Minireview *Highlight article*

Convalescent serum-derived exosomes: Attractive niche as COVID-19 diagnostic tool and vehicle for mRNA delivery

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Impact Statement

SARS-CoV-2 has spread across the world like a wildfire since its emergence in late 2019. COVID-19 symptoms overlap with several commonly occurring viral infections including influenza. Several cases even go undetected due to the asymptomatic nature of the disease. Diagnostic tools with higher sensitivity and accuracy are urgently needed to tackle this issue.

Abstract

The spread of SARS-CoV-2 over the entire world is more commonly known as COVID-19. COVID-19 has impacted society in every aspect of routine life. SARS-CoV-2 infection is often misdiagnosed as influenza or seasonal upper respiratory tract viral infections. General diagnostic tools can detect the viral antigen or isotypes of antibodies. However, inter- and intraindividual variations in antibody levels can cause false negatives in antibody immunoassays. On the contrary, the false-positive test results can also occur due to either cross-reactivity of the viral antigens or some other patient-related autoimmune factors. There is need for a cogent diagnostic tool with more specificity, selectivity, and reliability. Here, we have described the potential of convalescent serum-derived exosome as a diagnostic tool for the detection of SARS-CoV-2, even in asymptomatic patients, which is a limitation for currently practiced diagnostic tests throughout the globe. In addition, its potential as a vehicle for messenger RNA (mRNA) delivery is also emphasized.

Keywords: Convalescent serum, COVID-19, exosomes, SARS-CoV-2, diagnostic tool, mRNA

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Background about COVID-19

The presence of the deadly coronavirus disease 2019 (COVID-19) was noted in December 2019 in Wuhan City of China. COVID-19 has gained major attention in the therapeutic arena since it was declared a pandemic and it has impacted all aspects of life.[1–3] It is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[4–6] It is the seventh coronavirus to infect humans and is named COVID-19 by the World Health Organization (WHO).[7,8] Currently, there are more than 443 million confirmed cases with more than 5.9 million deaths registered globally due to the SARS-CoV-2

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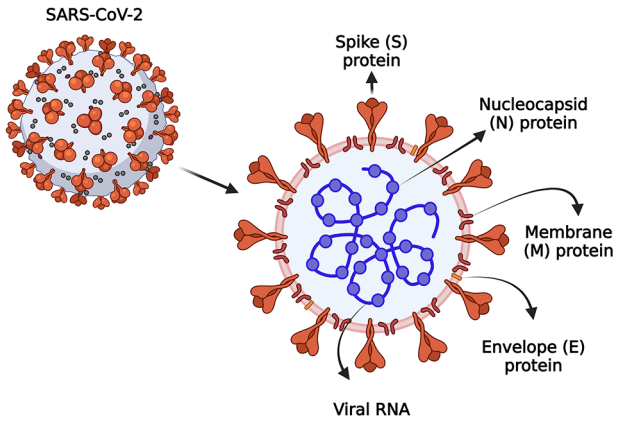

Figure 1. The structural representation of the SARS-CoV-2. SARS-CoV-2 is an RNA virus composed of various structural proteins such as nucleocapsid (N), envelope (E), membrane (M), and spike (S), which facilitate viral entry into the host, replication, assembly of virions, and pathogenesis, created with Biorender.com. (A color version of this figure is available in the online journal.)

infection.[9] Once the viral genome was encoded and the structural proteins were identified (Figure 1), the efforts were focused on the development of therapeutic agents, vaccines, as well as reliable and sensitive diagnostic tests.

The symptoms of COVID-19 include fever, body aches, sore throat, cough, body pain, difficulty in breathing, confusion, anosmia, loss of taste, and headache.[10] However, there are a lot of asymptomatic carriers globally which are difficult to identify. The new strain recently identified in England is responsible for additional symptoms like lethargy, dizziness, and diarrhea. The strain identified in Africa has modified viral spike proteins and is spreading at a much faster rate in various African regions. Although the majority of cases are mild, some can progress to pneumonia and lead to multiorgan failure. Various diagnostic tools that are being implemented for the detection of COVID-19 include serological tests, computerized tomography (CT) scans, magnetic resonance imaging (MRI), and the detection of viral antigens.[11,12] As the symptoms overlap with those of influenza and dengue, there is need for an effective diagnostic tool that can detect the presence of COVID-19 in symptomatic as well as asymptomatic patients. The present study describes currently used tools for COVID-19 detection, their limitations, and the potential use of exosomes for diagnosis as well as delivery of mRNA-based therapeutics.

