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Latest Insights on the Diagnostic Approaches and Treatment Strategies of COVID-19

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Abstract
Background: COVID-19 has emerged as the most serious pandemic in the 21st century to date. COVID-19 patients may develop various disease symptoms that hinder the accurate clinical diagnosis. ***Summary:*** Routine diagnosis of COVID-19 requires complementary investigations, including computed tomography, immunological assays, and molecular assays like real-time RT-PCR, loop-mediated isothermal amplification, metagenomic next-generation sequencing, and clusters of regularly interspaced short palindromic repeats-based assays. Clinically approved antiviral drugs available for the COVID-19 treatment are very limited. The most common measurements that enhance health condition and patients' viability are conservation fluid management, oxygen therapy, and antibiotics. Several therapeutic options have been developed or repurposed to prevent virus replication and/or modulate the immune response against virus infection. These options include various drugs that affect virus entry and membrane fusion, inhibit polymerase and protease activity, suppress the host pro-inflammatory cytokines, and utilize cell therapy approaches. ***Key Messages:*** In this review, we aimed to provide an up-to-date discussion on the current diagnostic options and therapeutic strategies used to control and manage COVID-19 in clinical and point-of-care settings.

Introduction

COVID-19 has developed as the most serious pandemic in the 21st century to date. The causative agent SARS-CoV-2 is classified as a member of genus *Betacoronavirus*, subfamily Coronavirinae, family Coronaviridae, and order Nidovirales [1]. A characteristic feature of SARS-CoV-2 is its transmission via close contact with infected persons by exposure to sneezing, coughing, respiratory droplets, and aerosols (airborne transmission) [2–4]. Routine diagnosis of COVID-19 is based on the epidemiological history, clin-

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ical manifestations, and some complementary investigations, including molecular detection of viral RNA, bedside/point-of-care (POC) testing, computed tomography (CT), and immunological assays like ELISA. Neverthelesss, SARS-CoV-2-infected patients may show a wide range of clinical symptoms such as dry cough, fever, loss of taste and/or smell, fatigue, headache, sore throat, myalgia, pneumonia, renal failure, diarrhea, septic shock, and others [5]. Therefore, complementary investigations are crucial for a better diagnosis of COVID-19 in clinical and POC settings [6]. Specimens for identifying SARS-CoV-2 infection may include nasopharyngeal swab/wash/aspirate, oropharyngeal swab, lower respiratory tract aspirate/lavage, sputum, whole blood, plasma, serum, urine, and stool [7, 8] (online suppl. Video; for all online suppl. material, see www.karger.com/doi/10.1159/000522336).

Unfortunately, the available and clinically approved antiviral drugs to prevent and/or treat COVID-19 are very limited [6, 9]. The alternative adjuvant treatments, including conservation fluid management, oxygen therapy, and antibiotic use against secondary bacterial infections, are the only measures to support the health condition and enhance the patient's viability [10]. Based on the genomic organization of SARS-CoV-2 [1] and the molecular mechanisms of infection [11], several prospective therapeutic targets are available for the development of efficient interventions against SARS-CoV-2 [6]. In the following sections, the different methods used for diagnosing COVID-19 and the approaches used for developing specific antiviral chemotherapy will be discussed according to the most recent available data.

Basic Structural and Functional Features of SARS-CoV-2

SARS-CoV-2, like most coronaviruses, is spherical or pleomorphic in shape with an average diameter of 65–125 nm [12]. The viral genome is typically composed of single-stranded positive-sense RNA that extends from 29.8 to 29.9 kb in length [1]. Viral RNA is wrapped with the nucleoprotein subunits to form a helical nucleocapsid structure. An outer envelope is formed by the budding of the viral nucleocapsid from the endoplasmic reticulum-Golgi intermediate compartment. Coronaviruses have a crown-shaped appearance as they possess club-shaped projections (spikes) of 20-nm length protruding from the viral envelope [13].

The genome of SARS-CoV-2 encodes for 4 structural proteins, including spike (S), membrane (M), envelope (E), and nucleoprotein (N), besides 23 nonstructural and accessory proteins [14]. RNA-dependent RNA polymerase (RdRp), in combination with other nonstructural proteins like nsp2 (RNA helicase) and nsp4, forms the polymerase complex that is crucial for RNA processing and virus replication [15]. RdRp plays a vital role in virus evolution since it controls the virus replication fidelity and consequently, the mutation rate and adaptation to sudden changes of host populations and new environmental conditions [16]. Other accessory proteins, rare in RNA viruses, were also described to gather at the cell membrane during virus budding from the host cell. These proteins include 2′-*O*-ribose methyltransferase, putative sequence-specific endo-RNAse, 3′–5′ exonuclease, and ADP ribose 10-phosphatase [17].

S protein is a type I membrane glycoprotein mediating virus binding to host cell and membrane fusion and is crucial for determining host susceptibility and transmissibility [18]. Two subunits have been described for S protein, including S1, which has four core domains $S1_{A–D}$ responsible for cell attachment and S2, which is implicated in membrane fusion and virus entry into the host cell [19]. M glycoprotein has a long C-terminus cytoplasmic domain, a short N-terminus domain protruding externally, and 3 transmembrane domains spanning the viral envelope [20]. This protein represents a chief organizer of virus assembly and budding by interaction with other structural proteins [21]. The E protein is the minor membrane protein engaged in different virus replication stages, particularly during assembly and envelope formation [22]. N protein is distinct from the other structural proteins of SARS-CoV-2 binding to the viral RNA forming the nucleocapsid [23]. The protein also augments RNA synthesis and folding and influences cell cycle and protein translation in the host cell [24, 25]. M, E, and N proteins interact to stabilize the RNA-nucleoprotein complex within the internal core of virions, triggering virus assembly and budding [26].

