399.024 and informed written consent had been provided by all patients.

2.2 CT Examination and Image Analysis

Chest CT, especially high-resolution CT (HRCT), is a promising imaging tool for monitoring the disease and can detect small areas of GGOs [25]. Radiologists can easily evaluate the pneumonia severity qualitatively or semi-quantitatively by visual scoring [9]. A 16-row detector CT scanner (General Electric; Windsor, CT, USA) was used for all CT scan examinations. The protocol was based on COVID-19 low-dose thoracic CT scanning as follows:

- Tube voltage – 100 kVp, 120 mA
- Slice thickness – 2.5 mm
- Reconstruction interval – 1.25 mm
- Pitch – 1.75
- Speed 35 mm/rot
- Detector configuration – 16*1.25
- CT dose index (CTDI) – 3.5 mGy

For performing non-contrast chest CT scan images, patients were in the supine position at full inspiration. The CT findings were evaluated by two experienced chest radiologists who were blinded to the clinical data. The inter-rater coefficient agreement between the two radiologists was r=0.98 (p-Value <0.0001). Following characteristics based on the Fleischner Society Nomenclature recommendations and similar studies [26, 27], the lesions were examined and a thin-section CT involvement score for determining the extent of lesions was assigned on the basis of all abnormal areas involved [28]. The range of scores was between 0 and 5 and was assigned to each lobe and classified as follows: score 0, 0% involvement; score 1, less than 5% involvement; score 2, 5–25% involvement; score 3, 26–49% involvement; score 4, 50–75% involvement; and score 5, greater than 75% involvement. Thus, each lobe had a score of 0–5, with a total possible score of 0–25 (there are 5 lobes in lungs).

2.3 Statistical Analysis

Comparisons Between Two Groups

The mean ± standard deviation (SD) was used for describing data for continuous variables. For categorical variables, frequency and percentage were reported. For comparison between numbers of involved lobes and age in two groups, the Mann-Whitney U test and independent T-test were performed. In addition, evaluation of the

association between categorical variables was achieved using the Chi-square test. All analyses were done using the R software, version 3.6.3.

Deep Learning Analyzing and Classification

Deep learning neural networks were utilized to classify patients into critical or non-critical cases. First the input variables (age, gender, lesion distribution, lesion type, presence of diffuse opacity, and number of involved lobe) were scaled by using the following formula [29]:

$$\frac{X - X_{\min}}{X_{\max} - X_{\min}}$$

The data were split into two groups as training (70%) and validation (30%) sets. Following this, a supervised back propagation model with 3 layers was fitted to the data. The first layer was input data, and the next two sets were the hidden layers with 5 and 3 nodes, respectively. The utilized activation function was logistic. All analyses were performed using the neuralnet package in R 3.6.3. After fitting the data, the performance of the prediction was checked using indices such as accuracy, sensitivity, specificity, true positive rate (TPR), and true negative rate (TNR) [30].

3 Results

3.1 Demographic Characteristics and Chest CT Findings

From 1,078 confirmed patients of COVID-19, 169 cases were critical (65 in ICU and 104 died) and 909 were non-critical cases. Out of the total cases, 737 (68.4%) were male, and the mean ± SD age of subjects was 53±14.37 years (range: 14–92 years). The mean age in the critical group was significantly higher than the non-critical group (61.24±13.48 vs. 51.47±14.02, $p<0.001$). But there were no statistical differences between the two groups in terms of gender ($p=0.179$). According to chest CT scans, bilateral and multifocal involvement was observed in 86.4% of the participants, with 97.6 and 84.3% reported in critical and non-critical cases, respectively. The results of comparing all variables between the two groups are shown in Table 11.1.

3.2 Deep Learning Analyzing Results

Approximately 70% (n=754) of total patients were utilized for training and 30% (n=324) for the validation test. Age, gender, and CT scan findings were modeled by the deep learning prediction method. Poor classifications were found in the validation data by using age, gender, lesion type, and underlying disease. All of these classified the patients as non-critical cases.

Lesion distribution, presence of diffuse opacity, and number of involved lobes with diffuse opacities had acceptable predictions. Table 11.2 shows the properties of prediction for the critical or non-critical cases in the validation data part using the mentioned variables. The best accuracy of prediction was seen for presence of diffuse opacities and lesion distribution (both=0.91, 95% CI: 0.83-0.99) followed by number of involved lobes (0.89, 95% CI: 0.80-0.98). The largest sensitivity was achieved using lesion distribution (0.74, 95% CI: 0.55-0.93) followed by number of involved lobes (0.71, 95% CI: 0.51-0.91). The lowest sensitivity was found for presence of diffuse opacities (0.66, 95% CI: 0.44-0.0.88). The largest specificity was found for presence of diffuse opacities (0.95, 95% CI: 0.9-1) followed by lesion distribution (0.94, 95% CI: 0.88-1) and number of involved lobes (0.93, 95% CI: 0.87-0.99). The TNR for lesion distribution and presence of diffuse opacities were similar (both 0.95, 95% CI: 0.9-1), and the TNR of the number of involved lobes was lower than other two variables (0.94, 95% CI: 0.88-1). The largest TPR was seen for lesion distribution (0.69, 95% CI: 0.47-0.91), followed by the number of involved lobes (0.68, 95% CI: 0.46-0.90), and then by the presence of diffuse opacity (0.66, 95% CI: 0.88-1).

The three variables were considered in a deep learning model combined (Fig. 11.1). The accuracy of the validation data was 0.89 (95% CI: 0.79-0.99). The sensitivity and specificity were

Table 11.1 Demographic characteristics and chest CT findings in critical and non-critical patients with COVID-19 (* indicates significant p-values)

Variables	Critical patients (n=169)	Non-critical patients (n=909)	Total patients (n=1078)	p-value
Age				
Mean ± SD (range)	61.24±13.48 (25-92)	51.47±14.02 (14-91)	53±14.37 (14-92)	<0.001*
Gender (%)				
Male	123 (72.8)	614 (67.5)	737 (68.4)	0.179
Female	46 (27.2)	295 (32.5)	341 (31.6)	
Lesions distribution (%)				
Bilateral + multifocal	165 (97.6)	766 (84.3)	931 (86.4)	<0.001*
others	4 (2.4)	143 (15.7)	147 (13.6)	
Presence diffuse opacity (%)				
Yes	118 (69.8)	63 (6.9)	181 (16.8)	<0.001*
No	51 (30.2)	846 (93.1)	897 (83.2)	
Number of involved lobes with diffuse opacities (%)				
1	1 (0.6)	5 (0.6)	6 (0.6)	1
2	33 (19.5)	10 (1.8)	49 (4.5)	<0.001*
3	35 (20.7)	15 (1.7)	50 (4.6)	<0.001*
4	30 (17.8)	13 (1.4)	43 (4)	<0.001*
5	19 (11.2)	14 (1.5)	33 (3.1)	<0.001*
Total opacity score				
Mean ± SD	13.71±6.26	4.86±3.52	6.24±5.19	<0.001*

Table 11.2 Performance of the prediction in validation data part according to univariate models using mentioned variables

	Accuracy	TPR	TNR	Specificity	Sensitivity
Lesion distribution	0.68	0.69	0.95	0.94	0.74
Presence diffuse opacity	0.91	0.66	0.95	0.95	0.66
Number of involved lobe	0.89	0.68	0.94	0.93	0.71

0.71(95% CI: 0.51-0.91) and 0.93(95% CI: 0.87-0.96), respectively. The TPR and TNR were 0.7(95% CI: 0.50-0.92) and 0.94 (95% CI: 0.88-1), respectively (Fig. 11.2).

4 Discussion

We have analyzed the chest CT features of 1,078 patients with COVID-19 disease in critical and non-critical cases with the aim of improving prediction of patient severity and outcomes. According to studies and clinical experiences, critical patients have poorer prognosis and higher mortality than non-critical patients [31, 32]. Chest CT examination in patients with COVID-19 is useful for imparting a deeper understanding of the more critical cases and to better inform clinical diagnosis and treatment [33]. For reduction of the complications and mortality of these cases, the most appropriate treatment of critical cases is needed. Therefore, it is important to specify the critical cases and detect the related factors and symptoms of this patient group.

Here, we have used a deep learning neural network for predictive classification of patients into two groups of critical and non-critical cases. First of all, univariate analyses were performed and all

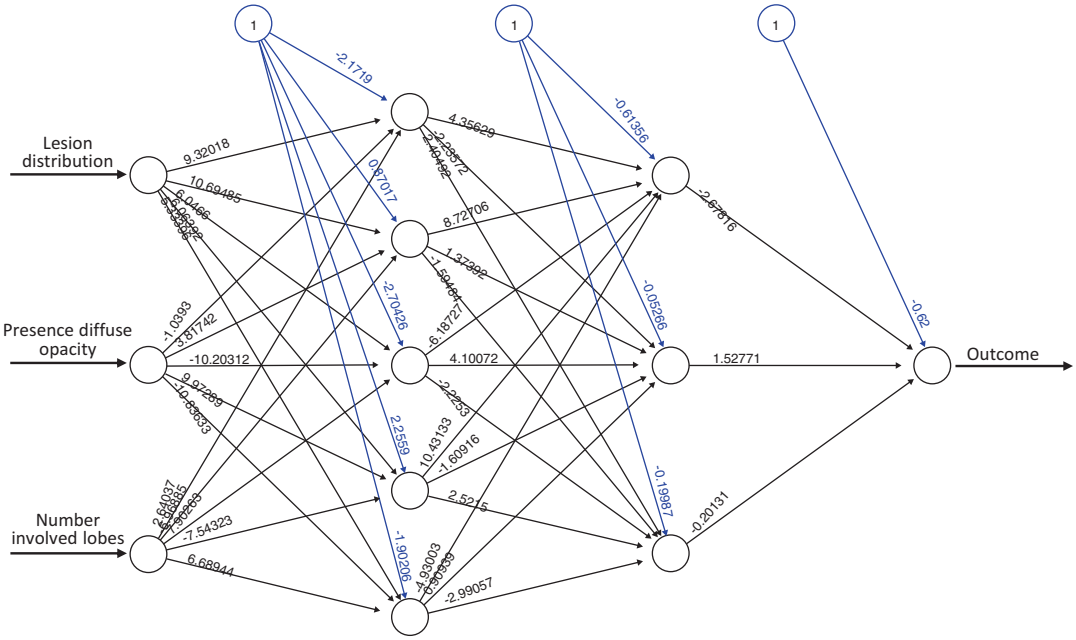

Fig. 11.1 Neural network model to classified patients in to critical or non-critical ones

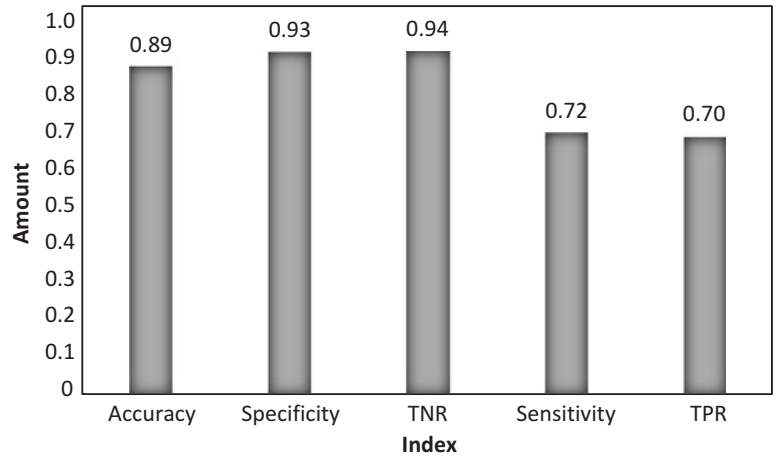

Fig. 11.2 Amount of indexes to evaluate the performance of predictions using CT scan features

variables were entered into the model one at a time. From all of the variables, lesion distribution, presence of diffuse opacity, and number of involved lobes with diffuse opacities had a good fit and classified patients correctly. Other studies have shown the power of CT imaging for identifying the status of COVID-19 patients. Consistent with our findings, lesion distribution was also found to be important with good discriminative power for classifying COVID-19 patients. Wang and colleagues found that lesions rapidly progressed in 75% of patients 6–9 days after admission [34]. Also these lesions distinctly resolved in 76.9% of the cases after 10–14 days of admission [34]. Another study from China reported that 83% patients had more than two lobes involved

and patchy GGOs and consolidation of lesions co-existing in 47% of cases [35]. These results were also aligned with our studies.

In deep learning analyses, checking the performance of the prediction is important. In this manuscript, this was achieved via comparison of the indices. In the univariate analyses with three different models and three different factors, the number of involved lobes with diffuse opacities was a significant factor with an accuracy of 0.89, TNR of 0.94, and TPR of 0.68. In addition, the presence of diffuse opacity had an accuracy of 0.91, TNR of 0.95, and TPR of 0.66. Finally, lesion distribution had an accuracy of 0.91, TNR of 0.95, and TPR of 0.69. This all of these were good predictors of non-severe cases but less so for severity.

After entering all three variables into our multivariate model, we found that the accuracy and TNR were similar to those parameters in the univariate model but the TPR was higher with a value of 0.70. This suggested that the multivariate model was better at identifying the severe cases compared to the three univariate models.

Anotherne study reported a sensitivity of 0.80–0.90 and a specificity of 0.83–0.96 for CT images for the diagnosis of lung lesions [36–39]. However, there were other CT image variables that were significant in other studies which were not found to be significant in our deep learning model. This could be due to the high precision of this model, meaning only the most important factors indicated significant results.

The large sample size was one of the strengths of this study. However, there are several limitations which should be noted. Due to novelty of the COVID-19 disease, lack of information, and long-term CT data, we could only perform a retrospective analysis. In addition, the times of chest CT examination after symptom onset were inconsistent, which made it difficult to summarize the CT appearances that could reflect the whole course of the disease. Thus further studies are needed, potentially employing the use of other biomarkers, such as laboratory analytes and demographic characteristics in an algorithm. This could make prediction more accurate. For example, circulating markers related to the cytokine storm could be employed, such as C-reactive protein, D-dimer, procalcitonin, interleukin interleukin-6, interferon γ-induced protein 10 kDa (IP-10), and monocyte chemoattractant protein-1MCP-1 [40]. Demographic features could also be used such as age, gender, body mass index, and the presence of co-morbidities such as diabetes and metabolic syndrome [41].

5 Conclusions

In conclusion, our study showed that chest CT examination was effective in detecting pulmonary parenchymal abnormalities in the natural course of COVID-19. According to our deep learning neural networks lesion distribution, the presence diffuse opacities and number of involved lobes with diffuse opacities can distinguish critical from non-critical patients. This is helpful for the judgment of clinical condition and has important clinical value for the diagnosis and follow-up of COVID-19 pneumonia. The value of this becomes apparent considering the recent development of potential new ways of improving patient outcomes. For example, the Recovery Trial showed a reduction in mortality with dexamethasone treatment and the Adaptive COVID-19 Treatment Trial showed that treatment with Remdesivir reduced the time of patient recovery by 4 days [42]. In addition, the global response to the COVID-19 crisis has led to the fastest development of efficacious vaccines in history, with recent reports of promising candidates which have already been staged for rapid deployment. Through these combined efforts, it is hoped that the effects of this current pandemic will begin to ease in 2021 and we will also lay the groundwork to minimize or stop the spread of future potentially devastating outbreaks of similar pathogens.

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Part IV

Treatments and Vaccines

12

Using Ozone Therapy as an Option for Treatment of COVID-19 Patients: A Scoping Review

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Abstract

Recent investigations are seeking a novel treatment to control the new pandemic of coronavirus 19 (COVID-19). The aim of this systematic review was to study the effect of ozone therapy on COVID-19 patients and the available supporting evidence. Electronic databases including MEDLINE (via PubMed), EMBASE, Cochrane Library (CENTRAL), and TRIP, clinical trial registries, and preprint sources were searched for published evidence-based articles. In addition, manual searching was conducted for articles published up to April 6, 2020, using MeSH and free text keywords with no language limitation. Articles were screened, categorized, and extracted for relative data. Data were reported in a descriptive manner. Among 234 articles, 9 were selected for review of the inclusion criteria. No published original articles were found regarding the efficacy of ozone therapy on COVID-19. Five review studies were found in which the potential role of systemic ozone therapy was concluded to be effective in controlling COVID-19 because of its antiviral, oxygenation, anti-inflammatory, oxidation balancing, and immunomodulation effects. Three ongoing clinical trials were registered in China. A preliminary report of an ongoing study in Italy on 46 patients (11 intu-

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bated and 35 non-intubated) showed that in 39 (84%) of the patients, an improvement was seen. In spite of the promising background data, as well as the expert opinions and a preliminary report indicating the effectiveness of ozone, there is still not enough evidence to confirm this as a viable treatment option for COVID-19.

Keywords

Ozone therapy · Autohemotherapy · COVID-19 · SARS-CoV-2 · Systematic review

1 Introduction

Novel coronavirus disease (COVID-19), which is responsible for causing severe acute respiratory syndrome, was first identified in December 2019 in Wuhan and later became a large global outbreak and major public health issue [1, 2]. The disease is caused by SARS-CoV-2, which is an enveloped non-segmented positive-sense RNA virus [3]. This virus causes flu-like symptoms such fever, dry cough, dyspnea, and fatigue. Further reported symptoms are sputum production, headache, hemoptysis, diarrhea, dyspnea, and lymphopenia, as well as gastrointestinal symptoms [4–6]. Pneumonia is the main presentation of the illness ranging from normal O_2 saturation to significant lung injury and hypoxemia due to severe acute respiratory syndrome, which can be life-threatening [7]. Therefore, complementary oxygenation has been applied to save lives.

In the absence of any approved efficient treatment of this new disease, there is a growing interest in testing novel and old methods. In the mid-nineteenth century, ozone therapy was approved in medical community [8]. Ozone is a triatomic oxygen (O_3) that acts as an oxidizing agent and inactivates bacteria, fungus, and viruses as a disinfectant and as a therapeutic approach [8, 9]. Water disinfection, treatment of various infectious diseases, wound healing, and boosting the immune system with minimal side effects are the well-known applications of this therapy [10]. Recent studies have shown that this method has some efficacy in the treatment of pulmonary and vascular diseases [11–13]. The mechanism involves the reaction of ozone with biological substrates leading to the synthesis of signal transducers [10, 14]. It also increases the concentration of oxygenated hemoglobin and improves oxygenation in tissues [15]. This can be an advantage over other treatment options of hypoxic conditions, such as those seen in COVID-19 patients. For these reasons, a number of studies have evaluated the potential of using ozone therapy as a novel treatment for COVID-19 patients.

The objective of this scoping review was to provide a clear understanding of the current evidence regarding ozone therapy as a treatment option for COVID-19. Also, we have briefly reviewed the effects of ozone therapy on other viral infections and respiratory diseases, and investigated the possible mechanisms of actions as it relates to the known effects of COVID-19 disease.

2 Methods

2.1 Inclusion Criteria

All studies and primary reports, which assessed the effects of ozone therapy on COVID-19, were included in this review regardless of study designs. Because of the novelty of the project and the small amount of available data, no exclusion criteria were applied to avoid losing data and maximize comprehensiveness.

2.2 Search Strategy

The search process was done independently by two authors (FP and SR). The electronic databases including MEDLINE (via PubMed), EMBASE, Cochrane Library (CENTRAL), and TRIP were searched for evidence-based articles published until April 6, 2020. In addition, in order to ensure the comprehensive search of clinical trials (unpublished or ongoing), the follow-

ing databases were searched: clinicaltrials.gov, the Iranian clinical trial registry, the Chinese clinical trial registry, the Italian clinical trial registry, and clinicaltrialsregister.eu. Furthermore, for retrieving unpublished articles, Google scholar was also searched. The main keywords used for the review (Both MeSH or free text) were ozone, ozone therapy, coronavirus, COVID-19, SARS-CoV-2, antiviral, and viral infection. No language limitation was applied, and data other than English were translated using translation websites and applications.

We also searched through the references of the selected papers. Preprint articles were searched via BioRxiv, Medrxiv. Manual searching was also undertaken to ensure comprehensiveness, which involved the use of Google and scientific web pages to obtain further articles and information about this topic. In order to obtain the results of ongoing trials and webpage reports, e-mail messages were sent to authors.

Search results were assembled in EndNote X7 and duplicate references were removed. The first screening was performed on titles and abstracts, and the secondary screening was completed after reviewing full texts to include related articles.

3 Results

3.1 Extraction of Results

All 234 hits from the initial search were screened by title and abstract and 72 studies were selected for further evaluation. Also, relevant studies were included considering the concept of review and the set scopes (Fig. 12.1). Nine studies were obtained concerning both COVID-19 and ozone therapy, including 5 hypothetical reviews with expert opinions indicating potential effects of ozone therapy as a COVID-18 treatment (Table 12.1). Three ongoing clinical trials were registered in China, and a preliminary report of a trial was generated in Italy. No articles with relevant new data were found. Because of the novelty of the issues and to ensure comprehensiveness, related studies, but non-COVID-18-associated, were also reviewed. Along these lines, 7 studies assessing the role of ozone therapy in medicine, in addition to 3 clinical trials on respiratory diseases, were included. In addition, 11 studies recognized the potential role of ozone as a treatment of viral infection. Three clinical trials are currently underway in China to study the effect of ozonated autohemotherapy but no results have been released yet. The information on these trials is listed in Table 12.2.

There have also been primary reports by the Scientific Society of Ozone Oxygen Therapy (SIOOT) in Italian hospitals for the use of oxygen-ozone therapy in the treatment of COVID-19. Based on the second SIOOT report on this therapy applied to 46 patients with confirmed COVID-19 infections, 39 out of 46 (84.7%, including 11 intubated and 35 non-intubated patients), showed improvement of clinical symptoms. Among the patients, the number of extubated patients was 6, 3 were currently intubated, and 28 did not need intubation. Four of the intubated patients died due to bacterial infection, septic shock, pulmonary emboli, or myocarditis. Five patients became free of the virus, as determined by two negative swabs [16–18].

3.2 Protocols

Different protocols are available to supply ozone: major autohemotherapy (MAH); ozonized saline solution (O3SS); extracorporeal blood oxygenation-ozonation (EBOO); and a variant of the minor autohemotherapy (MiAH). All clinical protocols should be defined in accordance with the standard dosage and procedures described in the Madrid Ozone Therapy Report [19].

