SARS-CoV-2 and its New Variants

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Since the beginning of 2020, the new pandemic caused by SARS-CoV-2 and named coronavirus disease 19 (COVID 19) has changed our socio-economic life. In just a few months, SARS-CoV-2 was able to spread worldwide at an unprecedented speed, causing hundreds of thousands of deaths, especially among the weakest part of the population. Indeed, especially at the beginning of this pandemic, many reports highlighted how people, suffering from other pathologies, such as hypertension, cardiovascular diseases, and diabetes, are more at risk of severe outcomes if infected. Although this pandemic has put the entire academic world to the test, it has also been a year of intense research and many important contributions have advanced our understanding of SARS-CoV-2 origin, its molecular structure and its mechanism of infection. Unfortunately, despite this great effort, we are still a long way from fully understanding how SARS-CoV-2 dysregulates organismal physiology and whether the current vaccines will be able to protect us from possible future pandemics.

Keywords: COVID19 ; SARS-CoV-2 ; coronavirus

1. Introduction

The last two decades have been punctuated by the sudden appearance of viruses able to quickly spread among continents and cause large-scale pandemics, such as SARS-CoV in 2003, MERS-COV in 2012, and SARS-CoV-2 since the beginning of 2020 [1]. At present, one year from the start of the COVID-19 pandemic, the efforts of the academic world and pharmaceutical industry have resulted in the development of better therapies and the production of multiple vaccines that hold promise for a better future. Unfortunately, despite this great effort, we are still far from fully understanding the mechanisms that SARS-CoV-2 uses to dysregulate many physiological pathways causing hyperactivation of the immune response and multiorgan dysfunction [2]. In fact, COVID-19 is not only a lung disease, and unravelling the molecular pathways dysregulated by SARS-CoV-2 in different organs will help to develop specific therapies [3]. Moreover, the appearance of new SARS-CoV-2 variants is constantly raising the question of whether the currently available vaccines have the potential to prevent possible future pandemics. Here, we first discuss what we have learned about SARS-CoV-2's origin, mechanism of infection, and how its sequence has changed, collecting the available information about the impact of SARS-CoV-2 new variants. We will then focus on the main role that the dysregulation of its receptor ACE2 plays in determining disease progression, especially in the organs that seem to be more affected. We will discuss how the dysregulation of the Renin–angiotensin aldosterone system (RAAS) and Kinin–Kallikrein system (KKS) [4] can, in part, explain the overproduction of proinflammatory cytokines and the coagulopathy seen in severe cases of COVID-19. Furthermore, we will describe the available therapies offered at present, and how the advancement in our understanding of SARS-CoV-2 infection is leading to the development of new treatments. Finally, we will discuss how the current vaccination campaign taking place worldwide is affecting the rate of infection and death, especially among the weakest part of the population.

2. SARS-CoV-2 Origin and Molecular Structure

Coronaviruses (CoV) are enveloped positive-stranded RNA viruses belonging to the Coronaviridae family. Four members of this family—CoV-NL63, -229E, -OC43, and -HKU1—have already been identified in humans and known to cause endemic mild respiratory tract infection, although fatal outcomes have been reported, particularly in immunocompromised patients [5]. In the last twenty years, three newly identified members of this family have been responsible for causing epidemic severe acute respiratory syndrome (SARS), from which they have been named SARS-CoV, middle east respiratory syndrome (MERS), and SARS-CoV-2 [6,7], the latter of which is responsible for the ongoing outbreak. Several studies have demonstrated that the natural reservoir host for these viruses is bats [8]; usually, infection of an intermediate species occurs prior to transmission to humans (Figure 1).

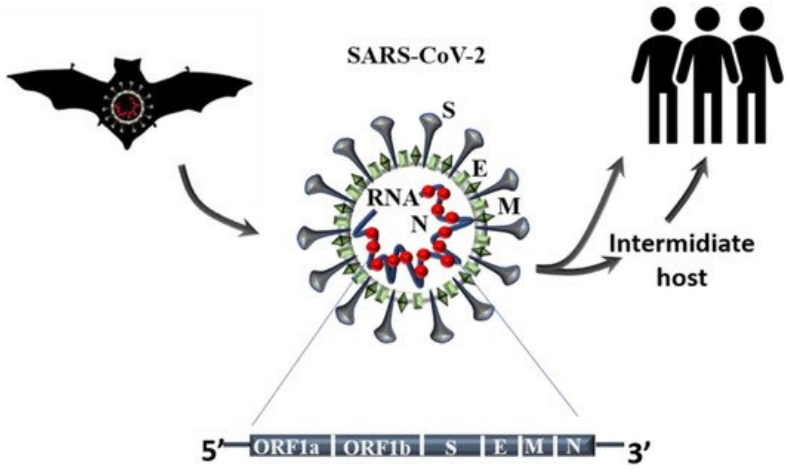

Figure 1. SARS-CoV-2 origin and molecular structure. The natural reservoir of the new betacoronavirus Sars-CoV-2 has been demonstrated to be bats and thought to spread to humans through an intermediate host. The viral RNA is associated with the N proteins that are involved in the key process of infection such as transcription, replication, and packaging. The lipid membrane that protects the viral RNA contains structural proteins such as membrane (M) and envelope proteins (E). The spike glycoprotein (S), through its receptor-binding domain, is responsible for the recognition of the host cell receptor. The picture shows a simplification of the viral genome.