Current Diagnostic Measures for COVID-19

Besides CT, which is critical for the initial diagnosis of COVID-19, the currently available diagnostic tests are divided into two major classes. The first class includes the molecular assays used for the identification of SARS-CoV-2 RNA in clinical specimens, such as real-time RT-PCR (rRT-PCR), next-generation sequencing (NGS), clusters of regularly interspaced short palindromic re-

Table 1. Contemporary diagnostic tools for SARS-CoV-2: advantages and disadvantages

Diagnostic tool	Common sample types	Advantages	Disadvantages
rRT-PCR	Sputum Bronchial aspirates Pharyngeal, nasal, and anal swabs Bronchoalveolar lavage fluid Blood Feces [8–9]	Sensitive and specific [29–43] Rapid: 1.5 h/run Closed system reaction (limited false-positive results) [30] Targets most viral protein genes Structural (S, E, M, N) Nonstructural (RdRp, ORF1a/b) [31–34]	Requires well-equipped labs Infrastructure is lacking in developing countries [33] Requires a satisfactory threshold of virus concentration in the specimen Delay of 5 days for switching from premier negative to positive results [93] False-negative results due to improper handling [94] Expensive [94]
RT-LAMP	Swabs (nasopharyngeal, oropharyngeal, anterior nares, MTN Nasal and bronchial aspirates Bronchoalveolar lavage fluid	Sensitive and specific [95] Rapid (less than an hour) Simple one-step amplification technique Performed at constant temperature (60–65°C) No need for sophisticated equipment Low-cost reagents (stable at room temp) Simple detection of positive results (color, turbidity, and fluorescence)	Four to six primers
CRISPR	Respiratory samples	Sensitive and specific (100%) [43] Short turnaround time (40 min) No false-positive results	Off-target results [94]
mNGS	Respiratory samples	High throughput [96] Unbiased nature	Long turnaround time (about 20 h) [43] Relatively short reads [96]
SHERLOCK	Respiratory sample Serum Urine Saliva Blood	A simple and rapid technique Single sample handling step Performed at a single temperature Visual result interpretation No need for sample extraction Suitable for home testing [46]	Technically demanding RNA/DNA oligonucleotides and reaction mixtures are not commercially available Multistep nucleic acid amplification Less useful in gene expression profiling [97]
Serological tests	Blood Plasma Serum	Easy to perform Require little instrumentation Do not need technical proficiency Easy sample collection Less potential hazard [98]	Less sensitive and specific Cannot detect infection in early stages [98] Do not discriminate between infection and vaccination Cross-reactivity with other coronaviruses [33]
Chest CT	Chest	Noninvasive [33–99] Screens even asymptomatic people Sensitivity (86–98%) [93]	Expensive Requires technical expertise [33] Results may confuse with other infections

MTN, mid-turbinate nasal.

peats (CRISPR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), specific high-sensitivity enzymatic reporter unlocking (SHERLOCK), and nucleic acid sequence-based amplification (Table 1) [27]. However, the second class comprises the immunological assays used for detecting virus-specific antibodies in patients' sera [28].

rRT-PCR Assays

rRT-PCR is the gold standard for detecting a wide range of pathogens, including SARS-COV-2, because of its sensitivity, specificity, and rapidity [28]. Scientists have used several targets like RdRp, ORF1a/b, E, N, and S genes to detect SARS-CoV-2 by rRT-PCR. Testing two molecular targets in parallel are mostly recommended to avoid cross-reactivity with other human coronaviruses

and reduce the potential of false-negative results caused by the generation of new virus variants over time [29]. Studies have utilized conserved sequences from S and RdRp [29], E and RdRp genes [30], and ORF1b and N genes for the development of reliable rRT-PCR assays. It was suggested that the ideal design for a SARS-CoV-2 rRT-PCR assay is to target at least one conserved region and one specific region [29]. The WHO recommends using a gene-based assay for initial screening and RdRp gene-based assay for result confirmation [29, 30], whereas the CDC identified two loci in the N gene for getting the best results [31].

Genome analysis of a wide array of SARS-CoV-2 strains has identified three genes: RdRp, E, and N, as highly conserved. The developed assays that target these genes have shown variable analytical sensitivities, where E- and RdRp-based assays were highly sensitive (detection limit of 3.9 and 3.6 copies per reaction, respectively), and N-based assays were the least sensitive (8.3 copies per reaction) [30, 32]. Similarly, a Chinese group has compared the results of three assays targeting RdRp, S, and N ORFs using 273 clinical samples from confirmed cases and confirmed that RdRp is the most sensitive and specific for the use in laboratory diagnosis of COVID-19 [33].

A notable drawback of rRT-PCR is that it could not determine the infection early enough to apply the control measures, particularly in asymptomatic patients [32]. The sensitivity of the assay also depends on the type of sample used for testing. In a cross-sectional study that involved 1,070 specimens collected from 205 confirmed cases, the highest sensitivity was obtained with bronchoalveolar lavage (93%), followed by sputum (72%), nasal swabs (63%), and pharyngeal swab (32%) [32]. The viral RNA was also detected in the feces but not in the urine of infected patients. Evidence also suggested using the saliva as an alternative sample with less need for protection facilities; however, it still needs validation.