Types of SARS-CoV-2 variants in COVID-19

After the emergence of SARS-CoV-2 in 2019, several variants of SARS-CoV-2 have been observed all over the world, including South Africa, India, the United Kingdom, Brazil, and so on.[6] SARS-CoV-2 variants complicate the recovery of COVID-19 patients. Therefore, there is a need to understand the biology of these variants. The genetic mutations in SARS-CoV-2 are responsible for the generation of new variants. The mutation rate is generally higher in RNA viruses like coronaviruses. B.1.1.7 is a UK variant and it is resistant to the monoclonal antibodies against N-terminal of the spike protein. A total of 23 mutations were found in the UK variant. The South African variant (B.1.351) has shown 21 mutations, with many variations in the spike protein. The Brazil variant (P.1) was detected with 17 mutations and has a higher transmission rate. Moreover, other variants of SARS-CoV-2 have been emerging, including B.1.617 in India (Figure 2).[13]

Diagnostic and detection methods for COVID-19

Following the onset of COVID-19 symptoms, the first step is to detect the presence or absence of SARS-CoV-2. Many patients test positive despite being asymptomatic, due to testing requirements as per the government norms. Rapid antigen detection test is one of the easiest first-line detection mechanisms but its reliability has been questioned. Therefore, real-time reverse transcription-polymerase chain reaction (RT–PCR) is recommended for the cogent early detection (Figure 3).[14,15]

RT-PCR test mainly focuses on the detection of the viral nucleic acid or its fragments present in the upper respiratory tract of the patient. Improving test sensitivity and specificity is essential to reduce the number of false-positive or false-negative cases.[16] More sophisticated enzyme-linked immunosorbent assay (ELISA) can detect viral spike protein as well as the presence of IgG and IgM.[17] In addition, there are next-generation sequencing-based assays that are commercially viable and more reliable than the other 430 different types of nucleic acid detection assays in the market. More than 170 and 430 immunoassays are being developed for the detection of viral antigens and antibodies, respectively.

Limitations of current diagnostic tools

Although there are several immunoassays in development for the diagnosis of COVID-19, most of them have shown very poor sensitivity or reliability of the test outcome.[18] Assay formats, antigens to target (S and N proteins, as well as the subunits of SARS-CoV-2), isotypes of antibodies to detect (IgA, IgM, IgG, and complete antibodies), the diagnostic testing window, as well as inter- and intraindividual variation in antibody levels can all cause false negatives in antibody immunoassays.[19] Moreover, false-positive test results are mainly due to either cross-reactivity of the viral antigens or some other patient-related autoimmune factors (Figure 3).

In order to improve the false-positive and false-negative results, there is need for a highly sensitive and specific diagnostic test. CRISPRCas13a has recently obtained a lot of attention because of its ability to remove amplification steps while also shedding light on point-of-care (POC) diagnostics and, as a result, removing any potential background signal from the amplification method. Selection of the particular epitope and isotope is also very crucial for the development of a reliable and specific diagnostic test (Figure 4).[20–22] Combining RT-PCR and IgM-IgG antibody tests would result in more reliable and sensitive methods to detect the SARS-CoV-2 virus. However, there is a need to think outside the box and develop some diagnostic tools having more accuracy and sensitivity of detection with added advantages of the existing methods. Currently, none of the clinical or laboratory

