COVID-19: Main findings after a year and half of unease and the proper scientific progress (Review)

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Abstract. Since the emergence of the disease in late December 2019, numerous studies have been published to date regarding clinical, laboratory and treatment aspects associated with COVID-19. The present study attempts to compare and unify the clinical, para-clinical and therapeutic aspects that have come to light regarding coronavirus disease-19 (COVID 19), mainly in adults. Between April 2020 and September 2021, a comprehensive systematic literature review was performed, which we added to from our own medical experiences. The search was performed on the PubMed, Scopus and Google Scholar databases, comprising studies with analyzable data that were identified alongside studies and documents containing general scientific data. All published studies were written in English, and were from different countries. A 95% confidence interval (CI95) was also calculated for almost each study using the Wilson formula. When compared with preliminary reports between December 2019 and January 2020, the most frequent symptoms were still identified as being fever (68.6%; CI95: 67.5-69.7) and cough (72.7%; CI95: 71.7-73.8). Nevertheless, asymptomatic cases also increased (by 21.4%; CI95: 16.6-27.1). Severe and critical cases accounted for 10.4% (CI95: 9.6-11.1) of all cases. The mean fatality rate was found to be 4% (CI95: 3.6-4.5). The primary co-morbidity found was hypertension (28.9%; CI95: 27-30.8), followed by other underlying cardiovascular diseases (15.4%; CI95: 13.9-16.9) and diabetes (14.5%; CI95: 13.1-16.1). The majority of studies showed lower white blood cell numbers with neutropenia and lymphopenia, and lower platelet levels. The levels of the biomarkers C-reaction protein and erythrocyte sedimentation rate were positive in all studied cases alongside other lab tests, such as examining the D-dimer levels and those of other hepatic, cardiac and renal injury markers. The procalcitonin level was also found to be elevated in many cases, resulting in high usage of antibiotics (83.7%; CI95: 81.2-85.9). Approximately 31.6% (CI95: 29.1-34.1) of the patients required non-invasive ventilation, whereas 9.9% (CI95: 8.1-12.1) of the patients were intubated or placed on extracorporeal membrane oxygenation. The most used antivirals were ribavirin (67.3%; CI95: 63.4-70.9), oseltamivir (52.5%; CI95: 49.4-55.5) and Arbidol™ (34.5%; CI95: 32-37.1). General admittance to the intensive care unit was ~7.2% (CI95: 6.5-7.9) of patients.

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Abbreviations: SARS, severe acute respiratory syndrome; MERS, middle-eastern respiratory syndrome; ACE2, angiotensin-converting enzyme 2; CNS, central nervous system; ARDS, acute respiratory distress syndrome; MODS, multiple organ dysfunction syndrome; COPD, chronic obstructive pulmonary disease; WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; PCT, procalcitonin; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CK-MB, creatine kinase MB; BUN, blood urea nitrogen; CT, computed tomography; GGO, ground-glass opacities; NAAT, nucleic acid amplification test

Key words: SARS-CoV-2, clinical findings, laboratory analysis, cytokines, therapies

Contents

1. Introduction

The present coronavirus disease had its inception in December 2019 in a cluster of cases in the city of Wuhan, Hubei Province, China. On March 11 2020, a global pandemic was declared by the World Health Organization (WHO), with >114 countries affected and with >118,000 confirmed cases and 4,000 cases of mortality (1).

The causative agent of this outbreak, named severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), is an RNA virus of the *Coronaviridae* family, *Betacoronavirus* genus, alongside the other 2 well-known viruses, SARS-CoV and MERS-CoV. Coronavirus of the acute respiratory syndrome (SARS-CoV) in the Middle East (MERS-CoV) and SARS-CoV-2 have appeared in the human population in the last 20 years and are extremely pathogenic. Other coronaviruses, such as HCoV-229E and HCoV-OC43, are also known, which, together with the most recently identified HCoV-NL63 and HCoV-HKU1, are responsible for seasonal infections. These are usually mild, occur in the respiratory tract, and are associated with the symptoms of a common cold. The initial stages of coronavirus infection feature the specific binding of coronavirus spike (S) protein to cellular entry receptors, which have been identified for several coronaviruses, including human aminopeptidase N (APN; HCoV-229E), angiotensin-converting enzyme-2 (ACE2; HCoV-NL63, SARS-CoV and SARS-CoV-2) and dipeptidyl peptidase 4 (DPP4; MERS-CoV) (2). SARS-CoV-2 invades cells using ACE2 as a receptor. ACE2-mediated degradation of angiotensin II (Ang II) has an important role in the pathogenesis of severe lung failure after a viral infection, and the severity of the virus infection is closely associated with the maturity and binding capacity of ACE2 (3). Thus, it is assumed that a lower level of ACE2 and a lower binding capacity for SARS-CoV-2 should be major factors leading to the absence of any clinical manifestations with asymptomatic infections. It has been specified that a specific mild immune response is elicited via SARS-CoV-2 invasion in asymptomatic patients (3).

Following molecular evolutionary analyses of the SARS-CoV-2 reference genome, it was concluded that SARS-CoV-2 originated from the viruses of non-human mammals, for example bats, via selection by recombination and purification. Coronavirus genomes are able to accumulate new variants by recombination between divergent strains living in different host species (4).

SARS-CoV-2 binds to the alveolar epithelium, activating both the adaptive immune system and the innate immune system. This leads to the release of a large amount of cytokines, including interleukin-6 (IL-6). Cytokine release syndrome (CRS) has been documented for many patients with severe coronavirus disease-19 (COVID-19), and has led to several deaths.

One of the main mediators of CRS is IL-6. TZM, the IL-6R antagonist, has proven itself to be useful for the management of the so-called 'cytokine storm' (CS) that has been observed in patients with COVID-19 (5).

The CS is characterized by an increased level of inflammatory markers, especially cytokines. TZM has been used as an investigative agent against SARS-CoV-2 (6). CRS represents the most feared and serious complication of patients with COVID-19, due to an excessive immune response reaction to the virus, triggered by inflammatory cell infiltration in the lungs, activation of T-helper 1 reactions, and abundant release of proinflammatory cytokines into the circulation (7). The majority of studies have shown the presence of venous thromboembolisms and microthrombi in arterioles and venules in deceased patients with COVID-19, and CRS led to multiple organ dysfunction syndrome (MODS) and disseminated intravascular coagulation. As a result, certain researchers consider that regulating the CS early on using immunomodulators, corticosteroids and cytokine antagonists is critical for lowering the mortality rate of these individuals (8).

Cytokines may be functionally characterized as polypeptides that are secreted by leukocytes and other cells that act predominantly on hematopoietic cells, and their effects include the modulation of immune and inflammatory responses (9). Among the most important cytokines that modulate immune and inflammatory processes are IL-1 through to IL-13, hematopoiesis-stimulating factors, interferons (IFNs), tumor necrosis factors, growth factors and suppressive factors. They are small cell-signaling protein molecules that fulfill crucial roles in regulating the immune system through modulating immune and inflammatory responses. These polypeptides are secreted in order to activate an inflammatory process for the purposes of eliminating a virus present in the body, and the protein molecules that participate in this process include chemokines, IFNs, interleukins, lymphokines and tumor necrosis factors (10).

On the other hand, when their number exceeds the limit that would be beneficial for the body, cytokines are capable of creating a strong inflammatory reaction, which in certain cases can be fatal (10). Therefore, many patients with severe forms of COVID-19 that are admitted into intensive care units (ICU) present with very high levels of inflammatory markers following blood tests, including D-dimers. According to a meta-analysis based on 18 research studies, a very large increase in this protein of approximately 4-fold presents an increased risk of mortality, and this should be considered when performing blood tests on patients (10).