A combined Influenza SARS-CoV-2 Multiplex rRT-PCR Assay (Flu SC2) was launched by the CDC in July 2020 to detect SARS-CoV-2, influenza A, and influenza B viruses simultaneously in upper or lower respiratory specimens. The assay is extremely sensitive and can be used as a powerful tool to evaluate specimens from patients in the acute phase of infection [34]. The Flu SC2 Multiplex Assay allows public health laboratories to run three tests in a single reaction tube with fewer test reagents and higher throughput. Flu SC2 is currently under improvement, utilizing sequence data that were not available during the first release of the assay [34].

Reverse Transcription Loop-Mediated Isothermal Amplification

RT-LAMP is a one-step RNA amplification technique used for the diagnosis of many infectious diseases. The advantages of RT-LAMP over RT-PCR are shown in Figure 1. The assay is performed at a constant temperature between 60 and 65°C, and the amplified target is identified using simple methods like visual inspection of color change or turbidity, measurement of fluorescence, and agarose gel electrophoresis [35]. Recently, several research groups have developed optimized RT-LAMP systems as rapid and straightforward choices for COVID-19 diagnosis in regions lacking the facilities and equipment [36–41]. The developed assays use primer sets that target conserved sequences in different genomic regions of SARS-CoV-2 (either separately or combined), including ORF1ab, S, and N genes. In particular, the primers are designed to target the RdRp region of ORF1ab and have shown higher amplification efficiency and specificity [39].

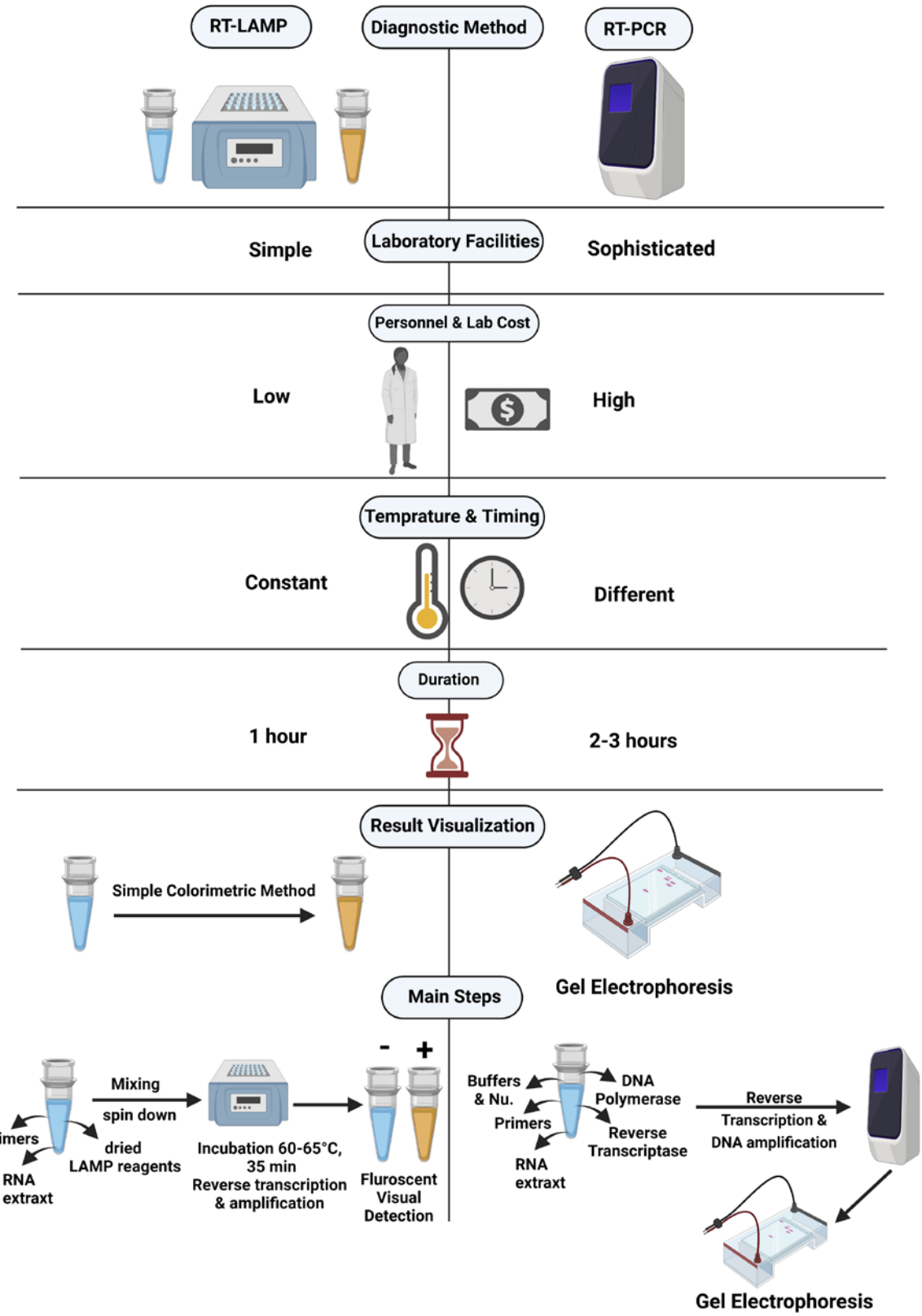
The RT-LAMP assays could amplify the specific sequences in a single-step process without the need for RNA extraction. Also, obtained results are quick with high specificity, sensitivity, and minimum cross-reactivity [40]. It was reported that the colorimetric LAMP is a quantitative method [41]. In two different studies, there was a high degree of compatibility between the results of RT-LAMP and the gold standard rRT-PCR (89.9% and 100%) [36, 38]. The technique was also adapted for use with the commercial Eppendorf PCR tubes combined with the 3D-printed incubation chamber [41].

