REVIEW

A NEW ERA FOR THE THERAPEUTIC MANAGEMENT OF THE ONGOING COVID-19 PANDEMIC

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Abstract

Three zoonotic coronaviruses emerged at the beginning of the XXI century: SARS-CoV (Severe Acute Respiratory Syndrome coronavirus), Middle-East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV2 (previously known as 2019-nCoV), the etiologic agent of COVID 19 (Coronavirus disease) pandemic. The outbreak of the COVID-19 started in Wuhan, Hubei province, China, in December 2019 and has spread extremely fast worldwide. The World Health Organization declared it a pandemic on 11 March 2020. The present study is reviewing the background of human coronaviruses, the potential viral reservoirs, the main genomic and pathogenic aspects of SARS-CoV-2 and the actual data on the immunopathological mechanisms of COVID 19, looking towards therapeutic approaches for SARS-CoV2 infection.

Rezumat

Începutul acestui secol este marcat de trei sindroame respiratorii acute, două cu caracter epidemic/endemic și unul pandemic aflat în desfasurare. Toate acestea sunt cauzate de trei coronavirusuri: coronavirusul sindromului acut respirator sever (SARS-CoV), coronavirusul sindromului respirator din Orientul Mijlociu (MERS-CoV) și SARS-CoV2 (anterior cunoscut sub numele de 2019-nCoV). Boala cauzată de SARS-CoV2, denumită COVID-19, a început în Wuhan, provincia Hubei, China, în decembrie 2019 și s-a răspândit extrem de rapid în întreaga lume. Organizația Mondială a Sănătății a declarat pandemie infecția SARS-CoV2 la 11 martie 2020. Prezentul studiu analizează caracteristicile coronavirusurilor, rezervoarele potențiale care au transmis virusurile către populațiile umane, aspectele genomice și patogenice ale noului coronavirus, și datele actuale legate de mecanismul imunopatologic al bolii, încearcând să contureze o perspectivă cât mai clară asupra abordărilor terapeutice ale infecției cu SARS-CoV2.

Keywords: coronaviruses, SARS-CoV-2, COVID-19, pandemics, immunopathogenicity, therapy, antivirals, outbreak

Introduction

Coronaviruses (CoVs) comprise a vast viral family that infects a huge variety of species, from avian to mammalian, including humans, causing respiratory, gastroenteric and sometimes even CNS (central nervous system) disorders [14]. Until 2002 CoVs were studied mainly for research or veterinary purposes, due to the mild symptoms associated with human CoVs. However, the world's vision upon the virulence of coronaviruses changed in 2002, when a zoonotic betacoronavirus named SARS-CoV emerged in Southern China, and caused a global SARS epidemic, with more than 8,000 human cases and 774 deaths (mortality rate: 9.5%), until its disappearance in 2004 [16]. Precisely ten years later, another zoonotic betacoronavirus called MERS-CoV (Middle-East respiratory syndrome coronavirus) emerged in the Middle East, causing 2521 cases and 919 deaths (mortality rate: 35%) [2]. Genome sequencing and molecular epidemiology studies proved, in both cases, the spillover of animal viruses to humans, followed by secondary human-to-human transmission. The Asian wild animal markets facilitated the inter-species transmission; therefore scientists pointed out that "*it is likely that more members of CoVs will emerge in the years to come*" [56]. Unfortunately, the predictions were correct, and a novel highly contagious virus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) emerged in 2019 and is now causing the biggest pandemic of the modern era - known as Coronavirus Disease 2019 (COVID-19). Recent studies have shown that

acute respiratory distress is the main cause of death during severe SARS-CoV2 infection, with a hyper-inflammatory syndrome caused by a cytokine storm as the underlying mechanism, suggesting that the immune system homeostasis plays an important role in the clinical evolution of COVID-19 [12, 15]. The present study is reviewing the background of human coronaviruses, the potential reservoirs that interacted with humans, the main characteristics of the first two epidemics from the 21st century, namely SARS and MERS. A literature survey on the main genomic and pathogenic aspects of SARS-CoV2 was conducted and the actual data on the immuno-pathological mechanisms of COVID 19 were summarized in order to gain an insight on the present therapeutic approaches for SARS-CoV2 infection.