CRS often arises in pediatric rheumatic diseases, typically in systemic juvenile idiopathic arthritis and childhood-onset systemic lupus erythematosus. This pathological condition is also known as macrophage activation syndrome in the pediatric field (11). Furthermore, in infectious diseases caused by Gram-negative bacteria, lipopolysaccharides, components of

the outer membrane of Gram-negative bacteria, induce CSs during sepsis (12).

Genome sequencing has revealed an ~86 and 50% similarity of SARS-CoV-2 to SARS-CoV and MERS-CoV, respectively. This feature helps to explain the similar clinical findings that have been reported for the two viruses, mainly involving the respiratory system. As already mentioned above, both the first SARS-CoV and the novel SARS-CoV-2 utilize the ACE2 receptor for cell penetration, whereas MERS-CoV recruits the cell-surface enzyme DPP4. Both ACE2 and DPP4 are found on respiratory cells (13-15).

2. Etiology, epidemiology and timeline of events

The origin of this disease is thought to be zoonotic in nature, as it has been revealed that more than 90% of its genome is identical with that of a coronavirus typically found in bats (16,17). Therefore, it is considered that the original place of transmission may have been the seafood market in the city of Wuhan, where humans could have been exposed to such potential carriers (16,17). After the original exposure, the spread was mainly effected via human-to-human transmission through Flugge droplets (13,14,17), although the presence of the virus can also be found in enterocytes, and thus in stool samples (14,18). Large public events, such as the Chinese New Year, and the long incubation period ensured that the virus was able to spread, initially among citizens from the Hubei region and later throughout the whole country. Afterwards, it spread to other Asian countries, such as South Korea, Japan, Thailand and others. By February 26, when more cases had been reported outside of China, the disease could be found in Asia Minor (where it heavily hit Iran), Oceania, Africa, North and South America and Europe (where it heavily hit Italy and Spain) (19). On March 11, WHO declared that COVID-19 had reached the pandemic stage (1). By the beginning of April, many countries had already entered a state of emergency, taking social distancing and preventive measures, limiting border passing, even setting up quarantine zones for the regions with the highest infection and imposing isolation, self-quarantine, curfews and stay-at-home orders (20). In April, the number of confirmed cases had surpassed the million-mark threshold. In addition, by this time, the first vaccine and antibody treatment studies were planned to begin (21). Data on SARS-CoV and MERS-CoV were consulted, as these coronaviruses had caused epidemics with mortality rates of ~9.5 and 34.4%; respectively. Given that the third highly epidemic disease detected was COVID-19, it was stated then that the mortality rate was lower compared with SARS and MERS, although the data were shown to vary from country to country. According to WHO statistics, there were 45,678,440 (as of 1 November 2020) confirmed cases in 219 countries due to the high transmission capacity of SARS-CoV-2 (22). SARS-CoV-2 primarily affects the respiratory system, and then the heart, liver and kidneys. It was not clear whether the viral infection directly results in organ or tissue damage, as observed in patients with COVID-19 (23).

Most celebrations such as Easter, Ramadan and other religious and public events had been scaled down. Most major events, such as the Wimbledon tournament or the E3 convention, were cancelled altogether. The most relevant method of asymptomatic infection is close contact with patients who have been diagnosed or suspected (3). During the initial phase of the COVID-19 outbreak, a data set was obtained from 1,099 patients with laboratory-confirmed COVID-19 from 552 hospitals in 30 provinces in China on January 29, 2020. Only 2% of patients had a history involving any contact with animals; over three-quarters of them had either visited Wuhan or were residents. Therefore, it was not possible to predict focal patterns or the source of infection from their study. The incubation period of SARS-CoV-2 was found to be of 1-12 days' duration; however, the median incubation period was 4 days. The most common symptoms encountered were fever (43% at hospitalization and 88.7% during hospitalization), cough (67.8%), diarrhea (3.8%) and fatigue. SARS-CoV-2 was detected in the saliva, blood, sputum and urine prior to the development of viral pneumonia; certain of the patients did not develop pneumonia at all. Asymptomatic people were potential sources of SARS-CoV-2 infection, thereby controlling the transmission dynamics of the current outbreak (24). Due to the lockdowns, the global economy suffered, with many businesses entering bankruptcy, applying for state financial aid and laying off employees. In view of this, many countries started to slowly open up their economies after April 15, 2021. Since this time, protests against lockdown have erupted in many places, including the United States of America (21).

The beginning of May saw a decrease in the numbers of cases in the Far East countries, yet the total number of cases and deaths had exceeded 3 million and 220,000 marks, respectively (25).

By the middle of May, many countries began lessening their crisis measures, considering the bad state of the economy; free movement was allowed, while the use of masks and social distancing remained. By this time, a new condition was linked to COVID-19 and children from North America and Europe, called the Multisystem Inflammatory Syndrome (26,27).

By the beginning of June, the total number of cases exceeded 6 million, and the number of mortalities was over 370,000 (28).

Coronavirus strain. The virion structure of the coronavirus consists of four major structural proteins: Tip, envelope, membrane and nucleocapsid (29).

Alpha coronavirus. The alpha coronavirus consists of two human pathogenic viral strains: HCoV-229E and HCoV-NL63. HCoV-229E uses APN as the primary receptor for entry into the host cell, whereas HCoV-NL63 uses ACE-2 receptors to enter the host cell (29).

Beta coronavirus. The two bat viruses that are part of this genus are MERS-CoV and SARS-CoV. Additionally, HCoV-OC43 and HCoV-HKU1 are two non-SARS-CoV species included in this genus that probably use sialic acid residues as receptors, and which were shown to have hemagglutinin esterase activity. ACE2 acts as one of the major SARS-CoV receptors for enty into the host cell (29).

Gamma coronavirus. This includes avian viruses, such as chicken pox, which provides a representative example of the group. It can cause respiratory and reproductive tract diseases

in chickens (29). Swine delta coronavirus (PDCoV) is part of the genus Deltacoronavirus. This type of virus leads to gastrointestinal symptoms in piglets, with transient viremia, which results in dehydration and death. The virus infects the hairy epithelial cells of the entire large intestine, and the jejunum and ileum are the main sites of infection. In the majority of published studies, it has been mentioned that PDCoV uses APN host protein as the input receptor. Human cells have also been reported to be prone to PDCoV6 infection (30).

The Delta variant is ~60% more transmissible than the Alpha variant. The Delta variant is moderately resistant to vaccines, especially in people who have received only a single dose (31). It has been mentioned that the risk of hospitalization is higher in people infected with the delta variant. This variant has been spreading rapidly, especially through schools in England (32).