3.3 Mechanism of Ozone as a Treatment

Ozone is an important gas in nature with high solubility in water. Ozone can be formed from three basic sources of energy-driven reactions: chemical electrolysis, electrical discharge, and ultraviolet light radiation. Based on the findings of numerous scientific studies, ozone has a high

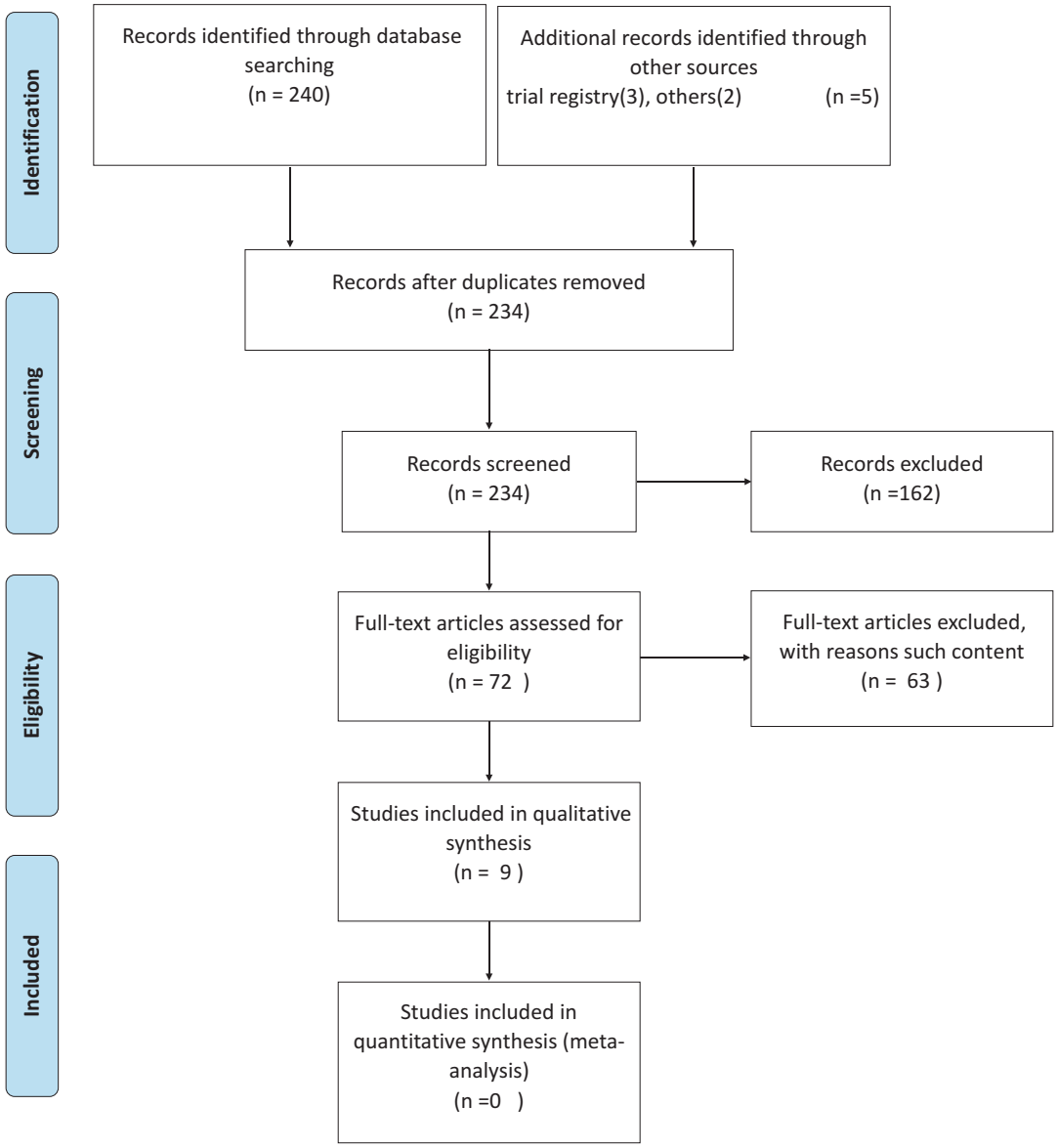

Fig. 12.1 PRISMA flowchart of selection process (Moher 2009)

ability to oxidize substances. The biological basis of ozone therapy is its reaction with proteins, amino acids, and unsaturated fatty acids, which are found in blood and cell membranes. The four fundamental products of this action are ozonides, aldehydes, peroxides, and hydrogen peroxide [20]. The interaction of ozone with unsaturated cell membrane fatty acids in the intestinal mucosa (rectal administration) or blood cells (in the extracorporeal blood–ozone mixture, during autohemotherapy) produces aldehyde and hydroxy-hydroperoxide (ozone-peroxide), which forms H_2O_2 and second aldehyde—4-hydroxynonenal (4-HNE). These substances act as second messengers and prompt a further adaptive response [21, 22]. If ozone is used at specific therapeutic doses, it stimulates nuclear factor erythroid 2-related factor 2 (NRF2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and balances the antioxidant

Table 12.1 Characteristics of published articles on COVID-19

References	Year	Country	Aims and purpose	Type of study	Key findings
Galoforo et al. [31]	2020	Italy	Indicate the potential effects of ozone as a COVID-19 treatment	Review article- expert opinion	Noticeable effect of ozone on virus and immune system
Rowen & Robins [29]	2020	USA	Predicting the position of ozone therapy for COVID-19	Review article- expert opinion	(1) Ozone -therapy as low-cost solution (2) Ozone oxides cysteine-rich virus
Valdenassi et al. [16]	2020	Italy	Demonstrate the potential effect of ozone on COVID-19	Review article- expert opinion	Ozone as immune modulator and improves oxygen circulation
Schwartz et al. [19]	2020	Spain	Investigate the potential effect of ozone therapy on COVID-19	Review- expert opinion	Guides to use ozone as COVID-19 treatment
Eren et al. [30]	2020	Turkey	Potential effect of ozone on SARS-COV-2	Review- expert opinion	Ozone reacts with thiol groups in COVID-19

Table 12.2 Characteristics of registered clinical trials

Registration no.	Title	Setting	Study design	Primary purpose	Recruitment status
ChiCTR2000030165	Clinical study for ozonated autohemotherapy for treatment of COVID-19	Tianjin, China	Non-randomized controlled trial	Chest CT	Recruiting
				Whole blood cell analysis	
				Recovery rate	
				Oxygenation index	
				Inflammatory response index	
ChiCTR2000030102	A multicenter randomized controlled trial for ozone autohemotherapy in the treatment of novel coronavirus pneumonia (COVID-19)	Tianjin, China	RCT (phase 0)	• Chest imaging • RNA test of COVID-19 • Time to remission / disappearance of primary symptoms • Completely antipyretic time • Incidence of medical complications during hospitalization • Blood oxygen saturation • Time to SARS-CoV-2 RT-PCR negativity	Recruiting
ChiCTR2000030006	A randomized controlled trial for the efficacy of ozonated autohemotherapy for treatment COVID-19	China, Wuhan	RCT	• Recovery rate	Recruiting

system. In addition, ozonides may suppress the cytokine storm. Ozone can also activate the cellular and humoral immune systems, increase proliferation of immunocompetent cells, and synthesize immunoglobulins, in addition to enhancing phagocytosis. It also stimulates signal transduction molecules via NRF2 and causes immune system improvement [23–25].

Ozone also stimulates aerobic metabolism as an anti-hypoxic agent. In addition, it activates protein synthesis and boosts cell metabolism. Ozone causes the synthesis of interleukins, leukotrienes, prostaglandins, and immunoglobulins which have an important biological role in the treatment process [26–28]. The main mechanisms behind ozone therapy are shown in Fig. 12.2 [28].

Focusing on coronaviruses, ozonized blood suppresses the cytokine storm in the body and improves the oxygen circulation [16, 19, 29]. Coronaviruses, including SARS-CoV-2, are rich in cysteine amino acid residues, which were essential for viral activity. It has been suggested that this property may make SARS-CoV-2 susceptible to ozone therapy because of the high oxidation capability of the ozone [29, 30]. Because of the capacity of ozone for full oxidation of substances such as the sulfhydryl groups of cysteine residues, ozone therapy could be an inexpensive and safe treatment of COVID-19 disease [29, 31].

3.4 Ozone Therapy and Viral Infections

The disinfectant effect of ozone on viruses as well as bacteria, spores, and fungus has been studied [32, 33]. Hudson et al. evaluated the

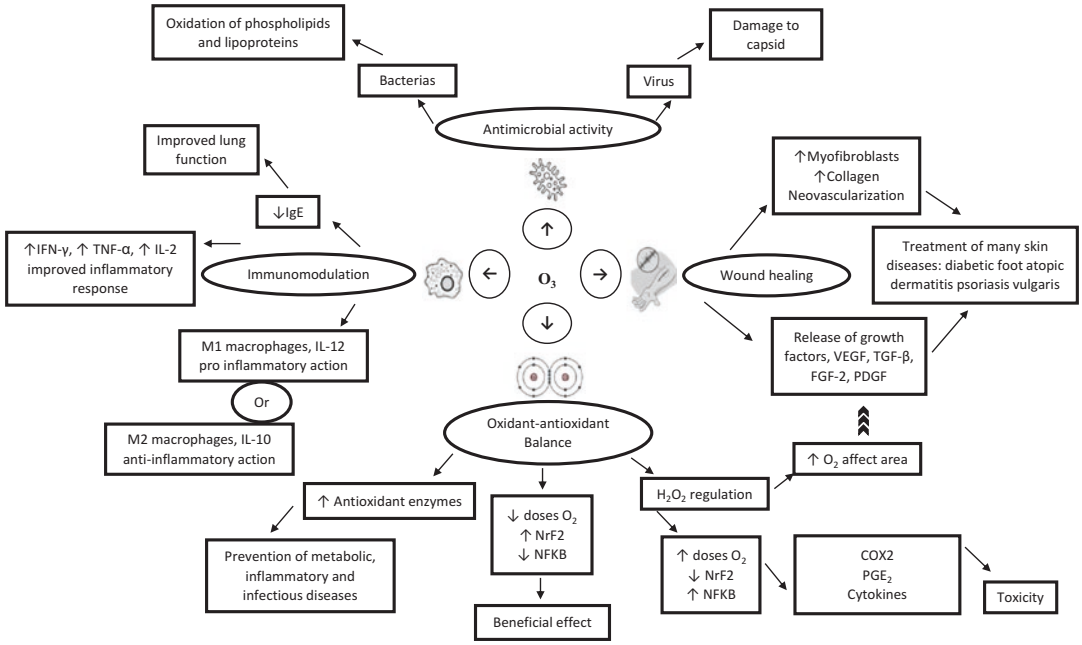

Fig. 12.2 The main mechanism behind ozone treatment (Pivotto et al. [28])

decontaminating effect of ozone on 12 different viruses via a mobile apparatus [34]. Influenza H3N2, herpes simplex virus type 1 (HSV), rhinovirus types 1A and 14, adenovirus types 3 and 11, sindbis virus (SINV), yellow fever virus (YFV), vesicular stomatitis virus (VSV), poliovirus (PV, vaccine strain), and vaccinia virus (VV) were tested on different surfaces and biological fluids. The results showed that inactivation occurred by factors of at least 1000-fold. Short periods of high humidity (>90% relative humidity) with peak ozone gas concentration (20–25 ppm) provided the optimum antiviral activity. Another study also confirmed that ozone exposure can similarly inactivate norovirus from surfaces, as confirmed by quantitative reverse transcriptase real-time polymerase chain reaction (RT-PCR) [35]. Maler and Chu studied the disinfectant efficacy of ozone in combination with ultraviolet treatment in of the polyoma virus [36]. Again, this revealed a significant reduction in the number of viruses and viable cells.

In addition to surface decontamination, some studies have evaluated the therapeutic effects of systemic ozone therapy for viral diseases. Ozone could affect both enveloped [herpes simplex virus type-1 strain McIntyre (HSV-1), vaccinia strain Elstree (VAC), vesicular stomatitis virus strain Indiana (VSV), influenza A strain (H1N1) A/WS/33], and non-enveloped [human adenovirus type 2 (Ad2)] viruses [16, 31]. In vitro studies examined ozone-treated serum for potential inactivation capability against HIV and the associated cytotoxic products [37]. This showed that ozone inactivated HIV-1 in a dose-dependent manner. The antiviral mechanisms observed included disruption of viral particles, reverse transcriptase inactivation, and disability to bind to receptors of target cells, all without apparent cytotoxicity [37, 38].

Rowen et al. administered combinations of ozone therapies to 5 patients with Ebola hemorrhagic fever [39]. In this study, direct intravenous ozone gas was infused at 55 μg/mL in 20–40 mL. Rectal ozone was also administered at a concentration of 36 μg/mL with a volume between 150 and 350 mL. Ozone water was made by bubbling ozone gas at approximately 70 μg/mL into the water, and this was taken orally for 15 min at a volume of 300–500 mL. All patients experienced full remission of symptoms within 2–4 days. Possible antiviral mechanisms included

oxidation of sulfhydryl groups to disulfide which inactivated viral entry, attachment of the lipid envelope, oxidation of viral surface glycoproteins, and stimulation of the immune system. However, the effectiveness of ozone still requires confirmation in clinical trials.

3.5 Ozone Therapy and Respiratory System

Ozone cannot be inhaled directly because of its toxicity and irritancy via this route. However, it can be administered via the systemic circulation for lung diseases. Following are three clinical trials which studied the effect of ozone therapy on different respiratory diseases.

Asthma

Hernandez et al. recruited 113 asthmatic patients and divided them into three groups [14]. Two groups were treated by major ozone autohemotherapy (MAHT) for 3 cycles and 12 total sessions using an ozone dose of 4 mg (20 mg/mL per mL blood and 200 mL total volume) in the first group or 8 mg in the second group. The third group received ozone rectal insufflations (RIs) for 3 cycles over 20 sessions using an ozone dose of 10 mg (50 mg/mL and 200 mL total gas volume). Functional vital capacity (FVC) was increased significantly (2.34 vs. 2.99 L) in the 8 mg MAHT group while no significant changes were observed in the other groups. Improvement in symptoms such as dyspnea, wheezing, and medication was observed respectively in 8 mg MAHT, 10 mg RI, and 4 mg MAHT groups. The anti-inflammatory indicators IgE and HLA-DR were decreased, while increments in antioxidant agents of glutathione pathways were achieved with all treatments but more prominent in the 8 mg MAHT group. These findings indicated immunomodulatory and oxidative stress regulation effects of ozone therapy.

Chronic Obstructive Pulmonary Disease (COPD)

Fifty patients affected by moderate/severe COPD were enrolled in a clinical trial [40]. Half of the patients received major ozonated autohemotherapy, and the other half were entered as the control group. The protocol was as follows: 225 mL of blood was vacuumed from the antecubital vein into a sterile citrated glass bottle at a 9:1 ratio. Then 225 mL of 20 μg/mL ozone was added and mixed for 5 min and re-infused 15–20 min later. The only observed complication was facial redness in some of patients. Significant improvements were seen in the 6 min walking test, and dyspnea was observed in the ozone therapy group. However, there were no significant differences in resting arterial gas and pulmonary function tests.

Pulmonary Emphysema

Colunga et al. published a trial in Spanish studying the effect of ozone therapy on pulmonary emphysema and spirometry parameters [41]. Sixty-four patients were randomly assigned to treatment with either rectal ozone therapy, ozone therapy, or control (no treatment). At baseline, the intervention group had significantly lower values of forced expiratory volume in the first second (fEV1) and fEV1/forced vital capacity. However, at the end of the treatment period, these parameters were similar in the three groups with no difference in other spirometric parameters.

4 Discussion

This systematic review assessed the role and efficacy of ozone therapy as a potential treatment for COVID-19 disease, which shows dyspnea and respiratory distress as major features. Based on the extracted studies, it seems feasible that ozone therapy may be a suitable approach in the treatment of these aspects of COVID-disease [16, 19, 25, 29–31]. The studies showed that ozone therapy can be effective by 4 main routes: (1) virus inactivation; (2) modification of oxidative stress along with reduction of inflammation and apoptosis; (3) increased blood flow and tissue oxygenation; and (4) improvement of the immune response by stimulation of interferon gamma (IFN-γ) and pro-inflammatory cytokines [16, 19, 29, 31].

A number of studies in this review described a role of ozone therapy in infectious diseases. Ozone is known as a disinfectant and therapeutic

substance in medicine [13]. In addition, some of the studies indicated the importance of ozone in the treatment of respiratory Illnesses such as COPD, lung fibrosis, pulmonary inflammation, and other lung diseases[14, 22]. The efficiency of ozone therapy in diabetic wounds, asthma, heart disease, cancer, and antibiotic-resistant diseases like methicillin-resistant Staphylococcus aureus (MRSA) and HIV has been demonstrated previously. The side effects of ozone include the probability of interference via the increased nitric oxide (NO) production subsequent to stimulation of antioxidant enzymes. In addition, ozone therapy may cause transmission disorder because NO can cause a further increase in NO levels [8, 22, 42]. However, there is not enough evidence supporting this possibility.

This review was limited by the lack of direct data concerning the effects of ozone therapy in COVID-19 disease.

5 Conclusions and Future Perspective

According to the evidence, it is postulated the ozone therapy is potentially effective in the treatment of COVID-19 disease. As no direct data were available, much of the information in this review has been extrapolated from of other viruses and respiratory conditions. Thus, there is still not sufficient evidence to endorse the efficacy of ozone therapy as a potential novel COVID-10 treatment. This will require at least partial completion of the ongoing clinical studies and establishment of further follow-up studies to address any gaps in the data.

Conflict of interest The authors declare no conflict of interest

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COVID-19, Coenzyme Q10 and Selenium

13

I. R. Hargreaves and D. Mantle

Abstract

In COVID-19 infection, a balance must be achieved in immune defence against the virus without precipitating a cytokine storm, which is responsible for lung injury and respiratory distress in severe cases. The initial immune response and the subsequent resolution of inflammation are likely to be dependent on nutritional status, as one contributing factor. Here, we have reviewed the potential link between two specific nutrients, coenzyme Q10 (CoQ10) and selenium, with effects on oxidative stress and inflammation in viral infection. We conclude that both reagents show promise in the treatment of patients with COVID-19 disease. This could give particular relevance over the next several months as promising vaccines are deployed to minimise the COVID-19 spread and as a potential preventative or mitigating approach for future epidemics and pandemics.

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Keywords

COVID-19 · SARS-CoV-2 · Coenzyme Q10 · CoQ10 · Selenium · Supplement

1 Introduction

There is currently no effective treatment for COVID-19 (Coronavirus disease 2019), since antibiotics are ineffective against viral infections, and vaccines against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which causes COVID-19 have only recently become available to a significant extent in the first few months of 2021. The best defence against such viral infections is therefore an optimally functioning immune system. An important factor determining immune function and resistance to infection is an individual's nutritional status. In order to determine, on a rational basis, whether nutritional supplementation could be potentially beneficial with regard to SARS-CoV-2 infection, one must first consider how the immune system functions.

In simplistic terms, the immune system is comprised of two parts, the innate immune system and the adaptive immune system. As the name implies, the innate immune system is present and operational from birth, providing a rapid

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and non-specific first-line response to invading microorganisms. The innate immune system comprises a number of components, the most prominent being phagocytic cells (macrophages, neutrophils, natural killer cells) which destroy engulfed invading microorganisms via the generation of free radicals. Subsequent to the innate response, the adaptive immune system may be activated, depending on the severity of infection. Adaptive immunity is a slower responding but more specific form of immune defence, involving B and T lymphocytes. B lymphocytes produce antibodies to neutralise specific antigens, whereas T lymphocytes have a role in destroying infected host cells. Communication between the various cell types of the two branches of the immune system is facilitated via cytokines, protein chemical messenger molecules.

There is a common misconception that inflammation, which involves the release of pro-inflammatory cytokine substances, is a wholly negative process within the body. However, inflammation is the body's normal response to infection or injury and is essential for tissue healing, although this process should resolve following the initial immune response to viral infection [1]. This resolution occurs via negative feedback mechanisms involving the generation of specific molecules such as resolvins, protectins, and maresins [2].

In COVID-19 infection, a balance must therefore be achieved in immune defence against the virus, without precipitating the so-called cytokine storm, the uncontrolled release of pro-inflammatory cytokines responsible for lung injury and respiratory distress in severely affected patients [3]. Both the initial immune response to viral infection and the mechanism for subsequent resolution of inflammation are therefore dependent on an individual's nutritional status. In this article, we have reviewed the link between two specific nutrients, coenzyme Q10 (CoQ10) and selenium, and free radical-induced oxidative stress (an imbalance between reactive oxygen generation and antioxidant capacity), inflammation, and virus infection, with regard to the potential benefits of CoQ10 and selenium supplementation in the treatment of patients with COVID-19.

2 CoQ10

CoQ10 is a lipid soluble molecule comprising a central benzoquinone moiety, to which is attached a 10-unit polyisoprenoid lipid tail. The benzoquinone ring contains redox active sites, while the polyisoprenoid chain is responsible for positioning the CoQ10 molecule within the mid-plane of the lipid bilayer of various cell membrane types [4]. CoQ10 is usually described as a vitamin-like substance, although by definition, CoQ10 is not a vitamin since it is produced by various tissues within the human body [5].

CoQ10 has a number of vital cellular functions, particularly within mitochondria, but also elsewhere within the cell [4]. Within mitochondria, CoQ10 has a key role as an electron carrier transferring electrons derived from complex I and II to complex III, ensuring a continuous passage of electrons within the mitochondrial electron transport chain which is required for the process of oxidative phosphorylation with the concomitant product of ATP [5]. CoQ10 serves as an important lipid soluble antioxidant protecting cellular membranes, both mitochondrial and extra-mitochondrial organelles (Golgi apparatus, lysosomes, endoplasmic reticulum, peroxisomes) together with circulatory lipoproteins against free radical-induced oxidative damage [4]. The antioxidant function of CoQ10 is attributed to its fully reduced ubiquinol form [4, 5]. In addition to acting as an antioxidant directly, CoQ10 is also involved in the regeneration of the antioxidants vitamin C and vitamin E, respectively [6]. CoQ10 has also been reported to be involved in the mediation of inflammation by its ability to regulate the expression of genes involved in this process [7]. A recent study also indicated that CoQ10 plays a central role in maintaining the acidic environment of the lysosomal compartment [8]. CoQ10 exists in both oxidised (ubiquinone) and reduced (ubiquinol) forms, and the normal functioning of CoQ10 involves continual inter-conversion between these two forms [4].