Historical and evolutionary overview of corona viruses

Coronaviruses (CoVs) were reported more than 70 years ago as being of zoonotic origin, but their pathogenic mechanisms were blurred for a long period of time, since their human infections were known to cause only mild illness, mainly with symptoms of common cold [13]. In 1968 the term "coronavirus" was established and in 1975, the International Committee on the Taxonomy of Viruses settled the *Coronaviridae* family, as a member of *Nidovirales* order, comprising enveloped, positive single stranded RNA viruses with a diameter of 100 - 160 nm [9].
Coronaviridae members are grouped into four genera, namely *Alpha-, Beta-, Gamma-* and *Deltacoronaviridae* (Table I). *Alphacoronaviruses* affect swines, felines, dogs, bats, two human viruses causing mild infections have been described: HCoV-NL63 and HCoV-229E. *Betacoronaviruses* comprise a large number of viruses infecting mammals, as well as 5 human pathogens: HCoV-OC43, HCoV-HKU1, and the three viruses that caused important human epidemics: SARS-CoV, MERS-CoV and SARS-CoV-2. *Gammacoronaviridae* includes viruses specific for whales and birds and *Deltacoronaviridae* includes viruses isolated from various mammalian and avian species [9, 13, 22].
Coronaviruses are causing diseases in multiple animal species, including rats, mice, chickens, turkeys, calves, dogs, cats, rabbits and pigs [53]. Piglets were recently affected by a novel syndrome, named swine acute diarrhoea syndrome (SADS), caused most probably by a bat coronavirus, named SADS-CoV.
Human Coronaviruses. There are evidences and controversies regarding the origin of human CoV, but all seem to have originated in animals. HCoV-NL63, HCoV-229E, SARS and MERS seem to have bat origins, while HCoV-OC43 and HKU1 are possibly originating in rodents. Viruses in the family *Coronaviridae* are RNA viruses with high sequence

variations, favouring recombinations, mutations and emergence of new strains with variable virulence and extended hosts range [49].
HCoV-NL63, HCoV-229E, HCov-OC43, HKU1 are human CoVs responsible for mild forms of respiratory infections and only rarely severe infections in children and older persons [19].
Human CoVs OC43 and 229E were involved in the aetiology of common cold. HCoV-229E has moderate infectiveness and is especially prone for people with immune deficiencies. It was first isolated in 1967, seemingly transmitted to humans through the *Camelidae* species. It shares 65% nucleotide-level genomic similarity with HCoV-NL63, described in 2003. Interestingly, in 2007, a sequence related with HCoV-229E was identified in captive *Vicugna pacos* in California. Coronaviruses strains in *Hipposiderid* bats are also related with HCoV-229E [4, 5].
HCoV-HKU1, discovered in Hong Kong in 2004, in a patient with viral pneumonia, is taxonomically related to murine coronavirus MHV and *Sialodacryoadenits* virus of rats [18].
Outbreaks of severe respiratory syndromes caused by newly emerged human coronaviruses
The 21st century faced the emergence of three beta-coronaviruses associated with epidemic potential and severe human infections: SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle-East respiratory syndrome coronavirus) and SARS-CoV-2 [45].
SARS-CoV was identified in 2002 in Guangdong Province, China. The first infected human case was a 46-year-old man who presented fever for 9 days and severe shortness of breath; secondary person to person transmission was identified in members of his family [16, 63]. Investigations conducted by the Guangdong Provincial Center for Disease Control and Prevention led to the further identification of clusters of cases in six municipalities (Foshan, Jiangmen, Zhongshan, Guangzhou, Shenzhen and Zhaoqing) from November 2002 to mid-January 2003 [66]. The disease manifested as an atypical pneumonia, at the end of March 2003 a novel coronavirus was identified as the ethiologic agent of the syndrome and named SARS-CoV.
Genomic studies revealed that SARS-CoV originated in *Rhinolophus* bats, probably through recombinations between diverse bat strains found in caves in Yunnan province, China, and was transmitted to humans through an intermediate host represented by Palm Civets and raccoon dogs [19].
SARS cases were initially confined to China, but international transmission begun on February 15, when a physician from Guangdong Province, travelled to Hong Kong, developed a lethal form of the disease and caused an important number of secondary cases [16]. By July 2003 the World Health Organization (WHO) had recorded 8437 of cases in 26 countries

and 813 deaths associated to SARS-CoV. Three cases of SARS-CoV infection with no further transmission were reported in Romania at the end of March 2003 [16, 70].

MERS-CoV was identified in Saudi Arabia in June 2012, most probably originating from bat lineage C

beta coronaviruses and transmitted to humans through dromedary camel species [48]. An outbreak was reported in South Korea in 2015, the virus continues to circulate in the Middle East; until November 2019, 2494 cases (out of which 84.2% in Saudi Arabia) and 854 deaths were reported by WHO [7].