3. Structure and transmission

SARS-CoV-2 is an enveloped, single-stranded, positive sense RNA virus, with a diameter between 60 and 140 nm. Coronaviruses belong to the order Nidoviral in the family *Coronaviridae*. The *Coronavirinae* and *Torovirinae* subfamilies are divided according to family. As mentioned above, the subfamily *Coronavirinae* is further divided into four genera: Alpha-, Beta-, Gamma- and Deltacoronavirus. Phylogenic analysis has revealed that SARS-CoV-2 is closely related to the beta-coronaviruses. Similarly to other coronaviruses, the SARS-CoV-2 genome is a positive-stranded single-stranded RNA with a 5'-head and a 3'-untranslated region (3'-UTR) poly(A) tail. The length of the SARS-CoV-2 genome is <30 kb, in which there are 14 open reading frames (ORFs) that encode nonstructural proteins (NSPs) for virus replication and assembly processes, including the structural proteins, spike, envelope, membrane/matrix and nucleocapsid, in addition to accessory proteins. The first ORF contains ~65% of the viral genome and is translated into either pp1a (nsp1-11) or pp1ab (nsp1-16) polyprotein (22). It contains nine open reading frames (ORFs) and numerous accessory genes. Two-thirds of the viral RNA are used to encode replication and transcription proteins, and the remaining third encodes major structural proteins, which are the bilayer envelope, the membrane, the nucleocapsid and the cell-binding spikes (4,33). Similarly to the original SARS-CoV, with which it has high structural similarities, the virus binds to the ACE2 receptor. However, it has a higher affinity for this receptor, explaining why it is more contagious (16). It also explains why people with underlying respiratory and cardiovascular diseases are more susceptible to this infection. Other highly predisposed cells are the enterocytes and nasal epithelial cells. Less affected cells can be found in the urogenital system and central nervous system (CNS) (34). The main mode of transmission remains through droplets either directly from coughing, sneezing and talking, or indirectly by contact with possible infected objects and surfaces. A secondary route of infection might be through feces (18). It was initially considered that the pathogens are transported from the patient through larger droplets, which accumulate on the surfaces and are then transported to the host by dust rising from the dry droplets. The latest findings show that sneezing and dry cough produced by patients with COVID-19 generate droplets of size 0.6-100 μm, and the number of droplets increases in proportion to the cough rate. In excess of 97% of these drops tend to be <50 μm, and most of them are <10 μm. Pre- or asymptomatic patients may generate and emit large amounts of drops that are <1 μm in diameter through normal breathing and speech (35). Although still a matter of controversy, airborne spread (air suspension of smaller, lighter droplets) is possible (36,37). Consequently, the most frequently collected samples are nasal and oral swabs, sputum and saliva. Other respiratory samples are endotracheal aspirate and bronchoalveolar lavage, methods best used for intubated patients (38). These samples can subsequently be analyzed by reverse transcription (RT)-PCR assay, which is capable of identifying the proteins encoded by the OFRs (E, M, S, 1a and 1b protein-encoding genes) (15,33). However, the evidence suggests that sample tests collected from sputum and nasal swabs have a higher positivity rate than samples from other locations (38-40). Genital transmission (such as through semen, vertical transmission in pregnant women) seems improbable (38,41,42). Although blood-borne transmission is possible, it is still debated (38). Serological diagnoses, such as IgM and IgG antibody tests, are recommended only for screening purposes. Antibodies are detectable only after the incubation period of the virus, and are also liable to yield false positive results due to exposure to other coronaviruses (43-45).

4. Data search, selection and extraction

Studies from China, South Korea, India, Italy, Denmark and the USA have been selected, to which we have added our experiences and data. The main keywords searched for were 'coronavirus', 'COVID-19' or 'SARS-CoV-2', paired or not with secondary words including 'clinical', 'laboratory' or 'pathology'. The present article includes 25 studies that were available at the time.

The inclusion criteria were: i) the article needed to be a cohort, case-control or case-series study; ii) the study population contained RT-PCR-positive patients; and iii) the study reported on primary outcomes, clinical symptoms and signs, laboratory and imaging results, comorbidities and complications, and treatment options used.

The exclusion criteria were: i) the study comprised fewer than 7 patients; ii) duplicate studies; iii) studies that lacked data; and iv) studies that were focused on infection in children. The youngest individuals were aged 15 (46) and 16 (47,48) years, and fewer than 10 individuals in this age category were identified in all the studies. As such, the main focus of this article is on the adult population, defined as being over the age of 18. In the case of duplicate studies, the most informative or the most recent article was the one that was included.

A Microsoft Excel database was created and filled with data containing demographics, symptoms, complications, comorbidities, laboratory and imaging findings and used treatment plans.

Basic statistical analysis. Microsoft Excel was used for basic statistical analysis. A 95% confidence interval (CI95) was also calculated for each mean as a proportion by using the Wilson Score. Where the interquartile range (IQR) was provided, the formula for estimating standard deviation (SD) was used: SD=IQR/1.35. This was done to broaden the range for the laboratory results for

certain studies where it was not directly stated whether there were results that exceeded the normal lab limits.

5. Results

A total of 45 cohort, case-series and case control studies were identified, from which 25 (40,46-71) were selected for analysis. A total of 12,636 patients were identified for use in this study. The number of patients differed between categories and factors, as not all studies focused on exactly the same categories and factors.

Demographics. Out of the total of the 12,636 patients, there were 4,291 males (34%; CI95: 33.1-34.8) and 8,345 females (66%; CI95: 65.2-66.9). Out of the total number, the ages of 10,682 patients were able to be confirmed with certainty, of which only 923 (8.6%; CI95: 8.1-9.2) were above the age of 65.

According to Gupta *et al* (51) and Ruan *et al* (46), there were two individuals younger than 18. The study by Chen *et al* (48) stated there were at least 2 individuals younger than 18. However, the total of such individuals was no more than 10, which is too few to affect the statistical outcomes.

From 6,277 selectable patients, 650 (10.4%; CI95: 9.6-11.1) were classified as severe or critical cases. ICU admission was required for 423 out of 5,459 patients (7.7%; CI95: 7.1-8.5). The death rate was 4% (CI95: 3.6-4.5) across all studies.

There were 28 (5.4%; CI95: 3.7-7.7) pregnant women among 521 patients. Asymptomatic cases were clearly observed in 2 studies reporting 50 (21.4%; CI95: 16.6-27.1) out of 234 total patients.

There were 51 (11.9%; CI95: 9.2-15.3) current smokers among 428 patients and 45 (5.6%; CI95: 4.2-7.5) former smokers among 797 patients.

Salian *et al* (35) reported that biological aging was an optimal predictor of disease severity after they had performed biological age evaluations comprising chronological age and nine PhenoAge biomarkers [albumin, alkaline phosphatase, creatinine, log C-reactive protein (CRP), glucose, lymphocyte percentage, mean corpuscular volume, red blood cell distribution width and white blood cell count]. COVID-19 test-positivity and all-cause mortality were shown to be positively associated with accelerated aging 10-14 years prior to the COVID-19 pandemic [odds ratio (OR): 1.15 and 1.25, respectively, per 5-year acceleration].

Symptoms and signs. A total of 7,038 people had explicit varying data about their signs and symptoms. These findings are shown in Table I, and the data were collected from 18 stud ies (46,47,49-54,56-62,65,70,71).

Comorbidities. The main comorbidity findings are shown in Table II. These data were collected from 16 studies (40,46,50, 51,53,54,58,60,62-64,66,67,69-71).

Complications. The findings on complications are shown in Table III. These data were collected from 7 studies (46,49,55, 60,63,67,68).

Laboratory and imaging. The most frequently occurring and important laboratory and imaging findings are presented in Table IV, in which are recorded details both of patients with modified values and of studies that did not provide an exact number of cases, but which described the existence of such modifications. Pooled means and standard deviations are also provided for the data; however, studies with extensively negative values were omitted from this calculation. The data were collected from 17 studies, with varying emphases on the factors involved (40,46,48,50,51,53,55-60,66-70).

Regarding the blood tests, the erythrocyte sedimentation rate (ESR) and CRP values were increased in >80% of the patients, and lymphopenia was found in approximately half of the cases. Other important increased values in many patients were renal [blood urea nitrogen (BUN)=19.4%; creatinine=6.6%] and liver function [alanine aminotransferase (ALAT)=15.4%; aspartate aminotransferase (ASAT)=22.9%], cardiac markers [creatine kinase MB (CK-MB)=17.2%; troponins T/I=25.4%) and D-dimers (21.7%). Procalcitonin (PCT) values were increased in approx. 13.8% of patients.

The imaging factors are shown in Table V. There was only one study (47) in which a chest X-ray was done, which found only one individual with modification (4.8%; CI95: 0.8-22.7). In the other cases where imaging tests were performed, chest computed tomography (CT) showed uni- or bilateral ground-glass opacities (GGOs). Where progress was observed on the CT, the GGOs evolved into crazy-paving patterns and consolidations. These data were collected from 8 studies (48,49,51,53,59-60,62,71).