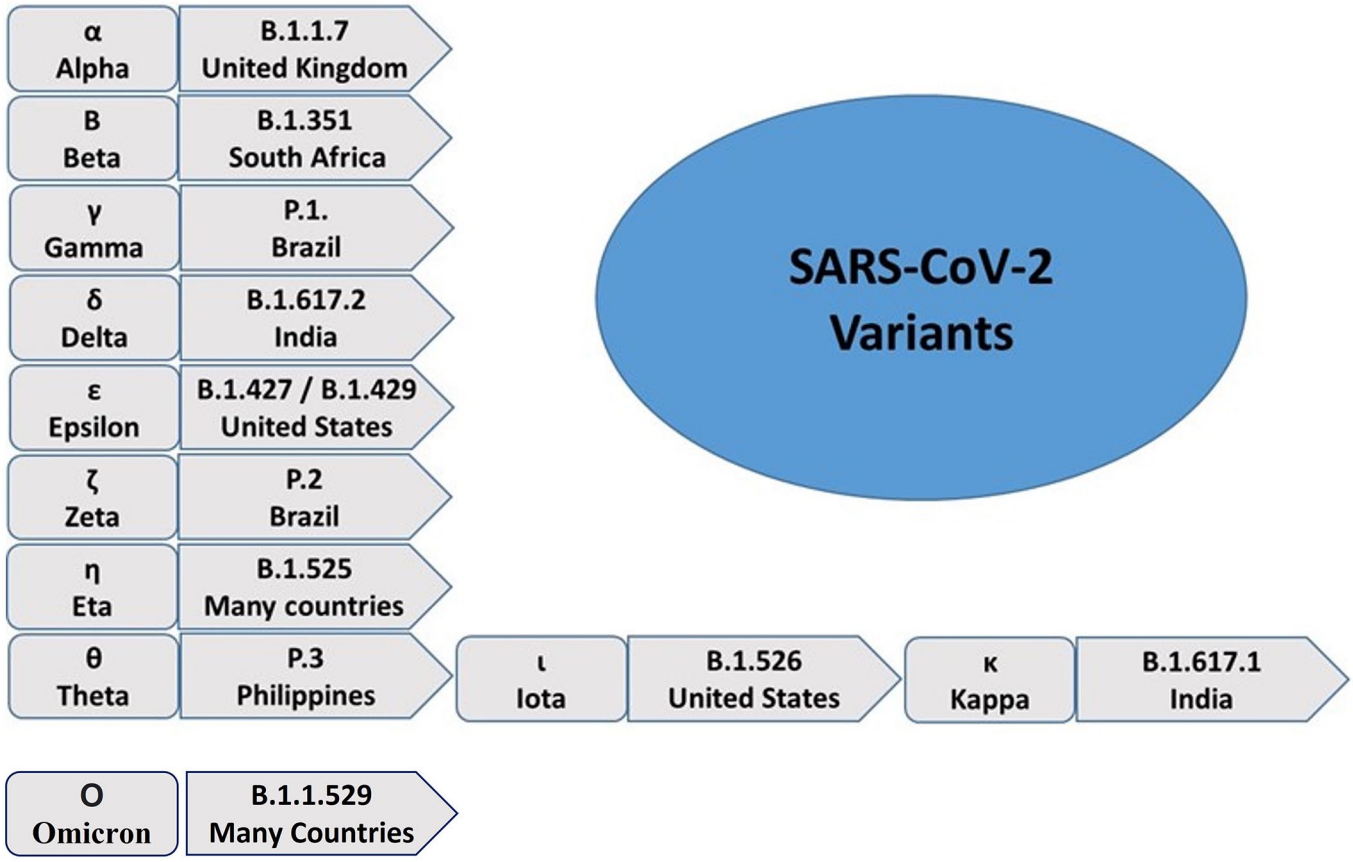

Figure 2. The reported variants of SARS-CoV-2. Several SARS-CoV-2 variants have been reported across the world since its emergence in December 2020. The most commonly found variants include B.1.1.7, B.1.351, P.1, B.1.617.2, B.1.427, B.1.429, P.2, B.1.525, P.3, B.1.526, B.1.617.1, and B.1.1.529. They have been designated with Greek letters ranging from Alpha to Kappa in that order. (A color version of this figure is available in the online journal.)

approaches available are reliable or precise enough to question the existing COVID-19 strain.

Exosome as a futuristic diagnostic tool for COVID-19

As there is no vaccine or a therapeutic regimen to cure COVID-19, convalescent plasma therapy is usually the first choice of treatment for patients. Convalescent serum is derived from recovered COVID-19 patients with high neutralizing antibody titers. Although the clinical advantage of convalescent plasma therapy in COVID-19 is still unknown, using antibody-containing plasma from recovered patients is a near-term choice that can be introduced rapidly.[23,24] It is a kind of passive immunization as opposed to vaccines, which trigger antibody production. Convalescent plasma provides immediate immunity. However, the mechanism of action is not very clear. Plasma also contains exosomes, other vesicles, proteins, lipoproteins, and so on, in addition to neutralizing antibodies.

Exosomes are formed through multivesicular bodies (MVB), which appear along the endocytic pathway, and are characterized by the presence of vesicles in their lumen (i.e. intraluminal vesicles) formed by inward budding from the limiting membrane. Exosomes contain various biological materials such as proteins, lipids, miRNA, mRNA, snRNA, and DNA.[25–27] Exosomes, in particular, are extracellular vesicles (EVs) that can carry viral DNA, RNA, and other biologic payloads,[25–31] and have great potential to be used as a diagnostic tool.[32,33] Several methods have been used to isolate exosomes, which are present in many extracellular body fluids such as serum, plasma, urine, and saliva. Exosomes are isolated from differential ultracentrifugation, ultrafiltration, precipitation, immunoaffinity capture, and size-exclusion chromatography.[34] Although many methods have been used, ultrafiltration is considered to be the gold standard.[35] Following the recovery of convalescent serum from patients, ultracentrifugation and filtration are carried out in order to isolate the exosomes. Convalescent serum-derived exosomes can be a cogent diagnostic tool for futuristic COVID-19 detection with more selectivity and specificity. Exosomes in the blood play a key role in cell-to-cell communication and are a promising new source of biomarkers for disease diagnosis and prognosis. Furthermore, exosomes have shown promise as biomarkers for disease status and treatment outcomes.[36,37] Proteomic profiling of exosomes may help identify the differential biomarkers. Furthermore, cluster analysis can pinpoint the differentially expressed proteins which could be the potential biomarkers for noninvasive COVID-19 detection. A growing body of evidence suggests that exosomes proved to be a potential biomarker for conditions like cancers, viral diseases, and some autoimmune disorders.[38–40] Proteomic analysis of patient-derived exosomes have detected many