Table I

Coronaviruses, hosts and year of discovery

Genus	Species	Natural hosts	Year discovered
	***Human coronavirus HCoV-229E**	**bats**	**1966**
	***Human coronavirus HCoV-NL63**	**palm civets, bats**	**2004**
α-coronavirus	Alphacoronavirus 1 (Transmissible gastroenteritis virus of swine, Porcine transmissible gastroenteritis virus, Feline infectious peritonitis virus, Canine coronavirus, and Feline coronavirus) Bat coronavirus 1A Mi-BatCoV-1A AFCD62 Bat coronavirus 1B Mi-BatCoV-1B AFCD307 Bat coronavirus CDPHE15 Bat coronavirus Hi-Bat CoV-HKU10 Bat coronavirus Ro-Bat CoV-HKU10 Feline infectious peritonitis virus FIPV Ferret coronavirus Lucheng Rn rat coronavirus	Miniopterus bat coronavirus 1 Miniopterus bat coronavirus HKU6, HKU7, HKU8 Mink coronavirus 1 Myotis ricketti alphacoronavirus Sax-2011 NL63-related bat coronavirus strain BtKYNL63-9b Nyctalus velutinus alphacoronavirus SC-2013 Porcine epidemic diarrhoea virus PEDV Porcine respiratory coronavirus PRCV ISU-1 Rhinolophus bat coronavirus Rh-Bat-CoV HKU2 Rhinolophus ferrumequinum alphacoronavirus HuB-2013 Scotophilus bat coronavirus Sc-BatCoV 512 Transmissible gastroenteritis virus TGEV Purdue	
β-coronavirus	***Human coronavirus HCoV-HKU1**	**mice**	**2005**
	***Human coronavirus HCoV-OC43**	**cattle**	**1967**
	***Middle East respiratory syndrome coronavirus MERS-CoV**	**Bats/ camels**	**2012**
	***Severe acute respiratory syndrome coronavirus SARS-Cov**	**Bats/ palm civets**	**2003**
	SARS CoV-2	**Bats**	**2019**
	AntelopeCov Bat Hp-betacoronavirus Zhejiang 2013 Betacoronavirus 1 Bovine coronavirus BCoV China Rattus coronavirus HKU24 DcCoV UAE-HK23 ECov ErinaceousCoV Hedgehog coronavirus 1 KSA-CAMEL-363 Murine coronavirus Murine hepatitis virus MHV	NeoCoV PHEV Pipistrellus bat coronavirus Pi-BatCoV HKU5 Rat coronavirus RCoV Parker RbCoV HKU14 Rousettus bat coronavirus GCCDC1 Rousettus bat coronavirus Ro-Bat-CoV HKU9 SARSr-Rh-batCoV HKU3 Severe acute respiratory syndrome-related coronavirus SARSr-CiCov Tylonycteris bat coronavirus Ty-BatCoV HKU4	
γ-coronavirus	Avian coronavirus BdCoV HKU22 Beluga whale coronavirus BWCoV SW1 Infectious bronchitis virus IBV-partridge Infectious bronchitis virus IBV-peafowl Turkey coronavirus TCoV		
δ-coronavirus	Bulbul coronavirus BuCoV HKU11 Common moorhen coronavirus CmCoV HKU21 Coronavirus PorCoV HKU15 MRCoV HKU18 Munia coronavirus MunCoV HKU13 Night heron coronavirus NHCoV HKU19 SpCoV HKU17 ThCoV HKU12 White-eye coronavirus WeCoV HKU16 Wigeon coronavirus WiCoV HKU20		

The clinical picture of SARS and MERS is quite similar ranging from asymptomatic to mild and severe respiratory disease.

Initially, WHO reported that "*no individual symptom or cluster of symptoms has proven to be specific for a diagnosis of SARS*" [63], the most common presentation resembling influenza- with high fever (> 38.0°C), headaches, cough (initially dry), mild shortness of breath and general altered status. Symptomatic patients with MERS-CoV infection have an incubation period between 2 and 14 days, followed by fever, cough and shortness of breath, and sometimes gastrointestinal symptoms such as diarrhoea and abdominal pain. The disease can evolve to respiratory failure, requiring mechanical ventilation, with a median of 2 days between hospitalization and admission to the intensive care unit [2, 64].

SARS-CoV2, the new era of pandemic risks

Seventeen years after the epidemic of SARS-CoV and six years after the MERS-CoV outbreak, a new zoonotic betacoronavirus emerged, in December 2019, in Wuhan, Hubei Province, China and led to a tremendous number of confirmed infections worldwide [58]. The first cases reported to WHO were initially associated to a seafood market, but unrelated cases were also identified at the beginning of December 2019. Human to human transmission, *via* respiratory droplets or direct contacts with fomites are the main spreading ways [57].

