Review Article

A Concise Review of Major Challenges in the Vaccination, Diagnosis and Treatment of Novel Coronavirus Disease 2019

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ABSTRACT

The rapid dissemination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the 2019 coronavirus (COVID-19) pandemic on a global scale. An efficacious strategy to control the ongoing pandemic of the novel coronavirus disease (2019-nCoV) includes the rapid recognition of infected patients and the implementation of vaccination programs utilizing accurate and reliable methods. A variety of diagnostic techniques, including computed tomography (CT) scans, serological assays, and molecular methods, have been employed for the diagnosis of coronavirus disease 2019 (COVID-19). Furthermore, a variety of vaccines, antiviral drugs, and immunotherapies have been employed to combat the virus. This is of particular importance for patients diagnosed with SARS-CoV-2 infection who are at high risk of developing serious complications. The prognosis, diagnosis, vaccination, and treatment of COIVD-19 present a number of challenges, including variability in disease severity, the emergence of new variants, individual factors and immune responses, co-infections and complications, a lack of long-term data, psychological and social factors, the availability and accessibility of tests, the sensitivity and specificity of tests, variability in symptoms, mild or asymptomatic cases, a limited number of specific antiviral options, clinical heterogeneity, the lack of a universal treatment protocol, overburdened healthcare systems, the management of severe cases, long-term effects and post-COIVD-19 syndrome, vaccine hesitancy, global cooperation, and vaccine production capacity. This article presents an overview of the most recent advancements in the field of Coronavirus Disease 2019 (Covid-19), encompassing prognosis, diagnosis, vaccination, and therapy. It is of the utmost importance to consult reliable sources such as national health authorities and the World Health Organization (WHO) in order to obtain the latest information on the vaccination of individuals against the novel coronavirus, including details on eligibility, availability, and recommended protocols in any specific region. The range of available treatment options and strategies is subject to ongoing evolution. Healthcare professionals and researchers are assiduously striving to surmount the challenges inherent to therapy and to enhance the outcomes for those affected by SARS-CoV-2 infection. It is imperative that randomized clinical studies be conducted with the objective of identifying the most appropriate and proven treatment in order to reduce the prevalence of SARS-CoV-2 infection and to prevent the occurrence of future pandemics.

Keywords: Coronavirus Disease 2019, Prognosis, Vaccination, Diagnosis, Antiviral Therapy.

1. Context

Despite the identification of the novel coronavirus disease 2019 (COVID-19) genomics and proteomics, the host response details to the virus remain unclear (1). The implementation of effective control policies, a combination of measured and rational reuse of existing treatments, the development of novel drugs, and the establishment of medical toxicology guidelines have all contributed to a reduction in the mortality and morbidity associated with the novel coronavirus disease, SARS-CoV-2 (2). Vaccination represents a pivotal strategy for controlling the spread of the disease and mitigating its impact on individuals and communities. These vaccines, including mRNA vaccines (such as Pfizer-BioNTech and Moderna) (4, 5), viral vector vaccines (such as AstraZeneca and Johnson & Johnson) (6), protein subunit vaccines (such as Novavax) (7), and inactivated virus (CoronaVac, BBIBP-CorV and Covaxin) vaccines, have undergone rigorous testing and evaluation to ensure safety and efficacy (8). Initiatives such as COVAX are designed to facilitate the equitable distribution of vaccines, particularly in low- and middle-income countries where access may be constrained (9). It is imperative that governments, manufacturers, and logistics providers collaborate in order to guarantee the effective distribution of vaccines. It is noteworthy that with the advent of new variants, ongoing research and evaluation of vaccine effectiveness against these variants is being conducted (10). The aim of this review is to provide a summary of the latest advances and challenges in the prognosis, diagnosis, vaccination, and eradication of COIVD-19.

2. Diagnostic Approaches of COVID-19

The global impact of the SARS-CoV-2 pandemic has prompted scientists to rapidly develop accurate diagnostic methods to hinder the spread of infection. In the wake of the elucidation of the chemical and structural attributes of SARS CoV-2, a multitude of researchers, sectors, and organizations have been engaged in the pursuit and advancement of precise diagnostic methodologies for the identification of COIVD-19, as delineated in the following sections (11).

2.1. Reverse Transcriptase Polymerase chain reaction (RT-PCR)-based assays

The Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have employed a multitude of authorized molecular tests for the expeditious detection of SARS-CoV-2. Among these, RT-PCR represents the gold standard technique for the detection of SARS-CoV-2 (12). This technique allows for the rapid (1-2.5 hours) detection of viral genomic material through the use of primers and probes. However, RT-PCR is an expensive, time-consuming process that requires the expertise of trained professionals and access to sophisticated laboratory equipment. Furthermore, RT-PCR is not regarded as a rapid test due to the necessity of multiple temperature cycles in comparison to other available tests. This method is not useful for determining the history of SARS-CoV-2 infection in individuals (13). Modified RT-PCR assays, including the Seegene Allplex 2019-nCoV (South Korea), which is capable of detecting SARS-CoV-2 without the need for RNA extraction in specimens transported to UTM (Universal Transfer medium) or water, the Cobas® Liat® (Roche Molecular Systems, USA), the Xpert® XpressSARS-CoV-2 (Cepheid, USA), and the ID NOW™ (Abbott, USA), have been licensed for emergency use by the FDA (14).