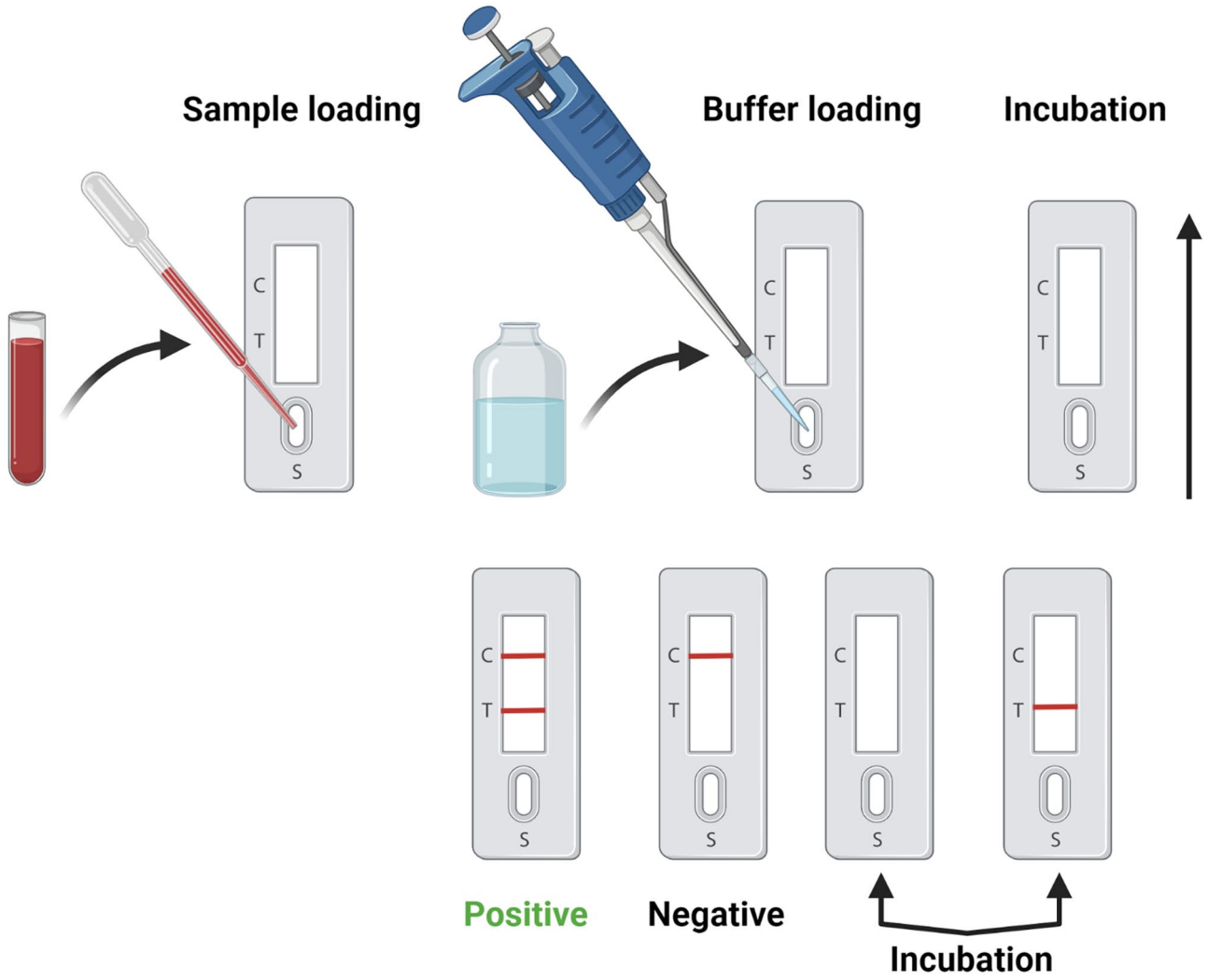

Figure 3. SARS-CoV-2 serological testing. Serological testing for SARS-CoV-2 detection involves a series of steps including sample/buffer loading, incubation, antibody-antigen recognition, SARS-CoV-2 / control antibody detection, and data interpretation, created with Biorender.com. (A color version of this figure is available in the online journal.)