SARS-CoV2 is the best human-adapted betacoronavirus, with high transmissibility and important mortality, causing more than 1.1 million cases and more than 62,700 deaths, in 209 countries (Figure 1), as of April 5, 2020 [69].
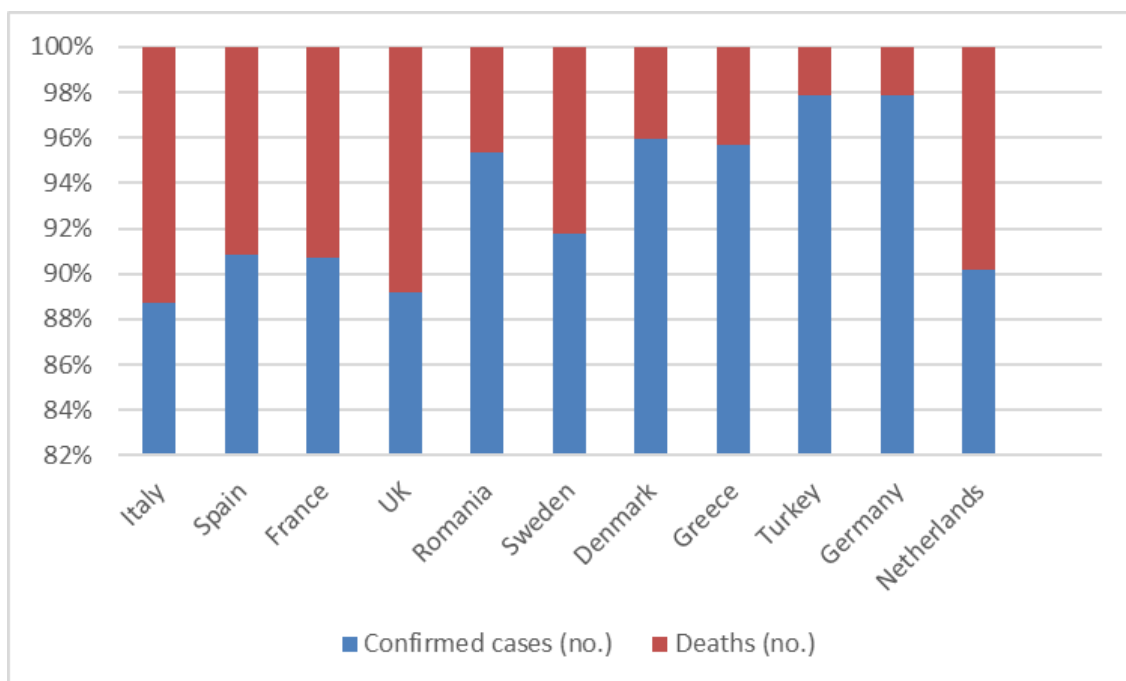

Figure 1.
Statistics on the number of confirmed cases and deaths at the time of publication according to status according to Johns Hopkins University, New York, USA

SARS-CoV2 displays 96.3% genomic similitude to a *Rhinolophus* bat coronavirus, isolated in Yunnan province, China in 2015, nevertheless, it is believed that human transmission has probably involved a still unidentified intermediate host. Pangolins are harbouring multiple lineages of coronaviruses similar to SARS-CoV2 and are under intense scrutiny as reservoirs or intermediate hosts for the human spillover [35].

The clinical spectrum of SARS-CoV2 infections seems to be wide, from asymptomatic to mild upper respiratory tract illness (fever and cough, followed by sputum production and fatigue [23]) and severe viral

pneumonia. Gastrointestinal manifestations (diarrhoea), dysgeusia and anosmia were reported as early symptoms. During the SARS-CoV and MERS-CoV pandemics, and today, in bursting SARS-CoV2 pandemic, many studies have been carried out for a better understanding of coronaviruses characteristics, transmission and possible treatments [34, 36, 43].

Viral structure of SARS-CoV2 and potential therapeutic targets

SARS-CoV2 is a member of *Coronaviridae* family, genus *Betacoronaviridae*, subgenus *Sarbecovirus*, species *SARS related coronaviruses* [26]. The phylo-

genetic studies demonstrated that SARS-CoV2 is more closely related to SARS-CoV than to MERS-CoV, with genomic similarities of 79% and respectively 50% [1, 23, 42].

SARS-CoV2 is an enveloped, positive-sense single-stranded RNA virus. The main viral structural proteins of SARS-CoV2, are presented in Figure 2:

- spike protein (S), which interacts with the host receptor initiating the infection;
- envelope protein (E) which plays a central role in virus morphogenesis and assembly, ion channel activity, induces cell apoptosis and activates the inflammasome;
- membrane protein (M), involved in virus morphogenesis and assembly;
- nucleocapsid protein (N) which packages the positive strand viral RNA genome and is essential for RNA transcription, viral replication as well as the virion assembly [22].

The essential role of spike protein and virus entry to the host cells

The spike protein projections detected by electron microscopy gave a crown-*like* appearance, and hence the name *corona*virus. The S protein is the main viral antigenic component, and several vaccine candidates based on this protein are studied in preclinical and phase I clinical trials.