2.2. Loop-Mediated Isothermal Amplification (LAMP) Based Assay

Loop-mediated isothermal amplification (LAMP) is a highly sensitive, specific, accurate, inexpensive, and rapid technique for the detection of the coronavirus. Some studies have demonstrated that LAMP exhibits a 100-fold sensitivity compared to conventional RT-PCR methods for the successful diagnosis of SARS-CoV-2 infection (15). The findings of the Parker et al. studies have indicated that LAMP is a highly specific method for diagnosing SARS-CoV-2 without cross-reactivity with other human pathogens (16). To compare RT-LAMP and RT-PCR, Yang et al. performed visual detection and colorimetry to detect SARS-CoV-2 after RNA purification in 130 samples, demonstrating that LAMP has higher rapidity, convenient analysis, and similar sensitivity to RT-PCR (17). In consequence, the LAMP method yielded the same level of clinical diagnostic efficacy as the RT-PCR technique. De Oliveira et al. employed a polystyrene-toner (PS-T) centrifugal microfluidic device for the molecular detection of SARS-CoV-2, which is manually operated via a fidget spinner with automated and integrated colorimetric detection. Amplification was conducted for a period of 10 minutes at a temperature of 72°C in a microwave apparatus with a capacity of 5 µL. In a separate study conducted by Inaba et al., 124 nasopharyngeal specimens from 24 patients diagnosed with Coronavirus Disease 2019 (Covid-19) were analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) methods. By day 9, the RT-LAMP method demonstrated a 92.8% positive result, with 100% specificity and sensitivity compared to RT-qPCR. However, after 10 days, the positivity rate for RT-LAMP declined to less than 25%, and the concordance of positivity between the two approaches was less than 60%. The results demonstrated that RT-LAMP could be employed as an alternative methodology to RT-qPCR for the diagnosis of SARS-CoV-2 infection during the acute phase of the disease (18). In a recently published study, Amaral et al. developed a method for visually detecting less than 100 copies of the SARS-CoV-2 viral genome in a single-tube experiment based on RT-LAMP in 30 minutes. A comparison was conducted between the assay and RT-PCR, the gold standard test for the detection of SARS-CoV-2, using 177 nasopharyngeal RNA samples. The RT-LAMP assay has been demonstrated to exhibit high

specificity and sensitivity (>95% and 100%) for viral loads above 100 copies (19).

2.3. CRISPR Cas Systems for COVID-19 Diagnosis

CRISPR represents a rapid, specific, and sensitive diagnostic technique for the detection of SARS-CoV-2 RNA. The primary proteins utilized for the determination of CRISPR effectors in CRISPR-Cas systems include Cas12 and Cas13. The use of Cas9 and Cas9 effectors (20, 21) for the identification of other pathogens has already been demonstrated. A recently developed CRISPR-Cas12a-based DETECTR assay has been designed for the detection of SARS-CoV-2 RNA in biological specimens. The DETECTR assay is based on the reverse transcription-loop-mediated isothermal amplification (RT-LAMP) method for the production of Cas12a target single-stranded nucleic acids using RNA extracted from samples. The cleavage of single-stranded DNA reporter molecules by Cas12a ssDNase and trans-cleavage activity, followed by visualization through a fluorescent reader or lateral flow strip, allows for the identification of the virus in approximately 30 to 40 minutes (22). A novel method for identifying SARS-CoV-2 was developed, namely CRISPR-top (a CRISPR-mediated test). This method simultaneously combines target pre-amplification with CRISPR/Cas12b-mediated detection in a single reaction, performed at a constant temperature. The CRISPR-top method for identifying SARS-CoV-2 operates at 59°C for 40 minutes, requiring minimal equipment, and targets the viral ORF1ab and nucleoprotein genes. The specificity of the CRISPR-top assay among non CoV-2 clinical samples was 100%. In specimens positive for SARS-CoV-2, the assay yielded 73.1% positive results by fluorescence readout and 67.3% positive results by lateral-flow readout (23). Hybridization of the Np aptamer and an activator strand resulted in the priming of an arched probe. In the presence of the SARS-CoV-2 Np, the activator strand can be released from the arced probe due to the specific interaction between the aptamer and the target, which then activates the trans-cleavage activity of the CRISPR-Cas12a system. Subsequently, the polyA-MB reporters were cleaved from the electrode surface, resulting in a reduction in current differential pulse voltammetry at a potential of −0.27 V. This electrochemical aptasensor demonstrated efficient performance for the detection of SARS-CoV-2 Np in concentrations ranging from 50 pg mL-1 to 100 ng mL-1, with a limit of detection as low as 16.5 pg mL-1 within 30 minutes (24). The potential of CRISPR-Cas technologies for the detection of SARS-CoV-2 is illustrated in Figure 1. The principal disadvantages of the CRISPR-Cas system in the diagnosis of viral diseases include the occurrence of false positives, a limited range of detection, time-consuming results, and high costs. Consequently, the specificity of the guide RNA precludes the diagnosis of mutations (25) (Figure 1). CRISPR-Cas technologies for diagnosis of SARS-CoV-2 infection: (A) SHERLOCK; (B) DETECTR; (C) FELUDA. The use of CRISPR-Cas technologies for the genomic detection of the novel coronavirus (2019-nCoV), also known as SARS-CoV-2. In accordance with the aforementioned, three common systems have been illustrated. In the SHERLOCK system, RNA is extracted and RT-RPA and transcription of complementary sequences are also performed. Following cleavage of the target by CRISPR-Cas 15a, the signal is released, resulting in the appearance of two bands on the paper strip. In the DETECTR system, following the extraction of RNA, RT-LAMP is conducted for the complementary sequence, resulting in its detachment and signal release. Similarly, the FELUDA system employs CRISPR-Cas9 in a manner analogous to the SHERLOCK system.