Typical radiographic discoveries on X-ray or CT images showed that pulmonary involvement was bilateral. It was most commonly located in the posterior lung areas. The common feature was ground glass that occurs bilaterally. These represent areas of active interstitial inflammation in the subsegmental areas of consolidation. After the fifth clinical day, high-density mass shadows and lesions were able to be distinguished. Uncommon symptoms identified were cavities, discrete lung nodules, pleural effusions, emphysema and fibrosis (5).

Nucleic acid amplification test (NAAT). Through the assistance of NAAT, it is possible to diagnose an active COVID-19 infection. RT-PCR testing is used for the detection of SARS-CoV-2 RNA in the upper respiratory tract. NAAT tests target the SARS-CoV-2 nucleocapsid, envelope and spike genes, in addition to regions in the first ORF (orf1a and orf1b) and RNA-dependent RNA polymerase (72).

Antigen detection. Antigen detection tests detect the presence of SARS-CoV-2 viral proteins in respiratory samples. Most tests require samples to be taken from the nasal cavity or nasopharynx. Immunity-based technologies with different detection variations are used, such as side-flow sandwich immunoassays, microfluidic immunofluorescence analyses and digital chromatographic immunoassays (72).

Antibody detection. Non-quantitative detection of antibodies is a method suitable for determining the rate of attack in a given population. A change in antibody titer can be detected by semi-quantitative or quantitative assays that are able to quantify the level of antibody production. It is not considered the primary test of choice for acute infection, although it may have a role in diagnosing it.

Table I. Average sign and symptoms findings.

Frequency	Finding	Reported/total	Mean (%)	CI95
Most frequent	Coughing	4,924/6,770	72.7	71.7-73.8
(≥66.6%)	Fever (+/-chills)	4,831/7,038	68.6	67.5-69.7
Frequent	Muscle pains	3,366/5,785	58.2	56.9-59.5
(≥50%, <66.6%)	Headaches	3,201/5,775	55.4	54.1-56.7
Moderately frequent	Dyspnea	2,422/6,509	37.2	36-38.4
(≥33.3%, <50%)	Sore throat	1,958/5,533	35.4	34.1-36.7
Uncommon	Sputum	238/797	29.9	26.8-33.1
(≥25%, <33.3%)	Diarrhea	1,630/5,647	28.9	27.7-30.1
	Fatigue	221/882	25.1	22.3-28
Rare	Nausea and vomiting	824/4,965	16.6	16.5-18.4
(≥15%, <25%)	Taste and smell loss	824/4,965	16.6	15.6-17.7
Rarest	Nasal congestion and rhinorrhea	786/5,637	13.9	13.1-14.9
(<15%)	Chest pain/tightness	172/1,348	12.8	11.1-14.6
	Abdominal pain	638/5,145	12.4	11.5-13.3

CI95, 95% confidence interval.

Table II. Average comorbidity findings.

Frequency	Finding	Examples	Reported/ total	Mean (%)	CI95
Most frequent (≥25%)	Hypertension	N/A	632/2,190	28.9	27-30.8
Frequent (≥12.5%, <25%)	Other cardiovascular diseases	Myocarditis, infarction history, valvulopathies	334/2,173	15.4	13.9-16.9
	Diabetes	N/A	317/2,183	14.5	13.1-16.1
Moderately frequent (≥5%, <12.5%)	Other respiratory diseases	Emphysema, asthma	96/999	9.6	7.9-11.6
	Neurological and cerebrovascular diseases	Stroke history, peripheral neuropathy	130/1,648	7.9	6.7-9.3
	Psychiatric diseases	Anxiety, depression, alcohol abuse	30/395	7.6	5.4-10.6
	Compromised immunity status	Immunosuppressive therapy, HIV	16/211	7.6	4.7-12
Rare (<5%)	Cancer	N/A	67/1,446	4.6	3.7-5.8
	Digestive diseases	Gastritis, ulcer	30/653	4.6	3.2-6.5
	Renal dysfunction	N/A	77/1,972	3.9	3.1-4.9
	Other diseases	Rheumatic, pregnancy related and bone conditions	24/612	3.9	2.6-5.8
	Liver diseases	B/C virus hepatitis, cirrhosis	57/1,637	3.5	2.7-4.5
	Hematologic diseases	N/A[a]	13/486	2.7	1.6-3.5
	COPD	N/A	39/1,549	2.5	1.8-3.4
	Endocrine diseases	Hypo- and hyperthyroidism	8/388	2.1	1-4

[a]N/A, no explicit examples were given. COPD, chronic obstructive pulmonary disease; CI95, 95% confidence interval.

Antibody tests typically target two SARS-CoV-2 antigens: The nucleocapsid protein or the spike protein. The detection technology also differs. For laboratory tests, enzyme-linked immunosorbent assays and chemiluminescence immunoassays are typically used (72).

6. Risk factors

Entities that had a P-value ≤0.05 when comparing mild/moderate cases with severe/critical cases were considered risk factors. In this manner, older age was reported in 7 studies, male sex in

Table III. Average complications data.

Frequency	Finding	Examples	Reported/total	Mean (%)	CI95
Most frequent (≥25%)	Other cardiovascular diseases	Myocardial injury, arrhythmias	143/415	36.9	32.4-41.6
	Other respiratory diseases	Pneumonia, emphysema	270/749	36	32.7-39.5
	ARDS	N/A	172/660	26.1	22.9-29.5
Frequent (≥12.5%, <25%)	Liver injury	N/A	51/276	18.5	14.3-23.5
	MODS	N/A	14/92	15.2	9.3-23.9
Moderately frequent (>5%, <12.5%)	Secondary infection or sepsis	N/A	54/473	11.4	8.9-14.6
	Kidney injury	N/A	54/565	9.6	7.4-12.3
Rare (<5%)	Other/unrelated	Pregnancy-associated, intestinal hemorrhage	3/99	3	1-8.5

ARDS, acute respiratory distress syndrome, MODS, multiple organ dysfunction syndrome; CI95, 95% confidence interval.

Table IV. Most important laboratory findings.

Findings	Pooled mean	Pooled SD	Modification	Reported/ total	Mean (%)	CI95	Reported/ total	Mean (%)	CI95
Total WBC ($x10^9$/l)	6.00	3.15	Leukopenia	13/144	9	5.4-14.8	11/13	85	57.8-95.7
			Leukocytosis	32/577	5.5	4-7.7	7/13	54	29.1-76.8
Neutrophils ($x10^9$/l)	3.24	2.14	Neutropenia	14/123	11.4	6.9-18.2	7/7	100	64.6-100
			Neutrophilia	113/577	19.6	16.6-23	3/8	38	13.7-69.4
Lymphocytes ($x10^9$/l)	1.24	0.75	Lymphopenia	322/645	49.9	46.1-53.8	12/12	100	75.7-100
			Lymphocytosis	1/70	1.4	0.3-7.7	1/9	11	2-43.5
Platelets ($x10^3$/nl)	194.15	78.44	Thrombocytopenia	84/706	11.9	9.7-14.5	9/10	90	59.6-98.2
			Thrombocytosis	2/53	3.8	1-12.8	1/9	11	2-43.5
ESR (mm/H)	69.95	30.65	Elevated	372/409	91	87.8-93.4	2/2	100	34.2-100
CRP (mg/dl^3)	128.91	58.8	Elevated	498/616	80.8	77.5-83.8	8/8	100	67.6-100
D-Dimers (μg/ml)	5	3.43	Elevated	86/396	21.7	17.9-26	3/3	100	43.8-100
PCT (ng/ml)	0.23	0.18	Elevated	48/348	13.8	10.6-17.8	4/5	80	37.6-96.4
ALAT (U/l)	42.76	35.04	Elevated	86/560	15.4	12.6-18.6	6/8	75	41-93
ASAT (U/l)	49.45	33.44	Elevated	128/560	22.9	19.6-26.5	6/7	86	49-97
CK-MB (μg/l)	1.70	1.23	Elevated	60/349	17.2	13.6-21.5	2/3	67	21-94
Troponin I/T (μg/l)	0.02	0.02	Elevated	134/527	25.4	21.9-29.3	2/3	67	21-94
Creatinine (μmol/l)	69.18	31.94	Elevated	30/453	6.6	4.7-9.3	7/7	100	65-100
BUN (mmol/l)	5.62	3.3	Elevated	73/376	19.4	15.7-23.7	6/7	71	36-92

WBC, white blood cells; ES, erythrocyte sedimentation rate; CRP, C-reactive protein; PCT, procalcitonin; ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; CK-MB, creatine kinase MB; BUN, blood urea nitrogen; CI95, 95% confidence interval.