The spike protein is responsible for virus entry in the human cells. The mechanism is based on the interaction of the S1 protein domain with the human angiotensin converting enzyme 2 (ACE2) receptor expressed in the epithelial cells in the lung, intestine, kidney and blood vessels [33, 56]. In a recent study, Hao Xu *et al*. demonstrated that the receptor is highly expressed in oral cavity (mouth and tongue) which enables an easy access of the virus in the host cells [65].
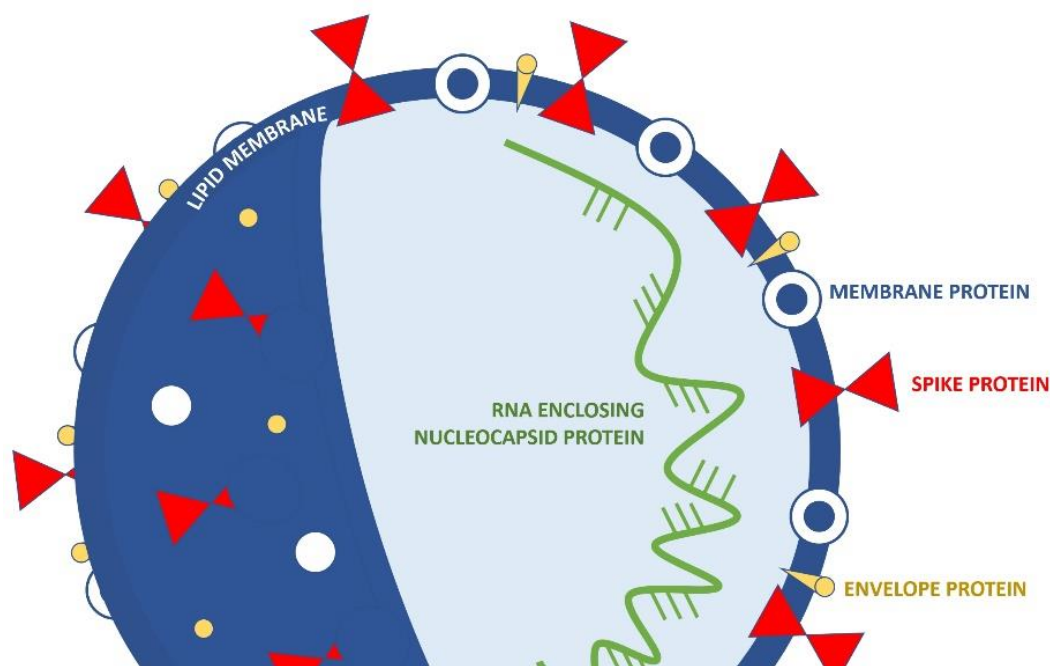

Figure 2.
The typical structure of Coronavirus
The coronavirus genome encodes a spike protein, an envelope protein, a membrane protein, and a nucleocapsid protein. Among them, the spike protein is the most important surface membrane protein of the coronavirus

Recent studies sustain a higher affinity of SARS-CoV-2 spike protein for ACE2 receptor compared to SARS-CoV, potentially explaining the increased infectiveness of this new virus [59]. Several conformational modifications in the Spike protein of SARS-CoV2 have facilitated human transmission (Figure 3) [1, 54]: (1) mutations in the receptor-binding domain, similar to the ones identified in the pangolin SARS-CoV2 related coronaviruses; (2) insertion of a polybasic cleavage site between the two subunits of the spike protein- a modification also reported for high pathogenicity avian influenza

strains that can be transmitted to humans, probably related to a broader cellular tropism; (3) insertion of a leading proline in the polybasic cleavage site that predicts addition of O-linked glycans near the cleavage site, a potential immune evasion mechanism. In humans, ACE2, a monocarboxypeptidase, involved in the renin-angiotensin signalling pathways, is present in two main forms: (1) a protein with a trans-membrane domain anchoring for an extra-cellular domain that acts seemingly as the main entry site for SARS-CoV-2 and (2) a soluble protein without the anchor domain, which allows the protein to circulate

through blood. Several antibodies and other compounds can block the receptor or can induce an unfavourable ACE2 conformation for viral binding or fusion [38]. *In vitro* studies suggest that the soluble ACE2 protein could act as a competitive interceptor of SARS-CoV2 [6].

The overexpression of ACE2 receptor increases the severity of the COVID-19 disease and several unproved concerns were raised related to an o a higher risk of fatal outcomes in patients with diabetes, hypertension or those who are under the treatment with ACE inhibitors [37]. The current guidelines do not sustain discontinuation of ACE2 inhibitors or angiotensin receptor blockers.

ACE2 gene has been shown to be under epigenetic control. The disruptions in ACE2 methylation rates relate to the clinical severity, modulation of this mechanism is studied in order to decrease COVID-19 morbidity in the elderly [17, 20].

The SARS-CoV2 spike protein consists of two sub-units (S1 that contains the receptor binding domain-RBD and S2 involved in the envelope fusion with the cell membrane). A proteolytic cleavage of the two subunits (priming of S protein) is required for cell entry. Recent studies demonstrated that a cellular trans-membrane serine protease TMPRSS2 is involved in this process. Based on *in vitro* results, an inhibitor of TMPRSS2 activity - camostat mesylate, already approved in Japan for unrelated indications, was proposed for SARS-CoV2 infection treatment [28].

During the viral life cycle, endosomal transportation, a pH-dependent step, is involved in the further release of the viral genome into the cytoplasm. The viral genome acts as an mRNA being recognized by the host cell ribosomes and the viral proteins are translated and transported to the Golgi apparatus. The genome is replicated through a viral RNA-dependent RNA polymerase (RdRP or RNA replicase), considered one of the best targets for future therapeutic options [67].