Metagenomic NGS and CRISPR

Metagenomic NGS, high-throughput sequencing, is now playing a pivotal role in diagnosing unexplained pathological conditions, particularly those caused by atypical causative agents. This method has many applications including genome sequencing (>1 million base pairs per single run), diagnosis of cancer, inheritable disorders, and infectious diseases, besides large-scale recognition of novel viruses and virus strains. In conjunction with the bioinformatic tools, the metagenomic NGS has greatly inspired the contemporary viral pathogenesis and diagnostics, especially during the current pandemic [42]. NGS has recently been utilized in routine screening of viral genomes for genetic drifts due to its high sensitivity [43]. At the start of COVID-19 outbreak,

Fig. 1. Advantages of using RT-LAMP over RT-PCR as a diagnostic method for SARS-CoV-2 detection.

(For figure see next page.)


RT-LAMP
Diagnostic Method
RT-PCR
Simple
Laboratory Facilities
Sophisticated
Personnel & Lab Cost
Low
High
Temprature & Timing
Constant
Different
Duration
1 hour
2-3 hours
Result Visualization
Simple Colorimetric Method
Gel Electrophoresis
Main Steps
Primers
RNA extraxt
Mixing
spin down
dried LAMP reagents
Incubation 60-65°C, 35 min
Reverse transcription & amplification
- +
Fluroscent Visual Detection
Buffers & Nu.
Primers
RNA extraxt
DNA Polymerase
Reverse Transcriptase
Reverse Transcription & DNA amplification
Gel Electrophoresis

1

the patients' samples of acute respiratory distress syndrome (ARDS) indicated negative for all suspected pathogens. NGS was the only tool that enabled scientists to identify the etiological pathogen as novel coronavirus [44]. Yet, the costs of NGS chemicals and equipment limit its use as a laboratory-standard diagnostic technique [42].

CRISPR/Cas is a powerful gene-editing tool and a promising treatment for many diseases. Different kinds of Cas proteins were demonstrated, among which Cas12a and Cas13a are efficient in diagnostic purposes, and Cas9 is specialized in gene editing [45]. A CRISPR/Cas12-based assay was developed to detect SARS-CoV-2 in clinical samples and was termed SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter. Another isothermal CRISPR/Cas13-based assay (CRISPR-nCoV) was developed and compared to rRT-PCR and NGS [43]. CRISPR-nCoV had a sensitivity and specificity of 100% compared to the reference assays. The assay seems a promising diagnostic tool that offers a shorter turnaround time, even in under-resourced settings with no need to use thermal cyclers [43].

SHERLOCK is an innovative technique that involves amplifying specific viral RNA sequences, followed by detecting the amplicons using CRISPR-mediated collateral reporter unlocking [32, 46]. An RNA guide sequence, together with Cas13a, binds specifically to the amplified fragments forming a complex [47]. This binding stimulates activation of Cas13a to cleave adjacent fluorophore-quencher probes emitting fluorescence. SHERLOCK was used to distinguish Zika virus from dengue virus in the serum and urine in concentrations lower than 2,000 copies/mL. It could also detect several bacterial strains and identify mutations in cell-free tumor DNA [48].

SHERLOCK Testing in One Pot is a modified method based on SHERLOCK's use in a simple platform. The technique does not require RNA extraction and is performed in a single step using a single reaction temperature. STOPCovid was recently developed for use in POC settings and household facilities to face the growing need for COVID-19 diagnostics [46]. The developed assay utilizes lysis buffer (QuickExtract) to release SARS-CoV-2 RNA, which is detected after amplification, RNA binding, and Cas13a activation on a commercially available paper dipstick by the naked eye [46].

Immunological Testing

Immunological testing, particularly serological assays, is relatively inexpensive, easier to perform, and requires fewer tools and less technical proficiency than nucleic acid-based assays. However, a recognizable level of antibodies cannot be detected before elapse of several days to weeks of infection. Therefore, serological tests are not recommended to diagnose acute SARS-CoV-2 infections except in very rare conditions [49]. They may be used for confirming or excluding the infection in late stages [50].

Serological tests are mainly used to determine former infection with SARS-CoV-2 and help diagnose clinically suspicious patients with negative PCR results. Patients are considered positive when they have recognizable IgM antibodies or an increased IgG titer (4× or more) in the convalescence sera compared to the acute phase sera [51]. The antibody dynamics of SARS-CoV-2 are very similar to most acute viral infections, where the IgG level increases as the IgM level decreases [31]. In general, IgM starts to appear in the patient's serum by the 5th day postinfection, whereas IgG does not develop before the 14th day [51]. Likely, several serological tests have been authorized for use, under the emergency, by the US FDA for SARS-CoV-2 diagnosis. The majority of currently authorized serological tests detect IgM and IgG in infected persons' blood by ELISA, lateral flow cassettes, or chemiluminescence platforms [52].

Other assays that determine cellular markers and immune cells' levels are also described as practical approaches for COVID-19 diagnosis. Patients mostly show higher D-dimer and C-reactive protein levels and low levels of leukocytes, lymphocytes, and blood platelets [53]. There is much to be determined about the value of these parameters and serological testing in monitoring and diagnosing COVID-19.