2 studies, and smoker status in 1 study. Regarding the symptoms, these were reported as dyspnea in 4 studies, fever and fatigue in 2 studies, and coughing, chest pains and headaches in 1 study.

Underlying diseases associated with high risk were cardiac comorbidities (including hypertension) in 5 studies, diabetes in 4 studies, renal, hematological and cancerous comorbidities in 3 studies, respiratory diseases (including COPD) and hepatic comorbidities in 2 studies, and neurological/cerebrovascular and endocrine disease in 1 study.

Complications with high risk were noted as follows: Sepsis in 3 studies, acute respiratory distress syndrome (ARDS) and kidney injury in 2 studies, and cardiac and liver conditions in 1 study. CT images that correlated with high risk were crazy-paving patterns and consolidations in 2 studies.

Laboratory findings with high risk were lymphocytopenia and high CRP (in 8 studies each), thrombocytopenia and elevated PCT in 6 studies each, leukocytosis, elevated renal markers (creatinine, BUN) and cardiac troponins in 5 studies

Table V. Main imaging findings.

Findings	Modification	Reported/total	Mean (%)	CI95
Unilateral GGO on CT	Present	438/796	55	51.6-58.4
Bilateral GGO on CT	Present	578/818	70.7	67.4-73.7
Crazy-paving patterns	Present	94/629	14.9	12.4-17.9
Consolidations	Present	126/580	21.7	18.6-25.3

GGO: ground-glass opacities; CT, computed tomography; CI95, 95% confidence interval.

each, ASAT in 4 studies, neutrophilia and high CK-MB in 3 studies each, and D-dimers and ALAT in 2 studies each.

As such, the most important (found in >5 studies) risk factors were thereby identified as: Older age, cardiac underlying diseases (including hypertension), leukocytosis and lymphocytopenia, thrombocytopenia, elevated CRP, PCT, cardiac troponins, creatinine and BUN.

Moderately important risk factors (found in at least 2 studies) were male sex, diabetes, lung (including COPD), hepatic, renal, hematological/neoplastic underlying diseases/immunosuppressed status, dyspnea, fever, fatigue, sepsis and secondary infection, neutrophilia, elevated CK-MB and ASAT.

Other risk factors were stated in 1 study, and these also merit further study. These factors were smoker status, neurological/endocrine underlying diseases, headaches, coughing and chest tightness, ARDS, cardiac, renal and hepatic underlying diseases, elevated D-dimers and ALAT, and crazy-paving patterns and/or consolidations.

The host factors of COVID-19, being a new infectious disease, are the most important to identify in order to determine the severity and progression of the disease. In severe cases of COVID-19, the major risk factors are: Age, male gender, obesity, smoking and chronic comorbid conditions, such as hypertension, type 2 diabetes and others. The most significant risk factor worldwide for severe COVID-19 disease and its negative health outcomes is age. Immunity is the most important consideration in the host-pathogen interaction in any infectious disease. This involves three distinct, but related key issues: Vulnerability, immune response, and protection and potential immune pathology. In the majority of cases, the immune response due to previous exposure to the same pathogen or by vaccination with the same dominant antigen may provide partial immune protection through immune memory. The level of vulnerability also implies innate immunity, which is independent of antigen-specific immune responses and other physiological protective mechanisms. However, since SARS-CoV-2 is a newly identified coronavirus with no previous immune response, the entire population is susceptible without effective immunity to it (73).

7. Treatment

General treatment measures used were non-invasive ventilation (31.6%, 411/1,302; CI95: 29.1-34.1), invasive ventilation (9.9%, 83/839; CI95: 8.1-12.1), antibiotics (84%, 813/968; CI95: 81.5-86.2), antifungals (44%, 165/375; CI95: 39.1-49.1), IFN therapy (13.2%, 98/745; CI95: 10.9-15.8) and corticotherapy (49.1%, 648/1319; CI95: 46.4-51.8). Non-steroid anti-inflammatories were described in only one study (9.2%, 17/184; CI95: 5.8-14.3).

Antivirals used in more than one study were Ribavirin (67.3%, 409/608; CI95: 63.4-70.9), Arbidol/Umifenovir (34.5%, 467/1,353; CI95: 32-37.1), Oseltamivir (52.5%; CI 95: 531/1,012) and Kaletra (Lopinivir + Ritonavir) (16.8%, 125/745; CI95: 14.3-19.6).

Antiviral therapies used in only one study included Ganciclovir (71.2%, 203/323; CI95: 66-75.9), Ribavirin + Oseltamivir (13.6%, 25/184; CI95: 9.4-19.3), Oseltamivir + Kaletra (11.4%, 21/184; CI95: 7.6-16.8), IFN-α + Danuravir (12.5%, 23/184; CI95: 8.5-18.1), Arbidol + IFN therapy (4.6%, 11/238; CI95: 2.6-8.1) and Kaletra + IFN therapy (40.8%, 97/238; CI95: 34.7-47.1).

The antimalarial drug Cloroquine was used in 2 studies (26.3%, 84/319, CI95: 21.8-31.4), of which 1 studied high-vs. low-dose effects (69).

We have found that certain treatments were preferred to be used in severe patients (described by low P-values when compared to mild cases). These included O_2 administration (in 3 studies), antifungal treatment (in 1 study), Kaletra with or without IFN therapy (in 2 studies), and corticotherapy (in 3 studies).

8. Our experience

In Romania, the first patient detected with Sars-Cov2 infection was in Timisoara, being treated at the 'Victor Babes' Infectious Diseases Hospital. From February 2020 until October 2021, this hospital has been designated for the treatment of patients with Sars-Cov2 infection. Patients were both adults and children, with mild to severe-critical forms. The treatments varied according to each wave and form of the disease.

During the first wave, 1,099 patients were hospitalized, out of which 111 died and 120 were treated in ICU. The main symptoms were fever, cough, anosmia, myalgias, dysphonia and dysphagia. A total of 25% of the patients required oxygen therapy during hospitalization. The treatment protocol included antivirals [Lopinavir/Ritonavir (200 mg/50 mg) or Darunavir (800 mg) + Ritonavir (100 mg) or (Darunavir 800 mg) + Cobicistat (150 mg]), which was combined with hydroxychloroquine, anticoagulant and anti-inflammatory drugs. The treatment was determined by the form of the disease, the patient's symptoms and the interactions of the antiviral drugs with the drugs used by the patient.