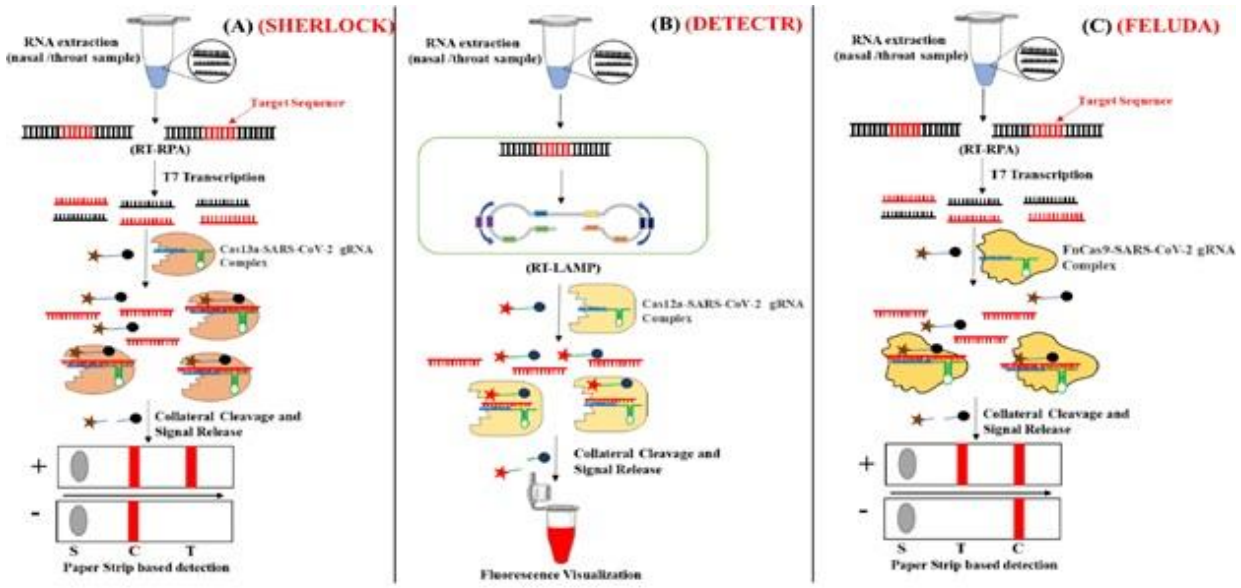

Figure 1. The CRISPR-Cas technologies for COVID-19 genomic detection. Accordingly, three common systems have been depicted. In SHERLOCK system, RNA is extracted and RT-RPA and transcription of complementary sequences is also performed and its cleavage by CRISPR-Cas 15a, the signal is released causing appearance of two bands on paper strip. In DETECTR system, after RNA extraction, RT-LAMP is conducted for complementary sequence which results in its detachment and signal release. In FELUDA system being similar to SHERLUCK system, CRISPR Cas 9 is applied.

2.4. Point-of-Care Testing

Point-of-care (POC) technologies are a rapidly evolving category of diagnostic tests for the novel coronavirus disease (Covid-19). These tests are characterized by several key attributes, including rapidity, decentralization, cost-effectiveness, and sensitivity. Lateral flow assays (LFAs) are a common point-of-care (POC) diagnostic system that play a pivotal role in the management of the ongoing pandemic of the novel coronavirus disease (Covid-19) in developed countries and resource-limited settings. A substantial body of research has been conducted with the aim of developing LFA-based diagnostic technologies for the rapid diagnosis of SARS-CoV-2 infection. Some of these studies have resulted in the development of commercial test kits (27). An engineered graphene-based electrical-electrochemical point-of-care (POC) biosensor for serological diagnostics of SARS-CoV-2 was developed. The device was designed to quantify immunoglobulin G (IgG) levels in patient samples. The biosensor was based on a SARS-CoV-2 receptor binding domain bioconjugate immobilized onto the surface of the device. The graphene basal plane is integrated with the following characteristics: high charge carrier mobility, low intrinsic resistance, high conductivity, and interfacial sensitivity to capacitance changes. This point-of-care device offers several advantages over serological methods, such as ELISA and other immunochromatographic approaches. These include a relatively short analysis time (approximately 15 minutes), a low limit of detection (LOD) of 1.0 pg mL-1, and a simple sample preparation process (28). Beduk et al. developed a miniaturized LSG-based electrochemical sensing system for the detection of SARS-CoV-2, which was combined with three-dimensional gold nanostructures. The surface of the electrode was initially modified through the application of electrochemical techniques, including X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) characterization. This was followed by the introduction of the SARS-CoV-2 spike protein antibody. The platform was integrated into a handheld point-of-care (POC) detection system, enabling the convenient and straightforward utilisation of a user-friendly diagnostic tool. This was achieved through the platform's accessibility, straightforward operation, and systematic data management. The analytical characteristics of the electrochemical immunoassay were assessed through the standard solution of S-protein in the range of 5.0-500 ng/mL, with a detection limit of 2.9 ng/mL. A clinical study was conducted on 23 patients with confirmed SARS-CoV-2 infection, with the results compared to those obtained using commercial diagnostic tools, namely RT-PCR and ELISA. The results were obtained more rapidly, offering a potential solution for next-generation POC applications. In recent years, a multitude of enhanced LFA methods with isothermal amplification have been developed with the objective of controlling the spread of the novel coronavirus, SARS-CoV-2, which causes the disease known as Coronavirus Disease 2019 (Covid-19). Yu et al. developed a multiplex LFA as an alternative to conventional RT-qPCR for the simultaneous diagnosis of three SARS-CoV-2 genomic sections (i.e., ORF3a, RdRp, and N genes) in 30 minutes. This was achieved by the PCR product obtained through single-tube RT-PCR (30). Xia et al. described an ultrasensitive field-deployable technique for diagnosing the SARS-CoV-2 gene, which employs RT-enzymatic recombinase amplification. In this study, a multienzyme RT-ERA reaction system, comprising ribonuclease inhibitor, reverse transcriptase, polymerase, recombinase, single-stranded DNA-binding protein, nuclease, and creatine kinase, was employed for the diagnosis of the target RNA (31).

2.5. Other Genetic and Nano-technological Methods

Next-Generation Sequencing (NGS) is a high-throughput DNA sequencing technology that enables the rapid and cost-effective sequencing of large amounts of DNA or RNA molecules. This technology is being used to uncover the genetic basis of diseases, discover novel biomarkers, and gain new insights for precision medicine (32). In this process, millions of DNA fragments are sequenced concurrently. Next-generation sequencing (NGS) has the potential to enhance our understanding of genetic variations, gene expression patterns, and disease mechanisms (33). Digital PCR offers several advantages over traditional quantitative PCR (qPCR), including higher sensitivity, improved precision, reduced dependence on reference standards, and greater robustness in the presence of PCR inhibitors. These characteristics render digital PCR a particularly advantageous technology for applications such as the detection of low-frequency mutations, the precise measurement of gene expression, the quantification of viral loads, and environmental monitoring (34). Nanopore sequencing represents a revolutionary advancement in next-generation sequencing (NGS) technology, enabling real-time analysis of DNA and RNA molecules (35). The method is based on the principle of passing nucleic acid strands through nanoscale pores and measuring the changes in electrical current as individual bases move through the pore. In nanopore sequencing, a biological sample containing DNA or RNA is prepared and introduced to a nanopore sequencing device (Table 1).