Computed Tomography

Despite the availability of many sensitive and specific diagnostic assays for SARS-CoV-2, chest CT is still significant as an initial screening tool [53–57]. A chest CT scan is a cross-sectional image taken across a patient's chest at different angles. Several scientists claimed that chest CT is more credible than rRT-PCR in COVID-19 diagnosis since rRT-PCR may render false-positive and/or false-negative results [58]. Others confirmed that even asymptomatic patients could be screened by chest CT [54]. It was concluded that patients with intermediate symptoms who display inconspicuous chest X-ray findings are positive COVID-19 even if their rRT-PCR results are negative [55]. Recently, the Fleischner Society declared three main scenarios for using imaging as a primary diagnostic tool, including (a) cases with mild respiratory features yet have risk factors for disease progression, (b) cases presenting with moderate to severe features of COVID-19, and (c) cases with moderate to severe

symptoms with limited testing resources and within a high frequency of disease [59].

Chest CT mostly shows peripheral, bilateral, and ground-glass opacity in COVID-19 patients [54]. However, the imaging pattern varies according to the disease stage and severity [60]. Chest CT exhibits interstitial changes and multiple small plaques in the early stages, besides common patterns like lymphadenopathy, cystic changes, pleural effusion, bronchiectasis, and nodules [54]. In more advanced stages, an infiltrating opacity is recognized with lung consolidation (solid or fluid material in compressible lung tissue), multiple bilateral ground-glass opacity, and fibrous stripes or interstitial thickening [54, 61].

Breathe Print

The COVID-19 breath test has developed to exploit the advances in sensor technologies for breath diagnostics based on the crystallo-chemical principle of selective gas detection [62–64]. An electronic signature can be generated based on the presence and concentration of a specific chemical compound (biomarker) in a single exhaled breath, as opposed to other nose technologies that use nonselective sensors and sample the entire breath for patterns drawn by machine learning algorithms [65]. Exline et al. provided the first study to use a nanosensor breathalyzer to detect viral infection from exhaled "breathe prints" in critically ill patients. The test is noninvasive and quick. Due to epidemiological concerns and regulations regarding research staff exposure, the analysis was not done at the bedside, but it could be done easily in clinical practice [66]. This COVID-19 sensor captures the interaction and relative ratio of two distinct gases (NO and ammonia) in temporal information that two selective sensors cannot capture, unlike gas chromatography, where ammonia is often absorbed by stainless steel [67]. This unique technology utilizes pure γ-phase tungsten trioxide's semiconducting, catalytic, and gas-sensing properties, as well as the redox reactions between the two biomarkers in the presence of the sensor [66].

Available Therapeutics and Treatment Options

Since the beginning of the COVID-19 pandemic, most countries and pharmaceutical companies worldwide have spent much time, effort, and expense developing effective vaccines and specific antiviral drugs against SARS-CoV-2. Concurrently, they tested a wide range of existing and approved drugs, particularly those that showed promising results with other viral infections like SARS, MERS, Ebola, flu, and AIDS [68]. Four classes of drugs have now been described according to their mode of action as shown in Figure 2 including (i) viral entry and membrane fusion inhibitors, (ii) protease inhibitors, (iii) RdRp inhibitors, and (vi) immunomodulatory agents.

The S protein of SARS-CoV-2 anchors to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of epithelial cells [69]. The S protein is primed and activated by the host cell protease, transmembrane serine protease 2 (TMPRSS2), for virus entry [70]. Numerous candidate drugs including umifenovir, camostat mesylate, ACE inhibitors (ACEis), angiotensin receptor-1 blockers (ARBs), soluble recombinant human ACE2 (rhACE2), chloroquine phosphate, and hydroxychloroquine sulfate have been tested to disrupt virus attachment and fusion with cell membranes (Table 2A). Umifenovir, a broad-spectrum antiviral used as a prophylaxis against influenza, is currently approved in China and Russia but not FDA-licensed [63, 64]. Camostat mesylate has shown ability to inhibit SARS-CoV and MERS-CoV replication in cell culture and partial blockage of SARS-CoV-2 replication in Caco-2 and Vero-TMPRSS2 cells [71]. Several nonrandomized studies were conducted to confirm the safety and efficacy of both drugs in the treatment of COVID-19 [71–73]. However, the use of limited sample size and the contradictory results obtained discouraged their routine use in therapeutic purposes.

ACEis and ARBs are common drugs for the treatment of hypertension and heart failure [74]. During the SARS-CoV outbreak, it was predicted that prolonged treatment with these drugs might protect from severe lung injury due to the elevation of ACE2 expression [75]. Conversely, several scientists claimed that frequent use of ACEis or ARBs could increase the likelihood of severe COVID-19 in patients with cardiovascular diseases since they upsurge the ability of SARS-CoV-2 to enter permissive cells. The latter prospect was disproved by two recent studies, which showed no evidence for increased severity or mortality of COVID-19 in 1,200 and 362 patients regularly treated with ACEis or ARBs [76]. rhACE2 is also proposed as a bait receptor that traps virus particles and prevent them from binding with cell-surface receptors [77]. It was recently announced by APEIRON Biologics AG that APN01, synonymous with rhACE2, was used to treat 200 COVID-19 patients in Austria, Germany, and Denmark [78].