During the second wave, at 'Victor Babes' Infectious Diseases Hospital, 1,603 patients were hospitalized, out of which 274 patients died and 1,329 were discharged. Most of the deceased people (171) were over 70 years old. Out of the total number, 49 were children under 18 years old. The distribution by sex was as follows: 715 men and 614 women. Among the hospitalized patients, 151 presented with a mild form of the disease, 564 with a moderate form, 614 with a severe form and 103 with a critical form, in need of intensive care. Of the hospitalized patients, 814 patients needed oxygen therapy and 103 were intubated. The main symptoms were dyspnea, marked asthenia, fever, dry cough and odynophagia. The patients were treated with antiviral medication [Lopinavir/Ritonavir (200 mg/50 mg), Darunavir (800 mg) + Cobicistat (150 mg), Darunavir (800 mg) + Ritonavir (100 mg), Favipiravir (200 mg), Remdesivir (100 mg)], and immunomodulatory medication (Anakinra and Tocilizumab). The therapeutic scheme was established according to the lung damage, biological analyses and oxygen saturation in the atmospheric air. Compared with the first wave, the main infections were moderate to severe forms, affecting mainly elderly patients with multiple comorbidities (hypertension, diabetes, kidney failure, stroke and dementia).

In the present review, we have also investigated other case studies found in different parts of the world and presented in the literature that compared the first two waves (46,47,49-54, 56-62,65,70,71). In the second wave, non-invasive mechanical ventilation and corticoids were used more frequently, whereas invasive mechanical ventilation, conventional oxygen therapy and anticoagulants were used less frequently. Other therapies were Lopinavir, Ritonavir, and hydroxychloroquine for patients in the first wave, and Remdesivir and Tocilizumab for those in the second wave (74).

Another study also found a decreased proportion of patients required invasive mechanical breathing, with a concomitant lower rate of thrombotic events compared with the first wave. During the second wave, the time between ICU admission and tracheal intubation was greater. The two waves did not differ significantly in terms of ICU mortality (50 vs. 52%; P=0.96) or ICU stay duration (75).

In another study conducted on 204 patients during the first wave and 264 during the second one, results regarding the risks of death were assessed. In the two groups of patients from the first two waves, the most important factors functioning as determinants of death were determined (74). Age, fever, dyspnea, acute respiratory distress syndrome, type 2 diabetes mellitus and cancer were found to be important during the first wave, whereas age, sex, smoking habits, acute respiratory distress syndrome and chronic neurological diseases were found to be important during the second wave.

During the third wave, at 'Victor Babes' Infectious Diseases Hospital, 1,711 patients were hospitalized, of which 329 died and 1,382 were discharged. Out of these patients, 120 had a mild form of the disease, 499 had a moderate form, 619 had a severe form and 144 required intensive care treatment. Most deaths (180) occurred in the 40-70 age group bracket. The sex distribution was 761 men and 621 women. The main symptoms were dyspnea, marked fatigue, dry cough, insomnia and fever. Unlike the second wave, in the third wave the patients aged between 40-70 years were mainly affected. They had >50% lung damage, and the highest number of deaths was registered among them. Our hospital had 20 patients who had silent pneumonia, although the lung damage was >60%; they needed a low oxygen flow. Sixty-five children were also hospitalized, 20 of whom had developed lung damage and were in need of oxygen therapy.

Patients received antiviral treatment with Favipiravir (200 mg) or Remdesivir (100 mg), corticotherapy (Solumedrol was used in patients with lung damage >50% and Dexamethasone), anticoagulant, immunomodulators (Anakinra or Tocilizumab), vitamin D3 in severe forms (10,000 IU/day) and oxygen therapy.

In a study conducted in Spain on 89 ICU patients, it was concluded that during the third wave, there was a tendency to use corticosteroids, noninvasive mechanical ventilation, high-flow nasal oxygen and awake prone positioning, albeit with lower use of mechanical ventilation compared with the first and second waves (76).

Between March 29 and May 23 2021, a study conducted in England found that Delta variant infections were more likely to result in hospitalization than Alpha variant infections. The study comprised 196 patients with the Delta variation who were taken to the hospital, 47 (24%) of whom were admitted more than 21 days following their first vaccination. The results were similar to a Danish national analysis of hospitalization risk related to the Alpha variant between January 1 and March 28 2021, and 4 cases up to June 27 2021, which also included individuals with the Delta variant to support these findings. It was concluded that the Delta variation was linked to an increased likelihood of hospitalization (77).

The current fourth wave worldwide is dominated by the much more contagious Delta strain and with a shorter incubation period. From July 2021 until September 26, 2021, 195 patients were hospitalized at 'Victor Babes' Infectious Diseases Hospital. Of these patients, 53 have died and 13 were hospitalized in the ICU. Out of the total number of patients, only 10 were vaccinated, with mild-moderate forms. The treatment used is similar to that of the third wave. The mortality rate is higher in patients over 70 years, with 36 deaths. Of these patients, 10 had a mild form of the disease, 47 had a moderate form and 65 had a severe form.

9. Considerations

In 2003, the province of Guangdong, China was ground zero for what is called SARS-CoV. Coronavirus 2 (SARS-CoV-2) is responsible for the coronavirus disease (COVID-19). This is the latest outbreak of a respiratory pathogen. This outbreak has had a substantial socio-economical impact, being the third major outbreak of coronavirus in the last 20 years (56). The patients experienced fever, diarrhea, coughing, dyspnea and pneumonia, which led to ARDS. This outbreak was relatively short-lived, as the mortality rate was quite high (9.6%), the infection rate low (basic reproduction number, R_0=2-3) and it mainly affected young adults (85%) (15,78). The second outbreak was caused by MERS-CoV in 2012, which displayed the same symptoms, but with the addition of renal failure. The fatality rates were quite high, as it progressed into severe disease in less than a week. The mortality rate was 36% (R_0=0.7), and half the patients were over 50 (16). It is also

thought that these viruses were not infectious unless symptoms were present, after the incubation period, which was typically around 1 week (15,78).

Nowadays, we are facing the third outbreak, a pandemic disease similar in symptomatology with the previous ones, which is not surprising given they all originate from the same family and genus. SARS-CoV and SARS-CoV-2 have a high genomic resemblance (~86%) and use the ACE2 receptor for penetration into host cells; however, in COVID-19, the affinity towards this receptor was shown to be even higher (16,78).

SARS-CoV-2 primarily affects the lungs and the upper respiratory tract, yet it also exerts effects on other systems as well. In the digestive tract, it is able to infect the enterocytes, resulting in diarrhea and the possibility of infection by stool samples (34). Due to the high creatinine and BUN levels in these patients, it may be assumed that the kidneys suffer damage during infection, and that this should be treated as an acute kidney injury (26,34,79).

In the CNS, some viral load has been found in the cerebrospinal fluid, and even in cerebral tissue from autopsy. This could be explained by assuming the virus can use the route of neuronal transport. Headaches have been shown to be quite frequent. Dizziness and confusion have also been observed. Loss of taste and smell may appear due to the infection of the olfactory cells (34,80).

Another entity that the virus affects is the inflammatory cascade, which may be responsible for complications such as ARDS, MODS and microcoagulation dysfunctions. In these cases, for critical patients it would be good to introduce immunosuppressive medications, especially IL-6 blockers (34,81).

Microcoagulation dysfunction can be explained by the fact that the platelet numbers are lower in Patients with COVID-19. D-dimer levels, which are related to fibrin destruction, are high, especially in severe cases (34).

Epidemiologically speaking, the virus is less deadly, which has been borne out by the analysis presented in this study (4.9%) and in others before it. It still has R_0=2-3; however, it was speculated that the virus could be transmitted even before the appearance of the first symptoms (82). Viral load is highest immediately after onset, with greater quantities in the nose, which explains why nasal swabs are superior in terms of sample collection. It is thought that the nasal viral load is often high in asymptomatic cases as well (38,82).

An interesting particularity is that this disease is more severe in older individuals (>65 years old) and in people with underlying conditions. Infection of children still exists; however, they seem to experience only mild symptoms and a very low mortality rate (60).