2.6. Antiviral Drugs

The efficacy of Remdesivir in the treatment of severe cases of SARS-CoV-2 infection was demonstrated in primary studies (36). In a randomly selected study of 1,063 patients, 538 patients were assigned to receive Remdesivir and 521 patients were assigned to receive a placebo. The mean recovery time for patients receiving Remdesivir and placebo was 11 days (95% confidence interval [CI], 9 to 12) and 15 days (95% confidence interval, 13 to 19), respectively (37). Another study indicated that 68% of severe patients with coronavirus disease 2019 (Covid-19) exhibited clinical improvement following treatment with Remdesivir (36).

Table 1. The advantages and disadvantages of each COVID-19 diagnostic test

Diagnostic test	Advantages	Disadvantages
RT-PCR	High sensitivity, specificity and rapidity, quantitative analysis,	High costs, Susceptibility to contamination, complexity, limited information,
LAMP	High sensitivity, simplicity, rapidity and specificity, cost-effectiveness	Technical limitations, Primer design challenges, contamination risk, Operator-dependency
CRISPR Cas	High specificity and sensitivity, rapid detection, portability (SHERLOCK or DETECTR systems)	Off-target effects, detection limitations for certain strains or variants, technical requirements, Regulatory challenges
CT scan	Visual representation, high sensitivity, rapidity, identification of complications	Specificity concerns, Radiation exposure, Resource-intensity, Overuse and unnecessary imaging
Serology	Detection of past infections, Screening for population immunity, Longevity of antibodies, Complement to other diagnostic methods	Timing of detection, inability to distinguish between active and past infections, false negatives and false positives, limited information on immunity
Next-Generation Sequencing (NGS)	rapid sequencing, detection of specific mutations or variants,	High costs, unaffordability, technical requirements
Digital PCR (dPCR)	High sensitivity, specificity and accuracy, precise quantification of viral nucleic acids, monitoring viral load,	High costs, unaffordability, technical requirements
Mass Spectrometry	rapid and accurate identification of viral proteins or peptides,	High costs, timing of detection, technical requirements, unaffordability
Microarray Technology	simultaneous detection of multiple viral targets	High costs, timing of detection, technical requirements, unaffordability, contamination risk
Nanopore Sequencing	portable and rapid real-time sequencing of viral genomes	Error rates, costs, sample preparation concerns, read length variability, bioinformatics challenges

Favipiravir has been demonstrated to inhibit virus replication (38), and has been inferred to be effective against hemorrhagic fever RNA viruses such as filoviruses, flaviviruses, bunyaviruses, and arenaviruses (39). The combined use of lopinavir and ritonavir has been demonstrated to inhibit the activity of protease enzymes, including 3CL protease, which are responsible for cleaving viral polypeptides and disrupting the replication of SARS-CoV or MERS-CoV viruses (40, 41). Ivermectin, an approved anti-parasitic agent, has demonstrated a pronounced anti-SARS-CoV-2 effect (42).

2.7. Immunotherapeutic Approaches

2.7.1. Immune Modulating Treatments; Anti-Inflammatory Agents

Tocilizumab, an anti-IL-6 receptor antibody, binds to membrane-bound and soluble IL-6 receptors, thereby suppressing the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway and the production of downstream inflammatory molecules (43, 44). Blockade of the IL-1 pathway is employed in the treatment of certain conditions characterised by hyperinflammation. Anakinra is a recombinant human IL-1 receptor antagonist that has been approved for the treatment of rheumatoid arthritis, Still disease, and Cryopyrin-Associated Periodic Syndromes (CAPS).

2.7.2. Passive Immunization & Convalescent Plasma

At present, passive immunity is achieved through the administration of antigen-specific monoclonal antibodies (mAbs) or polyclonal antibodies derived from nonhuman or human blood products. The primary source of antiserum is polyclonal antibodies obtained from immunized animals. However, there is a risk of "serum sickness," which is caused by the immune response to antibodies of nonhuman origin in recipients, particularly in repetitive encounters. The utilization of convalescent plasma from human patients serves to mitigate the aforementioned risks. By employing accurate screening techniques, such as determining the presence of pathogens and measuring antibody titers and neutralization capacity, convalescent plasma therapy (CPT) may prove an effective and safe option. CPT involves using plasma derived from individuals who have previously been infected and recovered, generating antibodies against the infection. The transmission of convalescent plasma may prove an effective method of preventing infection and reducing the clinical severity of illness in individuals recently infected with the virus (45). An efficacious and secure therapeutic alternative for the treatment and post-exposure prophylaxis of SARS-CoV-2 infection is derived plasma from recovered patients (46). In a study of 173 patients infected with SARS-CoV-2, the presence of antibodies in serial plasma samples was investigated, and the seroconversion rates for antibodies, including IgM and IgG, were indicated at 93.1%, 82.7%, and 64.7%, respectively. In patients within their first week since onset, the percentage of antibodies was less than 40 %, with a rapid increase to 100.0 % (Ab), 94.3 % (IgM), and 79.8 % (IgG) by the fifteenth day after the initiation of symptoms. These findings provide substantial practical support in the diagnosis and management of COVID-19 patients with coronavirus disease 2019 (1).