The progeny virions are released from the host cell through exocytosis.
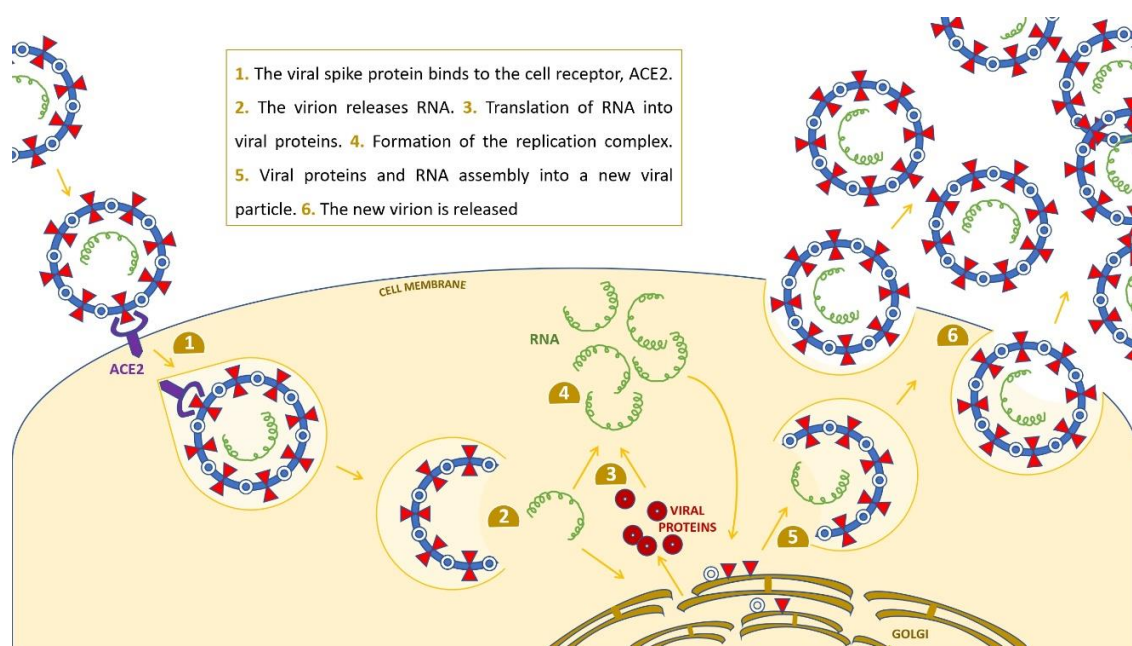

Figure 3.
The life cycle of SARS-CoV2 in host cells

Immunopathological mechanism of disease

Antigen presentation
The virus infects mainly epithelial cells, but also immune cells like local macrophages or dendritic cells, which further activate the anti-viral immune response. These cells are acting as antigen presenting cells, processing and presenting the viral peptides through major histocompatibility complex/ human leukocyte antigen (HLA) to specific cytotoxic lymphocytes T [40, 51]. In the initial SARS infections, several HLA variants were associated with an increased host susceptibility for infection (like HLA-B*0703,

HLA-B*4601, HLA-DR B1*1202), while other HLA alleles (HLA-DR0301, HLA-Cw1502 and HLA-A*0201) were correlated with a protective effect against the disease [39, 41, 52]. Their role in SARS-CoV2 infection is not yet elucidated, but should be further investigated.

Cellular and humoral immunity
Antigen presentation is followed by the activation of humoral and cellular immune responses [8, 50]. Humoral immune response is based on the activation of lymphocytes B to release specific IgM antibodies, starting in the first 7-10 days and continuing for 12

weeks, followed by a secretion of IgG antibodies specific to the viral S- and N-proteins. The cellular immune response is less investigated and the actual knowledge is based on the previous SARS infections [57]. In the case of SARS-CoV2 infected patients, the peripheral blood analyses revealed a decreased number of CD4+ and CD8+ lymphocytes but in a hyper-active status. A significantly reduced number of lymphocytes T was also reported in critically-ill patients. Memory T cells, important for the development of future vaccines, were detected in some patients six years after SARS infections, but less is known for COVID-19 patients [34].

Clinical and immunological features correlated with the "cytokine storm" syndrome in critically ill patients with COVID-19

The main cause of death among the critically ill patients diagnosed with COVID-19 is acute respiratory distress syndrome (ARDS), a type of respiratory failure associated with a widespread inflammation in the lungs [29].

Acute inflammation with increased pulmonary micro-vascular permeability and neutrophils accumulation in the lungs is frequently reported in these patients. Activated neutrophils release a wide variety of cytotoxic molecules and pro-inflammatory cytokines, inducing the formations of neutrophil extracellular traps (NETs) activating autophagy, apoptosis and causing severe lung tissue injury [3, 38].