In the beginning, it was thought that COVID-19 infected men more than women, a ratio that appears to have reversed (male/female ratio=34/66%) and is not statistically significant anymore. However, men continue to have a higher chance of mortality, although it is a matter of contention whether this is due to the virus itself or due to the fact that men tend to have greater comorbidities than women.

Another feature that is a contentious matter is that the virus is not capable of vertical transmission, although newborns may be infected through blood or Flugge droplets immediately after birth. It also appears that semen contains no viral load. However, given that the placenta and testes are rich in ACE2 receptors, this hypothesis requires further study (40,41,79).

Clinical findings. The present study has shown that fever is still one of the most frequent symptoms. However, we have shown that its importance has reduced (68.6%) since the preliminary studies performed in December-January, when it was the most important feature, found in 93.1% of the patients (83). This may be due to a selection bias at that time, which excluded asymptomatic cases, which have been shown to exist (21.4% in the present study). No reduction in the incidence of other symptoms has been reported or observed.

Another study conducted by Jiang *et al* (83) showed that symptoms that have increased in incidence are coughing (72.7 vs. 66.8%), headaches (55.4 vs. 8.5%), rhinorrhea and nasal congestion (13.9 vs. 4%), sore throat (35.4 vs. 11.1%), nausea and vomiting (17.4 vs. 6.3%) and diarrhea (28.9 vs. 7.3%).

Symptoms that have maintained about the same level of incidence (<5% difference) are dyspnea (37.2 vs. 33.5%) and sputum production (29.9 vs. 25.4%).

Complications that vary highly comparing between the present study and that of Jiang *et al* (83) are ARDS (26.1 vs. 20.1%) and cardiac failure (27.9 vs. 15.6%). Lower variations (<5%) were identified for renal (9.6 vs. 5.8%) and secondary infection/sepsis (11.4 vs. 8.6%).

Underlying diseases that were observed to be found in higher-risk patients were hypertension (26.8%) and other cardiac diseases (16.9%), COPD (2.5%) and other respiratory conditions (9.6%), liver (26.3%) and kidney (3.9%) conditions, diabetes (14.3%), cancer (4.6%), hematological diseases (2.7%), immunosuppressed status (2.9%) and neurological or cerebrovascular disorders (7.9%). Another clinical feature found in this category of patients is their smoking status, either present smoker (5.8%) or former smoker (15.8%).

Other underlying diseases that were observed are endocrine (2.1%), psychiatric (7.6%), digestive diseases (4.6%) and unrelated conditions, including rheumatic, pregnancy or connective tissue issues (3.9%). Although anxiety, depression and dysfunctional coping mechanisms were found in infected patients in our study, the current outbreak seems to have taken a toll on the healthy general population as well, resulting in mass hysteria and depression (84,85).

Important aspects found in our study when making the risk assessment based on clinical findings are older age, cardiac, respiratory, renal, cancerous and immunosuppressive comorbidities, diabetes, dyspnea and sepsis or secondary infections. Patients with such risk factors should be watched more carefully, as their evolution tends to be more severe and require more medical attention.

Paraclinical findings. In the present outbreak, it was deemed that chest X-rays have very limited use. There was only one study in which this procedure was performed, and it recorded only one positive pneumonia case.

Chest CT is much more efficient and can detect early-stage pneumonia. The most common pattern was GGOs, both bilateral (75.7%) and unilateral (62.7%). These abnormalities tend to be found peripherally, and evolve into consolidations and crazy-paving patterns (17). Although imaging findings are

important, they can be considered nonspecific, and therefore other investigations must also be performed (86).

During the laboratory tests, it was revealed that mildly affected patients experienced a lower white blood cell count (9%), whereas more severe patients experienced a higher count (5.5%). A drop in the number of lymphocytes (49.9%) was observed, which was more marked in more severe cases. Neutrophil counts were lower in mild cases (11.4%), and higher in severe cases (19.6%). Thrombocytopenia was also of moderate importance (11.9%), and worse in severe cases. Systemic inflammatory markers, including ESR (91%) and CRP (80.8%), were elevated, and the higher their values were, the more closely they were associated with the more severe cases.

PCT, which is an indicator of secondary bacterial and fungal infection and/or sepsis, was high in 13.8% of the cases. D-dimer levels were also increased (21.7%), and were observed to be the highest in severe cases.

Liver and renal functions were also found to have deteriorated, as the transaminase duo of ALAT (15.4%) and ASAT (22.9%) and creatinine (6.6%)-BUN (19.4%) were elevated, especially in more severe cases. The cardiac markers CK-MB (17.2%) and troponins I and T (25.4%) were elevated, findings which allude to myocardial injury and a higher risk of death.

Features identified in the present study which are important when making the risk assessment based on paraclinical findings are leukocytosis with neutrophilia and especially lymphopenia, elevated levels of CRP and PCT, which also indicate secondary bacterial or fungal infection, elevated cardiac markers troponins I/T and isoenzyme CK-MB, elevated renal markers (serum creatinine and BUN) and elevated ASAT. Patients with such factors should be watched more carefully, as their evolution tends to be more severe and require more medical attention.

For the routine clinical diagnosis of COVID-19, data on the epidemiological history and clinical manifestations are used. The diagnosis is confirmed by a variety of laboratory methods. Chest CT, NAAT and serological techniques are also used. The diagnosis of SARS-CoV-2 infection can also be made by nasopharyngeal and/or oropharyngeal swab, bronchoalveolar lavage fluid, sputum, bronchial aspirate or blood (87).

RT-PCR is a method routinely used for RNA detection. It is a fast technique, with the results being visible in a few hours. This technique is based on two consecutive reactions: i) conversion of RNA into complementary DNA (cDNA) through the action of a reverse transcription enzyme; and ii) amplification of the cDNA sample by PCR using gene-specific primers and fluorescently labeled hydrolytic probes. RT-PCR is currently the best method for detecting SARS-Cov-2 due to its ability to directly measure the viral genomic components rather than secondary biomarkers such as antigens or antibodies (88).

Treatment considerations. Basic treatment options against COVID-19 are meant to maintain homeostasis and ease the symptoms. Ventilation is very important, especially in severe cases with ARDS or heavy dyspnea. As such, both invasive and non-invasive oxygenation methods have been used (89). Venous access should also be considered for intravenous (IV) solutions, as many patients suffer from complications. Non-steroid anti-inflammatory drugs can be administered; however, corticosteroids seem to have a more pronounced effect.

Corticotherapy should also be used in severe cases, as it reduces reactions like ARDS and MODS, which are mostly sequels of an intense inflammatory response (37). In these patients, it was found that the level of IL-6 was high, which tends to create a positive feedback loop. Another medication that can be administered is IFN-α, which lessens the inflammatory response caused by IL-6 (67). Another IL-6 inhibitor that has shown promise is Tocilizumab (33,90).

Besides antiviral therapy, antifungal and broad-spectrum antibiotic treatments are also vastly important. It should be noted that many patients suffered from rudimentary secondary infections to sepsis and septic shock, a fact backed-up by heightened CRP and PCT levels. These treatments may also be applied prophylactically.

Antivirals can be used as monotherapy or in combinations. Monotherapy treatment options are Ribavirin, Arbidol (Umifenovir), Oseltamivir, Danuravir and Ganciclovir, whereas combination treatments include Kaletra (Lopinavir + Ritonavir), Kaletra + Oseltamivir and Oseltamivir + Ribavirin.

Due to intense promotion by the US media, chloroquine and hydroxychloroquine were proposed to be used in emergencies. Although *in vitro* studies have shown promising results, these antimalarial drugs have limited use *in vivo* due to a very low toxicity index (20 mg/kg). Usual side effects include ophthalmic manifestations and elongated QTc, resulting in ventricular fibrillation and tachycardia (91,92). The study (70) also looked into low (2x450 mg/day plus placebo, total dose 2.7 g) vs. high (2x600 mg/day, total dose: 12 g) dose effects, and these investigators needed to retract patients from the high-dose group due to cardiac complications. As such, the recommendation would be to use lower doses of these drugs (57).