To date, all evidence suggests that SARS-CoV-2 is the result of an infection from an intermediate host and not a result of laboratory manipulation [8]. In fact, since the first outbreak in 2002, a considerable number of SARS-like viruses have been identified in bats and shown to have the capacity to infect humans [7], supporting the hypothesis that potential future outbreaks are possible. The diversity in the coronavirus family is generally believed to be due to the lack of proofreading activity of the RNA-dependent RNA polymerase (RdRp) which is necessary for viral RNA replication. Recent data have instead shown that RdRp of both SARS-CoV and SARS-CoV-2 has proofreading activities and a decrease in the replication fidelity is probably due to mutations in a specific exon [9]. Indeed, although SARS-CoV-2 is closely related to SARS-CoV, sharing 96% of its identity (Table 1), genomic analysis has demonstrated that mutations in the spike glycoprotein of SARS-CoV-2 increased the affinity for its receptor ACE2 [8].

Table 1. Percentage of RdRp identity related to SARS-CoV-2 and different family of Coronavirus. Sequence reference are respectively YP_009725307, QHR63299.1, QDF43819.1, NP_828869.1, YP_009047223.1, AIW52769.1, YP_459941.1, AIW52828.1, YP_009555260.1.

SARS-CoV-2	Bat CoV	BtRs-BetaCoV/YN2018A	SARS -CoV	MERS	hCoV 229E	hCoV HKU1	hCoV NL63	hCoV OC43
	99%	96%	96%	70%	58%	67%	59%	66%

3. The Effect of Sars-Cov-2 New Variants

Structurally, the spike glycoprotein is formed by two subunits, named S1 and S2. The S1 subunit is responsible for receptor binding and can be further divided into N terminal-subunit (NTD) and the receptor-binding domain (RBD). The S2 subunit is necessary for the membrane fusion between the virus envelop and the late endosome membrane. Moreover, the genomic structure of the viral RNA, 2.9kb in length [10], contains genes necessary for its replication, such as the RdRp and helicase (HEL), genes encoding structural proteins such as membrane (M), nucleocapsid (N) and envelope protein (E) and, as we discussed above, the spike glycoprotein (S), responsible for host recognition [8].

In the months of 2020, more attention was focused on the identification of new SARS-CoV-2 variants, some of which have caused an unexpected increase in COVID-19 cases. For instance, the variant 501Y.V1 (B.1.1.7), known also as the English variant, has been associated with an increased infectivity and high transmission through populations, possibly due to an enhanced affinity for its receptor ACE2. At the same time, in South Africa, another variant called 501Y.V2 (B1.351) is spreading widely through the population [11,12,13]. These two variants share a mutation in the RBD of the spike protein, with the South African variant having two additional mutations, E484K and K417N, that allow the immune escape of SARS-CoV-2 and, in particular, to the neutralizing antibodies [12,13]. Another variant identified in Brazil (P.1 (501Y.V3)), shows three mutations in the RBD, shared with south African variants, but the infectivity rate of this variant is still under investigation (Figure 2). At this point, the most relevant issue is whether the available COVID-19 vaccines will be able to protect the population from these and new variants that will be identified. It is worth remembering that the three main

vaccines, Pfizer BioNTech, Moderna, and Oxford AstraZeneca, which have already been administered to millions of people, target the spike protein. Some tests are underway to evaluate their effectiveness against these variants, even if the spike protein is a large protein and a lot of mutations would be needed to completely escape the immune system [11].