Monoclonal antibodies (MAbs) are generated using human phage libraries, immunized animals, and memory B cells from patients [72]. MAbs are more effective than

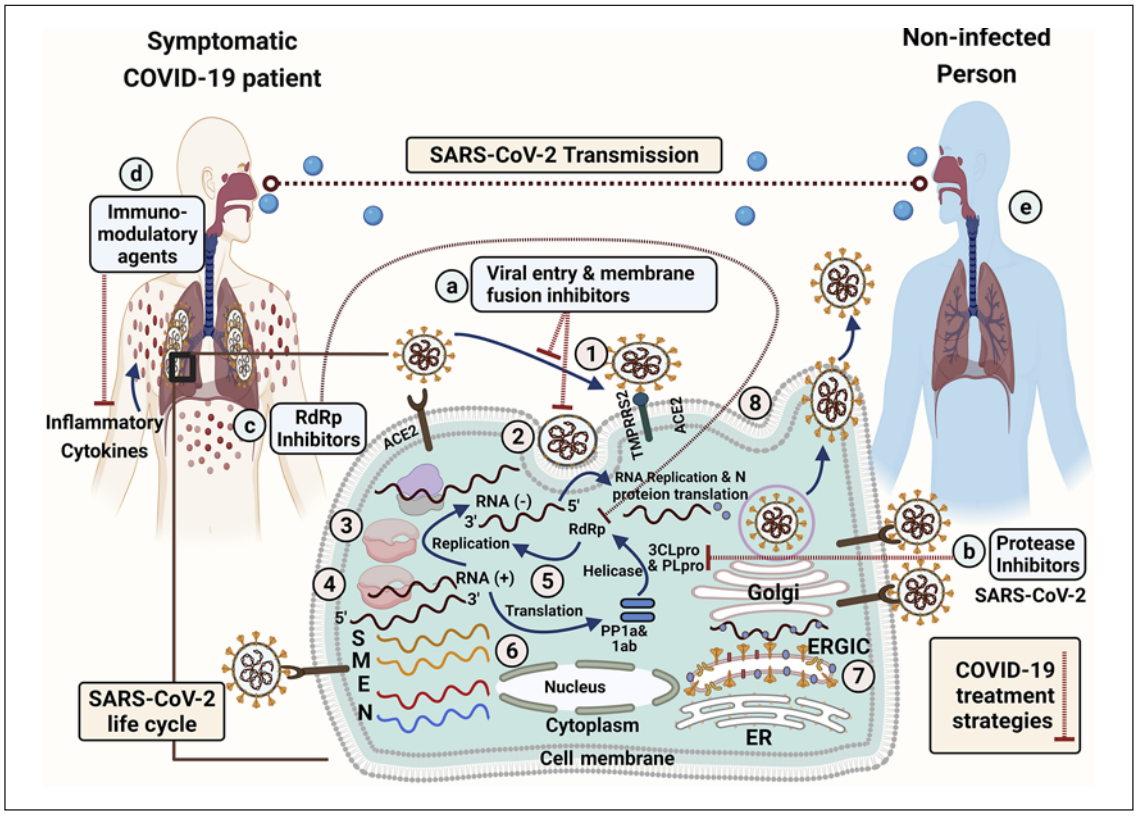


Fig. 2. Schematic representation of SARS-CoV-2 life cycle and the treatment strategies for COVID-19 pandemic. An infected person with COVID-19 can infect another person through their respiratory droplets, sneezing, and coughing. The SARS-CoV-2 life cycle includes (1) binding of SARS-CoV-2 spike protein with ACE2 receptor on the host cell's surface with the aid of the host protease enzyme TMPRSS2; (2) virus entry by fusion with the plasma membrane or by endocytosis mediated by endosome formation; (3) translation of the viral machinery of replication at cytoplasmic membranes; (4, 5) genomic and sub-genomic mRNA transcription through coordinated processes of continuous/discontinuous RNA synthesis determined by the viral replicase and mediated by viral proteases (e.g., 3CLpro and PLpro) and RdRP; (6) translation of structural and non-structural proteins by viral-encoded enzymes and host cell machinery; (7) virions components are assembled at the ERGIC and obtain their membrane envelope; and (8) viruses are directed through the host secretory pathway and released by budding from the plasma membrane. The treatment options for COVID-19 include (a) viral entry and membrane fusion inhibitors (e.g., umifenovir, camostat mesylate, ACEi, ARBs, rhACE2, hydroxychloroquine); (b) protease inhibitors (e.g., LPV/r, darunavir/cobicistat, and disulfiram); (c) RdRp inhibitors (e.g., RDV, favipiravir, and ribavirin); and (d) immunomodulatory agents (e.g., tocilizumab and sarilumab, anakinra, nitazoxanide, thalidomide, and corticosteroid). Blue arrows for the subsequent step and a red "T" sign for inhibition. ERGIC, endoplasmic reticulum/Golgi intermediate compartment.

using convalescent plasma in treatment of COVID-19 patients since they can be produced in greater quantities and possess reduced risk of antibody-dependent enhancement [73]. REGN-COV2 is a novel MAb cocktail that binds to the RBD of S1 or S2 subunits of SARS-CoV-2 spike protein to prevent virus entrance into a host cell. A double-blind, randomized controlled clinical trial using REGN-COV2 has shown reduction in the viral load, particularly in patients whose immune response did start to respond yet [74]. B38, H4, and CR3022 are three other MAbs that may be effective against SARS-CoV-2 in future trials [75, 79].