The daily requirement for CoQ10 is not known with certainty, but has been estimated to be approximately 500 mg/day, based on a total body pool of 2000 mg and average tissue turnover time

of 4 days [9]. A small amount of CoQ10 (approximately 5 mg) is obtained from the daily diet, with most of the daily requirement being synthesised within the body [9]. CoQ10 is produced in many tissues, with the liver being the principal site of production based on organ mass and metabolic activity level. Optimal production occurs in an individual when they are around 25 years of age, after which production steadily declines, with the production level at age 65 being approximately 50% of that at age 25 [10]. In addition to the effect of ageing, CoQ10 levels are also reduced by certain prescribed drugs (particularly statins), and in a variety of diseases [11]. Indeed, a study by Becker et al. reported that early statin usage appeared to be associated with increased risk of post-stroke infection [12]. However, controversy exists at present as to whether statin induced lowering of circulatory cholesterol levels may predispose individuals to developing COVID-19 [13].

CoQ10 therefore has a number of cellular functions of potential relevance to the immune system. Firstly, there is the role of CoQ10 in cellular energy supply. Since the immune response has intensive energy requirements, an adequate supply of CoQ10 is required to enable the various cell types of the immune system to function optimally. Secondly, is the role of CoQ10 as an antioxidant, in protecting cells from free radical-induced oxidative damage, since phagocytic cells destroy invading pathogens via the production of free radicals. The antioxidant action of CoQ10 may protect phagocytic cells from self-destruction caused by their secretion of free radicals. Finally, CoQ10 is able to moderate directly the action of genes involved in inflammation and may have a role in controlling the release of pro-inflammatory cytokines [7].

A recent study by Ghosh et al. reported the ability of SARS-CoV-2 to utilise a lysosomal-dependent exocytosis pathway for viral release into the extracellular environment from the host cell [14]. However, as a consequence of the viral exploitation of this process, SARS-CoV-2 de-acidifies the lysosome by an as of yet unknown mechanism. This de-acidification was reported to decrease lysosomal hydrolytic enzyme activity in addition to impairing the antigen presentation role of the organelle in the adaptive immune response. Therefore, the possibility arises that therapeutic strategies aimed at reversing the de-acidification of the lysosome may enhance the immune response against the virus and mitigate the spread of infection. In view of the essential role that CoQ10 plays in maintaining lysosomal acidification [8], CoQ10 supplementation may be an appropriate therapeutic strategy to consider in the treatment of COVID-19. Figure 13.1 summarises the functions of CoQ10 in the immune function.

3 Selenium

Selenium is a trace element obtained from the normal diet. In the UK, a deficiency of selenium in soil is manifest upwards through the food chain, such that the average UK diet contains only about half of the recommended selenium intake of 70 μg per day. Selenium is essential for the normal functioning of both the innate and adaptive immune systems. In the innate immune system, selenium is required for the differentiation, motility, and action of neutrophils and macrophages, for the production of antimicrobial proteins, and for recovery from inflammation. In the adaptive immune system, selenium is required for the differentiation and proliferation of lymphocytes, for cytokine production, and for antibody production (Fig. 13.2). Adequate levels of selenium are important for initiating immunity, but they are also involved in regulating excessive immune responses and chronic inflammation [15]. It is also of note that selenium reduces the formation of thrombosis in the blood vessels. Blood coagulation disorders leading to the formation of micro-clots (particularly in the kidneys) are a significant cause of death in patients with COVID-19 [16].

Of particular note is the synergistic interaction at the cellular level between CoQ10 and selenium. In addition to its role as a cofactor of the antioxidant enzyme glutathione peroxidase, one of the major functions of selenium is as a component of the enzyme thioredoxin reductase, which

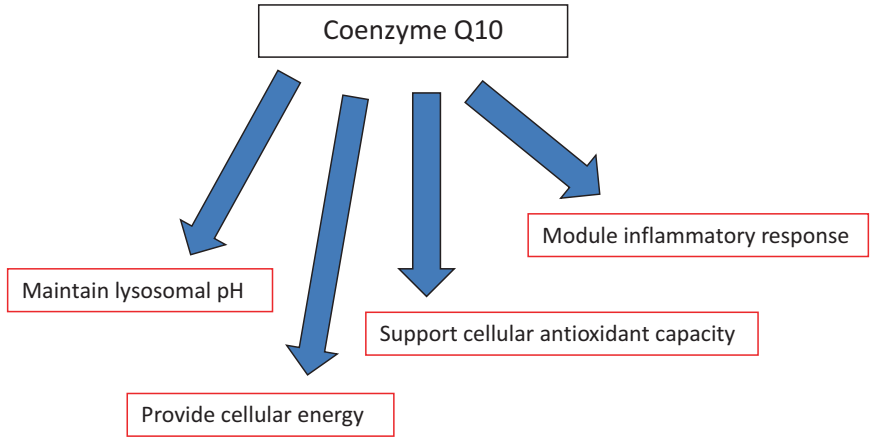

Fig. 13.1 Functions of coenzyme Q10 in the immune system

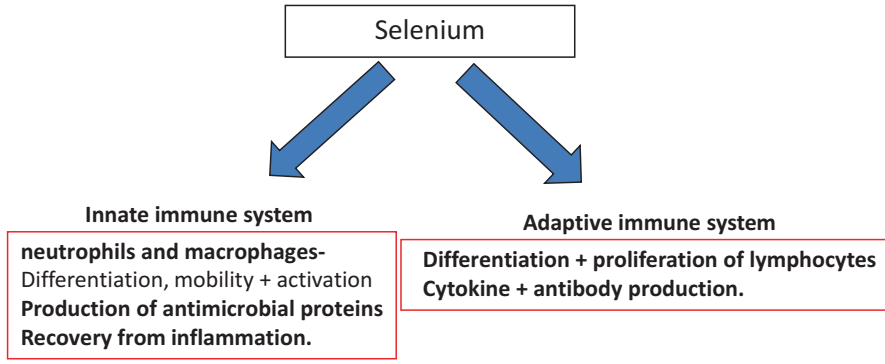

Fig. 13.2 Min roles of selenium in the immune system

is required for recycling of the ubiquinol from the CoQ10 molecule [17]. Supplementation with CoQ10 alone is therefore likely to be sub-optimal in effect if the level of selenium is also deficient, although this has yet to be confirmed or refuted in patient studies [17].

4 Oxidative Stress, Inflammation and Virus Infection

4.1 Oxidative Stress

Free radicals are highly reactive chemical species produced in all cells as an unwanted by-product of normal cell metabolism, with the potential to cause damage in a wide range of tissues. The body is protected from such damage by antioxidants, including vitamin C, vitamin E, CoQ10, and selenium-containing proteins. The body is also exposed to free radicals produced by external agents, including infectious microorganisms. Free radical production is a characteristic of infection by a wide range of viruses, including influenza-type viruses [18]. The additional free radicals generated by viruses have the potential to overwhelm the cellular antioxidant defence, with harmful consequences for the cells. This imbalance between free radical generation and defence is known as oxidative stress.

Oxidative stress is especially likely to occur in individuals with sub-optimal levels of CoQ10 or selenoproteins, resulting from impaired endogenous synthesis or dietary deficiency respectively. Dietary deficiency of selenium is known to occur

in various countries worldwide (including Europe), but particularly in certain regions of China. This is thought to be one reason why many viral infections, including the current coronavirus pandemic, originate from China. Dietary selenium deficiency resulting in oxidative stress in the host can alter the viral genome, such that a normally benign or mildly pathogenic virus becomes highly virulent. Once such mutations occur, even individuals with a normal diet become susceptible to infection by the newly pathogenic viral strain.

Some of the most compelling evidence regarding the detrimental effect of selenium deficiency and susceptibility to viral infection relates to Keshan disease, a heart condition affecting the population in regions of China with selenium deficient soils. Keshan disease results from the effects of infection by an endemic coxsackievirus, and dietary supplementation with selenium was shown to completely prevent development of this disorder, by elevating host anti-viral immunity and preventing viral mutations that can lead to increased virulence.

4.2 Inflammation

Inflammation is part of the body's normal response to injury or infection. The inflammatory process is characterised by increased blood flow to the affected tissue, macrophage infiltration, and the subsequent release of a range of chemical mediators (cytokines) involved in tissue repair. Cytokines can be both pro-inflammatory and anti-inflammatory. Examples of pro-inflammatory cytokines involved in tissue repair include interleukin-1 (IL-1), IL-6, and tumour necrosis factor (TNF). Under normal circumstances, the inflammatory process is then switched off via a negative feedback loop mechanism involving anti-inflammatory cytokines such as IL-10 and IL-11. When this does not occur, uncontrolled inflammation results, which in turn has been implicated in a variety of degenerative disorders, as well as in patients more severely affected following COVID-19 infection.

A proportion of patients infected with COVID-19 are subject to uncontrolled inflammation, the so-called cytokine storm, which can result in multi-organ damage and failure, particularly in the lungs, heart, liver, and kidneys. COVID-19 patients subject to such organ damage typically have sustained high circulating levels of IL-1, IL-6, and TNF [19]. One area of COVID-19 research which to date has received relatively little attention is the role of nutrients in the inflammation negative feedback loop. There is evidence that a number of nutrients, including CoQ10 and selenium [15], as well as vitamin D3 [20] and beta 1,3/1,6 glucans [21], have important roles in mediating the inflammation negative feedback mechanism. There is also evidence that levels of these nutrients, especially selenium and vitamin D [22, 23], may be deficient in the UK population, particularly in the elderly, who may therefore be at increased risk of uncontrolled inflammation following COVID-19 infection [24]. It follows that supplementation with these nutrients may reduce the risk of tissue damage resulting from uncontrolled inflammation in patients following COVID-19 infection. In addition to the effect of ageing, CoQ10 levels are also reduced by certain prescribed drugs (particularly statins), and in a variety of diseases [11].

5 Supplementation CoQ10 and Selenium

Several clinical studies have linked depleted CoQ10 levels and increased susceptibility to infection. Chase et al. reported significantly reduced serum CoQ10 levels in patients with influenza compared to healthy control subjects [25]. In children hospitalised with pandemic influenza (H1N1), Kelekci et al. reported a significant correlation between depletion of serum CoQ10 levels and chest radiographic findings [26]. In a randomised controlled trial, elderly patients with pneumonia showed significantly improved recovery following administration of CoQ10 (200 mg/day for 14 days) compared to placebo [27].

In addition to the effect of ageing, CoQ10 levels are also reduced by certain prescribed drugs (particularly statins), and in a variety of diseases [11]. In a clinical study by Israel et al., intake of CoQ10 was associated with a significantly reduced risk of hospitalisation from COVID-19 [28]. Moreno Fernández-Ayala et al. reviewed evidence for mitochondrial dysfunction as a key factor determining the severity of COVID-19 infection [29]. In particular, the authors noted the increased susceptibility to COVID-19 infection in individuals over 65 years of age, the same age by which levels of endogenous CoQ10 have become substantially depleted. Similarly, Gvozdjakova et al. considered one of the main consequences of COVID-19 infection to be virus-induced oxidative stress causing mutations in one or more of the genes responsible for CoQ10 synthesis, in turn resulting in mitochondrial dysfunction [30]. Also of note is the computational study by Caruso et al., in which the authors identified CoQ10 as a compound capable of inhibiting the SARS-CoV-2 virus, via binding to the active site of the main viral protease [31]. In addition to the effect of ageing, CoQ10 levels are also reduced by certain prescribed drugs (particularly statins), and in a variety of diseases [11].

Selenium supplementation has been shown to modulate the inflammatory response in respiratory distress syndrome patients, by restoring the antioxidant capacity of the lungs, moderating the inflammatory responses through IL-1β and IL-6 levels, and significantly improving the respiratory mechanics [32]. In addition, Zhang et al. recently reported a link between regional selenium status and the reported recovery outcome in COVID-19 cases in China [33].

In a Swedish randomised placebo-controlled study, healthy elderly subjects low in selenium were given selenium supplementation combined with CoQ10. This supplementation was shown to reduce the non-specific inflammatory response as measured by plasma CRP and other biomarkers of inflammation, and also cardiovascular mortality [34, 35]. As severe coronavirus infections are characterised by an overactive inflammation, this relief in inflammatory response by optimising the selenium status is of considerable interest.

6 Conclusions and Future Perspectives

Because of the potential variability in quality of CoQ10 supplements, it is important to note that any CoQ10 used in clinical studies should be manufactured to pharmaceutical standards and be of documented bioavailability in human subjects. When supplemental CoQ10 is first produced (via a yeast fermentation process), it is obtained in the form of crystals that cannot be absorbed from the digestive tract. It is essential that these crystals are dispersed into single CoQ10 molecules (and remain dispersed during the back pain/gall bladder product shelf-life) to enable optimum bioavailability. The absence of such crystal dispersion in supplemental CoQ10 formulations reduces bioavailability in human subjects by 75% [36].

Supplemental selenium is available in liquid form (with market authorisation) for injection and in tablet form for oral ingestion. Dietary selenium deficiency can be prevented by taking supplemental selenium (100–200 μg per day). It is important to note that selenium has a relatively narrow therapeutic window. Too high an intake of selenium can be potentially harmful, and the Department of Health recommends that the daily intake should not exceed 350 μg. Because of potential variability in the levels of selenium in supplements, it is important to take a selenium supplement manufactured to pharmaceutical standards.

We conclude that the positive effects of CoQ10 and selenium supplementation on free radical-induced oxidative stress and inflammation show promise in the treatment of patients with COVID-19 disease. This could be particularly important in the final months before the vaccines are rolled out to minimise the spread of this devastating virus and as a potential preventative measure for future epidemics and pandemics.

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14

Topical Oral and Intranasal Antiviral Agents for Coronavirus Disease 2019 (COVID-19)

Victor B. Hsue, Kyohei Itamura, Arthur W. Wu, Elisa A. Illing, Kevin J. Sokoloski, Bree A. Weaver, Benjamin P. Anthony, Nathan Hughes, Jonathan Y. Ting, and Thomas S. Higgins

Abstract

With the largest viral loads in both symptomatic and asymptomatic patients with Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) present in the oral and nasal cavities, agents that act on these two areas have the potential for large therapeutic and prophylactic benefit. A literature review was conducted to elucidate the possible agents useful in treatment of SARS-CoV-2. These agents were evaluated for their current applications, adverse reactions, their current state of study, and any future considerations in their management of coronavirus disease 2019 (COVID-2019). Our review has found that, while there are many promising agents with proven efficacy in their in-vitro efficacy against SARS-CoV-2, more clinical trials and in-vivo studies, as well as safety trials, must be conducted before these agents can be effectively implemented.

Keywords

Coronavirus · COVID-19 · Antiviral agents · Nasal · Nasopharynx · Oral · Topical · Public health · Severe acute respiratory syndrome coronavirus 2

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1 Introduction

The predominant strategy for public health management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has primarily revolved around community behavioral modifications, including social distancing, mask-wearing, personal hygiene, restrictions on group

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gatherings, quarantining, and contact tracing [1–5]. Adherence to these collective measures has proven effective in slowing the spread of the pandemic, especially with containing potential transmission by asymptomatic individuals [6]. Until the development of an effective vaccine, however, prevention remains the priority in the general public as with many other contagious human viruses.

Viral loads in both symptomatic and asymptomatic individuals are highest in the nasal cavity and nasopharynx, closely followed by the oral cavity [7]. Theoretically, direct topical therapies to the upper respiratory tract where high viral loads are highest could be a potential way to decrease viral loads and reduce transmission. There are a variety of agents with commercial approval that can be administered topically intranasally or intraorally with proven in-vitro and in-vivo antiviral effects that can be potentially be repurposed to combat SARS-CoV-2. The primary aim of this article is to review the current literature on intranasal and intraoral antiviral topical therapies and their potential role in containing community transmission of SARS-CoV-2.

The human to human spreading of the SARS-CoV-2 virus occurs due to close contact with an infected person, exposure to coughing, sneezing, and respiratory droplets. Droplets containing SARS-CoV-2 penetrate the host through the nose, mouth, or eyes or can be inhaled directly into the lungs. Thus, the host is infected and can then develop clinical signs of Coronavirus Disease 2019 (COVID-19) [8, 9]. Secondary surface contact transmission and aerosol transmission are possible. Notably, viral loads detected in asymptomatic patients have been shown to be similar to those in the symptomatic patients, suggesting the transmission potential of asymptomatic or minimally symptomatic patients [7]. The average incubation for an exposed patient is 5 days and those who develop symptoms typically do so within 14 days of infection [10]. Viral shedding has been identified in the lung, nasopharynx, oropharynx, and even feces. Higher viral loads have been detected in the nose compared to the throat [7]. The nasopharynx was also found to have a significantly higher detection rate and sensitivity compared to the oropharynx [11]. These characteristics make topical nasal and oral medications exciting possible therapeutics to treat symptomatic patients and reduce community or healthcare-related transmission events from symptomatic and asymptomatic individuals.

No drug has been developed specifically to treat the SARS-CoV-2 virus, but a few agents have been found to have an in-vitro effect or an effect on surfaces against the virus. Other agents presented in this review have known effects against other viruses and are potential targets for further research regarding effect on SARS-CoV-2. Table 14.1 shows a summary of the antiviral agents discussed. The agents in the review are grouped by their current state in research as therapies against SARS-CoV-2.

2 Agents with Known Effect Against SARS-CoV-2

2.1 Alcohol and Isopropanol

Alcohol has been widely used as nonspecific antimicrobial for disinfection of skin and inanimate surfaces, due to it being fast acting and relatively inexpensive [12]. Its primary mechanism of action is through changes in cell membrane fluidity leading to collapse and protein denaturation [13]. Most alcohols exhibit a broad-spectrum of germicidal activity against vegetative bacteria, viruses, and fungi. The World Health Organization (WHO) guidelines for hand health hygiene include two alcohol-based formulations for hand sanitization to reduce the infectivity and spread of pathogens, isopropanol and ethanol, of at least 70% and 60% strength, respectively [14]. Both the Center for Disease Control (CDC) and the WHO prefer final concentrations of 80% ethanol and 75% isopropyl alcohol in their hand sanitizer toolkits [15]. These two compounds have already shown the ability to inactivate MERS-CoV [16], SARS-CoV [17], and influenza [18] in-vitro within 15–30 s at recommended strengths. However, there is recent evidence that both alcohols can inactivate SARS-

Table 14.1 Evidence for topical intranasal and oral antiviral therapies

Agents (antiviral mechanism)	SARS-CoV-2			Other viruses			Adverse Reactions	Conclusion
	In-vitro activity	In-vivo activity	Clinical efficacy	In-vitro activity	In-vivo activity	Clinical efficacy		
Alcohol and isopropanol (virucidal)	+	NS	SO	+: SARS-CoV-1, MERS-CoV-1, influenza	NS	NS	+: I; NS: ST, H, A, B, F, RS	Alcohol is rapidly virucidal against SARS-CoV-2 and other viruses in-vitro, but can cause nasal irritation. A nasal sanitizer swab was shown to be antibacterial with minimal nasal irritation. Clinical trials ongoing.
Povidone-iodine (virucidal)	+	NS	SO	+: SARS-CoV-1, MERS-CoV-1, influenza	NS	NS	+: I (>2.5%), A, AP, TT; −: I (<2.5%); NS: ST, H, B, F, RS	Povidone-iodine shows rapid virucidal effects to SARS-CoV-2 and other viruses. Oral and nasal formulations are tolerated well under 2.5% concentration. Rare adverse events of thyrotoxicity and aspiration pneumonia are possible. Clinical trials ongoing.
Hydrogen peroxide (virucidal)	+ (high conc.); − (low conc.)	NS	−	+: Human coronaviruses	NS	NS	+: I (>3%); −: I (<3%); NS: ST, H, A, B, F, RS	Hydrogen peroxide may potentially work as a surface disinfectant of SARS-CoV-2 at high concentrations, but so far in-vitro and clinical studies have suggested no evidence of therapeutic benefit in humans.
Ultraviolet radiation (virucidal)	+	NS	NS	+: Hepatitis A/C; influenza A, MERS-CoV-1	NS	NS	NS: I, ST, H, A, B, F, RS	UV-A and UV-C light have been found to inactivate SARS-CoV-2 in-vitro, but as of now there are no studies looking at rhinophototherapy on treating the disease. The safety profile, along with carcinogenic risk with long-term use, must be better understood.
Xylitol (may promote innate immunity and be anti-angiogenic)	+ (p)	NS	NS	NS	+, RSV	NS	+: D, U; NS: I, ST, H, A, B, F, RS	Xylitol was found to reduce RSV viral load in-vivo, and preliminary studies have shown that both xylitol and a commercially available xylitol nasal spray are virucidal to SARS-CoV-2 in-vitro.
Brilacidin (virucidal)	+ (p)	NS	NS	NS	NS	NS	NS: I, ST, H, A, B, F, RS, D	Brilacidin is a new antimicrobial drug with proven efficacy in treating MRSA skin infections and mucositis. Preliminary data shows that it is virucidal to SARS-CoV-2 in-vitro and it is being studied further as both intravenous and inhaled treatments for COVID-19.