A recent clinical study including COVID-19 patients with respiratory failure hospitalized in a clinic in Wuhan, China reported increased levels of the following cytokines: IL-1B, IL-1RA, IL-7, IL-8, IL-9, IL-10, IFNγ, interferon-γ-inducible protein (IP10), fibroblast growth factor (FGF), tumor necrosis factor (TNFα), vascular endothelial growth factor (VEGF), granulocyte-macrophage colony stimulating factor (GMCSF), monocyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), granulocyte-colony stimulating factor (G-CSF), platelet derived growth factor (PDGF).[29]. These are associated with a sudden deterioration of health status, decreased level of lymphocytes in peripheral blood and lymphoid organs, infiltration of innate immune cells in lung tissues, atrophy of spleen and lymph nodes and sometimes, multiple organ failure [29, 63]. A constant monitoring of the inflammatory status (high neutrophile to lymphocytes ratio, decreasing platelets, increased erythrocyte sedimentation rate, ferritin, D-dimers, protein C-reactive) is helpful for implementing the best therapeutic options.

Foresight upon the therapeutic approaches to SARS-CoV2 infection

Until now there are no vaccines against SARS CoV-2 and no specific antivirals available.

Currently, for the minor forms, the treatment is symptomatic, for the mild to severe ones several drug molecules are being used (Table II), based solely on their *in vitro* activity and on limited clinical experience. Their real efficacy is not yet well established and several clinical trials are ongoing [12, 44, 46].

Table II

Current therapeutic options for the treatment of SARS-CoV2 infection

Therapeutic agent	Pharmacological classification	Mechanism of action
Lopinavir/ritonavir [11, 47]	HIV protease inhibitors	Unknown, recent clinical trial in severely ill patients did not sustain their efficacy
Remdesivir [47, 55, 62]	Nucleotide analogue prodrug	Interaction with SARS-CoV2 polymerase
Chloroquine/Hydroxychloroquine [47, 55, 61]	Antimalarial	Inhibit the pH-dependent viral replication steps, interference with ACE2 receptor glycosylation, immunomodulation
Metformin [68]	Hypoglycaemic	The molecule of metformin ionizes, generating a cationic structure that melts the phospholipidic membrane of the virus
Azithromycin [24]	Antibacterial macrolide	Prevents bacterial superinfections modulates cytokine production associated with respiratory viral infections- administered in combination with hydroxychloroquine
Tocilizumab [47, 63]	Recombinant humanized monoclonal antibody	Interleukin (IL)-6 receptor antagonist, inhibition of IL-6 signalling pathway
COVID-19 convalescent plasma [21, 47]	Passive immunity	May contain neutralizing antibodies against SARS-CoV-2
Camostat mesylate [28]	Serine protease inhibitor,	Inhibitor of TMPRSS2 – involved in viral infectivity activation
Ivermectin [10]	Antiparasitic drug	Inhibit importin dependent nuclear transport of viral protein

The use of glucocorticoids is controversial, since data already published signals that the potential benefits are counterbalanced by the side effects. Furthermore, several clinical reports evidenced no effective results to support these agents as a therapy for the novel viral pneumonia [63].

Nevertheless, the recent clinical experience favours the early administration of low corticosteroid doses (starting at the end of the first week of treatment), in order to prevent the hyperinflammation associated with high mortality rates.

The most promising antiviral candidate is **Remdesivir,** an RNA polymerase inhibitor, exhibiting a broad antiviral spectrum. Remdesivir was initially developed for Ebola haemorrhagic fever treatment, but demonstrated both *in vitro* and *in vivo*, activity against SARS-CoV and MERS-CoV. A recent study has compared the efficacy of the lopinavir, ritonavir, interferon β and remdesivir in cell culture, demonstrating that remdesivir has the most significant effect against MERS-CoV [27]. In United States, the efficacy of remdesivir is tested in an adaptive double-blinded, placebo-controlled trial for patients with pneumonia and hypoxia and in two randomized-label trials for patients with radiographic evidence of pneumonia and oxygen saturation of ≤ 94% on room air. Patients from areas where clinical trials are not conducted have received remdesivir on an uncontrolled compassionate-use basis. The manufacturer is currently transitioning the emergency access of remedesivir from individual compassionate-use requests to an expanded-access program [60]. Another RNA polymerase inhibitor, **Favipiravir,** is currently tested in China and Japan.

Tocilizumab is a recombinant humanized monoclonal antibody interleukin (IL)-6 receptor antagonist, used in rheumatic diseases. Its use in COVID 19 is based on the potential blocking of the cytokine storm, through inhibition of the IL-6 signalling pathway. Until now there are reports on the favourable effect of 400 mg i.v. administered tocilizumab for a limited number of COVID-19 severe patients, new data are required to clarify its efficacy [63].

A study made by Justin Stebbing *et al.*, demonstrated that combining a kinase inhibitor - **Baricitinib** with other antivirals (lopinavir/ritonavir and remdesivir) could reduce viral replication, viral infectivity and the aberrant host inflammatory response. Using artificial intelligence-derived knowledge graphs, Baricitinib was identified as a NAK (Numb-associated kinase) inhibitor with a particular affinity for AAK1 (adaptor protein complex 2 (AP2) associated kinase 1) and GAK (cyclin G-associated kinase). Other investigational compounds from these drug classes are tested for severely ill patients, for which the host's inflammatory response becomes a major cause of lung injury [49].