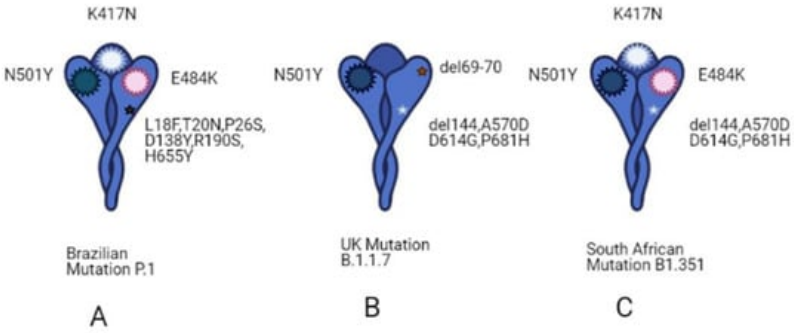

Figure 2. Schematic representation of spike variants. (**A**) Brazilian mutation called P.1, that shares three mutations in the RBD domain of spike protein with South African variants (N501Y, E484K and K417T); P.1 has 17 amino acid changes, nine of which are in its spike protein (L18F, T20N, P26S, D138Y, R190S, H655Y). (**B**) English mutation called B1.1.7 has a mutation (N501Y) in the RBD of the spike protein like P.1 and B1.351 variants. Additionally, amino acid deletions were found within the N-terminal domain (NTD) of spike protein, important for efficient entry into host cells. (**C**) South African mutation called B1. 351, shows a mutation in spike protein (N501Y, E484K and K417T) and several changes in NTD spike domain (A570D, D614G, P681H), including amino acid deletion (del144). [14] Created with BioRender.com.

4. Potential Treatments for COVID19: The Need to Find Multiple Therapeutic Options

The clinical reports published since the first outbreaks in December 2020 have indicated which part of the population is more at risk. In Table 2 we have summarized the findings of several studies, where advancing age [35], male sex, and the presence of comorbidities such as hypertension, diabetes, and cardiovascular disease seem to represent the main risk factors for severe outcomes [36]. Recently, obesity has been indicated as an additional risk factor, which can promote severe outcome in subjects infected with SARS-CoV-2. Kompaniyets and colleagues have reported a relationship between body mass index (BMI) and severe infection, probably due to the high level of inflammation characteristic of obese people [37].

Table 2. Percentage of COVID19 patients with pre-existing conditions identified in the reported clinical studies.

Clinical Report	Nr Cases	Age	Males	Females	CVD	Diabetes	Hypertension
Zhonghua Liu Xing 2020 [38]	44672	30–69 (77.8%)	51.4%	48.6%	4.2%	1.1%	12.8%
Xie J et al., 2020 [39]	168	>50	75%	25%	18.5%	25%	50%
Guan WJ et al., 2020 [21]	1099	>50 (56%)	58.1%	41.9%	2.5%	7.4%	15%
Huang C et al., 2020 [40]	41	49 median	73%	27%	15%	20%	15%
Zhang JJ et al., 2020 [41]	140	57 median	50.7%	49.3%	not specified	12.1%	30%
Li Q et al., 2020 [42]	425	59 median	56%	44%		not specified	
Wang D et al., 2020 [43]	138	56 median	54.3%	45.7%	10.1%	14.5%	31.2%
Chen N et al., 2020 [44]	99	55.5 median	67.7%	32.3%	40%	13%	3%
Shi H et al., 2020 [45]	81	49.5 median	52%	48%	10%	12%	15%
Liang WH 2020 [46]	1590	48.9 median	57.3%	42.7%	3.7%	8.2%	16.9%

In fact, after one year in this pandemic, although COVID19 has been shown to spread among young and middle-aged people, where only a small percentage develop severe symptoms [47,48], indicating that our efforts should be concentrated towards finding better therapies in subjects that are often treated for other pathologies. It is crucial to understand to what extent underlying diseases and their treatment could represent a risk factor for severe outcomes. Another important aspect to note is the risk of concomitant bacterial infection, which has previously been shown to exacerbate the symptoms of influenza viruses [49].

Today, there are no specific treatments for COVID-19 and all strategies being applied are entirely supportive [3], with many clinical trials underway that have not yet offered definitive support to any particular treatment. Since the beginning of this pandemic, different classes of drugs or treatments have been tested against SARS-CoV-2 infection, including anti-viral (AV) drugs, anti-inflammatory (AI), monoclonal antibody (MA), plasma therapy (PT) and cell-based therapy (CT).

At the time of writing, the website clinicaltrial.gov reports more than five thousand clinical trials underway worldwide (Figure 3), of which 980 have been completed.

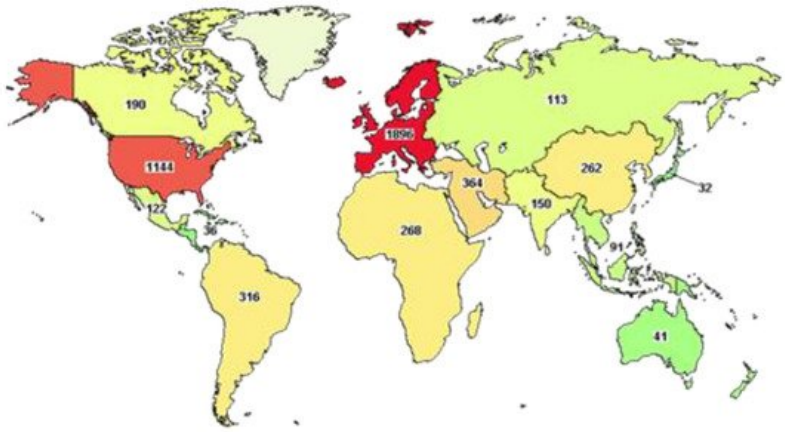

Figure 3. Map of COVID19 clinical trials. This map has been obtained by using clinicaltrial.gov searching for COVID-19 clinical trials for the age 65 and above.