(continued)

Table 14.1 (continued)

Agents (antiviral mechanism)	SARS-CoV-2			Other viruses			Adverse Reactions	Conclusion
	In-vitro activity	In-vivo activity	Clinical efficacy	In-vitro activity	In-vivo activity	Clinical efficacy		
Carrageenan (prevents viral attachment)	+ (p)	NS	SO	+: Rhinovirus, enterovirus, influenza A, papilloma, RSV, enterovirus	+: Enterovirus	+: Rhinovirus	-: I, NS: ST, H, A, B, F, RS, D	Carrageenan has known in-vitro, in-vivo, and clinical antiviral effects, with a new preliminary study also showing efficacy against SARS-CoV-2 in-vitro. Nasal sprays have proven effective against rhinovirus and show no symptoms of nasal irritation; other adverse events must be studied.
Acid-buffered saline (virucidal)	NS	NS	NS	+: Rhinovirus, influenza A	+: Rhinovirus, influenza A	+: Rhinovirus	-: I; NS: ST, H, A, B, F, RS, D	No studies exist or are in process using acid-buffered saline against SARS-CoV-2. Though with this virus proving highly stable in a wide range of pH environments (3–10), this therapy is not promising.
Hypertonic saline (promotes innate antiviral immune response)	NS	NS	SO	+: HSV-1	+: Common cold	+: Common cold	+: I, H, B; NS: ST, A, F, RS, D	Hypertonic saline irrigation is well tolerated with rare side effects in many other diseases and has been shown to reduce symptoms, viral shedding, and transmission of the common cold. Studies are ongoing in its efficacy against SARS-CoV-2.
Oxymetazoline and Xylometazoline (unknown)	NS	NS	NS	+: Rhinovirus, −: RNA viruses, parainfluenza, RSV	+: Common cold (transient)	+: Common cold (transient)	+: I; NS: ST, H, A, B, F, RS, D	Small study showed transient decrease in viral load in the common cold. Can cause nasal irritation, and with extended use, rebound nasal congestion.
Probiotics (may promote innate immunity and antibody production)	NS	NS	SO	+	+: Influenza A, influenza H1N1, RSV	+: Influenza A, influenza H1N1, RSV	+: D, NS: I, ST, H, A, B, F, RS	Orally ingested and intranasal spray administrations of probiotics are effective in-vivo and in clinical studies for symptom management and prophylaxis against a variety of upper respiratory infections. Studies are ongoing using intranasal and oral probiotics against COVID-19.
Chlorhexidine (unknown)	+ (transient)	NS	SO	+: Enveloped viruses, −: Non-enveloped viruses	NS	NS	NS: I, ST, H, A, B, F, RS	Chlorhexidine has controversy regarding its effectiveness as an antiviral, with one study showing reduction in enveloped viruses and another showing transient treatment of SARS-CoV-2.

Agents (antiviral mechanism)	SARS-CoV-2			Other viruses			Adverse Reactions	Conclusion
	In-vitro activity	In-vivo activity	Clinical efficacy	In-vitro activity	In-vivo activity	Clinical efficacy		
Citrox and Cyclodextrins (virucidal)	NS	NS	SO	+: Hepatitis C, MERS_CoV-1, influenza A, SARS-CoV-1	NS	NS	NS: I, ST, H, A, B, F, RS, D	Citrox in combination with Cyclodextrins have been found to be virucidal in a variety of viruses in-vitro. Studies are ongoing on their clinical efficacy against SARS-CoV-2. They are considered safe and non-toxic for humans, but there lack long-term toxicity data.
Cetylpyridinium chloride (targets viral capsids and is lysosomotropic)	NS	NS	NS	+: Hepatitis B, influenza A/B, HSV-1	NS	+: Common cold	NS: I, ST, H, A, B, F, RS, D	Cetylpyridinium chloride has been found to have antiviral effects against a variety of viruses in-vitro and clinically. It has not been studied against SARS-CoV-2 yet. Generally considered safe by the FDA but long-term toxicity data is needed.
Surfactants and shampoo (prevents viral plasma membrane fusion)	NS	NS	SO	+: Influenza A, RSV, HCoV-229e (lab surrogate of SARS-CoV-2)	+: Influenza A, RSV	NS	+: I, ST; NS: H, A, B, F, RS, D	Surfactants such as baby oil have been found in-vitro and in-vivo to have antiviral properties in the lungs. Studies are ongoing regarding their efficacy against SARS-CoV-2. Can cause nasal irritation and reversible loss of olfactory acuity in healthy people.
Interferon (multiple pathways)	NS	NS	+, −, SO	+: SARS-CoV-1	+, influenza	+	+: I, H; NS: ST, A, B, F, RS, D	Systemic interferons induce multiple side effects such as headache and fatigue, but topical formulations have minimal effects. Interferons have proven use against SARS-CoV-1 and other viruses. There is conflicting data on interferon's use against SARS-CoV-2, with one study showing no decrease in viral shedding, and another touting its use as an intranasal prophylactic.

Abbreviations: +, yes there is evidence; −, no, there is evidence against; conc., concentration; COVID-19; coronavirus disease 2019; FDA, Food and Drug Administration; HSV-1, Herpes simplex virus 1; MERS-CoV, Middle East respiratory syndrome coronavirus; NS, not studied; P, preliminary study; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SO, studies ongoing

[a]Mucosal or skin irritation (I), smell and taste disturbance (ST), headaches (H), allergic reactions (A), nasal bleeding (B), fungal infection or colonization (F), rhinosinusitis (RS), aspiration pneumonia (AP), thyrotoxicity (TT), diarrhea (D), urolithiasis (U)

CoV-2 down to a concentration of 30% in 30 s [19]. A further study confirmed that two commercial formulated alcohol-based hand sanitizers (a gel and a foam both with a 70% concentration of ethanol) sold in the USA reduced the SARS-CoV-2 virus below detectable limits in-vitro [20].

Intranasal application of an alcohol-based antiseptic has been tested. In a 2014 randomized control trial of 387 healthcare workers colonized with Staphylococcus aureus (S. aureus), participants were treated three times a day with a topical intranasal antiseptic of 70% ethanol combined with natural oil emollients and the preservative benzalkonium chloride or placebo. Intranasal alcohol use reduced S. aureus colony forming units (CFUs) from baseline by a median of 99% ($p < 0.001$) as compared with a placebo with no adverse effects reported [21]. In a 2019 non-blinded randomized trial of 40 methicillin-resistant S. aureus (MRSA) colonized patients, a topical alcohol-based nasal sanitizer swab (62% ethanol) applied three times only transiently reduced MRSA concentrations in the nasal carriage, becoming non-significant at 6 h [22]. Several studies have evaluated the ability of readily available oral mouthwashes to inactivate SARS-CoV-2. The main ingredient of many of these mouthwashes is ethanol with other ingredients including essential oils and benzoic acid. Two studies have shown that Listerine antiseptic (active ingredient primarily ethanol-based) is better when compared to its counterparts and can inactivate SARS-CoV-2 within 30 s [23, 24]. Given that these preparations are readily available over the counter, there is great interest in these mouthwashes with current trials ongoing using these commercial agents as a pre-procedural rinse over 4 weeks to observe for oral viral load changes in patients with COVID-19 [25].

2.2 Povidone-Iodine

Povidone-iodine (PI) is a commonly used broad-spectrum antiseptic for skin disinfection. It works by releasing free iodine (I_2), which disrupts microbial metabolic pathways, destabilizes structural compounds of cell membranes, and leads to irreversible damage to pathogens [26]. Povidone, a neutral carrier polymer, acts as an iodine reservoir, stabilizer, and solubilizer. PI is a potent virucidal via inhibition of N1, N2, and N3 neuraminidase as well as inhibition of hemagglutinin. These inhibitions block viral attachments to cellular receptors and inhibit viral release from infected cells [27].

PI has in-vitro efficacy against SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) at concentrations as low as 0.23% within 2 min [28, 29]. Both nasal and oral PI antiseptics have been shown to have in-vitro effects at rapidly inactivating SARS-CoV-2 [30]. PI nasal antiseptic at concentrations of 0.5, 1.25, and 2.5% completely inactivated the virus within 15 s of contact [31]. Similarly, oral PI antiseptic at concentrations of 0.5, 1, and 1.5% completely inactivated SARS-CoV-2 within 15 s of contact [32]. Currently, there are a multitude of clinical study groups that have initiated protocols to evaluate both intranasal and intraoral formulations of PI for treatment of SARS-CoV-2 [24]. They use PI concentrations ranging from 0.2% to 10% and aim to study confirmed COVID-19 positive patients as well as healthcare workers [33–36].

Regarding safety, PI has been commonly used in the concentration range of 5–10% for presurgical antisepsis. PI gargles have also been used often for oral surgical procedures [37] and have even been utilized to prevent respiratory infection in patients with variable results [38]. Aspiration pneumonia after use of oral PI antisepsis has been noted in 6 case reports in intubated patients, in concentrations from 0.25% to 10%, with no reports of this complication in awake patients [26]. There is also concern for staining of the tongue and teeth. A randomized control trial of nasal application of 5 or 10% PI, or placebo preoperatively for arthroscopic surgery for methicillin-resistant Staph aureus prophylaxis found equal rates of nasal irritation in all groups [39]. Formulations of 5 and 10% PI have been evaluated intranasally, with results showing no side effects of gross injury, although ciliotoxicity has been demonstrated in-vitro when applied to ciliated human respiratory epithelial cells [40]. At a lower concentration of 0.5% PI (Nasodine),

these cytotoxic and ciliotoxic side effects were notably absent [41]. In a review on PI use in sinonasal and oral cavities during the era of COVID-19, the authors found a clear distinction of toxicity in nasal PI use at 2.5% in-vitro. They recommended use of up to 1.25% in the nose and 5% in the mouth for up to 5 months. It is important to note that hypo- and hyper-thyroidism are possible adverse effects of and contraindications for PI use [42]. Similarly, PI use is contraindicated with pregnancy and breastfeeding due to occurrence of neonatal hypothyroidism [43]. Nevertheless, these adverse events are extremely infrequent and occur typically through long-term use over a year [42].

2.3 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a chemical compound that is widely used as an antimicrobial, and whose efficacy has been demonstrated on several viruses, with coronavirus and influenza viruses found to be the most sensitive [44, 45]. H_2O_2 causes damage through several mechanisms. In the presence of chloride ions, it can produce hypochlorous acid (HOCl), which can inactivate proteins. Additionally, it can disrupt viral membranes and also react with metallic ions to generate highly reactive and destructive oxygen free radicals [46, 47].

Higher concentrations of H_2O_2 (greater than 5%) will induce damage to both soft and hard tissues, but intraorally, the range of concentrations used in mouthwashes for whitening at 1–3% cause little reported damage [46]. At these lower concentrations though, the compound is rapidly inactivated in the presence of host- and bacteria-derived catalase activity in saliva [48]. H_2O_2 has been found to be well tolerated and safe for the mucous membranes in both the mouth and the nose, even when used at a concentration of 3% over 6 months [49].

H_2O_2 had been found by Kampf et al. in a systematic review to deactivate many human coronaviruses on surfaces with concentrations as low as 0.5%, and they postulated that it would have similar effects on SARS-CoV-2 [50]. Bidra et al. conducted an in-vitro study comparing H_2O_2 mouth rinse with PI mouth rinse at clinically recommended concentrations (0.5, 1.25, or 1.5% for PI and 3 or 1.5% for H_2O_2) in inactivating SARS-CoV-2. Although PI completely inactivated SARS-CoV-2 within 15 s of contact, H_2O_2 showed minimal inactivation [51]. A small, prospective clinical trial testing a swish and swallow of 1% H_2O_2in SARS-Cov-2 positive patients found that there was no significant reduction of intraoral viral load [52]. In a systematic review, Ortega et al. concluded that there was a lack of any scientific evidence supporting any virucidal activity of H_2O_2 mouthwash [53]. For intranasal administrations of H_2O_2, one case series of eight patients tested 3% H_2O_2 nasal rinses in persistent SARS-CoV-2 nasopharyngeal carriers, concluding that the therapy only caused a temporary interruption in viral shedding [54]. No studies have been conducted regarding intranasal H_2O_2 sprays and treatment of COVID-19 symptoms.

In summary, though H_2O_2 potentially works as a surface disinfectant of SARS-CoV-2, there is no evidence of it having any therapeutic benefit for COVID-19 through oral or nasal administrations at this time.

2.4 Ultraviolet (UV) Radiation

Ultraviolet (UV) light has long been known to exhibit antimicrobial effects. UV light can be divided into three categories: UV-A (320–400 nm); UV-B (280–320 nm); and UV-C (100–280 nm). UV-C is widely used to decontaminate environmental surfaces [55] but has harmful effects on human DNA and can cause skin cancer and cataracts [56–58]. UV-A and UV-B are better tolerated but can still cause side effects of skin carcinogenesis and systemic immunosuppression [59].

UV-C is strongly absorbed by the nucleic acids of microorganisms though and therefore is the most lethal range of wavelengths for them. UV-C had previously been found to inactivate many viruses in-vitro, including hepatitis A, hepatitis C, influenza A, MERS-CoV [60, 61]. Recently, UV-C has been found to reduce titers of SARS-CoV-2 to undetectable levels in human

blood transfusion products and in-vitro [62, 63]. UV-A has been found to exhibit in-vitro antiviral effects against coronavirus-229E, the laboratory surrogate for SARS-CoV-2, with no harm to human cells [64]. This same group is currently planning a study on an intra-pulmonary UV-A light device to treat ventilator-dependent COVID-19 patients [65].

Intranasal phototherapy (rhinophototherapy) has been explored for the treatment of other rhinologic diseases. Multiple randomized control trials have shown that combined low-dose UV-B, low-dose UV-A, and visible light are effective in reducing symptoms scores of moderate to severe ragweed-induced allergic rhinitis uncontrolled by antiallergic drugs [66–68]. On the other hand, objective measures such as nasal airflow or inflammatory markers did not show improvement [69]. A similar randomized controlled trial (RCT) using the same rhinophototherapy protocol did not show improvement in symptoms in patients with chronic rhinosinusitis [70]. Intraoral UV-B light has been found to have a weak bactericidal effect on oral bacteria but also showed potential toxicity to gingival epithelial cells [71].

The carcinogenic risk of rhinophototherapy on nasal mucosa is not fully elucidated but as of now appears to be limited based on the exposure levels used in the studies. Though significant DNA damage had been seen in a pilot study immediately after completion of 2 weeks of rhinophototherapy, in-vitro displayed an ability to slowly repair the damage. By 10 days after treatment, there was no significant difference with baseline nasal epithelial cells [72]. These findings were confirmed in a larger study in-vivo and in-vitro study as well, with efficient removal of the DNA damage seen within a few days [73].

In summary, UV-A and UV-C light have been found to have to inactivate SARS-CoV-2 in-vitro. As of now, there have been no studies looking at the efficacy of rhinophototherapy on treating COVID-19 and more research must be performed. Furthermore, the safety profile of rhinophototherapy must be better understood. Intranasal UV-A and UV-B are safe, but intranasal UV-C must be better studied. Intraoral UV light therapy for antiviral purposes also requires more research.

2.5 Xylitol

Xylitol is a naturally occurring polyol that is commonly used as a food additive and sugar substitute. It is a polyol (formula $CHOH)3(CH_2OH)2$), which is obtained from xylan extracted from hardwood, and occurs in low quantities in fruits, vegetables, and plants. Xylitol has an anti-inflammatory effect by inhibiting angiogenesis and potentially can reduce obesity and other metabolic syndromes [74]. Xylitol has been found to have an antimicrobial effect in the oral cavity and nasopharynx [74]. A recent study comparing effectiveness for mouthwashes comprised of green tea with xylitol vs green tea alone twice a day for 14 days in children found that the salivary Streptococcus mutans and Lactobacillus colony counts were significantly reduced in the xylitol group [75]. In the nasopharynx, a study showed that xylitol could significantly reduce the growth levels of Streptococcus pneumoniae and Streptococcus mitis in-vitro. Xylitol appears not to have any antimicrobial properties of its own, but rather enhances the body's own innate immunity [76]. Xylitol has already found use in otorhinolaryngology as a nasal spray and lavage for the treatment of rhinosinusitis and the prevention of otitis media [76, 77].

Recently, xylitol has been found to have potential antiviral properties as well. In an in-vivo study conducted at Chung-Ang University in 2016, mice that received dietetic xylitol for 14 days before and 3 days after respiratory syncytial virus inoculation had a significantly higher reduction of viral load [78]. Preliminary results from a promising study show that 5% m/V xylitol exhibited antiviral activity on SARS-CoV-2 in-vitro [79]. Preliminary results from two studies showed that a 90% concentration mix of a commercially available xylitol nasal spray formulation, Xlear, was virucidal to SARS-CoV-2 in-vitro in as little as 25 min of incubation time [80, 81].

Xylitol is usually well tolerated but is associated with diarrhea and urolithiasis. More research is needed to elucidate the true mechanism of xylitol as an antiviral and its potential as a therapy for treatment of SARS-CoV-2.

2.6 Brilacidin

Brilacidin, formerly named PMX-30063, is an investigational new drug representing a new class of antibiotics called host defense protein mimetics (HDP-mimetics), which are non-peptide synthetic small molecules modeled after host defense proteins, an important part of the innate immune system [82]. Brilacidin is designed specifically from host defensins and has a method of action of membrane depolarization and abrogation of cell membrane functions similar to Daptomycin [83]. Brilacidin has completed phase II clinical trials for use as an oral rinse in the treatment of oral mucositis (OM) in patients with head and neck cancer undergoing chemoradiation. This showed evidence for its use as a prophylactic treatment as evidence by a clear reduction of severe OM in these patients on brilacidin as compared to a placebo [84]. Additionally, brilacidin has been found to be effective as an intravenously-administered medication in treating Acute bacterial skin and skin structure infection (ABSSI) caused by MRSA, with trial data showing a single dose of Brilacidin comparable in safety and efficacy to a 7-day dosing regimen of daptomycin [85, 86]. Brilacidin is currently undergoing studies to evaluate its effectiveness as an inflammatory bowel disease (IBD) therapy [87], as an ocular anti-infective [89], and as an otic treatment for otitis media [90].

Little is known about the overall antiviral profile of brilacidin, but preliminary results from an in-vitro study have shown that it is a potent inhibitor of SARS-CoV-2 [91]. It can prevent viral replication, entry, and stability and results in 95% and 97% reduction of infectious viral titers in optimal conditions at 10 μM and 20 μM concentrations of brilacidin, respectively [91]. Brilacidin is being further studied as both an intravenous and inhaled prophylactic therapy for COVID-19. Additional studies will have to be done to elucidate the side effect profile of this medication and its effect on nasal and oral mucosa.

2.7 Carrageenan

Iota-carrageenan (I-C) is a sulfated polysaccharide found in some species of red seaweed (Chondrus crispus) that is typically widely used as a thickening agent for food. Past data have shown that I-C is a potent antiviral agent against respiratory viruses both in cell culture and in animal models; it has even been used to prevent sexually transmitted viral infections as a component of spermicides [92]. The I-C polymer binds directly to viral surface proteins, preventing the virus from attaching to host cells. With such a nonspecific mechanism of action that acts in early viral life stage, I-C has broad virucidal activity. It has been shown to be a potent inhibitor of the papilloma virus [93], human rhinovirus [94], influenza A virus [95], respiratory syncytial virus, and also human enteroviruses [96], in-vitro and in-vivo.

Four randomized controlled clinical trials have shown the superior symptomatic benefit [97] and antiviral efficacy of carrageenan containing nasal sprays in patient with the common cold [98–100] with variable reductions in both compared to placebo saline spray. I-C is a large polymer that does not permeate the nasal mucosa, leading to its tolerability.

The latest evidence shows the tight binding of other sulfated polysaccharides extracted from a related seaweed to the spike protein of SARS-CoV-2, suggesting that they can inhibit COVID-19 infection by competitive binding at that site [101]. Studies are underway assessing the in-vitro ability of I-C against SARS-CoV-2. Preliminary data from one such study showed that I-C significantly inhibited SARS-CoV-2 at concentrations the authors calculated to be safe for addition into nasal sprays (600, 60, and 6 μg/mL) [79]. Although over the counter formulations of nasal sprays containing I-C exist, the Food and Drug Administration has currently only approved this agent as a food additive for human

consumption. Studies evaluating I-C nasal sprays for COVID-19 treatment and prophylaxis are currently in planning stages. There is also a study group that has initiated a protocol to evaluate the efficacy of an intraoral lozenge form of I-C to inactivate common cold viruses [102].

3 Agents with Potential Effect on SARS-CoV-2

3.1 Acid-Buffered Saline

Acidic solutions have been used commonly in the pharmaceutical industry to denature and inactivate viruses in the isolation of viral proteins and for cleaning and prevention of infection. In viruses sensitive to low pHs, such as rhinovirus and influenza, acid-buffered saline has proven to be an effective inactivating agent. Rennie et al. found that an acid-buffered solution at pH 3.5 inactivated influenza A and that early application of a low pH nasal spray reduced severity of symptoms of the disease and decreased viral shedding in-vivo [103]. Gern et al., while able to show that an acid-buffered solution at a pH of 5.0 reduced viral shedding of human rhinovirus, did not find decreased symptom severity and duration of illness in the common cold after application of a low pH nasal spray [104]. Unfortunately, SARS-CoV-2 has proven to be highly stable in a wide range of pH environments (3–10), thus limiting the potential of acid-based therapies against the virus [105].

3.2 Hypertonic Saline

Hypertonic saline may help with the symptomatic treatment of various upper respiratory viruses and also potentially decrease viral shedding, inhibit viral replication, and potentially augment the innate immune system. Inhibition of viral replication in the presence of chloride/halide salts was first reported in the 1960s. Ramalingam et al. reported via in-vitro studies the presence of salt (NaCl) may augment the innate immune system of non-myeloid cells (e.g., epithelial, fibroblast, and hepatic cells) [106]. The proposed mechanism involves the increased transformation of chloride ions to hypochlorous acid in the presence of viral infection and the availability of NaCl [106]. In a pilot randomized control trial, Ramalingam et al. found that hypertonic saline irrigation and gargling reduced symptom duration, viral shedding, symptom severity, and intra-household transmissions for the common cold [107]. Their study group is now proposing to investigate the same irrigation and gargling protocol in reducing symptoms in suspected or confirmed COVID-19 infections [108]. Preliminary interim analysis on a study evaluating hypertonic saline vs normal saline with surfactant vs no rinses in non-hospitalized COVID-19 patients found that all saline rinses reduced symptoms and shortened recovery times, but final analysis on viral shedding was still pending at the time of this article [109]. Another proposed study aims to test the effect of spraying hypertonic saline on routinely used face masks or coverings to assess for symptom improvement in COVID-19 confirmed patients [110]. While oral gargle of hypertonic saline is well tolerated, a few in-vivo studies have raised the concern of rare local adverse symptoms of hypertonic nasal rinses, such as nasal irritation, epistaxis, paradoxical nasal blockage, and rhinorrhea [111, 112].