2.7.3. Stem Cell Therapy for COVID-19

Stem cell therapy (SC) is regarded as a promising area of medicine, particularly in the fields of drug delivery and biotechnology. Its potential has been demonstrated in the treatment of various diseases, including neurodegenerative disorders, cardiovascular conditions, and diabetes. The

differentiation and self-replicating abilities of generic human cells are the result of specific functions that replace damaged or dysfunctional cells (48). Therapies utilising mesenchymal stem cells are considered to be amongst the most promising treatments based on cells due to their unique characteristics, including the ability to migrate, home, regulate the immune system, reduce inflammation and undergo multilineage differentiation (49). In 2020, Ling Tang and colleagues conducted a clinical trial investigating the efficacy of mesenchymal cell treatment in two patients with approved coronavirus disease 2019 (COVID-19) in Wuhan. Following MSC transplantation, elevated levels of immune markers, including lymphocytes and CD4+, and diminished levels of inflammatory markers, such as C-reactive protein and interleukin-6, have been observed. A primary support plan for patients with acute respiratory distress syndrome (ARDS) may include the use of a high-flow nasal cannula. Both patients who received mesenchymal stem cell transplantation exhibited a gradual decline in the fraction of inspired oxygen (FiO_2) and an increase in both the oxygen saturation (SaO_2) and partial pressure of oxygen (PO_2). Furthermore, the chest computed tomography of the patient demonstrated the absorption of exudate following the injection of mesenchymal SCs into the bilateral lung lesions. The clinical data pertaining to the treatment of patients with coronavirus disease 2019 (Covid-19) indicate that the transplantation of mesenchymal stem cells may represent a promising avenue for therapeutic intervention, particularly in patients with acute respiratory distress syndrome (ARDS) (50).

2.8. Membrane Fusion Inhibitors and Inhibitors of ACE-2 Receptor Connection

The receptor-binding domain (RBD) of S1 enables the virus to bind to the cellular receptor, which then forms a six-helix bundle (6-HB) across the two principal domains of S2, namely HR1 and HR2. This ultimately leads to the merging of the virus membrane (51). The role of the human angiotensin-converting enzyme 2 (ACE-2) as a cellular receptor for SARS-CoV-2 and the identification of the spike protein (S) as a fusion facilitator have been elucidated. In the S2 fusion subunit of the SARS-CoV-2, an increase in thermal stability and α-helicity in the heptad repeat 1 (HR1) sequence has been observed, which is indicative of a significant increase. Furthermore, a higher binding affinity to the respective heptad repeat 2 (HR2) site is indicated for SARS-CoV than for the HR1 sequence of S2. Based on the HR2 sequence, a lipopeptide binding inhibitor, designated IPB02, was developed. This inhibitor demonstrated remarkable efficacy in impeding the cell fusion of SARS-CoV-2 via the S protein, in addition to inhibiting virus-like transmission and activity. Furthermore, the IPB02 structure-activity relationship (SAR) was identified through the use of a truncated lipopeptide group, which serves to illustrate the crucial role that amino acid patterns play in both binding and antiviral capabilities. In 2020, this hypothesis was evaluated in a further trial at two London hospitals with multiethnic populations, using a consecutive cohort of 1,200 hospitalized patients with acute SARS-CoV-2 infection. The mean age of the patients was 68±17 years, 57% of whom were male, and 74% had at least one underlying condition. In general, during the initial 21-day period following the onset of symptoms, 34.6% of the patients (415 individuals) either achieved the primary endpoint of death or required organ support and were transferred to the intensive care unit. Of the 399 patients, 33.3% were prescribed either angiotensin-converting enzyme (ACE) inhibitors or angiotensin-converting enzyme-2 receptor blockers (ARBs). In patients hospitalized with SARS-CoV-2 infection who were receiving ACEi or ARB therapy, there was no evidence of increased severity of acute illness (52).

3. Challenges in the Prognosis, Diagnosis, Vaccination and Treatment of COVID-19

With regard to the availability of the vaccine, one of the principal challenges initially encountered was the limited supply of vaccines, coupled with the complexities of their manufacture and distribution on a global scale. This resulted in logistical difficulties, which in turn led to a shortage of doses, failing to meet the demand. In light of these considerations, the distribution of vaccines, particularly those requiring ultra-cold storage, such as the Pfizer-BioNTech vaccine, has proven to be a significant challenge. It has been essential to guarantee the appropriate management of the cold chain, the availability of suitable transportation infrastructure, and the effective coordination between the relevant stakeholders in order to reach remote locations and administer vaccines in an efficient manner (53). Furthermore, vaccine hesitancy, driven by misinformation and mistrust, has constituted a substantial impediment. Some individuals have expressed concerns regarding the safety, efficacy, and potential side effects of vaccines, which has resulted in a reluctance to undergo vaccination (54). Ensuring equitable access to vaccines across different regions and communities has proven to be a significant challenge. The existence of disparities in healthcare infrastructure, socio-economic factors, and vaccine distribution has resulted in unequal vaccine coverage, thereby exacerbating existing health inequalities. The emergence of new variants of the virus gives rise to concerns regarding the efficacy of existing vaccines against these variants. The ongoing challenges include the development and deployment of booster shots and the adaptation of vaccine strategies to address variant-specific challenges (55). The coordination of international efforts to guarantee a fair distribution of vaccines on a global scale has proven to be a significant challenge. The initial vaccine supply was secured by developed or high-income nations, which has underscored the necessity for global collaboration and equitable vaccine distribution. One of the most significant challenges has been the scaling up of vaccine production to meet the global demand. It is similarly crucial to expand manufacturing facilities and guarantee a dependable supply chain for raw materials and components, in order to enhance production capacity (56).