Chloroquine phosphate was promising in treating COVID-19 patients, compared to the control group, by improving lung imaging, advancing a negative infection transformation, and shortening the disease course [77]. The in vitro efficacy against SARS-CoV-2 was higher in hydroxychloroquine than chloroquine (EC_{50}: 0.72 vs. 5.47 μM) [77]. Complete virus clearance was reported in 6 patients concurrently treated with hydroxychloroquine and azithromycin within 5–6 days [79]. Nonetheless, a large-scale randomized clinical trial had recently shown that no notable difference in the hospital stay duration and the primary endpoint of mortality was observed

Table 2. Proposed therapeutic options for COVID-19

Drug	Common use	Target	Proposed mechanism of action
A. Viral entry and membrane fusion inhibitors			
Umifenovir	Broad-spectrum antiviral for prophylaxis against influenza	S protein/ACE2 interaction	Blocks membrane fusion – immunomodulatory effects (interferon induction and activation of macrophages)
Camostat mesylate	Pancreatitis and reflux disease	TMPRSS2	Serine protease inhibitor that blocks virus entry by targeting the endosomal proteolysis [81]
ACEi and ARBs	Hypertension and heart failure	ACE2 and angiotensin receptor 1	Elevates ACE2 expression rate and inhibits S-protein binding to ACE2 receptors on host cells
rhACE2		S protein	Acts as a circulating bait receptor that traps viral particles and prevents their subsequent binding to cell receptors
Chloroquine and hydroxychloroquine	Antimalarial drug	ACE2	Inhibits glycosylation of cell receptors – interfere with endosomal pH – immune-modulatory effect
B. Protease inhibitors			
LPV/r	Antiretroviral drug	$3CL^{pro}$ and PL^{pro}	Lopinavir inhibits virus proteases – ritonavir increases the half-life of lopinavir
Darunavir/cobicistat	Antiretroviral drug	$3CL^{pro}$ and PL^{pro}	Darunavir inhibits virus proteases – cobicistat is a boosting agent [72]
Disulfiram		PL^{pro}	Inhibits PL^{pro} protease
C. RdRp inhibitors			
RDV	Broad-spectrum antiviral for RNA viruses (e.g., Ebola virus)	RdRp	Adenosine analog that prevents viral RNA synthesis
Favipiravir	Antiviral for RNA viruses (e.g., influenza)	RdRp	Guanosine and adenosine analog that prevents RNA elongation
Ribavirin	Broad-spectrum antiviral for RNA viruses (e.g., respiratory syncytial virus, hepatitis C and E viruses, hantaviruses)	RdRp	In monophosphate form: guanosine analog that deactivates inosine monophosphate dehydrogenase In triphosphate form: obstructs polymerases and RNA capping
NHC; EIDD-1931	Antiviral for RNA viruses (e.g., hepatitis C, influenza, and human coronaviruses)	RdRp	Ribonucleoside analog – induction of C-to-U or U-to-C mutations during RNA replication affecting virus infectivity
D. Immunomodulatory agents			
Tocilizumab and sarilumab		IL-6	Antagonize IL-6 to reduce cytokine storm
Anakinra	Rheumatoid arthritis	IL-1	Antagonize IL-1 to reduce cytokine storm
Nitazoxanide	Antiparasitic drug	IL-6 and other pro-inflammatory cytokines	Upregulates innate antiviral mechanisms by amplifying cytoplasmic RNA sensing and INF-1 pathways [84] Inhibits pro-inflammatory cytokines (TNF-α, IL-2, IL-4, I-5, IL-6) [85]
Thalidomide	Sedative, multiple myeloma	IL-6, TNF-α	Reduces cytokine production [90]
Corticosteroid	A multitude of uses	Pro-inflammatory cytokines	Inhibit pro-inflammatory cytokines to reduce lung injury in ARDS patients [86]

ACEis, ACE inhibitors; ARBs, angiotensin receptor-1 blockers; rhACE2, soluble recombinant human ACE2; ACE2, angiotensin-converting enzyme 2; LPV/r, lopinavir/ritonavir; NHC; EIDD-1931, β-D-N4-hydroxycytidine; S protein, spike protein; TMPRSS2, transmembrane serine protease 2; 3CLpro, 3-chymotrypsin-like protease; PLpro, papain-like protease; IL, interleukin; TNF-α, tumor necrosis factor alpha.

when hydroxychloroquine was used in 1,542 patients as compared to 3,132 patients with standard care only [80]. Therefore, the WHO has lately declared discontinuation of hydroxychloroquine from COVID-19 treatment regimens [81].

The protease inhibitor is another group of drugs that has long been used for treatment of AIDS (Table 2B). Lopinavir is typically formulated in combination with ritonavir (LPV/r) under the brand name Kaletra®. The effect of LPV/r against human coronaviruses was previously confirmed in cell culture against SARS-CoV-1 and MERS-CoV [82] and recently against SARS-CoV-2 [83]. In a clinical trial, patients who received LPV/r (n = 99) showed significant clinical improvement and viral clearance after 14 days of treatment compared to patients who received standard care only (n = 100) [84]. Further studies have proven that the combination of LPV/r with interferon beta-1b and ribavirin are safe and superior to LPV/r alone in enhancing virus clearance, relieving symptoms, and facilitating discharge of patients with mild to moderate COVID-19 [85]. LPV/r is still an attractive candidate for the treatment of COVID-19 due to its commercial availability and large-scale producibility. Other protease inhibitors like darunavir (Prezista®) and disulfiram (Antabuse®) can inhibit replication of CoVs in vitro, and they did not show activity in clinically compatible concentrations [83]. Although the results of using protease inhibitor in the treatment of COVID-19 are discouraging till now, in silico studies of the protein-drug modeling showed a predictable strong interaction between HIV protease inhibitors and the active site of the SARS-CoV-2 protease. Hence, further studies on these compounds may provide a positive impact in the future [86].