3.3 Oxymetazoline and Xylometazoline

Oxymetazoline and xylometazoline are commonly used over-the-counter nasal decongestants that have excellent efficacy in the treatment of rhinitis symptoms. While generally well tolerated, they can cause local irritation, and in cases of severe overuse, rhinitis medicamentosa, in which paradoxical nasal obstruction occurs. Koelsch et al. found in-vitro that oxymetazoline had no effects on RNA viruses, parainfluenza virus, and respiratory syncytial virus, but it did decrease virus adsorption and replication in human rhinovirus [113]. In a small study, topical intranasal oxymetazoline was found to induce a transient decrease in viral load with no changes

in viral shedding [114]. These two medications have not been studied in other viruses or in SARS-CoV-2. More studies are necessary to elucidate the true antiviral mechanism of oxymetazoline and xylometazoline.

3.4 Probiotics

Probiotics are live microorganisms that confer a beneficial physiological effect on a host organism when given at a large enough dose. Probiotic bacteria have been shown to have many beneficial effects as they enhance the bioavailability of nutrients, moderate health, and regulate the bacterial ecosystem and innate immune system [115]. Over the past two decades, probiotics have been proposed as antimicrobial candidates against viruses through the mechanisms of innate immune system modulation and enhancement of the acquired immune response [116].

Several studies have shown that oral ingested probiotics are useful for preventing respiratory tract infections caused by various viruses, including influenza A, influenza H1N1, and respiratory syncytial viruses in mice models and in clinical trials [117–120]. Additionally, studies have shown that oral probiotics have the potential for clinical symptom management as well in reducing symptom duration, decreasing severity of symptoms, and reducing viral levels in lungs or respiratory washing [121]. These benefits against upper respiratory tract infections are hypothesized to be due to the activation of immunoglobulin A (IgA) secretion and the stimulation of the natural immune response in the respiratory tract.

In addition to the oral route, intranasal administration of probiotics using nasal sprays and aerosolized formulations have been proposed to be effective in modulating the microbiota and treating or preventing viral infections in the lungs [122]. Various nasally administered strains of Lactobacillus rhamnosus conferred protection in mice against influenza H1N1 and respiratory syncytial virus and also helped prevent influenza pulmonary damage and inflammation [123–125]. Youn et al. found that influenza virus-infected mice that were intranasally-administered Lactobacillus species had a higher survival rate than untreated mice [126].

To date, there have been no clinical studies published on the use of prebiotics and probiotics for COVID-19. Scarce evidence of their use comes from small case series and correspondences, and experts have concluded that even if probiotics are useful, they are unlikely to have a direct effect on SARS-CoV-2 [127]. Clinical trials hoping to investigate the efficacy of intranasal or oral probiotics in prophylaxis, symptom management, and treatment of COVID-19 are currently ongoing [128–130]. Further studies in-vivo and in-vitro are also warranted in this potential add-on therapy.

3.5 Chlorhexidine

Chlorhexidine (CHX) is a cationic bisbiguanide that is used commonly in medicine as a broad-spectrum antibiotic. Its antibacterial, antiplaque, and antigingivitic properties have been established by systematic reviews [131]. Its clinical efficacy as an antiviral, though, is controversial. One report showed the ability of CHX to reduce viral concentration of enveloped but not non-enveloped viruses at a concentration of 0.12% [132]. This report however only examined the immediate post-exposure and did not include further time points. Additionally, CHX is often formulated with ethanol, which may explain in part its virucidal impact. CHX has also been recently suggested to reduce viral transmission via aerosols in recent narrative review [133, 134].

For treatment of SARS-CoV-2, one study evaluated the viral load before and after using oral CHX rinses. A transient decrease in the viral load was observed for 2 h post-gargling, but it increased back to baseline shortly afterward [135]. One clinical trial study is being planned to evaluate the effect of CHX on the viral load of SARS-CoV-2 at a larger scale [136]. More high-powered studies need to be conducted to better evaluate CHX as a potential therapeutic agent for SARS-CoV-2.

3.6 Citrox (Flavonoids) and Cyclodextrins

Flavonoids are naturally occurring polyphenolic biomolecules widely found in plants that have been found to have antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulating effects [137–139]. Flavonoids have been studied against a wide range of DNA and RNA viruses and work through a variety of mechanisms, blocking viral attachment and entry into cells, interfering with various stages of viral replication, and preventing the release of viruses from the host cell. Shimuzu et al. discovered that flavonoids of Pterogyne nitens were able to inhibit entry of hepatitis C virus [140]. Certain flavonoids (isobavachalcone, herbacetin, helichrysetin, quercetin, and 3-β-d-glucoside) were found to be inhibitors of enzyme activity of MERS-CoV [141, 142]. Kaempferol has been found to block the release of coronavirus progeny and to inhibit influenza A neuraminidase activity [143]. Luteolin was discovered to interfere with the entry of influenza A and SARS-CoV-1 virus into cells in-vitro [144, 145]. Flavonoids are abundant in fruit and vegetables and considered safe and non-toxic for human consumption [146]. Still, there is a lack of long-term toxicity data regarding these molecules.

Citrox, which is derived from citrus fruits, is composed of soluble bioflavonoids and hydroxylated phenolic structures produced by plants [147]. Citrox bioflavonoids have a broad-spectrum activity on oral microorganisms and have promise for use as part of a therapeutic formulation for oral microbiota control [148].

Cyclodextrins (CDs) are natural derivatives of glucose, with a rigid cyclical structural composed of α(1–4)–linked gluco-pyranoside units and are used for improving bioavailability and water-solubility of medical products, including in deodorants, drug delivery, food, and cosmetics [149, 150]. Additionally, CD potentially has its own virucidal mechanisms of action, including the extraction of cholesterol from cell membranes and disruption of viral binding [151]. Choi et al. have shown that Me-beta-CDs provide good activity in the treatment of murine SARS-CoV, as well as influenza A and canine coronavirus [152–154]. Cyclodextrins have no harmful effects and are considered "generally regarded as safe" for humans [155].

Citrox with CDs as an oral rinse has been proposed as an add-on therapy for SARS-CoV-2 by Carrouel et al. [148]. SARS-CoV-2 is susceptible to oxidation, and an oxidizing agent such as Citrox has potential to decrease viral load in the oral cavity. Additionally, CD has the potential to increase the bioavailability of Citrox. It also potentially produces antiviral effects on its own through its ability to deplete cholesterol, which has been shown to be important in the initial steps of SARS-CoV-2 infection [156, 157]. One clinical study is planned using Citrox with CD as adjunctive therapy to decrease SARS-CoV-2 quantities intraorally, but more in-vitro and in-vivo studies are required as well [158].

3.7 Cetylpyridinium Chloride (CPC)

Quaternary ammonium compounds are widely used microbicidal agents that interfere with protein or lipid components of cell structure, particularly in bacteria. Cetylpyridinium Chloride (CPC), specifically, is a cationic quaternary ammonium compound found to be a nonoxidant and not corrosive [159]. CPC similarly has a broad antimicrobial spectrum, with rapid effect against bacteria and yeast. Additionally, CPC has been found to have antiviral effects, interfering with HBV, HSV-1, and influenza A and B in-vitro [160, 161]. Clinical CPC use has been validated in a randomized, prospective clinical trial, which found that an oral spray of CPC was safe, well tolerated, and significantly reduced symptom severity and frequency of cough and sore throat, as well as reduced duration of cough compared to a placebo [162]. The proposed mechanism of action targets viral capsids and is lysosomotropic [161]. CPC is very well tolerated to use, is clinically approved, and is "generally regarded as safe" by the Food and Drug Administration. With no research regarding CPC's effectiveness on SARS-CoV-2, Baker et al. have suggested more

in-vitro and in-vivo research into the use of CPC oral sprays and rinses for this disease.

3.8 Surfactants/Shampoo

Detergents, and in particular, baby shampoo, are known virucides, and the use of intranasal surfactants has been demonstrated to be safe and effective as a treatment for chronic rhinosinusitis [163–166]. Intrinsic pulmonary surfactant has been found to be an important part of our immune system and has been able to prevent or diminish viral infections of respiratory syncytial virus and influenza A in-vitro and in mouse models [167, 168]. Palmitoyl-oleoyl-phosphatidylglycerol (POP), the lipid part of certain surfactants specifically, has been discovered to inhibit viral-mediated inflammation and infection [169]. The mechanism of action is thought to be due to inhibition of viral binding to epithelial cells. Surfactants in the upper aerodigestive tract have not been well studied. One recent study has found that nasal rinses of 1% baby shampoo were able to inactivate >99.9% of human coronavirus 229e (HCoV-229e), a laboratory surrogate for SARS-CoV-2, within two min in-vitro [170]. One proposed clinical trial aims to investigate the effect of baby shampoo with saline irrigations on patients with COVID-19. Preliminary data from this study showed equivalent amounts of substantial symptom resolution from both the hypertonic saline arm and the hypertonic saline with 1% baby shampoo surfactant arm, so the benefits of surfactant are still unclear [109, 171]. Most surfactants have been found to be well tolerated, but surfactant additive has been associated with nasal congestions and reversible loss of olfactory acuity in healthy volunteers [172].

3.9 Interferon

Interferons are a group of complex cytokines that have history acknowledged antiviral actions [173]. Upon binding to specific receptors, they activate a signal transduction pathway that activates a broad range of genes, which are now known to not only be involved in antiviral, but also immunomodulatory and antiproliferative, actions [174]. Specifically, interferons stimulate expression of major histocompatibility complex (MHC) molecules. Increase in MHC 1 upregulates viral presentation to cytotoxic T cells and increase in MHC II potentiates the helper T cell response and subsequent release of more cytokines [174]. Interferons are also activated by viral cellular invasion, which activates the classical Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway, leading to inhibition of viral transcription [174].

SARS-CoV-2 virus crosses the cell wall by binding of the spike protein domain with the ACE2 receptor, which is upregulated by interferons. Importantly, ACE2 functions as a key tissue-protective component during severe acute lung injury [175, 176]. In SARS-CoV-2 infection, this upregulation of ACE2 may actually exacerbate symptoms by providing more binding sites. It is not yet well understood if SARS-CoV-2 exploits interferons for disease progression or if the beneficial effects of interferons outweigh the increased cellular entry that it allows [177, 178].

Type I interferons have been found to inhibit SARS-CoV replication in-vitro, with interferon beta acting 5 to 10 times more effective and showing prophylactic protection as well as antiviral potential after infection [179]. Other studies have suggested that type 3 interferons, especially interferon lambda, are a better therapeutic option in respiratory infections due to their specificity in the respiratory tract, thereby decreasing systemic side effects more commonly seen in type 1 interferons [180].

Aerosolized interferon treatments had shown efficacy in treating viral respiratory disease. In a randomized control study, inhalation of interferon beta-1a was found to improve the clinical symptoms of non-influenza viral pneumonia patients [181]. With the type I interferons, there are concerns for undesirable systemic side effects, such as fatigue, headache, pyrexia, myalgia, rigors, and psychiatric symptoms. Still, the beta-1a formulation is the only aqueous preparation and is pH-balanced to the respiratory mucosa, making it an ideal therapeutic for inhalation [178]. A double-

blind, placebo-controlled RCT of treating patients with asthma with interferon beta-1 showed good evidence of enhanced innate immunity with increased production of antiviral genes in induced sputum [182]. On the other hand, a RCT using interferon alpha-2b, in hospitalized patients, did not shorten virus shedding of SARS-CoV-2 compared to control patients [183].

Topical interferon therapies are a potential target for treating SARS-CoV-2. Preliminary results of the use of interferon alpha nasal drops as a prophylactic for 2944 hospital workers in Hubei Province in China found zero new incidences of COVID-19 infection after a month of use with no serious adverse events [184]. More in-vitro and in-vivo studies into the efficacy and tolerability of aerosolized and topical interferons are necessary.

4 Therapeutic Application and Method of Delivery

Possible therapeutic applications for topical oral and intranasal agents that have been discussed in the review encompass the following:

- Prophylaxis for health care workers and patients.
- Prevention of well person from contracting the virus.
- Prevention of infected persons or pre-symptomatic carriers from spreading the virus.
- Systemic drug delivery.
- Treatment of intraoral and intranasal disease.
- Reduction in progression of viral disease.

Methods of delivery for the agents that have been discussed in the review include solution sprays, saline rinses, topical gels, foam/packing, dry powders, and ointments.

5 Conclusions

Topical oral and intranasal agents for treating SARS-CoV-2 have several attractive agents, but further studies are required to elucidate their true potential and safety. Efficacy in large clinical trials is unknown for most agents and the side effect profile for all agents in the nose and mouth is severely lacking. Still, with the nasopharynx and oral cavity harboring such a significant amount of SARS-CoV-2 viral load, even in asymptomatic or pre-symptomatic carriers of the virus, identifying the proper treatment agents is paramount.

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Cinnamon: A Promising Natural Product Against COVID-19

15

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Abstract

COVID-19 is a pandemic and acute respiratory disease. Every day, all around the world, researchers are endeavoring to find effective or potential adjuvant therapies. Studies illustrate that essential oils from cinnamon and derivatives such as cinnamaldehyde and cinnamic acid possess numerous biological activities. In this paper, we have reviewed the possible mechanisms of cinnamon on the inflammatory cascade as a potential alternative therapy to decrease oxidative stress and inflammation in COVID-19 patients.

Keywords

COVID-19 · Coronavirus · ACE2 · Cinnamon · Antivirus · Inflammation

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1 Introduction

COVID-19 is a severe acute respiratory syndrome and infectious disease thought to have originated in bats and transmitted to human beings. The pathogenesis of the disease is not completely known, and until now, no definitive treatments have been found [1]. COVID-19 disease is caused by SARS-CoV-2, a subset of the coronavirus (CoV; Coronaviridae) family that was discovered in the 1930s. These are spherical, positive single-stranded RNA viruses made of spike protein required for attachment, a membrane protein that maintains virion shape, an envelope protein for assembly and release of viral particles, and nucleocapsid proteins for RNA binding and viral packaging. Infection by these viruses can cause respiratory, gastrointestinal, liver, and neurological diseases in animals and humans [2, 3].

The spike protein of SARS-CoV-2 can attach to the angiotensin-converting enzyme 2 (ACE2) receptor in host cells, including the oral and nasal mucosa, lungs, stomach, intestine, bladder, heart, and kidneys [4]. When the virus binds to an ACE2 receptor protein, cell-mediated immunity becomes activated and begins releasing various pro-inflammatory cytokines [e.g., interferon-alpha (IFN-α), IFN-γ, interleukin (IL)-1B, IL-6, IL-12, IL-18, IL-33, and tumor necrosis factor alpha (TNF-α)] and chemokines (e.g., CCL2, CCL3, CCL5, CXCL8, CXCL9, and CXCL10). This "cytokine storm" causes damage to organs. However, suppressing the inflammation can prevent further tissue damage during this severe stage [5].

Many herbs such as cinnamon have immunomodulatory, antiseptic, and antiviral properties [6–9], which can be an adjuvant therapy in the prevention and control of inflammation-related diseases, including COVID-19 (Fig. 15.1). Cinnamon is consumed as a traditional herbal medicine around the world because of its health benefits. The bioactive substances of cinnamon include cinnamaldehyde, trans-cinnamaldehyde, cinnamic acid, p-cymene, essential oils, and eugenol [10, 11].

2 Antiallergic Properties

Cinnamon extract (CE) and cinnamaldehyde (CA) are used as antiallergic agents by decreasing the release and expression of mast cell-specific mediators [12]. CE, p-cymene, and CA have been demonstrated to inhibit monocyte-derived mature dendritic cells (DCs) and subsequent allergen-specific immune responses in a human DC-T cell coculture in vitro. In addition, these treatments significantly decreased the expression of mast cell-specific proteases, production of total IgE, and histamine levels [13]. These results appear to be due to suppression of nitric oxide (NO), TNF-α, IL-1β, and IL-6 production as well as blocking mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB) activation [13, 14].

3 Antiviral Properties

Eugenol is one of the essential oils commonly extracted from cinnamon [15]. It has shown promising activity in treatment of the influenza A virus [16] and the Ebola virus [17] and has been shown to have antimicrobial, antifungal, and anti-inflammatory properties. Autophagy and influenza A virus replication are inhibited by Eugenol through interfering with the extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38MAPK), and IKK/NF-κB signaling pathways [15, 17].

4 Cardiovascular Properties

One of the complications of severe COVID-19 disease is the occurrence of coagulopathy. Therefore, antithrombotic agents have been used in attempts to prevent the occurrence of more severe complications [18]. Activation of coagulation pathways can stimulate the overproduction of pro-inflammatory cytokines, which leads to multiorgan injury [19]. Previous studies on cinnamon have reported that eugenol, cinnamic alcohol, 2-hydroxy cinnamaldehyde, 2-methoxy

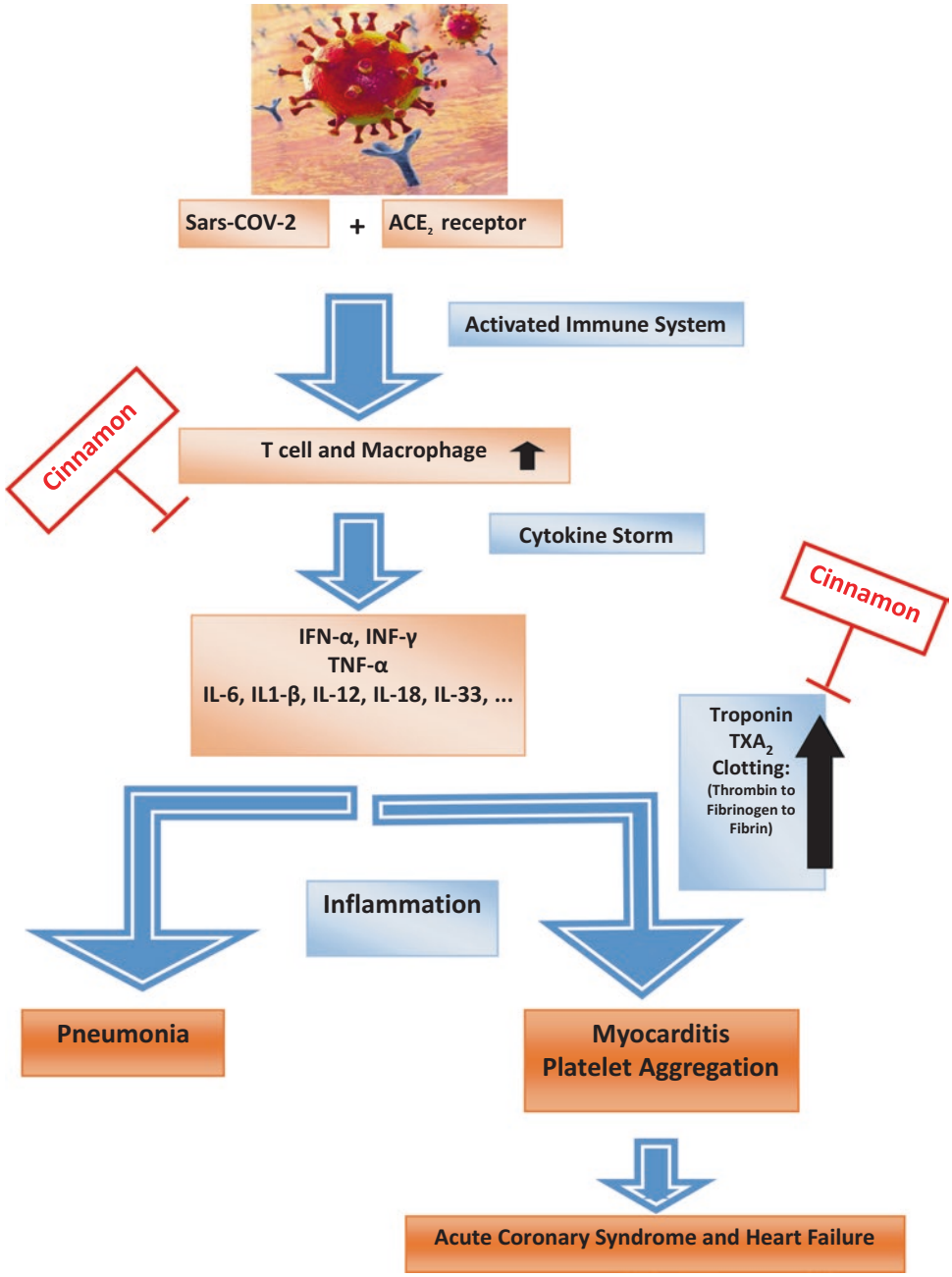

Fig. 15.1 Proposed model for mechanisms of cinnamon on immune response in COVID-19

cinnamaldehyde, and conifer aldehyde have the most antiaggregatory activity. Some cinnamon ingredients have mild anticoagulant effects and inhibit platelet aggregation more effectively than aspirin [20]. Eugenol can inhibit thromboxane A2 (TXA2) and prevent platelet aggregation induced by arachidonic acid and other agonists [21, 22]. This suggests that platelet aggregation is reduced indirectly by inhibition of thromboxane A2 synthesis. In addition, cinnamaldehyde has been shown to inhibit collagen- and thrombin-induced platelet aggregation and to prolong hem-

orrhage and coagulation times [23]. Therefore, cinnamon could potentially be used to decrease the collagen-epinephrine-induced acute pulmonary thromboembolism and the mortality rate [23, 24].

5 Safety of Cinnamon

According to the United States Food and Drug Administration (FDA), cinnamon is generally safe and well-tolerated in amounts commonly found in food. Also, cinnamon extract is safe and is exempt from toxicity data requirements by the US Environmental Protection Agency (EPA) [25].

6 Conclusions and Future Perspectives

Based on available evidence, the active ingredients in cinnamon have been shown to reduce the production of inflammatory cytokines such as IL6, IL-1β, and TNF-α by suppressing the expression of COX-2 and iNOS pathways [26–28]. These properties can regulate the release of inflammatory mediators and relieve pain in inflammatory diseases such as rheumatoid arthritis, diabetes, heart disease, cancer [29], and allergies [12] and neurological disorders such as Alzheimer's disease [30], allergic encephalomyelitis, multiple sclerosis [31], and migraine [32]. Therefore, it is possible that the beneficial effects of cinnamon on suppressing the inflammation could be used to control and prevent COVID-19 complications due to increasing pro-inflammatory cytokines and chemokines [33]. We hope that future studies will provide a clearer perspective on the effects of cinnamon as a potential treatment for COVID-19 disease.

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16 Curcumin and Piperine in COVID-19: A Promising Duo to the Rescue?