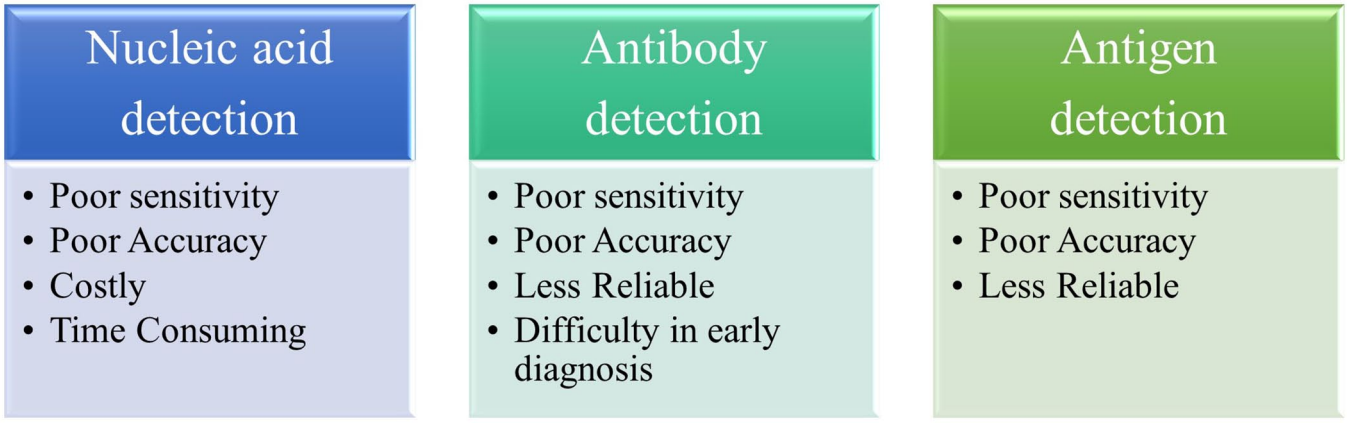

Figure 4. Limitations of current diagnostic tools. Current diagnostic tools for nucleic acid and, antibody and antigen detection have limited sensitivity, accuracy, and reliability. They are also expensive, time-consuming, and lack early detection capabilities. (A color version of this figure is available in the online journal.)

components involved in the immune response, pathology, and activation of the coagulation, and complement pathways associated with COVID-19-associated tissue damage and multiple organ dysfunctions.[41] It can also detect the asymptomatic patients acting as disease spreaders in society. In sum, the exosome will be the potential candidate for

the rapid and more reliable diagnostic method development for COVID-19.

Exosome-mediated mRNA delivery

To have therapeutic effects, mRNA molecules must reach certain target cells and create a sufficient amount of the desired proteins. However, targeted distribution and endosomal escape continue to be difficult challenges for mRNA delivery systems, emphasizing the need for safe and efficient mRNA delivery materials. Notably, lipid nanoparticles are used to deliver antigen mRNA in two approved coronavirus disease 2019 (COVID-19) vaccines: mRNA-1273 and BNT162b. Lipid nanoparticle–mRNA compositions must overcome various extracellular and intracellular obstacles in order to work *in vivo*. First, mRNA in physiological fluids must be shielded against nuclease destruction. Second, upon systemic injection, the formulation should avoid interception by the mononuclear phagocyte system and clearance by renal glomerular filtration. Third, lipid nanoparticle–mRNA systems must enter target tissues before being internalized by target cells. Finally, mRNA molecules must avoid endosomes in order to enter the cytoplasm, where translation takes place. All these features can be achieved by exosomes with least side effects and mRNA loss, as these vesicles are derived from humans.[42–46]

Exosomes have gained popularity in recent years due to their ability to facilitate intercellular communication via the targeted delivery of multimolecular cargo.[47,48] They are particularly useful in transporting mRNA molecules which are prone to degradation inside the cell. Other vehicles such as liposomes or nanoparticles have been previously used to carry mRNAs.[49] But these vehicles were found to have serious side effects.[50] On the contrary, exosomes are a part of the cell secretome, and are ubiquitous in the human body[51] and thus may serve as suitable candidates for delivering RNA-based therapeutics.[52]

In a recent study conducted by Tsai *et al.*, exosomes purified from cultured human cells were packed with mRNA encoding a carrier protein and multiple SARS-CoV-2 proteins including spike protein, before delivering them into *in vitro* and *in vivo* models at a dosage of 0.25 μg or 4 μg mRNA every 3 weeks (total three doses). Harvested tissues showed a prolonged (although modest, possibly due to the lower dose) CD4+ and CD8+ T-cell-mediated immune response against the N and S proteins for almost 8 weeks, following the final booster dose. In addition, CD4+ T cells demonstrated increased expression of interferon-gamma and lower expression of interleukin-4, indicating Th1-linked response.[53] While this study is yet to be peer-reviewed, it opens up a new avenue for the potential use of exosomes as delivery vehicles for mRNA-based drugs, including optimization of the exosome-RNA formulation conditions (Figure 5), scale-up of the dosage for studies in the larger animals, and determination of side effects.