Additionally, the prognosis of SARS-CoV-2 infection presents certain challenges that may influence the prediction of individual outcomes. The clinical presentation of SARS-CoV-2 infection can range from asymptomatic or mild symptoms to severe respiratory distress and organ failure. The wide spectrum of disease severity presents a significant challenge in accurately predicting the course and prognosis for individual patients (57). Individuals aged 65 and over, as well as those with underlying health conditions such as cardiovascular disease, diabetes, or immune system compromise, are at an elevated risk of developing severe illness and experiencing a poor prognosis (58). The emergence of new variants of the coronavirus introduces further complexity to the process of prognosis. Some variants may be associated with increased transmissibility or potential resistance to therapies, which can influence disease progression and outcomes. The variability in individuals' immune responses to the virus can influence disease progression and prognosis (57). Secondary infections or complications, such as pneumonia, blood clots, or organ damage, may ensue as a result of the initial infection with SARS-CoV-2, thereby further complicating the prognosis. Given that the disease is relatively new, there is a paucity of long-term data on recovery, long-term complications, and overall prognosis. Further research and follow-up studies are required to gain a deeper understanding of the long-term effects of the virus and to refine prognostic models. The psychological and social impacts of the novel coronavirus disease (2019-nCoV), including anxiety, depression, post-traumatic stress disorder (PTSD), and social support, can influence the overall prognosis and recovery of individuals (59). The diagnosis of SARS-CoV-2 infection has also presented a number of challenges. Initially, there were constraints in the availability and accessibility of testing globally. The availability of an adequate testing infrastructure, including the necessary test kits, laboratory capacity, and trained personnel, is of paramount importance to ensure the prompt and widespread implementation of testing (60). The accuracy of diagnostic tests (sensitivity and specificity) for SARS-CoV-2 is of paramount importance for the reliable detection of the virus. The potential for false-negative or false-positive results poses a challenge to accurate diagnosis. The timing of testing can have a significant impact on the accuracy of the results. The viral load may fluctuate at different stages of infection, and an individual may test negative at an early stage of the disease but subsequently test positive. The appropriate timing of testing, taking into account the presence of symptoms, exposure history, and the incubation period, is crucial for accurate diagnosis. The diagnosis of asymptomatic and mild cases of SARS-CoV-2 infection presents a significant challenge (61). Such individuals may not manifest the conventional symptoms or pursue medical care, rendering their identification and diagnosis challenging in the absence of extensive testing. The symptoms of the novel coronavirus disease (COVID-19) can vary considerably, from mild respiratory symptoms to severe pneumonia and multi-organ failure. Moreover, the treatment of patients with coronavirus disease 2019 (Covid-19) presents a number of significant challenges, including the following: The development of effective antiviral drugs that are specifically targeted at the virus has proven to be a significant challenge (62). The clinical manifestations of SARS-CoV-2 infection can range from mild respiratory symptoms to severe pneumonia and multi-organ failure. The treatment of patients exhibiting varying degrees of illness severity and complications necessitates the implementation of individualized approaches. Given the evolving nature of the disease and the variability in its clinical presentations, there is currently no universally accepted treatment protocol for coronavirus disease 2019 (Covid-19). Treatment decisions are frequently based on clinical judgment and guidelines that are subject to continuous updating in light of new evidence. During periods of increased case numbers, healthcare systems may be unable to cope, resulting in difficulties in providing the best possible care to all patients. The availability of limited resources, including hospital beds, ventilators, and healthcare personnel, can impede the timely and effective delivery of treatment (64).

4. Conclusions

The global impact of the SARS-CoV-2 outbreak on public health and the economy is significant and far-reaching. At present, there is no established strategy for the treatment or prevention of SARS-CoV-2 infection. However, a number of potential treatments have been proposed. The implementation of stringent quarantine measures and the administration of vaccines to the general public appear to be the only currently feasible and empirically validated strategies for reducing the rate of transmission. The prognosis for patients with coronavirus disease 2019 (COVID-19) is a complex process that requires the consideration of multiple factors. Healthcare professionals utilize clinical judgment, evidence-based guidelines, and available data to assess and communicate the probable outcomes for individual patients. It is of the utmost importance to seek the counsel of medical experts in order to obtain accurate and up-to-date information regarding the prognosis of patients infected with the novel coronavirus, SARS-CoV-2. It is of the utmost importance to consult reliable sources such as national health authorities and the World Health Organization (WHO) in order to obtain the latest information on the subject of the vaccination of patients with the novel coronavirus, including details on the criteria for eligibility, the availability of the vaccine, and the recommended protocols in any specific region. It is crucial to acknowledge the advancements that have been made in testing technologies and strategies. It is possible that testing guidelines and protocols have undergone evolution, and that new diagnostic methods have been developed to address some of these challenges. It is of the utmost importance to adhere to the most recent guidelines set forth by reputable health organizations in order to ensure an

accurate and timely diagnosis of the novel coronavirus disease (Covid-19). The range of available treatment options and strategies is subject to ongoing evolution. Healthcare professionals and researchers are assiduously striving to surmount the challenges inherent to therapeutic interventions and to enhance the outcomes for those afflicted with the SARS-CoV-2 infection. It is imperative that randomized clinical studies be conducted with the objective of identifying the most appropriate and proven treatment in order to reduce the prevalence of SARS-CoV-2 and to prevent the prevalence of any future pandemic.

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Authors' Contribution
N.B. R.H. and S.M. wrote the manuscript. A.G. wrote and edited the manuscript. All authors reviewed the manuscript.

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Not applicable.

Conflict of Interest
There is no conflict of interest by the authors.

Data Availability
The data used to support the findings of this study are included within the article.