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Abstract

COVID-19 is now pandemic throughout the world, and scientists are searching for effective therapies to prevent or treat the disease. The combination of curcumin and piperine is a potential option for the management of COVID-19 based on several mechanisms including antiviral, anti-inflammatory, immunomodulatory, antifibrotic, and antioxidant effects. Here, we describe the probable mechanism of curcumin-piperine against COVID-19. Administration of curcumin-piperine combination appears as a potential strategy to counterbalance the pathophysiological features of COVID-19 including inflammation. The optimal dose and duration of curcumin-piperine supplementation should be determined in the future.

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Keywords

Curcumin-piperine · Curcumin · Piperine · Coronavirus · COVID-19

1 Introduction

Coronaviruses are important human and animal pathogens. In late 2019, a new coronavirus (2019-nCoV), which causes severe respiratory infections, was detected in Wuhan, China. The disease spread rapidly and was observed worldwide by February 2020. This disease was named by the World Health Organization (WHO) as COVID-19 [1, 2]. Coronaviruses include a wide family of viruses ranging from the common cold to more serious diseases such as Middle East Respiratory Syndrome coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) [3, 4]. The 2019-nCoV (now classified as SARS-CoV-2) is a new virus that has not been previously detected in humans.

Coronaviruses cause respiratory, gastrointestinal, liver, and central nervous system diseases in humans, livestock, and wild animals. Bats have been identified as natural coronavirus carriers [5, 6]. Common signs and symptoms of infection with coronaviruses include fever, cough, shortness of breath, and respiratory problems. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death [5]. SARS is one of the most important complications of coronavirus infections. SARS-CoV originated in southern China in late 2002 and had high mortality and morbidity. Over a 6-month period from late 2002, more than 8000 people were infected with the disease and almost 800 died [5]. The disease posed a new threat to human health and a challenge to the production and prescription of antiviral drugs. These viruses are commonly known as respiratory and gastrointestinal diseases in humans and domestic animals. Although the etiology of this disease is not yet completely understood, the increased inflammation in the lungs is one of the main factors that adversely affect the disease [7, 8]. Therefore, in some cases, steroidal anti-inflammatory drugs have been used to reduce inflammation and help regulate the disease, which has been shown to reduce the strength of the immune system and thus reduce disease resistance and many adverse effects on the individual.

Due to the unexpected outbreak of the COVID-19 disease, no suitable treatment strategy has been introduced for it so far. Results of a recent randomized, double-blind, placebo-controlled study showed that nano-curcumin had a significant impact on reduction of inflammatory cytokines in COVID-19 patients [9]. Therefore, it seems that another novel form of curcumin, i.e., curcumin-piperine with several proven health benefits as a safe adjunct therapy, might be useful in treating COVID-19. The purpose of this manuscript was to introduce curcumin-piperine, a bioactive compound, as a novel potential pharmacological alternative for COVID-19 management.

2 Curcumin-Piperine

One of the adjuvant therapeutic strategies to alleviate inflammation and improve the condition of COVID-19 patients might come from natural sources, such as herbs or spices. Turmeric (*Curcuma longa* L.) has long been used in complementary medicine to alleviate several diseases. Curcumin is a phenolic compound extracted from turmeric with various pharmacological effects such as anti-inflammatory, antioxidative, antiviral, antibacterial, immune-modulating, and antitumor activities [10–19]. For this reason, curcumin has been tested as a potential natural substance to prevent or treat patients with chronic inflammatory diseases [20].

The health potential of curcumin has been suggested to be limited by instability, low aqueous solubility (<8 μg/mL in water) and subsequent low permeability and absorption, and rapid metabolism [21–23]. The combination of curcumin with an adjuvant has been utilized to overcome these potential limitations [24]. Along

these lines, the combination of curcumin with piperine has been shown to increase the bioavailability of curcumin by 2000%, reduce its hepatic conjugation and subsequent urinary excretion, and induce complementary therapeutic effects [25–29]. Piperine is an important alkaloid component extracted from long pepper (*Piper longum*) and black pepper (*Piper nigrum*) with anti-inflammatory and antioxidant effects. A recent systematic review showed that the antioxidative effects of curcumin in combination with piperine are greater than the curcumin alone [30].

3 Curcumin-Piperine and Virus-Induced Lung Injury

Preclinical studies have suggested that the administration of curcumin might have beneficial effects on viral pneumonia. As shown in Table 16.1, Avasarala et al. found that the daily administration of curcumin (50 mg/kg) 5 days prior to intranasal inoculation with reovirus 1/L-induced acute lung injury (ALI) in CBA/J mice could inhibit the activation of transcription factor nuclear factor-kappa B (NF-κB) and subsequently reduce pulmonary levels of interleukin-6 (IL-6), interferon gamma (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1), which are responsible for the development of acute respiratory distress syndrome (ARDS) in patients. The same researchers also found that curcumin intake could reduce pulmonary fibrosis through the suppression of transforming growth factor beta (TGF-ß) signaling and myofibroblast activation [31]. Likewise, Han et al. showed that the replication and disease severity of influenza A virus (IAV) were significantly reduced in mice treated with 100 mg/kg of curcumin every 8 h over a period of 6 days [32]. Their results in vivo showed that curcumin upregulated the expression of heme oxygenase-1 (HO-1) and reduced IAV-induced damage to the lung tissue through the suppression of IκBα activated NF-κB signaling. Another study also showed that the administration of curcumin (50 mg/kg and 150 mg/kg) twice a day for 6 days reduced the rate of mortality, concentrations of inflammatory mediators, IAV titer, and lung damage in mice infected with IAV [33]. Interestingly, curcumin intake could directly attenuate IAV activation, proliferation, and adsorption, as well as IAV-induced oxidative stress and inflammation through the activation of nuclear factor erythroid 2–related factor 2 (Nrf2) signaling, leading to NAD(P)H quinone oxidoreductase (NQO1), glutathione S-transferase A3 (GSTA3), IFN-β, and HO-1 production, and the inhibition of toll-like receptor (TLR) 2/4, p38/c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase (MAPK), and NF-κB signaling pathways (Table 16.1).

The authors of a systematic review conducted in 2020 concluded that curcumin can attenuate lung damage through inhibiting the recruitment of leukocytes and modulating inflammatory cytokines and chemokines in mouse models of bacterial pneumonia-induced ALI/ARDS [34]. The results of other studies have also suggested that piperine can ameliorate lipopolysaccharide-induced ALI, lung edema, production of inflammatory mediators such as IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), and myeloperoxidase (MPO) activity through blockade of NF-κB activation [35]. Taken together, these anti-inflammatory, antioxidant, and antiviral properties support the possibility that the curcumin-piperine combination would be a useful adjuvant therapeutic strategy to counteract respiratory failure in COVID-19 patients (Fig. 16.1).

4 Curcumin-Piperine and Thrombosis

In addition to respiratory failure, SARS-CoV-2 can increase the incidence of both arterial and venous thromboembolism [36]. Previous evidence has shown that curcumin as a neutral agent with anti-inflammatory activities can suppress platelet aggregation by blocking the cyclooxygenase (COX) activation and consequently inhibiting thromboxane biosynthesis [37]. Also, the findings of another study have indicated that the curcumin's antiplatelet aggregation activities can

Table 16.1 The effects of curcumin on virus-induced lung injury based on preclinical studies

Author, Year	Study design/ animal	Type of virus	Dosage and type of administration	Tissue or organ	Study duration	Results	Main conclusion
Avasarala et al. 2013 [31]	Animal study/ mouse	Reovirus 1/L-induced acute viral pneumonia	Curcumin (50 mg/kg)	Lung	5 days prior to intranasal inoculation	p65, IL-6, IL-10, IFNc, and MCP-1 expression reduced, which might be due to reduction in phosphorylated NFkB. Curcumin also modulated expression of key chemokines/cytokines implicated in development of acute lung injury/acute respiratory distress syndrome, from both the inflammatory infiltrate and whole lung tissue	Pathogenesis of viral pneumonia-induced ALI/ ARDS was lessened by curcumin
Han et al. 2018 [32]	Animal study/ mouse /in vivo	A/PR/8/34 (PR8) strain of influenza A virus (IAV; H1N1)	Curcumin (100 mg/kg)	Lung	2 h postinfection, followed by repeated readministration every 8 h for 6 days	IAV inhibited by curcumin in vitro. Curcumin alleviated disease severity following IAV infection. Expression of heme oxygenase-1 triggered by curcumin in vivo. IAV-induced injury to lung tissue was improved after curcumin administration. Immune response regulated by curcumin following IAV infection	Severe influenza pneumonia ameliorated via attenuating lung injury and regulating macrophage cytokine production after curcumin supplementation
Dai et al. 2018 [33]	Animal study/ mouse	IAV replication and influenza pneumonia	Curcumin (50 mg/kg and 150 mg/kg) twice a day	Lung	6 days	Survival rate significantly increased by curcumin. Curcumin improved inflammatory cytokines, lung IAV titer, and pulmonary histopathological changes after IAV infection	Curcumin directly inactivated IAV and inhibited IAV adsorption and replication

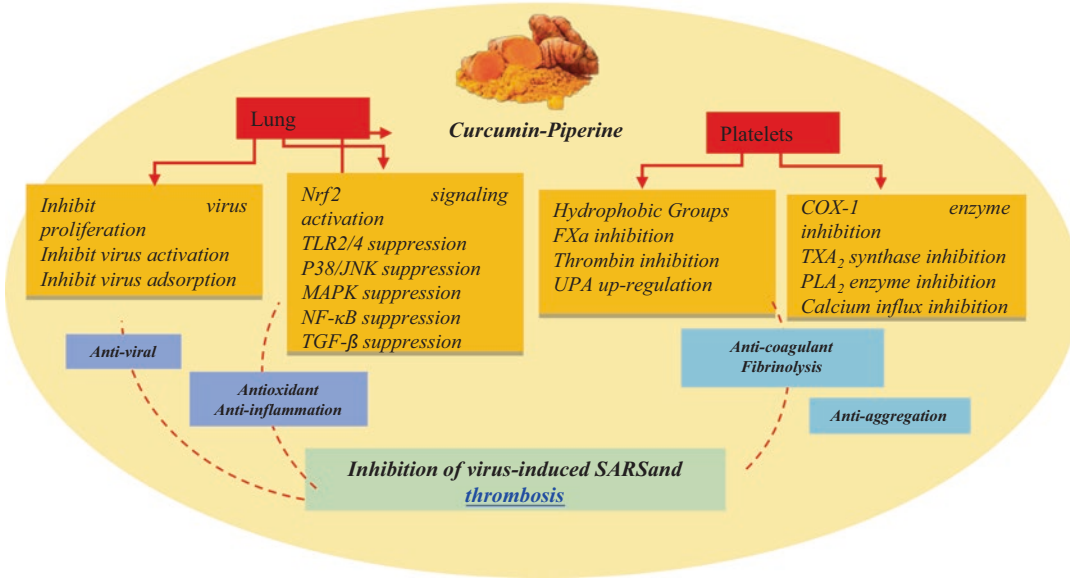

Fig. 16.1 Potential mechanisms of curcumin-piperine to inhibit SARS and thrombosis in patients with COVID-19. Abbreviations: nuclear factor-kappa B (NF-κB); severe acute respiratory syndrome (SARS); cyclooxygenase (COX); activated factor X (FX); urokinase-type plasminogen activator (uPA); phospholipase A2 (PLA2); thromboxane A2 (TXA2); nuclear factor erythroid 2-related factor 2 (Nrf2); toll-like receptor (TLR); c-Jun N-terminal kinases (JNK); mitogen-activated protein kinase (MAPK); transforming growth factor beta (TGF-ß)

be mediated by calcium influx blockade in platelets [38]. Curcumin also possesses anticoagulant activities due to its hydrophobic groups, such as an ortho-methoxy moiety [39, 40], and blockade of both activated factor Xa (FXa) and thrombin production [41]. It has also been reported that curcumin can promote fibrinolysis through upregulation of urokinase-type plasminogen activator (uPA) mRNA in fibroblast cells and consequently dissolving fibrin clots [42]. Furthermore, it was demonstrated that piperine exhibited antiplatelet aggregation activities through decreasing cytosolic phospholipase A_2 (PLA_2) activity and thromboxane A_2 (TXA_2) synthase activity without affecting COX-1 enzyme activity in platelets [43]. Taken together, the curcumin-piperine combination could act similar to antithrombotic drugs and offer a useful approach to counter thrombus formation in COVID-19 patients through three different mechanisms including antiplatelet aggregation, anticoagulant, and fibrinolytic actions (Fig. 16.1).

5 Safety of Curcumin-Piperine

Curcumin has been shown to be a safe and well-tolerated agent even at a high dosage of 8 g per day [44]. It should be noted that curcumin and its analogs have also been suggested as potential adjuvant treatments for cancers while being generally safe [45–48]. The curcumin-piperine combination is also nontoxic and safe with various therapeutic properties due to high bioavailability as compared with curcumin alone. Several review papers cover this topic in more detail [49–52]. In previous clinical trials, the oral administration of the curcumin-piperine compound at the daily dose of 500 or 1000 mg/day curcumin plus 5 or 10 mg/day piperine over a period of 8 weeks was well-tolerated without any serious side effects in patients with nonalcoholic fatty liver disease (NAFLD) and diabetes [53–60]. Likewise, in a recent clinical trial, it was found that administration of a capsule containing 500 milligram curcumin plus 5 milligram piperine was safe and

well-tolerated without any adverse reactions among critically ill patients who were admitted in intensive care unit (ICU) [61]. The amount of piperine in this compound is minor and functions by binding the glucuronidase enzyme in the intestine to allow better absorption of curcumin [62]. This small amount of piperine does not cause a high systemic concentration. Although several health benefits such as reduction in insulin resistance, improvement of hepatic steatosis, and immunomodulatory, antipyretic, and anti-inflammatory activities have been attributed to piperine [63, 64], it is assumed that the small amount of piperine in curcumin-piperine cosupplementation is not absorbed systemically [35, 65, 66] and, consequently, it might not have significant beneficial effects on health.

6 Conclusions and Future Perspectives

Based on the existing evidence, administration of the curcumin-piperine combination appears as a potential strategy to counterbalance the pathophysiological features of COVID-19 including inflammation. Thus far, studies evaluating the effect of the curcumin-piperine compound on coronaviruses are scarce. The optimal dose and duration of curcumin-piperine supplementation should be determined in the future. Currently, clinical trials are ongoing to assess the effects of curcumin-piperine in patients with COVID-19, and results of these studies will provide better information about the effectiveness of this combination.

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17

Association of In-hospital Use of Statins, Aspirin, and Renin-Angiotensin-Aldosterone Inhibitors with Mortality and ICU Admission Due to COVID-19

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Abstract

The exaggerated host response to Sars-CoV-2 plays an important role in COVID-19 pathology but provides a therapeutic opportunity until definitive virus targeted therapies and vaccines become available. Given a central role of endothelial dysfunction and systemic inflammation, repurposing ACE inhibitors

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(ACEIs), angiotensin receptor blockers (ARBs), statins, and aspirin has been of interest. In this retrospective, single-center study, we evaluated the primary outcomes of mortality and ICU admission in 587 hospitalized patients with documented COVID-19 with or without ACEIs, ARBs, statins, and aspirin. Atorvastatin was associated with reduced mortality, which persisted after adjusting for age, lockdown status, and other medications (OR: 0.18. 95% CI: 0.06–0.49, P = 0.001). ACEIs were also associated with reduced mortality in the crude model (OR: 0.20, CI: 0.06–0.66, P = 0.008), as ACEIs and ARBs were combined as a single group (OR: 0.35, CI: 0.16–0.75, P = 0.007), although ARBs alone did not reach statistical significance. There was no association between any medications and risk of ICU admission. Aspirin only achieved a significant association of reduced mortality in a subgroup of patients with diabetes in the crude model (OR: 0.17, CI: 0.04–0.80, P = 0.02). The reduced mortality observed with atorvastatin is consistent with other literature, and consideration should be given to atorvastatin as a COVID-19 treatment. While there was suggested benefit of ACEIs and ARBs in the present study, other studies are varied and further studies are warranted to recommend employing these medications as a treatment strategy. Nevertheless, this study combined with others continues to give credibility that ACEIs and ARBs are safe to continue in the setting of COVID-19.

Keywords

Coronavirus · COVID-19 · Statins · Angiotensin-converting enzyme inhibitors · Angiotensin II receptor blockers

1 Introduction

Approximately 9 months into the COVID-19 pandemic, the World Health Organization (WHO) published data demonstrating no benefit of four antiviral medications regarding mortality or risk of mechanical ventilation in patients infected with Sars-CoV-2 [1]. Included among these was remdesivir, the only FDA approved medication for COVID-19 in the USA. With virus-targeted therapies falling short and an unclear timeline for a safe and effective vaccine rollout, pivoting toward targeting the host response via repurposed medications offers a potentially effective, cost-effective, and easily employable interim treatment strategy.

An exaggerated host response to Sars-CoV-2 is largely attributed to the severity of the COVID-19 disease course [2–4]. Among the most severely ill patients, systemic viremia leads to infection of the endothelium, resulting in endothelial dysfunction [2]. Subsequent decreased endothelial barrier function, endothelial and epithelial apoptosis, inflammation, and coagulopathy ensue, which can lead to worsening respiratory distress and multiorgan failure [2–4]. Therefore, blunting the host response holds promise as an effective treatment strategy. Several common cardiovascular medications used in stabilization of chronic atherosclerotic disease achieve these ends. Of particular interest are ACE inhibitors (ACEI), angiotensin receptor blockers (ARBs), and statins. Aspirin is also of interest second to its anti-inflammatory activity as well as antiplatelet function.

Concerns over the theoretical potential for increased disease risk associated with ACEIs and ARBs spread rapidly early in the pandemic [5], and similar concerns were voiced regarding statins [6]. The basis for these concerns regarded the increased expression of ACE-2 caused by these medications, potentially increasing viral ports of entry due to the role of ACE-2 as the receptor site for Sars-CoV-2. Additionally, an association of hypolipidemia with COVID-19 fostered questions as to whether or not cholesterol lowering medications could be associated with an insufficient immune response to the virus [7].

Despite these concerns, the concept of ACEIs, ARBs, and statins providing survival benefit in infectious disease has been proposed for over a decade [8]. Some have postulated that these medications should be employed as therapeutics for

the current pandemic, acknowledging the role in stabilizing epithelial and endothelial barrier function, immunomodulatory effects, and multiple anti-inflammatory pathways [8]. Additionally, a recent paper provided in silico evidence of direct antiviral activity of statins on SARS-CoV-2 via binding to the main protease, thereby preventing viral replication [9]. While having a strong theoretical rationale for implementing use of these medications is important, the more important end point is to determine clinical relevance by decreasing illness severity and mortality.

Given the therapeutic potential and accessibility for real-life application of these medications, we sought to further explore their utility in COVID-19 patients.

2 Methods

2.1 Study Design and Participants

This retrospective, single-centered study was conducted in Baqiyatallah Hospital in Tehran, Iran, between February 15 and July 15, 2020. A total of 1,000 participants were diagnosed with COVID-19 according to WHO interim guidance. Upon admission, patients underwent chest computed tomography (CT) plus a nasopharyngeal swab test. All patients underwent nonenhanced chest CT examinations for detecting COVID-19 pneumonia in the supine position at full inspiration. All CT scan examinations were performed using an Optima 16-row detector CT scanner (General Electric; Boston, MA, USA). Since the scan results were readily available (compared to the swab test that required 24 h at the time the study was conducted), diagnosis was made based on CT results. Moreover, diagnosis of COVID-19 was confirmed by a positive reverse transcription–polymerase chain reaction (RT-PCR) assay of a specimen obtained by nasopharyngeal swab. All participants in the study were positive based on the two methods. Patients with incomplete medical records aged less than 18 or over 85 years, with pregnancy or severe medical conditions including acute lethal organ injury (i.e., acute coronary syndrome, acute stroke, and severe acute pancreatitis), were excluded. Additional exclusion criteria consisted of individuals with preexisting hypothyroidism or contraindications for statin use defined by serum creatine kinase (CK) or aminotransferase levels more than five times over the upper limit of normal (ULN) at admission. The study protocols were approved by the Ethics Committee of Baqiyatallah University of Medical Sciences, Tehran, Iran, with code (IR.BMSU.REC.1399.015), and the patients were enrolled after giving written informed consent.

2.2 Data Collection

All demographic and clinical characteristics, vital signs, laboratory tests, radiological reports, therapeutic interventions, and outcomes for this retrospective study were collected from medical records of patients with COVID-19. Laboratory data during hospitalization included routine blood tests for measurement of white blood cell count (WBC), lymphocytopenia (lymphocyte count), platelet count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), prothrombin time (PT), partial thromboplastin time (PTT), Creatinine (Cr), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and lactic acid dehydrogenase (LDH). Comorbid diseases such as chronic obstructive pulmonary disease (COPD), hypertension, diabetes, coronary heart disease, cerebrovascular disease, chronic liver disease, chronic kidney disease, cancers, and psychological disorders on admission were recoded for each patient. Government-directed semilockdown measures were accounted for in the analysis as well. In-hospital medications and life support interventions, including the classification of the drugs (atorvastatin, aspirin, and ACE/ARB), dosage, and course of treatment, were also extracted from medical records. Data were carefully reviewed and confirmed by an experienced medical team and cross-checked to guarantee accuracy.

2.3 Medication

Statins, potent lipid-lowering agents with anti-inflammatory effects, have been suggested as a therapeutic option for COVID-19. In the clinical setting, statins are often prescribed along with renin-angiotensin-aldosterone system (RAAS) blockers consisting of angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) predominantly used to treat hypertension, heart failure, and chronic kidney disease. In the current study, the atorvastatin regimen was used at a dose of 40–80 mg daily along with standard care of COVID-19 treatment. We additionally assessed for potential drug-drug interactions.

2.4 Outcomes

In-hospital mortality was defined as the primary outcome, and intensive care unit (ICU) admission was the secondary outcome.

2.5 Statistical Analysis

Categorical variables were described as frequency rates and percentages, and continuous variables were described using mean and standard deviation values. Means for continuous variables were compared using independent sample t tests when the data were normally distributed and of course an adequate sample size (otherwise, the Mann-Whitney test was used). Categorical variables were compared using the $\chi 2$ test as well as the Fisher exact test when the data were limited. In addition, crude logistic regression analysis was applied to ascertain the association between aspirin, ACEI, ARB, and atorvastatin recipients, as well as other clinical factors, with patient mortality and ICU admission. Adjusted regression analysis was also conducted to determine the association of the same factors. All statistical analyses were performed using R, version 3.6.3. $P < 0.05$ was considered statistically significant.