Notably, RdRp inhibitors showed promising outcomes in COVID-19 patients (Table 2C) [87–89]. Remdesivir (RDV, GS-5734, Gilead), for example, blocked SARS-CoV-2 infection at low concentrations (EC_{50} 0.77 μM) [88]. Animal studies in Rhesus macaques indicated that treatment with RDV is safe and effective compared to control animals [89]. The adverse effect of RDV on host cells is well-tolerated since human mitochondrial RdRp has a lower affinity to RDV than other adenosine analogs [90]. RDV was first utilized empirically with a 35-years-old COVID-19 patient in the USA and showed promising results [87]. Favipiravir (T-705, Avigan®), another RdRp inhibitor, has shown effective action against SARS-CoV-2 in Vero E6 cells at high concentration (EC_{50} 61.88 μM) [88], albeit some reports showed no effect even in concentrations up to 100 μM [91]. A clinical study conducted on 80 patients in China has shown that favipiravir is more effective than LPV/r with no profound adverse effect. Accordingly, favipiravir was approved in March 2020 for the treatment of COVID-19 in China [92]. Several clinical trials are now proceeding to evaluate the pharmacokinetics and antiviral activity of RDV and favipiravir either alone or in combination with other treatment options.

Other RdRp inhibitors, like β-D-N4-hydroxycytidine (EIDD-1931), were highly efficacious in preventing replication of SARS-CoV-1, SARS-CoV-2, and MERS-CoV in cell culture [93]. In contrary, ribavirin (Virazole®) showed limited activity against SARS-CoV-2 in cell culture and required high concentration to inhibit viral replication. It was found that ribavirin is 100-times less effective than RDV (EC_{50}: 109.5 μM) [88]. The use of ribavirin in COVID-19 patients was evaluated either alone (n = 111) or in combination with LPV/r (n = 41) in a clinical trial. The latter group showed no ARDS and mortalities. However, the use of high doses (1.2–2.4 g orally every 8 h) may exert a potential risk of toxicity in some patients [82].

Several collateral treatments are often used to decrease the severity and complications of COVID-19 and evade the inflammatory immune response developed in severe cases [94] (Table 2D). Drugs used to suppress the pro-inflammatory cytokines (e.g., interleukin [IL]-1, IL-2, IL-6, IL-8, TNF-α) such as MAbs (e.g., tocilizumab and sarilumab) and IL receptor inhibitors (e.g., anakinra) are widely emerged [60]. Anakinra (Kineret®) is currently proceeding in clinical trials in China, Italy, Spain, and Greece to evaluate the safety and efficacy of using anakinra in COVID-19 patients with respiratory distress [94]. Nitazoxanide has demonstrated antiviral activity against SARS-CoV-2 in Vero E6 cells (EC50: 2.12 μM at 48 h) [88]. It had a vital immune-modulatory role by amplifying cytoplasmic RNA sensing and INF-1 pathways [95]. Nitazoxanide was also able to restrain pro-inflammatory cytokines in peripheral blood mononuclear cells and inhibit IL-6 production in mice [96]. It is essential to include nitazoxanide in controlled-randomized clinical trials to conclude its potential for improving the health condition of COVID-19 patients.

Corticosteroid helps reduce lung inflammation and cytokine storm and avoid acute lung injury and ARDS. Nevertheless, corticosteroid treatment is often associated with delayed virus clearance and an elevated secondary infection rate [97]. In the UK's national clinical trial RECOVERY, the corticosteroid dexamethasone was evaluated in COVID-19 patients, including severe cases, and was found to have benefits. According to the preliminary

findings, the mortality rate for patients on ventilators was reduced by one-third, and for patients requiring only oxygen, the rate was cut by about one-fifth. The mortality rate was also decreased in another study conducted on 201 COVID-19 patients with ARDS after administering the corticosteroid drug methylprednisolone (46% vs. 61.8 in the control group) [98]. It was suggested that the reduction of severe illness and mortality rate are correlated to the early administration of low-dose methylprednisolone. The NIH, IDS, and other experts provided guidelines for using corticosteroids in COVID-19 patients based on the currently available information [99].

Cell therapies have demonstrated efficacy in treating conditions that were previously difficult to manage with conventional treatment modalities, including neurodegenerative, oncologic, and immunological disorders. Numerous cell therapy approaches have been studied such as induced pluripotent stem cells, mesenchymal stromal cells, and T cells [100–103]. In a variety of indications, safety and efficacy results of cell therapy have suggested that cell therapy may be exploited to treat COVID-19 patients [100]. As of 1 January 2021, clinicaltrials.gov listed several cell therapy-based clinical trials targeting COVID-19 pathology. Mesenchymal stromal cells account for approximately 71% of the cells used in cell therapy clinical trials, with the remainder consisting of natural killer cells, T cells, and early apoptotic cells. Approximately 88% of the clinical trials are nowadays in phases 1 and 2, with only one trial in phase 2/3 and one in phase 3 [100, 104].

Conclusion and Perspectives

COVID-19 is currently considered the most prominent health concern worldwide. Several options are available for sensitive and accurate diagnosis of COVID-19, among which rRT-PCR is the standard diagnostic tool in almost all countries. The rapid spread of SARS-CoV-2 and its burden on health and the economy has encouraged research and industrial foundations to pursuit effective therapeutic solutions for the disease. A group of medications has been developed or repurposed to help virus clearance, symptoms relief, and rapid discharge of patients from healthcare facilities. These drug candidates can inhibit virus entry, membrane fusion, viral RNA synthesis, or protease activity. Immunomodulatory agents and corticosteroids are proposed to be used in severe cases to suppress the cytokine storm induced by pro-inflammatory cytokines. Nevertheless, the safety and efficacy of these drugs require further evaluation in controlled randomized clinical trials.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

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