Exosomes: advantages and importance

Consistent evolution of the viral genome has made COVID-19 management quite difficult over the past few years. Based on the severity of disease, COVID-19 can be divided into asymptomatic or presymptomatic infection, mild, moderate, severe, and critical illness which is associated with intensive care unit (ICU) admission and mortality.[54,55] Considering the current devastating global scenario of COVID-19, there is an urgent need for the development of therapeutics that will be helpful in dealing with severe cases of COVID-19 with higher efficacy and recovery rates.[56,57] Furthermore, there is also an urge for a cogent diagnostic tool with more reliability, specificity, and sensitivity. Exosomes are becoming more widely accepted as contributors to a variety of diseases, and their potential as biomarkers and therapeutics is steadily gaining traction.[58,59]

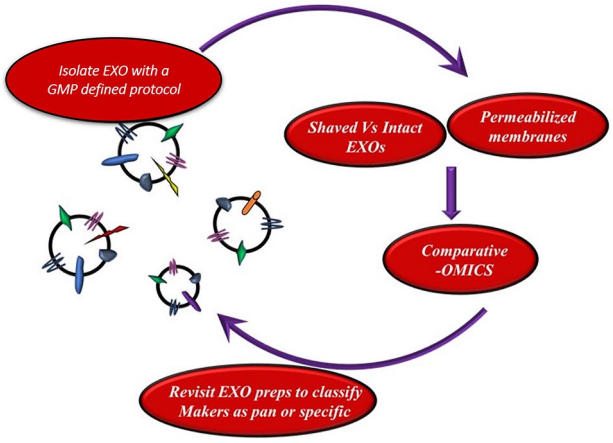

Figure 5. Schema for exosomal biomarker development. The optimization of the exosome-RNA formulation conditions. (A color version of this figure is available in the online journal.)

The exosome is a bilayer structure with the ability to carry the genetic material as well as the drug molecules for targeted delivery. Exosomes play a role in immune protection by activating immune responses. The exosomes produced in diseased cells have been shown to express specific proteins that can differentiate them from normal cells. In this way, exosomes may serve as useful biomarkers for disease diagnosis and monitoring (Figures 6 and 7).[60,61]

Exosomes and viruses share similar endosomal sorting pathways and mechanisms, giving them the ability to be used as a therapeutic to target, bind, and suppress the cellular uptake of a variety of viruses, including the novel SARS-CoV-2. Exosomal contents are relatively stable and safe against foreign proteases and other enzymes, making them valuable diagnostic tools. Accumulating evidence indicates that exosomal miRNA and lncRNA profiles in patients with particular pathologies vary from those in healthy people.[62,63]

Exosomes that exist naturally are best tolerated, have low immunogenicity and high extracellular fluid resilience.[57,64] These "naturally-equipped" nanovesicles could be used as drug delivery systems or be therapeutically targeted.[65–67] Exosomes have the potential to cross the blood–brain barrier, at least in certain circumstances, and may be used to deliver a variety of therapies such as small molecules, RNA therapies, proteins, viral gene therapy, and CRISPR gene editing.[68]