Vaccination is a good therapeutic option, since it provides long-term immunity. The target for vaccine development is protein S (i.e., the spike protein). It is expressed on the viral surface and it is susceptible to recognition by circulating antibodies. Vaccines designed against S proteins have proven to be the most effective. Existing strategies for designing an effective vaccine include full-length S protein preparations, RBD-only peptide, nanoparticles containing RBD DNA, nanoparticles containing RBD mRNA, inactivated virus, and recombinant viral vectors (93).

An effective vaccine for SARS-CoV-2 could prevent infection, disease or transmission. SARS-CoV-2 infection depends on several variables such as age, sex, ethnicity and comorbidities. The effects of infection have been shown to range from being asymptomatic to hospitalization with a requirement for respiratory support and mortality. The ability of infected individuals to transmit the infection when it is asymptomatic must be controlled. Infection control strategies are based exclusively on preventing transmission from symptomatic individuals, although these strategies are insufficient to interrupt SARS-CoV-2 transmission (94). Living organisms are used to make traditional vaccines that are attenuated or inactivated. The advantage of this vaccine is that it has a similarity to the natural infection, and thus a stronger and more lasting immune response can be effected. No data are currently available on the duration of immunity after SARS-CoV-2 infection. It has been said that a high level of immunity is conferred for a minimum of 1 year. These live vaccines may be risky in those with immunosuppression and fragile immune systems,

particularly for people at high risk of COVID-19 infection, such as the elderly. An alternative to these vaccines is inactivated virus vaccines. Their disadvantage is that they generally offer immunity with a shorter duration of action (95).

Certain data have been published, suggesting that the risk of harm is low with Remdesivir treatment, but in combination with corticosteroids it has a crucial effect. Studies have been performed on investigational agents, i.e., immunomodulators and monoclonal antibodies. In addition, in critically ill patients, an examination of the effects of Remdesivir in combination therapies using a placebo-controlled design would be informative (96).

As an RNA polymerase inhibitor, Remdesivir was originally developed for Ebola infections and was the first FDA-approved antiviral drug for COVID-19 therapy on October 22 2020. It terminates RNA synthesis and inhibits replication of the SARS-CoV-2 genome, which previously exhibited antiviral activity against SARS-CoV and MERS-CoV. Favipiravir, a guanine analogue, inhibits RNA polymerase and is currently used to treat influenza. A combination of Favipiravir and IFN-α (ChiCTR2000029600) or Baloxavir Marboxil (ChiCTR2000029544) has demonstrated anti-SARS-CoV activity in Vero E6 cells (23). Other antivirals currently in (re) use for COVID-19 therapy include the neuraminidase inhibitor, Oseltamivir. Oseltamivir has been used for influenza A and B viruses, as it requires neuraminidase to release the virus to host cells. SARS-CoV-2 does not express neuraminidase; therefore, combination therapy of Oseltamivir with protease inhibitors has been shown to be effective in acting against it (23). The mRNA vaccine combines the advantages of live attenuated vaccines, namely endogenous antigen expression and T cell induction with the remarkable safety profile of killed or subunit vaccines. mRNA vaccines are based on the supply of synthetic mRNA encoding one or more antigens in the cytoplasm of the host cell, and their expression leads to the generation of a sufficient amount of translated protein (97).

Lopinavir and Ritonavir are protease inhibitors that have been approved for the treatment of HIV-1 infection. Due to its poor oral bioavailability, Lopinavir is co-formulated with Ritonavir for increased drug solubility and improved antiviral activity. Lopinavir and Ritonavir were initially hypothesized to inhibit the 3-chymotrypsin protease of SARS-CoV and MERS-CoV, implying their potential use for the treatment of COVID-19. Lopinavir and Ritonavir have also been used in triple combination therapy with PEG-IFN-α and Ribavirin, or with chloroquine/hydroxychloroquine, in the treatment of COVID-19. Other clinical trials have shown that Lopinavir/Ritonavir is not able to inhibit SARS-CoV-2 proteases (ChiCTR2000029308). Other protease inhibitors that have been tried and reused as alternatives for COVID-19 clinical trials are Ritonavir in combination with ASC09 and Darunavir, an antiviral drug for the treatment of HIV/AIDS (23).

Vaccine strategies. As numbered points, the following vaccine strategies have been proposed:

i) Inactivated virus vaccines that contain physically or chemically inactive viruses and maintain the integrity of the virus particle that represents the immunogen (98).

ii) Vaccines with virus-like particles or nanoparticles in which structural viral proteins are co-expressed to form non-infectious particles as vaccine immunogens. These are similar to real virions, although they lack the genome of the virus (98).

iii) Vaccines for protein subunits that contain key viral proteins or peptides that can be manufactured *in vitro* in bacteria, yeast, insects or mammalian cells. Most candidates for the SARS-CoV vaccine rely on this strategy, both in the clinical and preclinical stages (98).

iv) Vaccines with viruses containing genes. They encode pathogenic antigen(s) and are cloned into non-replicating or replicating virus vectors (such as adenovirus) (98).

v) DNA and mRNA vaccines that have the advantage of rapid manufacture against emerging pathogens. In the case of DNA vaccines, the viral antigen(s) encoded by a recombinant DNA plasmid is produced in the host cells through a sequential transcription-translation process. mRNA vaccines instead are synthesized by *in vitro* transcription, leading to the production of viral antigen(s) in the cytoplasm by direct translation of proteins *in vivo* (98).

The main purpose in designing RNA-based vaccines was for their use in cancer and infectious diseases. This therapeutic approach is based on the synthesis of RNA chains, which encode the desired antigenic proteins and take advantage of the intrinsic immunogenicity of nucleic acids. To evade degradation by RNases, the RNA is encapsulated in nanoparticles or liposomes, and following endocytosis, the charge is delivered inside the target cells. The mRNA is then translated into immunogenic proteins through the cellular ribosomal apparatus (99). The mRNA vaccine is 94.1% effective in preventing COVID-19 disease, including severe disease. The reactions were transient local and systemic reactions, and no other problems were identified (100).

vi) The classic method of preparing a vaccine is based on a live microbe that has been attenuated so that it is not able to cause disease. Attenuated microbes have been used because they retain their ability to replicate *in vivo*, giving rise to a limited form of the disease. They are very effective in stimulating the immune system. Another advantage is that they induce a strong and persistent immune memory, which is effective in preventing infections. Hundreds of millions of people have been vaccinated with attenuated vaccines, and have been protected from serious diseases (99). Live attenuated antivirus vaccines are attenuated viruses generated by *in vitro* or *in vivo* passage or genetic reverse mutagenesis. The resulting virus becomes non-pathogenic or only weakly pathogenic; however, importantly, it still retains its immunogenicity via mimicking the live virus infection (98).

10. Conclusions

In the present review article, we have presented a summary of the current state of the COVID-19 pandemic and its main epidemiological, clinical, laboratory and radiological features. We have also noted some of the possible treatment options, many of which have been repurposed from the previous SARS-CoV and MERS-CoV outbreaks. This review includes 25 studies, and the conclusions are based on the information provided by them alone, since they were available at the time of submission of this article.

More case-controlled, experimental studies and randomized trials should be carried out in order to paint a more

accurate picture regarding the current situation, as well as any future virus outbreaks. Even as governments ease restrictions, preventive measures should still be followed carefully. We urge the use of protective equipment, especially individuals considered essential (healthcare personnel, couriers, store clerks and cleaners), the practice of social distancing, limitation of group activities and the continuing practice of proper washing and disinfection.

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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