References

1. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.
2. Chary MA, Barbuto AF, Izadmehr S, Hayes BD, Burns MM. COVID-19: therapeutics and their toxicities. Journal of Medical Toxicology. 2020;16(3):284-94.
3. Wagner CE, Saad-Roy CM, Grenfell BT. Modelling vaccination strategies for COVID-19. Nature Reviews Immunology. 2022;22(3):139-41.
4. Meo S, Bukhari I, Akram J, Meo A, Klonoff DC. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines. Eur Rev Med Pharmacol Sci. 2021:1663-9.
5. Ioannou GN, Locke ER, Green PK, Berry K. Comparison of Moderna versus Pfizer-BioNTech COVID-19 vaccine outcomes: A target trial emulation study in the US Veterans Affairs healthcare system. EClinicalMedicine. 2022;45.
6. Adjobimey T, Meyer J, Sollberg L, Bawolt M, Berens C, Kovačević P, et al. Comparison of IgA, IgG, and neutralizing antibody responses following immunization with Moderna, BioNTech, AstraZeneca, Sputnik-V, Johnson and Johnson, and Sinopharm's COVID-19 vaccines. Frontiers in Immunology. 2022;13:3094.
7. Heidary M, Kaviar VH, Shirani M, Ghanavati R, Motahar M, Sholeh M, et al. A comprehensive review of the protein subunit vaccines against COVID-19. Frontiers in microbiology. 2022;13:927306.
8. Yalçın TY, Topçu Dİ, Doğan Ö, Aydın S, Sarı N, Erol Ç, et al. Immunogenicity after two doses of inactivated virus vaccine in healthcare workers with and without previous COVID-19 infection: prospective observational study. Journal of medical virology. 2022;94(1):279-86.
9. Corey L, Mascola JR, Fauci AS, Collins FS. A strategic approach to COVID-19 vaccine R&D. Science. 2020;368(6494):948-50.
10. Araf Y, Akter F, Tang Yd, Fatemi R, Parvez MSA, Zheng C, et al. Omicron variant of SARS-CoV-2: genomics, transmissibility, and responses to current COVID-19 vaccines. Journal of medical virology. 2022;94(5):1825-32.
11. Guglielmi G. Rapid coronavirus tests: a guide for the perplexed. Nature. 2021;590(7845):202-5.
12. Kralik P, Ricchi M. A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. Frontiers in microbiology. 2017;8:108.
13. Yüce M, Filiztekin E, Özkaya KG. COVID-19 diagnosis—a review of current methods. Biosensors and Bioelectronics. 2021;172:112752.
14. Merindol N, Pépin G, Marchand C, Rheault M, Peterson C, Poirier A, et al. SARS-CoV-2 detection by direct rRT-PCR without RNA extraction. Journal of Clinical Virology. 2020;128:104423.
15. Kashir J, Yaqinuddin A. Loop mediated isothermal amplification (LAMP) assays as a rapid diagnostic for COVID-19. Medical hypotheses. 2020;141:109786.
16. Park G-S, Ku K, Baek S-H, Kim S-J, Kim SI, Kim B-T, et al. Development of reverse transcription loop-mediated isothermal amplification assays targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The Journal of Molecular Diagnostics. 2020;22(6):729-35.
17. Yang W, Dang X, Wang Q, Xu M, Zhao Q, Zhou Y, et al. Rapid detection of SARS-CoV-2 using reverse transcription RT-LAMP method. MedRxiv. 2020.
18. Inaba M, Higashimoto Y, Toyama Y, Horiguchi T, Hibino M, Iwata M, et al. Diagnostic accuracy of LAMP versus PCR over the course of SARS-CoV-2 infection. International Journal of Infectious Diseases. 2021;107:195-200.
19. Jiang M, Pan W, Arasthfer A, Fang W, Ling L, Fang H, et al. Development and validation of a rapid, single-step reverse transcriptase loop-mediated isothermal amplification (RT-LAMP) system potentially to be used for reliable and high-throughput screening of COVID-19. Frontiers in cellular and infection microbiology. 2020;10:331.
20. Yang W, Restrepo-Pérez L, Bengtson M, Heerema SJ, Birnie A, Van Der Torre J, et al. Detection of CRISPR-dCas9 on DNA with solid-state nanopores. Nano letters. 2018;18(10):6469-74.
21. Lee H, Choi J, Jeong E, Baek S, Kim HC, Chae J-H, et al. dCas9-mediated nanoelectrokinetic direct detection of target gene for liquid biopsy. Nano letters. 2018;18(12):7642-50.