Chloroquine, an oral drug used to treat malaria and **hydroxychloroquine**, used for the treatment of rheumatoid arthritis and lupus erythematosus have shown *in vitro* activity against SARS-CoV, SARS-CoV-2 and other beta-coronaviruses. Both drugs have shown beneficial virological effects in hospitalized patients, in small, non-randomized trials in China and several other countries. The antiviral mechanisms are still obscure, both drugs inhibit the pH-dependent viral replication steps and may alter viral protein formation by inducing endoplasmic reticulum stress [31] and hydroxychloroquine may act as an immunomodulatory drug, suppressing TNFα and IL6 overproduction. In United States hydroxychloroquine is used due to its wide availability, but caution must be exerted due to important cardiac toxicities [25, 60].

Metformin, a classic hypoglycaemic agent, has a dimethylbiguanide chemical structure that easily allows protonation. In addition to the formation of two positive guanidine ions, metformin can also protonate its primary, secondary and tertiary amines. Under anoxic metabolism, frequent in severely-ill COVID-19 patients, the acidic humoral environment is conducive to metformin being protonated, so the molecule itself turns into a cationic sphere that can directly damage the lipidic viral envelope. Furthermore, metformin and phospholipids from the inactivated virus can also form a surfactant, which can promote the expansion of the alveoli, prevent the collapse of the lungs, slow down the pathological changes of the hyaline membrane of the lungs, and help the patients' ventilation function. There are some reports, based on of limited clinical experience on Chinese patients infected with SARS CoV-2, showing promising results with 500 mg metformin three times a day, together with 100 mg vitamin C and 10 mg Vitamin B1, that might improve the antiviral mechanism of metformin, further strengthening the acidic environment *in vivo* and enhancing its bio-catalytic ability [68].

Recently, the SOLIDARITY clinical trial was enforced by WHO, it will compare the effectiveness of four antivirals/antiviral combinations: 1. remdesivir, 2. a combination of two HIV protease inhibitors: lopinavir and ritonavir, 3. the two protease inhibitors plus interferon beta, 4. chloroquine with standard of car*e* [72].

In Romania, the Ministry of Health has approved the therapeutic protocol for patients with SARS-CoV-2 on March 24th 2020 [70]. The following treatments are used:

- Mild cases, without pneumonia: *symptomatic treatment (paracetamol);*
- Slight impairment, without pneumonia or risk factors (> 65 years, co-morbidities: cardiovascular, liver, lung, diabetes): lopinavir/ritonavir or hydroxychloroquine;
- Mild impairment (*pneumonia without signs of severity*): hydroxychloroquine and lopinavir/ritonavir;
- Severe impairment: *hydroxychloroquine or remdesivir.* In the case of excessive inflammatory syndrome and organ dysfunction, *tocilizumab* is added.

Administration of hydroxychloroquine requires supervision o the cardiovascular toxicities (risk of long QT arrhythmias).

Critically ill patients require intubation, mechanical ventilation or non-invasive ventilation in case of respiratory failure and ARDS (acute respiratory distress syndrome). Experts recommend non-invasive ventilation

only in mild forms of respiratory distress, due to the potential risk of enhanced airborne transmission [30, 32]. For patients with ARDS without tissue hypoperfusion, mechanical ventilation is needed for more than 12 hours/day, with a volume between 4 to 6 mL/kg predicted body weight to reach a plateau pressure (Pplat) < 28 to 30 cm H2O. The use of paralytics is not recommended unless PaO2/FiO2 < 150 mmHg. Rapid sequence intubation and preoxygenation (100% O_2 for 5 minutes) should be performed via the continuous positive airway pressure (CPAP) method [12]. There are no clear recommendations regarding the administration of antibiotics, their use is limited to cases of severe infections, although several studies on the association of hydroxycloroquine and azithromycine are ongoing [11, 12].

Passive immunotherapy, using recovered patients' plasma has been recommended for severe and critical cases of COVID-19, based on the presence of neutralising antibodies against SARS-CoV-2. Nevertheless, concerns have been raised related to the potential risk for transfusion-transmitted infection and potential risk for severe disease due to antibody-dependent enhancement [21, 71, 72].

Conclusions

Although human coronaviruses were known to cause only mild respiratory infections, a dramatic change occurred over the last two decades. Humanity is presently facing a severe pandemic caused by the novel SARS-CoV2, a highly contagious virus, associated with increased mortality especially in older persons with comorbidities. The immunopathological features are correlated with a cytokine storm syndrome, causing severe lung tissue injury, with mimicry of vasculitis and thrombosis in severe cases of COVID-19. Several repurposed antivirals, immunomodulatory drugs and respiratory supportive therapies are currently the main choices for severe and critically ill patients. High-quality clinical trials are in development; their results will provide new data for the therapeutical management of the ongoing COVID-19 pandemic.

Acknowledgement

Conflict of interest

The authors declare no conflict of interest.

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