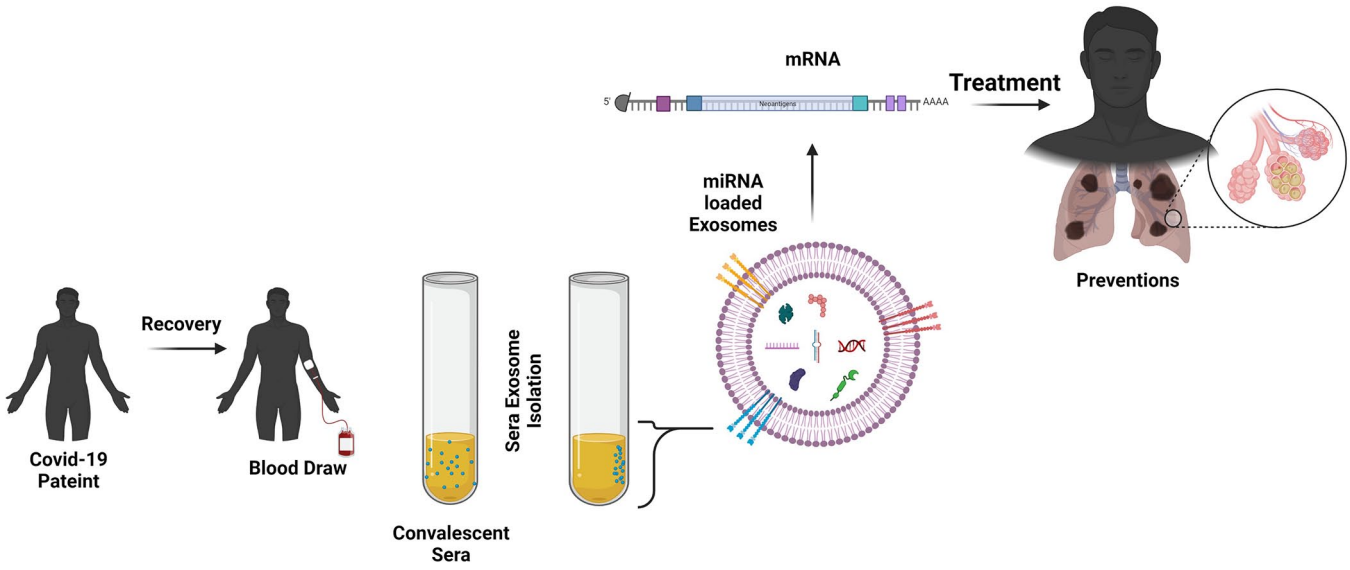

Figure 6. Convalescent serum-derived exosome-mediated mRNA delivery. Exosomes are isolated using convalescent serum obtained from a recovered COVID-19 patient, loaded with mRNA, and delivered to a patient as a potential treatment, created with Biorender.com. (A color version of this figure is available in the online journal.)

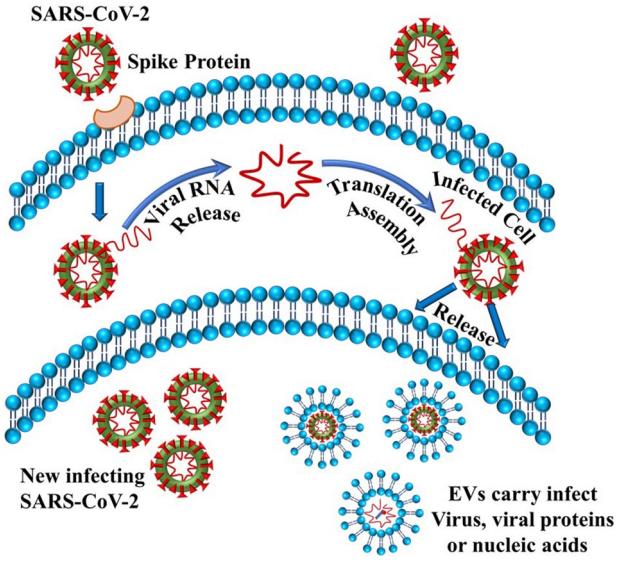

Figure 7. SARS-CoV-2 replication process using elements of exosomes. Viral replication occurs upon its entry into the host cell. Extracellular vesicles (EVs) such as exosomes capture these virions along with any viral protein or nucleic acids. (A color version of this figure is available in the online journal.)

Exosomes in clinical trials for the treatment of COVID-19

There are a total of 15 ongoing clinical trials, of which 7 are in the United States and 8 in Europe, where the potential of exosomes is being evaluated for the treatment of COVID-19. The virus-specific T cells (VSTs) in conjunction with interferon-gamma are engulfed in exosomes and evaluated in COVID-19 patients with pulmonary complications.[69]

Another clinical trial, a non-randomized open-label cohort study, was conducted to determine the safety and efficacy of exosomes (ExoFlo™) derived from allogeneic bone marrow mesenchymal stem cells as a treatment for severe COVID-19. From days 1–14, patients were tested for both protection and efficacy after receiving a single 15 mL intravenous dose of ExoFlo™. Within 72 h of ExoFlo administration, all safety endpoints were reached, with no adverse effects reported. The survival rate was found to be 83%. ExoFlo is a promising therapeutic candidate for extreme COVID-19 because of its safety profile and ability to restore oxygenation, downregulate cytokine storms, and restore immunity. ExoFlo's therapeutic potential might be assessed in future randomized controlled trials (RCTs).[68] Exosomes can control inflammation and regenerative processes by changing the concentration of anti-inflammatory cytokines and moving the immune cells to a regenerative secretome. Exosome inhalation has been shown to minimize inflammation and damage to lung tissue while also promoting regenerative processes. A pilot clinical trial involving the use of mesenchymal stem cells (MSCs) exosomes to treat severe novel coronavirus pneumonia (NCP) is ongoing in Wuhan, China (NCT04276987). This procedure aims to see whether exosome aerosol inhalation is safe and effective in treating critical patients hospitalized with NCP.