3 Results

From 1000 patient records, a total of 587 hospitalized patients were entered in this retrospective analysis according to inclusion criteria and completeness of datasets. Of these patients, 41 (7%) died and 80 (14.2%) were admitted to the ICU. Most patients (67.3%) were male aged 54.85 ± 13.84 years (mean ± sd). The demographic and clinical characteristics of COVID-19 patients receiving aspirin are presented in Table 17.1. The most frequent comorbidities were hypertension (29.6%), followed by diabetes (25.2%) and heart disease (18.6%). There was a significant difference in some demographic and clinical factors for both primary and secondary outcomes including: age, body temperature, breathing rate, and oxygen saturation. The most frequent significant comorbidities were heart disease and psychological disorders. According to the laboratory findings, polymorphonuclear leukocytes (Poly), Lymph, BUN, and CRP were significantly correlated with mortality, while WBC, Poly, and Lymph were correlated with admission in the ICU ward. Additionally, most of the CT scan features were associated with mortality and hospitalization in the ICU ward. The history of lockdown at the beginning of the pandemic also showed a protective association against mortality and ICU hospitalization. According to the results, 337 patients (39.4%) had used Aspirin, 172 (19.2%) had used ACEI, 191 (21.2%) had used ARB, and 326 (38.3%) had used Atorvastatin. Univariate analysis indicated that ACEI and atorvastatin significantly reduced the mortality rate, but aspirin and ABR use had no significant association with the mortality rate or ICU admission.

Logistic regression analysis was performed to determine the association of these multiple drugs on mortality and risk of ICU admission in both crude and adjusted models. Demographic factors including age and sex were included in the multivariate adjusted model. In addition, staying in lockdown was included in the model because of the significant protective observation on both outcomes. According to the crude model, ACEI had a

Table 17.1 Profile of patients infected with coronavirus pneumonia in 2019 in comparison to survival and ICU status (* indicates significant p-values)

Variables	Total	Survival status			ICU status		
		Death	Survived	*P*-value	ICU	NonICU	*P*-value
Clinical characteristics							
Sex	395 (67.3)	33 (80.5)	362 (66.3)	0.062	61 (76.2)	321 (66.5)	0.082
Age	54.85 ± 13.84	63.59 ± 14.14	54.19 ± 13.61	0.000*	59.02 ± 13.94	53.94 ± 13.73	0.002*
BMI	29.09 ± 11.77	28.33 ± 3.65	29.13 ± 12.05	0.727	30.96 ± 28.6	28.77 ± 6.59	0.531
Duration	6.55 ± 3.62	7.3 ± 4.49	6.52 ± 3.57	0.274	7.54 ± 5.1	6.36 ± 3.33	0.083
Body temperature	37.46 ± 1.01	37.81 ± 0.95	37.44 ± 1.01	0.029*	37.69 ± 1.02	37.43 ± 1	0.054*
Number of breaths	19.37 ± 5.82	23.38 ± 9.95	18.97 ± 5.1	0.042*	21.97 ± 8.57	18.98 ± 5.2	0.049*
Blood O_2 saturation	88.55 ± 10.96	79.46 ± 13.04	89.26 ± 10.47	0.000*	81.6 ± 13.17	89.6 ± 10.43	0.000*
Comorbidities							
Hypertension	172 (29.6)	16 (39)	156 (28.8)	0.168	28 (35.9)	138 (28.8)	0.200
Diabetes	147 (25.2)	13 (31.7)	134 (24.7)	0.317	24 (30.4)	119 (24.7)	0.287
Heart disease	109 (18.6)	10 (24.4)	99 (18.2)	0.323	22 (27.8)	84 (17.4)	0.028*
Lung disease	91 (15.6)	6 (14.6)	85 (15.6)	0.866	11 (14.1)	77 (15.9)	0.679
Kidney disease	55 (9.4)	6 (14.6)	49 (9)	0.260	9 (11.4)	42 (8.7)	0.439
Liver disease	32 (5.5)	1 (2.4)	31 (5.7)	0.718	4 (5.1)	28 (5.8)	1.000
Cancer	12 (2)	1 (2.4)	11 (2)	0.585	2 (2.5)	10 (2.1)	0.680
Chemotherapy	6 (1)	0 (0)	6 (1.1)	1.000	1 (1.3)	5 (1)	0.600
Mental disorders	328 (57.4)	9 (31)	319 (58.9)	0.003*	32 (47.8)	284 (59.2)	0.077
Laboratory findings							
WBC	6.48 ± 3.75	7.64 ± 4.02	6.49 ± 4.11	0.141	8.05 ± 3.84	6.36 ± 4.17	0.002*
RBC	4.89 ± 0.66	4.79 ± 0.86	4.9 ± 0.65	0.370	4.91 ± 0.7	4.89 ± 0.66	0.756
HB	14.3 ± 1.86	14.06 ± 2.34	14.32 ± 1.78	0.465	14.36 ± 2.2	14.31 ± 1.76	0.827
HCT	41.75 ± 5.01	41.08 ± 6.08	41.87 ± 4.86	0.403	42.16 ± 5.68	41.78 ± 4.87	0.568
MCV	85.96 ± 6.24	86.43 ± 5.76	85.89 ± 6.39	0.656	86.18 ± 6.96	85.98 ± 6.15	0.805
MCH	29.5 ± 3.55	29.5 ± 2.33	29.49 ± 4.11	0.992	29.33 ± 3.01	29.58 ± 4.17	0.647
Platelets	187.19 ± 76.68	200.21 ± 105.79	191.83 ± 80.18	0.593	199.55 ± 94.39	191.54 ± 80.44	0.475
Poly	72.74 ± 11.31	77.46 ± 14.28	71.7 ± 11.81	0.017*	78.22 ± 10.92	71.14 ± 12.04	0.000*
Lymph	21.55 ± 9.7	16.96 ± 10.1	22.06 ± 10.07	0.008*	17.63 ± 10.56	22.32 ± 10.02	0.001*
BUN	14.55 ± 7.52	18.63 ± 15.37	14.48 ± 6.81	0.006*	16.32 ± 11.31	14.58 ± 6.98	0.109
Creatinine	1.12 ± 0.63	1.33 ± 1.06	1.09 ± 0.66	0.077	1.16 ± 0.77	1.11 ± 0.69	0.604
SGOT	43.51 ± 35.1	57.13 ± 31.68	41.9 ± 37.16	0.056	60.2 ± 74.12	40.1 ± 27.82	0.097

(continued)

Table 17.1 (continued)

Variables	Total	Survival status			ICU status		
		Death	Survived	*P*-value	ICU	NonICU	*P*-value
SGPT	42.94 ± 34.48	55.04 ± 41.09	42.18 ± 34.21	0.086	55.85 ± 51.2	40.21 ± 29.95	0.066
ALK	192.05 ± 115.89	197.3 ± 110.82	185.21 ± 86.73	0.613	230.48 ± 173.11	181.25 ± 68.63	0.083
LDH	725.75 ± 660.5	965.96 ± 443.84	710.28 ± 771.9	0.118	917.89 ± 724.76	703.61 ± 774.79	0.120
NA	135.12 ± 8.61	135.82 ± 4.77	135.34 ± 9.34	0.793	135.43 ± 4.56	135.24 ± 9.74	0.880
Potassium K	4.34 ± 4.89	4.23 ± 0.58	4.44 ± 6.25	0.863	4.24 ± 0.51	4.47 ± 6.6	0.789
CRP	34.36 ± 34.3	46.33 ± 37.44	31.75 ± 33.16	0.036*	39.89 ± 38.28	31.89 ± 31.78	0.181
CT scan features							
Focal	437 (63.7)	23 (67.6)	221 (64.8)	0.741	35 (61.4)	198 (65.3)	0.568
Lobular	110 (16)	7 (20.6)	57 (16.7)	0.567	6 (10.5)	56 (18.5)	0.144
Both lungs	525 (76.5)	26 (76.5)	267 (78.3)	0.806	35 (61.4)	245 (80.9)	0.001*
Lymph nodes	36 (5.2)	5 (14.7)	17 (5)	0.039*	10 (17.5)	11 (3.6)	0.000*
Pleural effusion	25 (3.6)	3 (8.8)	10 (2.9)	0.104	5 (8.8)	7 (2.3)	0.027*
Cardiomegaly	55 (8)	5 (14.7)	28 (8.2)	0.204	10 (17.5)	21 (6.9)	0.017*
Crazy paving	47 (6.9)	8 (23.5)	21 (6.2)	0.002*	8 (14)	18 (5.9)	0.046*
Septal thickness	23 (3.4)	4 (11.8)	8 (2.3)	0.017*	7 (12.3)	5 (1.7)	0.001*
Calcification	33 (4.8)	5 (14.7)	17 (5)	0.039*	8 (14)	14 (4.6)	0.013*
Alveolar	35 (5.1)	5 (14.7)	14 (4.1)	0.021*	7 (12.3)	12 (4)	0.019*
Reverse halo	13 (1.9)	2 (6)	8 (2.3)	0.227	2 (3.5)	6 (2)	0.618
Drugs							
Aspirin	337 (39.4)	13 (29.3)	224 (43.2)	0.150	36 (50)	191 (40.7)	0.138
ACEI	172 (19.2)	3 (7.3)	153 (28.4)	0.003*	27 (34.2)	120 (25.1)	0.088
ARB	191 (21.2)	6 (14.6)	134 (24.6)	0.148	20 (40)	107 (22.2)	0.586
Atorvastatin	326 (38.3)	8 (19.5)	282 (54.9)	0.000*	39 (54.2)	243 (52)	0.736
Clinical symptoms	101 (17.3)	2 (4.9)	99 (18.2)	0.030*	10 (12.7)	89 (18.5)	0.210
Lockdown	378 (69.4)	1 (2.8)	377 (74.1)	0.000*	43 (12)	316 (70.1)	0.079

Data are expressed as mean ± SD and N (%)

For sex, "male" class was reported; and for dichotomous data, "yes" category was reported

Comorbidities: Heart disease included at least one of the cardiovascular diseases, angio, stent, CABG, and MI. Lung disease included at least one of the COPD and asthma. Kidney disease included at least one of the dialysis and kidney transplants

Mental disorders: These included at least one of the factors such as worry and anxiety, worry about infecting your loved ones, obsessive behaviors, sleep problems, and previous history of psychiatric disorders

Clinical symptoms: These included more than half of the following: dry cough, shortness of breath, chest pain, headache, weakness and fatigue, muscle aches, chills, runny nose, sore throat, diarrhea, nausea and vomiting, sputum production, olfactory sensation, dizziness, taste sensation, earache, and hemoptysis

Table 17.2 Association between mortality and type of drugs in crude and adjusted analysis

	Crude			Adjusted[a]		
Variables	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Aspirin	0.61	0.31–1.2	0.15	0.76	0.3–1.92	0.56
ACEI	0.20	0.06–0.66	0.008	0.47	0.11–1.96	0.29
ARB	0.53	0.22–1.27	0.15	0.36	0.11–1.18	0.09
ACEI&ARB	0.35	0.16–0.75	0.007	0.40	0.15–1.09	0.07
Atorvastatin	0.20	0.09–0.44	<0.001	0.18	0.06–0.49	0.001

[a]Adjusted for age, sex, Lockdown, and other drugs simultaneously

Table 17.3 Association between ICU admission and type of drugs in crude and adjusted analyses

	Crude			Adjusted[a]		
Variables	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Aspirin	1.45	0.89–2.39	0.14	1.13	0.64–1.97	0.67
ACEI	1.55	0.93–2.58	0.09	1.34	0.72–2.48	0.35
ARB	1.16	0.67–2.02	0.58	1.06	0.54–2.06	0.87
ACEI & ARB	1.15	0.71–1.85	0.57	1.05	0.61–1.83	0.85
Atorvastatin	1.09	0.66–1.79	0.74	1.00	0.58–1.74	0.99

[a]Adjusted for age, sex, Lockdown, and other drugs simultaneously

Table 17.4 Association between mortality and type of drugs in crude and adjusted analyses (for diabetic patients)

	Crude			Adjusted[a]		
Variables	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Aspirin	0.17	0.04–0.80	0.02	0.27	0.02–3.27	0.30
ACEI	–[b]			–[b]		
ARB	0.85	0.22–3.26	0.81	0.68	0.05–8.21	0.76
ACEI&ARB	0.35	0.09–1.34	0.12	0.59	0.5–6.88	0.68
Atorvastatin	–[b]			–[b]		

[a]Adjusted for age, sex, Lockdown, and other drugs simultaneously
[b]Model could not reach an estimation or CI due to low sample size or events

protective effect against COVID-19 mortality (OR: 0.20, 95% CI: 0.06–0.66). Patients who used ACEI or ARB also showed a significant decrease in mortality (OR: 0.35, CI: 0.16–0.75). Additionally, atorvastatin decreased the odds of death due to COVID-19 (OR: 0.20, CI: 0.09–0.44). In the adjusted model, only Atorvastatin significantly reduced the odds of death (OR: 0.18, CI: 0.06–0.49) (Table 17.2). Similarly, for the ICU admission outcome, both crude and adjusted analyses demonstrated that none of the drugs provided any protective association against mortality or odds of ICU admission (Table 17.3). We also performed a subgroup analysis for diabetic patients. Aspirin demonstrated a protection effect on mortality in the crude model (Table 17.4), but no other drugs reached significance for mortality or admission to the ICU in either the crude or adjusted model (Table 17.5). Neither atorvastatin nor ACEI reached any estimation for mortality since no diabetic patient receiving atorvastatin or ACEI died during the study.

4 Discussion

This study adds to the existing body of literature demonstrating that ACEIs, ARBs, and atorvastatin are safe medications in the setting of COVID-19 illness. Furthermore, the present data demonstrate that atorvastatin is associated with statistically significant mortality benefit, which is consistent with the broader body of literature. RAAS blockade as a combined group

Table 17.5 Association between ICU admission and type of drugs in crude and adjusted analyses (for diabetic patients)

	Crude			Adjusted[a]		
Variables	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Aspirin	0.86	0.35–2.08	0.74	0.96	0.37–2.49	0.94
ACEI	1.07	0.38–2.96	0.89	1.78	0.58–5.45	0.31
ARB	–[b]			1.15	0.40–3.27	0.79
ACEI& ARB	–[b]			1.23	0.48–3.15	0.66
Atorvastatin	0.71	0.29–1.72	0.45	0.71	0.27–1.83	0.48

[a]Adjusted for age, sex, lockdown, and other drugs simultaneously
[b]Model could not reach an estimation or CI due to low sample size or events

(ACEI + ARB) is highly suggestive of reduced mortality, as this reached significance in the crude model and approached significance in the adjusted model. This relationship appeared to be more heavily weighted by ACEIs as they were independently associated with mortality benefit in the crude model, whereas ARBs were not. Aspirin use did not demonstrate benefit within the entire cohort, although there was evidence of reduced mortality in a diabetic subgroup in the crude model, but not in the adjusted model. In this subgroup, combined RAAS inhibition no longer reached significance in the crude or adjusted models. It should be noted that odds ratios were not calculated in the diabetes subgroup using ACEIs or statins due to low data availability, although there were no deaths, which would suggest against any harm in this population. Finally, there was no association with any studied medication use and ICU admission.

A recent meta-analysis including six studies found a pooled 30% risk reduction of severe disease or mortality in COVID-19 patients taking statins [10]. While this result on its own is encouraging, there is reason to believe that the results potentially undercut true risk reduction. The two studies within this analysis that failed to demonstrate reduced risk had notable potential flaws [11, 12]. In particular, statin use was based on historical record, and there was no documentation of statins being administered in-hospital. In another preprint study, only 77% of hospitalized patients received their prescribed statins during admission, highlighting the importance ensuring hospital use as inclusion criteria [13]. Furthermore, abrupt discontinuation of statins upon admission poses a possible additional risk of rebound inflammation, further skewing results toward harm [8]. In response to this concern, we only included patients with documented in-hospital administration of statins.

Unreported statin type may also explain discrepant results between studies. Rossi et al observed that statins as a single medication class demonstrated no significant association with mortality in COVID-19 patients, although when separated into hydrophilic (pravastatin and rosuvastatin) versus lipophilic (simvastatin and atorvastatin) statins, there was a mortality benefit associated only with the lipophilic subclass [14]. The authors rationalized this observation by noting a broader tissue distribution of lipophilic statins and more profound anti-inflammatory activity. The present study included people solely taking atorvastatin, which may explain the magnitude of the reduced odds ratio. Notably, other studies isolating lipophilic statins also demonstrated benefits in disease severity or mortality [15].

The CORONADO study found elevated risk of death from COVID-19 in people with diabetes taking statins compared with those not taking statins in France, suggesting a divergent population specific effect of these medications [16]; However, this is in direct conflict with a paper by Saeed et al who demonstrated that statin use only significantly reduced mortality in diabetic patients and had no effect in nondiabetics [17]. Additionally, many of the studies that support statin use have a high percentage of diabetes in the study populations, including 25% in the present study and 34% in the study by Zhang [18]. Interestingly, Rosuvastatin is the most common prescribed statin in France, and this is discordant with prescribing patterns throughout most of

Europe and the USA where simvastatin is predominant [19]. Given the findings by Rossi et al. noted above, France's predilection for Rosuvastatin (a hydrophilic statin) may in part explain the CORONADO results. While Rossi did not demonstrate statistically significant harm with hydrophilic statin use in COVID-19 patients, there was a trend toward harm. It is possible, therefore, that the CORONADO study is not demonstrating population specific risk, but rather a within-class medication risk. In our study, we were unable to calculate an odds ratio for the diabetes subgroup taking statins, although it is worth noting that no deaths were observed in the atorvastatin-treated diabetic group.

There is a wide disparity in associations between ACEI and ARB use and COVID-19 severity and death [20]. Despite this, these medications are widely considered safe and several authoritative bodies have been vocal in recommending that they are continued during the pandemic [21, 22]. However, recommendations fall short of suggesting these as a COVID-19 treatment. While most reviews and meta-analyses do not conclude benefit, these largely have grouped studies that include a medication history of ACEI or ARB use as well as in-patient administration. As mentioned in the prior discussion on statins, this design potentially overrepresents actual medication use. Furthermore, ACEIs and ARBs are often discontinued in hospitalized patients second to concern for acute kidney injury and hypotension. Therefore, studies that do not specify in-hospital use are subject to inaccuracy as are the meta-analyses that combine these studies in their pooled results. Meanwhile, isolated studies that specify in-hospital use have largely suggested benefit [23–26], consistent with our results. The results of our study also suggest a greater benefit of ACEIs compared to ARBs although there are little comparable in-hospital data, and meta-analyses of pooled studies have come to conflicting conclusions [20, 27].

Comparatively few studies have evaluated the use of aspirin in COVID-19 patients. Although our results only suggested mortality benefit in the subpopulation of diabetes in the crude model, others have provided evidence of decreased ICU admission, mechanical ventilation, and in-hospital mortality [28].

5 Conclusion

In conclusion, statins appear to be a viable treatment option for patients with COVID-19 as more definitive solutions are developed. Despite this, some important questions remain. In particular, whether or not there is a within-class discrepancy between lipophilic and hydrophilic statins should be studied further in randomized controlled trials. If statins are employed as therapeutics in the interim, the existing evidence supports lipophilic over hydrophobic statins. The data for ACEIs and ARBs are supportive of use, albeit less compelling. This is true both within our own study results and within the broader literature. However, there are concerns over the quality of the existing studies and meta-analyses that may be minimizing a therapeutic benefit. In our study, any benefit of aspirin appears to be somewhat equivocal, although there is a paucity of data to make any firm conclusions.

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The Worldwide Effort to Develop Vaccines for COVID-19

18

Paul C. Guest and Susan E. Ozanne

Abstract

There have been recent encouraging reports about the development of vaccines for COVID-19. Given the scale and effects of this pandemic on public health and economies worldwide, there has been an unprecedented approach across the globe, leading to the emergence of vaccine candidates many times faster than the normal process would allow. This review gives up-to-date information as of November 28, 2020, on the latest developments in this area and covers the plans to roll out the most promising vaccines across the entire world to halt the spread of this devastating virus.

Keywords

COVID-19 · SARS-CoV-2 · Coronavirus · Spike protein · Receptor binding domain · Vaccine

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1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the third virus from the betacoronavirus genus to cause serious illness and death in humans, following the appearance of SARS-CoV in 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 [1]. SARS-CoV-2 shows high similarity to bat coronaviruses and SARS-CoV [2]. This is consistent with the idea that these coronaviruses may have originated from bats [3]. Furthermore, SARS-CoV-2 was initially described close to when the same virus occurred in lung samples from two dead Malayan pangolins, suggesting that these species may be a natural reservoir of the virus [4, 5].