22. Broughton JP, Deng X, Yu G, Fasching CL, Singh J, Streithorst J, et al. Rapid detection of 2019 novel coronavirus SARS-CoV-2 using a CRISPR-based DETECTR lateral flow assay. MedRxiv. 2020.
23. Li S, Huang J, Ren L, Jiang W, Wang M, Zhuang L, et al. A one-step, one-pot CRISPR nucleic acid detection platform (CRISPR-top): application for the diagnosis of COVID-19. Talanta. 2021:122591.
24. Han C, Li W, Li Q, Xing W, Luo H, Ji H, et al. CRISPR/Cas12a-Derived electrochemical aptasensor for ultrasensitive detection of COVID-19 nucleocapsid protein. Biosensors and Bioelectronics. 2021:113922.
25. Lou J, Wang B, Li J, Ni P, Jin Y, Chen S, et al. The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases. Mol Biol Rep. 2022;49(12):11301-11.
26. Sharma A, Balda S, Apreja M, Kataria K, Capalash N, Sharma P. COVID-19 diagnosis: current and future techniques. International Journal of Biological Macromolecules. 2021;193:1835-44.
27. Zhou Y, Wu Y, Ding L, Huang X, Xiong Y. Point-of-care COVID-19 diagnostics powered by lateral flow assay. TrAC Trends in Analytical Chemistry. 2021;145:116452.
28. Mattioli IA, Castro KR, Macedo LJ, Sedenho GC, Oliveira MN, Todeschini I, et al. Graphene-based hybrid electrical-electrochemical point-of-care device for serologic COVID-19 diagnosis. Biosensors and Bioelectronics. 2022;199:113866.
29. Beduk T, Beduk D, de Oliveira Filho JI, Zihnioglu F, Cicek C, Sertoz R, et al. Rapid Point-of-Care COVID-19 Diagnosis with a Gold-Nanoarchitecture-Assisted Laser-Scribed Graphene Biosensor. Analytical chemistry. 2021.
30. Yu S, Nimse SB, Kim J, Song K-S, Kim T. Development of a lateral flow strip membrane assay for rapid and sensitive detection of the SARS-CoV-2. Analytical chemistry. 2020;92(20):14139-44.
31. Xia S, Chen X. Single-copy sensitive, field-deployable, and simultaneous dual-gene detection of SARS-CoV-2 RNA via modified RT–RPA. Cell discovery. 2020;6(1):1-4.
32. Wensel CR, Pluznick JL, Salzberg SL, Sears CL. Next-generation sequencing: insights to advance clinical investigations of the microbiome. J Clin Invest. 2022;132(7).
33. Dotolo S, Esposito Abate R, Roma C, Guido D, Preziosi A, Tropea B, et al. Bioinformatics: from NGS data to biological complexity in variant detection and oncological clinical practice. Biomedicines. 2022;10(9):2074.
34. D'Alessandra Y, Valerio V, Moschetta D, Massaiu I, Bozzi M, Conte M, et al. Extraction-Free Absolute Quantification of Circulating miRNAs by Chip-Based Digital PCR. Biomedicines. 2022;10(6):1354.
35. Lewandowski K, Xu Y, Pullan ST, Lumley SF, Foster D, Sanderson N, et al. Metagenomic Nanopore Sequencing of Influenza Virus Direct from Clinical Respiratory Samples. J Clin Microbiol. 2019;58(1).
36. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, et al. Compassionate use of remdesivir for patients with severe Covid-19. New England Journal of Medicine. 2020;382(24):2327-36.
37. Gillenwater S, Rahaghi F, Hadeh A. Remdesivir for the treatment of covid-19-preliminary report. N Engl J Med. 2020;383(10):992.
38. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proceedings of the Japan Academy, Series B. 2017;93(7):449-63.
39. Du YX, Chen XP. Favipiravir: pharmacokinetics and concerns about clinical trials for 2019-nCoV infection. Clinical Pharmacology & Therapeutics. 2020;108(2):242-7.
40. Nukoolkarn V, Lee VS, Malaisree M, Aruksakulwong O, Hannongbua S. Molecular dynamic simulations analysis of ritronavir and lopinavir as SARS-CoV 3CLpro inhibitors. Journal of theoretical biology. 2008;254(4):861-7.
41. Yao TT, Qian JD, Zhu WY, Wang Y, Wang GQ. A systematic review of lopinavir therapy for SARS coronavirus and MERS coronavirus—A possible reference for coronavirus disease-19 treatment option. Journal of medical virology. 2020;92(6):556-63.
42. Abd-Elsalam S, Noor RA, Badawi R, Khalaf M, Esmail ES, Soliman S, et al. Clinical Study Evaluating the Efficacy of Ivermectin in COVID-19 Treatment: A Randomized Controlled Study. Journal of medical virology. 2021.
43. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). MedRxiv. 2020.
44. In Ah C, Sang Jin L, Won P, Sung Hwan P, Seung-cheol S, Han Joo B, et al. Effects of tocilizumab therapy on serum interleukin-33 and interleukin-6 levels in patients with rheumatoid arthritis. Archives of rheumatology. 2018;33(4):389.
45. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. Antimicrobial agents and chemotherapy. 1994;38(8):1695-702.
46. Bloch EM, Shoham S, Casadevall A, Sachais BS, Shaz B, Winters JL, et al. Deployment of convalescent plasma for the prevention and treatment of COVID-19. The Journal of clinical investigation. 2020;130(6):2757-65.
47. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clinical infectious diseases. 2020;71(16):2027-34.
48. Shende P, Bhandarkar S, Prabhakar B. Heat shock proteins and their protective roles in stem cell biology. Stem Cell Reviews and Reports. 2019;15(5):637-51.
49. Golchin A. Cell-based therapy for severe COVID-19 patients: clinical trials and cost-utility. Stem cell reviews and reports. 2021;17(1):56-62.
50. Tang L, Jiang Y, Zhu M, Chen L, Zhou X, Zhou C, et al. Clinical study using mesenchymal stem cells for the treatment of patients with severe COVID-19. Frontiers of medicine. 2020;14(5):664-73.
51. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new

coronavirus of probable bat origin. nature. 2020;579(7798):270-3.
52. Bean DM, Kraljevic Z, Searle T, Bendayan R, Kevin OG, Pickles A, et al. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers are not associated with severe COVID-19 infection in a multi-site UK acute hospital trust. European journal of heart failure. 2020;22(6):967-74.
53. Fahrni ML, Ismail IA, Refi DM, Almeman A, Yaakob NC, Saman KM, et al. Management of COVID-19 vaccines cold chain logistics: a scoping review. J Pharm Policy Pract. 2022;15(1):16.
54. Lin Y, Hu Z, Zhao Q, Alias H, Danaee M, Wong LP. Understanding COVID-19 vaccine demand and hesitancy: A nationwide online survey in China. PLoS Negl Trop Dis. 2020;14(12):e0008961.
55. Araf Y, Akter F, Tang YD, Fatemi R, Parvez MSA, Zheng C, et al. Omicron variant of SARS-CoV-2: Genomics, transmissibility, and responses to current COVID-19 vaccines. J Med Virol. 2022;94(5):1825-32.
56. Mukherjee S, Kalra K, Phelan AL. Expanding global vaccine manufacturing capacity: Strategic prioritization in small countries. PLOS Glob Public Health. 2023;3(6):e0002098.
57. Vakil MK, Mansoori Y, Al-Awsi GRL, Hosseinipour A, Ahsant S, Ahmadi S, et al. Individual genetic variability mainly of Proinflammatory cytokines, cytokine receptors, and toll-like receptors dictates pathophysiology of COVID-19 disease. J Med Virol. 2022;94(9):4088-96.
58. Goldman JD, Robinson PC, Uldrick TS, Ljungman P. COVID-19 in immunocompromised populations: implications for prognosis and repurposing of immunotherapies. J Immunother Cancer. 2021;9(6).
59. Samantaray NN, Kar N, Mishra SR. A follow-up study on treatment effects of cognitive-behavioral therapy on social anxiety disorder: Impact of COVID-19 fear during post-lockdown period. Psychiatry Res. 2022;310:114439.
60. Böger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. Am J Infect Control. 2021;49(1):21-9.
61. Maniruzzaman M, Islam MM, Ali MH, Mukerjee N, Maitra S, Kamal MA, et al. COVID-19 diagnostic methods in developing countries. Environ Sci Pollut Res Int. 2022;29(34):51384-97.
62. Rehman SU, Rehman SU, Yoo HH. COVID-19 challenges and its therapeutics. Biomed Pharmacother. 2021;142:112015.
63. Chilamakuri R, Agarwal S. COVID-19: Characteristics and Therapeutics. Cells. 2021;10(2).
64. Hosseinzadeh S, Ketabi S, Atighehchian A, Nazari R. Hospital bed capacity management during the COVID-19 outbreak using system dynamics: A case study in Amol public hospitals, Iran. International Journal of Healthcare Management. 2022:1-13.