Exosomes as therapeutics

Experimental research has shown that MSCs can minimize lung inflammation and pathological impairment caused by various forms of lung injury. Many researchers attribute MSC's anti-inflammatory properties to their secretome, which includes exosomes derived from MSC. Furthermore, exosomes have a powerful regenerative effect on various wounds, implying their usefulness in treating COVID-19 patients.

The use of convalescent blood products such as whole blood, plasma, or serum, pooled human immunoglobulin IgG, and polyclonal and monoclonal antibodies have also gained prominence.[38,70] Improvement of clinical symptoms, increased oxygen saturation, higher neutralizing antibody titers, reduced lung consolidation, and reduced viral load have been observed in response to the administration of

convalescent blood products.[39,40] However, the mechanism of action remains unknown. Some authors refer to it as an "empirical therapy" since most of the findings come from case studies with few participants, and the results, while encouraging, may be skewed due to the lack of a well-designed experimental setup. The presence of EVs in convalescent blood products could explain some of the therapeutic benefits including immunomodulation and wound healing in the lungs. Neutralizing antibodies, growth factors, and EVs can be passed to patients during plasma apheresis.[41,54,71] In addition, exosomes can induce the activation of intracellular signaling pathways which will help in the theranostics of COVID-19.

Platelet-derived EVs were shown to enhance cell proliferation, migration, and angiogenesis in wound healing models by activating phosphoinositide 3-kinase (PI3K)-mitogen-activated protein kinase (Akt) and mitogen-activated protein kinase (MAPK) TGF- and yes-associated protein interaction, as well as -Erk signaling (YAP),[39,41] which could help elucidate the mechanism by which convalescent plasma shows positive results. Another study found that engineered platelet-derived EVs loaded with TPCA-1 were successful in treating pneumonia.[55] In a mouse model targeting inflammatory sites, this treatment suppressed inflammation and reduced the local cytokine storm. Thus, platelet-derived EVs may be useful in developing new therapeutic strategies for COVID-19.

Conclusions

The SARS-CoV-2 pandemic is on an unsettling course. The health, humanitarian, social, and economic policies of each country will affect the pace and intensity of recovery. Treatment for COVID-19 is currently very limited, and there is no curative solution available. Rapid and early laboratory diagnosis is critical to detect infection and control the transmission. As per WHO, RT-PCR is the standard test for COVID-19 detection. Mismatches between primers, probes, and target sequences may result in reduced detection performance and false-negative results, although RT-PCR is optimized for conserved regions of the viral genome. Serological surveys may assist with the investigation of an existing epidemic as well as the retrospective evaluation of an attack intensity or spectrum of the outbreak. Rapid diagnosis is expected due to appropriate treatment strategies and necessary containment. While the general diagnostic tools have the capability to detect viral antigens or isotypes of antibodies, the diagnostic testing window, interindividual variation, and intraindividual variation in antibody levels can all cause false negatives in antibody immunoassays. On the contrary, the false-positive test results are mainly due to either cross-reactivity of the viral antigens or some other patient-related autoimmune factors. There is need for a cogent diagnostic tool with more specificity, selectivity, and reliability. Here, we have attempted to describe the potential of convalescent serum-derived exosome as a diagnostic tool for the detection of SARS-CoV-2 that can detect even asymptomatic patients, which is the limitation for the currently practiced diagnostic tests throughout the globe. There is a consistent change in the virus structure due to mutation which makes it difficult to detect and even cure by generating passive immunity by the vaccine. As the exosomes are derived from the serum of the COVID-19 recovered patient, it can differentially express the protein, a potential biomarker for the detection of COVID-19 infections even if the person is asymptomatic. The differential protein expression by the exosomes can be identified by the proteomic analysis for its potential use as a biomarker, checking the drug repurposing status, and evaluation of the newer therapeutic moieties. On the contrary, the lipid bilayer vesicle can also be used as a vehicle for drug delivery as well as non-immunogenic vaccine delivery, either DNA or mRNA. It has the machinery to easily carry the nucleic acid material with more stability which will become a boon for vaccine delivery. As indicated by Tsai *et al.*,[53] exosome-mediated mRNA delivery may soon turn into a reality as the second-generation vaccine for COVID-19 with fewer side effects and immunogenic complications.

AUTHORS' CONTRIBUTIONS

AK and PG equally contributed to the manuscript. VPC, MPJ, RM, DV, CV, PR, AL, NKK, and B-CA contributed to the preparation of the initial draft and subsequent revisions. All authors approved the final version of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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