SARS-CoV-2 causes coronavirus disease 2019 (COVID-19), which was first reported in December 2019 in Wuhan, China [6]. It is typically characterized by high fever, dry cough, difficulty in breathing, severe atypical pneumonia, and other symptoms such as gastrointestinal difficulties, as well as loss of smell and taste [7, 8]. Severe cases are often marked by a cytokine storm in blood sample analyses and the appearance of ground glass opacities with consolidation on lung computed tomography (CT) imaging [9]. As of November 28, 2020, there have been 62,618,683 confirmed COVID-19 cases and 1,458,944 deaths reported globally [10]. At the

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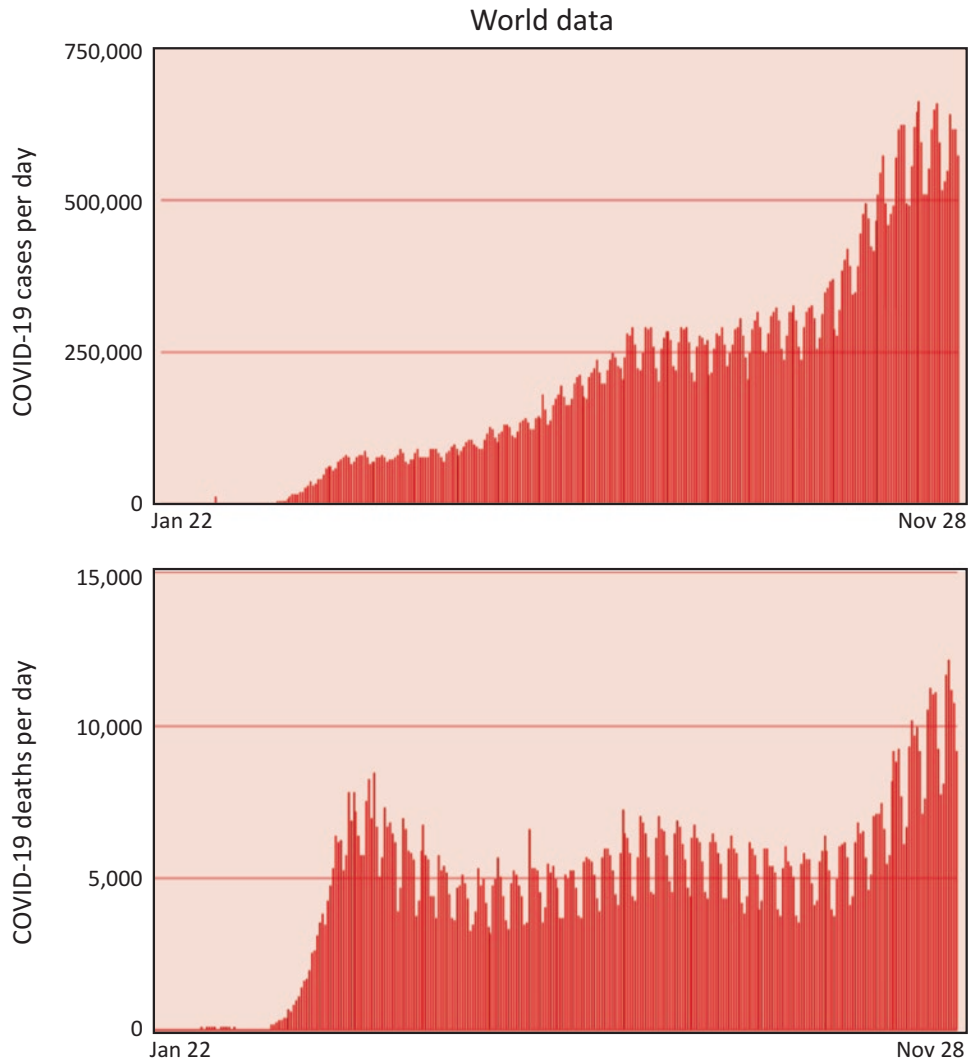

Fig. 18.1 World data showing COVID-19 cases (top) and deaths (bottom) as of Nov 28, 2020 [*World*]

present time, the daily rate of new cases and deaths is showing no signs of decrease (Fig. 18.1).

Given the scale and effects of this pandemic on public health and economies worldwide, there has been an unprecedented approach across the globe, to develop new treatment and vaccine candidates many times faster than the normal process allows [11–14].

As the most effective method for controlling the spread of COVID-19, this brief review focuses on the latest news in vaccine development. It will describe the main strategies involved in targeting the virus as well as the different methods involved in vaccine production. Finally, it will describe the main efforts that have already gone into fast-tracking dissemination of the top approved vaccine candidates around the globe.

2 The SARS-CoV-2 Spike Protein

SARS-CoV-2 is a solitary strand RNA virus of approximately 30 kb and four main proteins, termed envelope, nucleocapsid, membrane, and spike. The virus gains entry into host cells

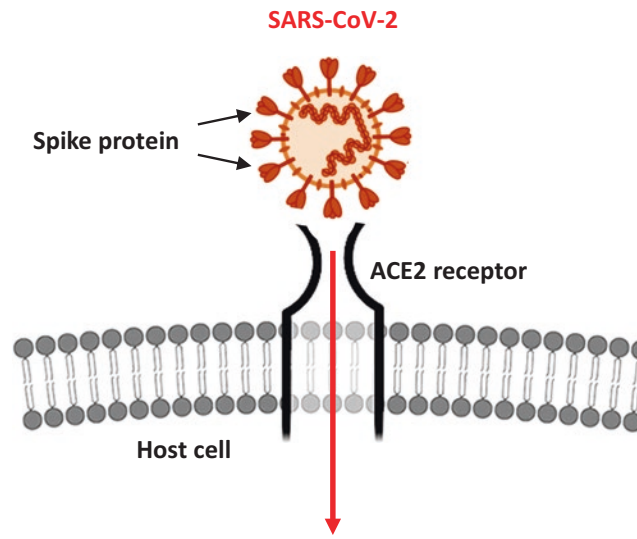

Fig. 18.2 Mechanism of SARS-CoV-2 entry into cells by binding of the spike protein to the host ACE2 receptor

through the concerted action of the transmembrane protease, serine 2 (TMPRSS2), and binding of the spike protein via the receptor binding domain to the angiotensin-converting enzyme 2 (ACE2) receptor (Fig. 18.2). This allows the virus to enter the cell by endocytosis and discharge the viral RNA into the cell cytosol. After this, the virus takes over the cellular machinery to reproduce itself and erupt from the cell via exocytosis, allowing the spread of the virus to other cells [15]. As the virus uses the spike protein for entering into host cells expressing ACE2, most researchers all over the world are targeting this interaction in different ways in the development of potential vaccines. A schematic of the viral genome and the structure of the spike protein are shown in Fig. 18.3.

3 SARS-CoV-2 Vaccine Candidates in Phase 3 Clinical Trials

There are many vaccine candidates worldwide in the effort to control COVID-19 disease. Many of these are being rapidly progressed, considering the global emergency. As of November 28, 2020, 10 of these vaccines are now in phase 3 clinical trials and several are already showing promise. These are shown in Table 18.1.

3.1 AZD1222 (Covishield)

AstraZeneca, the University of Oxford and the Serum Institute of India are in Phase 3 with AZD1222 (Covishield), a study which is being carried out internationally, including in India and the USA [14]. The vaccine uses a weakened adenovirus that causes a cold in chimpanzees and genetically modified to express the genetic code of the SARS-CoV-2 spike protein. Once inside the body, the host cells produce the spike protein that primes an immune response against the virus (Fig. 18.4). If the vaccinated person encounters the real virus, their immune system will produce neutralizing antibodies.

The Covishield preclinical data showed a humoral and cellular immune response in all participants, and the phase 3 trial (NCT04516746) is underway with more 40,000 participants enrolled. In addition, an inhaled version is undergoing testing in 30 people. Interim analysis of the phase 3 results showed an efficacy of 70.4% across two dosing regimens for 131 of the cases. When a half dose was used for the initial injection and a full dose for the second, the effectiveness increased to 90%. However, further studies will be required, including the analysis of multiple age groups. Preliminary results from the phase 1/2 trial showed that the vaccine had an acceptable safety profile with most patients producing an antibody

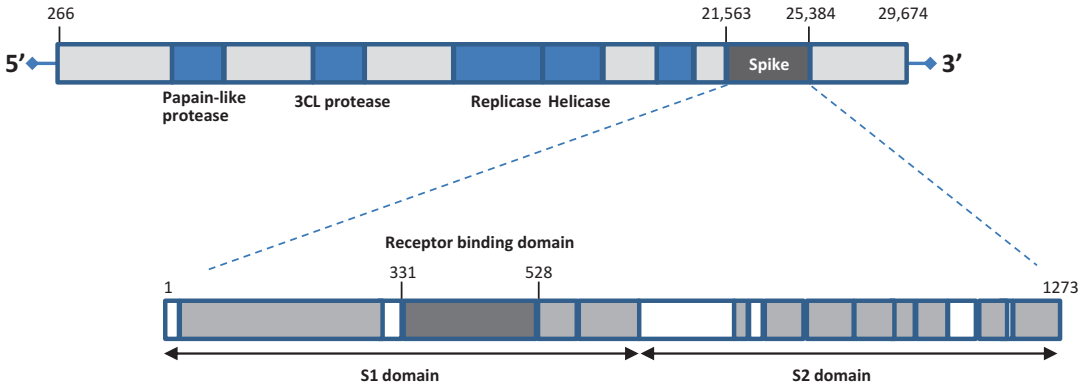

Fig. 18.3 Schematic of SARS-CoV-2 RNA sequence (top) and spike protein (bottom). The receptor binding domain is highlighted in the spike protein sequence. This is the region of the spike that targets the ACE2 receptor on host cells

Table 18.1 Vaccine candidates in phase 3 clinical trials

Candidate	Mechanism	Sponsor	Host institution	Results
AZD1222	Replication-deficient viral vector vaccine	University of Oxford; AstraZeneca; IQVIA; Serum Institute of India	University of Oxford, Jenner Institute	Interim results only
BNT162	mRNA-based vaccine	Pfizer, BioNTech	Multiple sites in Europe and North America	Interim results only
mRNA-1273	mRNA-based vaccine	Moderna	Kaiser Permanente Washington Health Research Institute	Interim results only
Sputnik V	Nonreplicating viral vector	Gamaleya Research Institute, Acellena Contract Drug Research and Development	Various	Interim results only – based on 20 patients
Ad5-nCoV	Recombinant vaccine	CanSino Biologics	Tongji Hospital; Wuhan, China	No phase 3 results available
CoronaVac	Inactivated vaccine	Sinovac	Sinovac Research and Development Co., Ltd.	No phase 3 results available
Covaxin	Inactivated vaccine	Bharat Biotech; National Institute of Virology		No phase 3 results available
JNJ-78436735	Nonreplicating viral vector	Johnson & Johnson	Johnson & Johnson	No phase 3 results available
No name	Inactivated vaccine	Wuhan Institute of Biological Products; China National Pharmaceutical Group (Sinopharm)	Henan Provincial Center for Disease Control and Prevention	No phase 3 results available
NVX-CoV2373	Nanoparticle vaccine	Novavax	Novavax	No phase 3 results available

response after the first dose and all patients showing a response after the second [16]. The EMA Human Medicines Committee (CHMP) and Health Canada had initiated a rolling review of this vaccine candidate to minimize the amount of time for making conclusions on its safety and effectiveness, and the Australian Therapeutic Good Administration (TGA) has already taken the first step in the process for approval. In the UK, the Medicines and Healthcare products Regulatory Agency (MHRA) has also begun an accelerated review of Covishield.

Four million doses of the vaccine will be available in the UK by the end of 2020, assuming

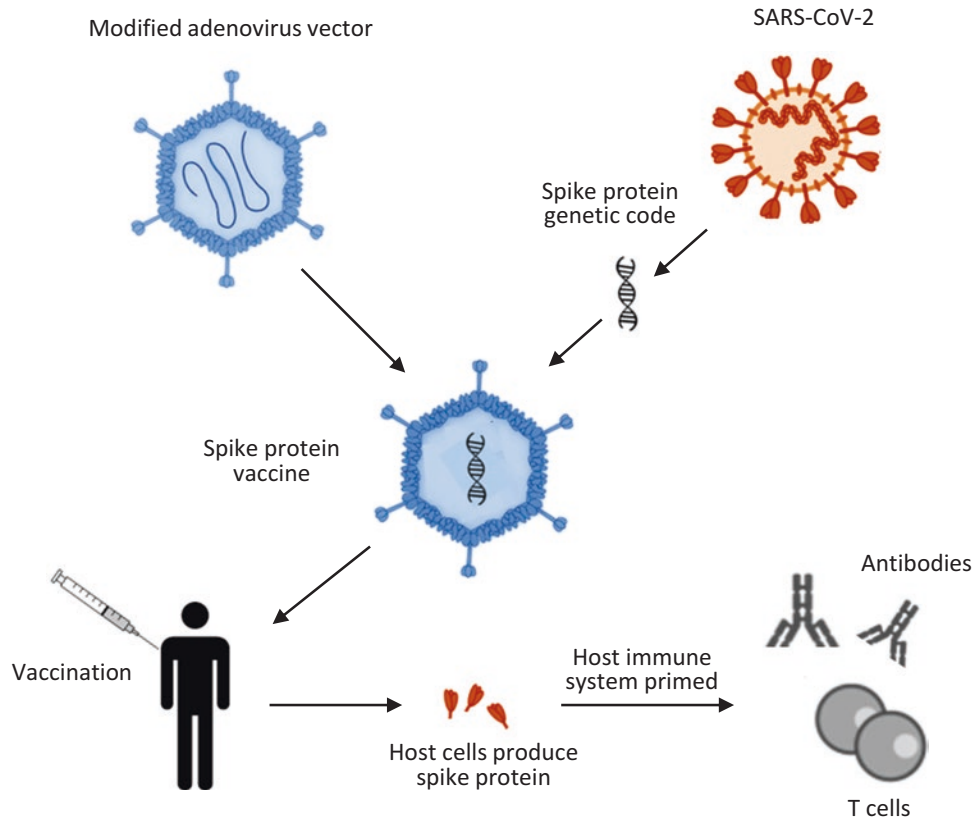

Fig. 18.4 Mechanism of AstraZeneca/University of Oxford viral vector-based vaccine

approval by the MHRA [17]. The UK government partially funded the development and, as of 2020, has preordered a total of 100 million doses to be shared between the four nations. Approximately 70 million of these will be administered by the end of March, 2021, which is hoped to be enough to vaccinate 35 million people. The remaining 30 million shots will be administered throughout 2021, which will be enough for another 15 million people. Furthermore, Astra Zeneca has estimated that it will produce 300 million doses around the world by the end of March, 2021. The Serum Institute of India has already produced 40 million doses and aims to increase this to 100 million by the end of December, and the overall aim is to produce one billion doses. Overall, the aim of Astra Zeneca and its global partners is to produce 3 billion doses by the end of 2021. Considering that this vaccine can be stored under refrigerated conditions, worldwide distribution should prove easier than other candidates below, which require more extreme freezer storage.

3.2 BNT162

Pfizer and BioNtech are currently running a phase 3 trial for BNT162, an mRNA-based vaccine [14]. This type of vaccine works by introduction of mRNA encoding the SARS-CoV-2 spike protein into a person's body, which allows the person's own cells to produce the spike protein that elicits an immune response (Fig. 18.5). On November 9th, they released interim results of 94 participants, which revealed that BNT162 showed greater than 90% efficacy in protecting volunteers from becoming infected by SARS-CoV-2 [18]. Their phase 1/2 data also showed robust immunogenicity of this candidate, [19, 20]

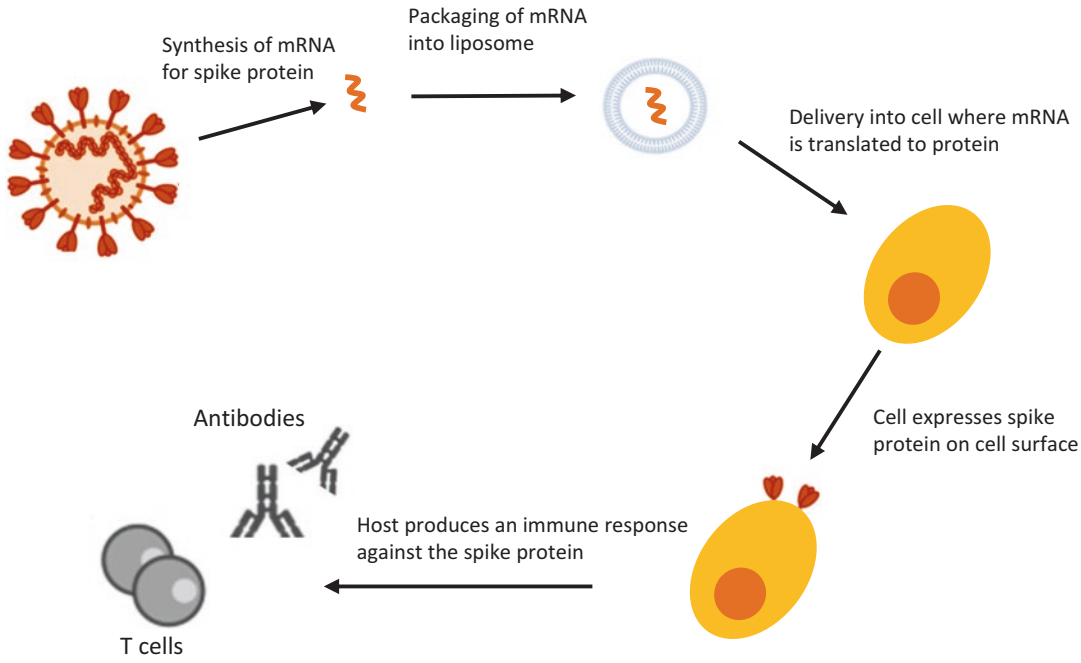

Fig. 18.5 Mechanism of Pfizer/BioNtech and Moderna mRNA-based vaccines

and a phase 1 trial showed only a few adverse effects [21]. Pfizer and BioNTech have now received Food and Drug Administration (FDA) fast-tracking for two BNT162 candidates. BNT162b2 is now in a phase 2/3 safety study due to the robust immune response and high tolerability. The FDA is also considering expanding the Phase 3 trial to include as many as 44,000 participants, the European Medicines Agency (EMA) had already initiated a rolling review of BNT162b2, to potentially bring approval forward, and such reviews have also been submitted in Australia, Canada, Europe, Japan, and the UK. Pfizer and BioNTech plan to file for emergency use authorization so that the vaccine may begin rolling out in December. Based on current projections, Pfizer and BioNtech expect to produce 50 million doses in 2020 and up to 1.3 billion doses by the end of 2021, globally [22]. In addition, Australia has received provisional determination from the Therapeutic Goods Administration (TGA) and China is seeking approval as well via the Shanghai Fosun Pharmaceutical Group. Distribution of this vaccine might prove to be more difficult than the AstraZeneca/University of Oxford candidate since it requires freezing storage conditions.

3.3 mRNA-1273

Moderna is conducting a phase 3 study to test mRNA-1273 as a potential mRNA-based COVID-19 vaccine in the COVE trial of more than 30,000 participants at 100 clinical research sites in the USA [14]. Interim analysis of data regarding 95 participants who developed symptomatic COVID-19 disease released on November 16th showed an efficacy of 94.5% with no severe cases in the vaccinated group compared to 11 in the placebo group [23]. In addition, a phase 1 dose escalation study showed that the mRNA-1273 vaccine induced immune responses in all participants with no serious safety concerns [24]. On November 17th, Moderna Announced a supply agreement with the UK and the EMA began its rolling review process to facilitate distribution if mRNA-1273 is approved [25]. On November 25th, they announced an advanced purchase agreement with the European Commission for an

initial 80 million doses of the vaccine [26]. In addition, the Medicines and Healthcare products Regulatory Agency (MHRA) initiated a rolling review to facilitate the approval process for the vaccine [27]. A similar process has begun in Switzerland via the Swissmedic regulator [28]. As above, this vaccine requires freezer storage conditions and therefore might prove difficult in worldwide distribution objectives.

3.4 Sputnik V

The Gamaleya Research Institute in Russia and Health Ministry of the Russian Federation are carrying out a phase 3 trial of 40,000 participants to evaluate a heterologous adenoviral vector-based vaccine against SARS-CoV-2, in Russia, Belarus, and the United Arab Emirates [14]. The vaccine was announced as 92% effective in an interim analysis of data from 20 participants. The Health Ministry has already approved Sputnik V although no trial data have been published as of November 27. This decision has been criticized as there are no data on safety and efficacy. However, two small phase 1/2 trials suggest that the vaccine induced a strong humoral and cellular immune response with a good safety profile [29, 30]. In light of this, a preliminary presubmission of the vaccine has been made in Brazil.

3.5 Other Candidates

Several other candidates are also in phase 3 studies, although none of these have reported efficacy data as of November 27, 2020 [14]. This includes the CanSino Biologics (China) vaccine that incorporates the adenovirus type 5 vector. They are carrying out a phase 3 study in Russia (500 participants across multiple study centers) as well as another phase 3 study including up to 40,000 participants internationally. An inactivated SARS-CoV-2 vaccine (CoronaVac) from the Chinese company Sinovac Life Sciences is being trialed in phase 3 studies in Brazil. Phase 1/2 trials of 743 volunteers showed that CoronaVac had a good safety and immunogenicity profile [31]. Sinopharm and the Wuhan Institute of Virology are carrying out a phase 3 trial in Peru, Morocco, and the United Arab Emirates using an inactivated COVID-19 vaccine candidate [14]. The vaccine has shown a good neutralizing antibody response in Phase 1/2 trials [32].

Bharat Biotech and the National Institute of Virology in India are in phase 3 studies with another inactivated vaccine called Covaxin of 26,000 participants [14]. Phase 1/2 and phase 3 trials are also underway. Johnson & Johnson is conducting a phase 3 trial with 30,000 volunteers called ENSEMBLE 2 using their recombinant spike protein JNJ-78436735 vaccine [14]. The preclinical data showed good immunogenicity and suggested protection against severe disease [33, 34]. Finally, the USA company Novavax will begin a phase 3 study of a recombinant spike protein nanoparticle vaccine candidate called NVX-CoV2373 in the UK, in up to 10,000 participants [14]. In a phase 1 study, participants who received the vaccine developed an antibody response at multiple doses with a favorable safety profile [35].

4 Conclusions and Future Perspectives

A number of vaccine candidates for SARS-CoV-2 have now shown promise in interim analyses of phase 3 clinical trials. These were produced in record time compared to the normal process of vaccine production, and procedures have already been put in place around the world to manufacture and distribute the most efficacious of these in anticipation rapid approval. This is critical as every day that passes without a vaccine for this disease results in substantial costs at both the public health and economic levels, worldwide. However, the knowledge that we have gained over the past several months about COVID-19 and the systems we have put in place to identify, manufacture, and distribute new treatments and vaccines will also provide important insights and strategic measures to successfully control future epidemics and pandemics.

Added Note As of March 11, 2021, the integrated effort to develop vaccines for COVID-19 has progressed rapidly with the authorization and distribution of more than one dozen vaccines around the world. In fact, several countries have now vaccinated more than 25% of their populations, such as Israel, United Arab Emirates, United Kingdom, Bahrain, United States of America, Chile and Serbia. To aid this process, the WHO is leading a global alliance called COVAX, which aims to accelerate the development and manufacturing of COVID-19 vaccines. This will facilitate access to all countries, prioritizing frontline healthcare workers, older adults, and those with underlying conditions in order to protect those most at risk of serious disease. The ultimate objective is to help the entire world in ending this devastating pandemic.

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