An advanced search shows that half of these trials (2554) are focused on subjects older than 65 years, as discussed above, demonstrating the urgency of finding better therapies for the strata of the population that has suffered more from the effects of this pandemic. Among these trials, only 17 have been completed and they include the use of Ivermectin (AV), alone or in combination with Doxycycline, Hydroxychloroquine monotherapy (AV) and in combination with Azithromycin (AI), Favipavir (AV), Remdesivir (AV), Convalescent Plasma. Ivermectin is an FDA-approved AV drug, known since 1981 and proven to work against different RNA viruses, such as Avian influenza A, Zika, yellow fever, dengue, among others, and recently shown to completely block SARS-CoV-2 replication in vitro. This drug is normally well tolerated, with minimal side effects and, hopefully, when published, the results of these clinical trials will advise its use in COVID-19 subjects [50]. Other AV drugs, Chloroquine and Hydroxychloroquine, have been shown to inhibit viral entry, but the clinical reports published are vague and often present many limitations, such as an inadequate number of patients and no medical or safety outcome being described [51,52]. It is also worth remembering that both drugs have been shown to cause severe side effects, such as cardiotoxicity and hypoglycemia [53,54] that, as described previously, could cause harm in particular subjects with cardiovascular disease or diabetes who are more at risk of developing severe COVID-19 infection. Furthermore, more precautions should be used in subjects with Glucose-6-phosphate dehydrogenase deficiency [55]. Remdesivir (AV), proposed as a treatment for Ebola, blocks viral RNA replication through the inhibition of RdRp and has been demonstrated to control SARS-CoV-2 infection in vitro and in vivo [56]. The final report published on 1062 patients demonstrated that Remdesivir improved recovery in adults with COVID-19 and the decrease in mortality rate was statistically significant [57]. Like Remdesivir, Favipavir (AV) is an antiviral drug, able to inhibit the RdRp and, although the data of the current clinical trial have not been published yet, early indications have demonstrated a decreased viral load in COVID-19 patients [58]. Different studies have shown that plasma from convalescent patients (CP) can also improve the clinical outcome in severely ill patients [59], although this may not be the best therapeutic strategy when an extremely high number of patients needs to be treated. Beyond the molecules just described, many other repurposed FDA-approved drugs have been used to treat SARS-CoV-2 infections. For the sake of space, and because the description of the molecular mechanisms specific to each drug is beyond the scope of this review, we have listed them in Table 3 and refer the readers to other works, where their mode of action is described in detail [60].

Table 3. Principal class of repurposed drugs involved into treatment of SARS-CoV-2.

Group	Drugs Name	Action
Anti-Inflammatory	1) Azithromycin	1) Immuno-modulatory effect;
	2) Tocilizumab	2) Humanized anti Il-6 receptor antibody, it bind soluble and membrane receptors blocking JAK-STAT pathways reducing inflammation;
	3) Corticosteroids	3) Helps dampens inflammation and other immune response;
	4) Thalidomide	4) Reduction of cytokine storm;
	5) Anakinra	5) Block IL-1;
	6) Rituxolitinib	6) JAK1 and JAK2 inhibitor;
	7) Bacitinib	7) Inhibits the kinase activities of JAK1 and JAK2.
Anti-Viral	1) Hydroxychloroquine;	1) Inhibit the virus entry into host cells increasing endosomal pH resulting in inhibition of membrane fusion between host cell and virus;
	2) Camostat;	2) Block viral maturation and entry into host cells;
	3) Remdesivir;	3) It terminates RNA synthesis and inhibits SARS-CoV-2 genome replication;
	4) Lopinavir;	4) Protease inhibitor, used in combination with ritonavir improving anti-viral activity;
	5) Ritonavir;	5) Used in combination with lopinavir;
	6) Favipiravir;	6) It is a guanine analogue, inhibits RNA polymerase;
	7) Umifenovir;	7) Inhibit viral and cellular membrane fusion;
	8) Ivermectin.	8) Block viral replication.
Monoclonal Antibody	1) Casirivimab;	1) Block viral entry into host cell;
	2) Imdevimab;	2) Block viral entry into host cell;
	3) Bamlanivimab.	3) Block viral entry into host cell.
Plasma Therapy	Immune serum (convalescent plasma)	Exploitation of virus-specific antibody
Cell- Based Therapy	1) Mesenchymal stem cell;	1) Ameliorate tissue regeneration;
	2) Natural Killer cell	2) Enhance immune response.