Pharmaceutical Sciences, 2020, 26(Suppl 1), S3-S11
doi:10.34172/PS.2020.34
https://ps.tbzmed.ac.ir/

Review Article

COVID-19: A Devastating Pandemic

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Article Info

Article History:
Received: 5 April 2020
Accepted: 27 April 2020
ePublished: 30 November 2020

Keywords:
-Novel coronavirus
-SARS-CoV-2
-SARS-CoV
-MERS-CoV
-COVID-19
-Pathogenesis

Abstract

Coronavirus disease 2019 (COVID-19) is a pandemic that was first reported in December 2019 in Wuhan, China. The disease is caused by virus SARS-CoV2. SARS-CoV2 has emerged from the highly pathogenic coronavirus in humans after Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) in the twenty-first century. Special efforts and attention to protect or reduce the transmission need to be applied in susceptible populations comprising elderly people, children, and health care providers. Different countries have implemented extensive measures to reduce person-to-person transmission of COVID-19 to regulate the current outbreak. In our review article, we provided a brief introduction to SARS-CoV2 and mentioned current knowledge on molecular immune pathogenesis, diagnosis, and treatment of COVID-19. Our work will help in offering novel insight and potential therapeutic targets for combating the SARS-CoV2 infection. Based on the research articles, we systemically discussed the characteristics of COVID-19 and provided some future aspects in the field of research.

Introduction

World health organization (WHO) on 11 March 2020 declared coronavirus disease 2019 (COVID-19) a global pandemic. COVID-19 has affected around 2160207 peoples (213 countries) and killed more than 146088 people worldwide to date. COVID-19 has spread like an epidemic after first come into sight in Wuhan, a Hubei province, China in 2019 December.[1] Coronavirus outbreak three deadly respiratory diseases specifically as COVID-19, the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS).[2-3] WHO announced and listed COVID-19 as Public Health Emergency of International Concern (PHEIC) On the 31st of January, 2020 which implicates that it stands risks globally and requires a coordinated response from countries at the international level. The detection of RNA SARS-CoV-2 plays an important role in early diagnosis of COVID-19, which will aid in controlling infection sources and prevent patients from disease infection. The expeditious and precise detection of a novel coronavirus consequently becomes significant. The recent progress in a field of molecular biology, the method of detecting nucleic acid has developed swiftly and turn into path-breaking technology for the detection of the virus. The mechanism based on polymerase chain reaction (PCR) indicated by quick detection, specificity and, high sensitivity regarded as the "gold standard" in virus detection.[4] Most of the developed countries are working to develop vaccine to neutralize the COVID-19 effect, but presently there is no specific antiviral agent developed to counter against COVID-19.[2,5] The genomic study and molecular mechanism of COVID-19 revealed various targets which can be use in different ways for therapeutic efficacy.[6]

Virology of SARS-CoV-2 (COVID-19)

Coronaviruses are enclosed viruses having a ssRNA genome of 26-32 kb.[4] Till now four genera of coronaviruses have been reported namely as α, β, γ and δ. Human coronaviruses are detected in β coronavirus (MERS-CoV, SARS-CoV, HCoV-OC43, and HCoV-HKU1) genera and α coronavirus (HCoV-229E and NL63).[7]

Sign and symptoms of COVID-19 patients reported with loss of appetite, olfaction, dysentery, taste, hyperpnea, cough, fever with acute respiratory distress syndrome (ARDS). The five patients infected with pneumonia were admitted to hospital between December18-29, 2019 which divulged the presence of β-CoV strain in all of them, which were unknown earlier.[8] The separated novel β-CoV divulges resemblance of 88% sequence of the genome of two bat-evolved severe acute syndromes (SARS) having the same attributes as coronaviruses, around 50% genomic sequence identical to MERS-CoV, bat-SL-CoVZC45, and bat-SL-CoVZXC21.[8-9] This novel β-CoV is classified as SARS-CoV-2 by International Virus Classification Commission.

The genome of SAR-CoV resembles typical CoVs and bears ten open reading frames (ORFs). The first ORFs, around

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2-3rd of viral RNA are expressed into two larger polyproteins. The two polyproteins i.e. pp1a and pp1ab of MERS-CoV and SARS-CoV prepared into 16 non-structural proteins (nsps) between nsp1 to nsp16, to form the viral replicas-transcriptase complex.[9] These non-structural proteins rearrange the membranes emanating from the rough endoplasmic reticulum (ER) into double-membrane bound vesicles where replication and transcription of the virus take place.[10-12] The four principal structural proteins like a spike (S), membrane (M), envelope (E), and nucleocapsid (N) genomic encoding is done by other ORFs of SARS-CoV2.

Just like SARS-CoV, scientists all over the globe have identified SARS-CoV2 also needs the angiotensin-converting enzyme-2 (ACE2) to enter into cells.[1,13] The pathogenesis of infection determines the binding of the virions with the receptor of the host cell. It is widely accepted now that SARS-CoV has an origin from bats and modified non-bat angiotensin-converting enzyme-2 alternative as the virus traverses species to attack human beings.[14] CD26 (Dipeptidyl peptidase 4) identified as functional receptor for MERS-CoV.[15] MERS-CoV is associated with DPP4, which facilitates the transfer of the viruses from one species to another.[16] A better understanding of the relation of receptors and proteases can help predict coronavirus infections and adaptability in humans.

Pathogenesis of COVID-19

Patients of COVID-19infections show symptoms including low leukocyte count, high fever, dry cough, dyspnea, myalgia, fatigue, and an indication of pneumonia which is a similar indication of SARS-CoV and MERS-CoV infections. A patient infected with COVID-19 for 5 days of fever exhibits cough, coarse breathing sound from both lungs, and body temperature of 39 °C. Severe infection of SAR-CoV might lead to pneumonia, and serious heart damage.[17-18]

COVID-19 Entry and Replication in Human

Coronavirus protein is a major determinant of entry of virus into host cells.[2] The envelope S glycoprotein of SARS-CoV and SARS-CoV-212,[19] attaches to the cellular receptor, angiotensin-converting enzyme-2, SARS-CoV attaches to C-type lectin (also called L-Sign) and MERS-CoV attaches to DPP4.[15,20] Direct membrane fusion between the virus and plasma membrane is identified in SARS-CoV.[21-22] Belouzard et al.[22] suggested that an important proteolytic cleavage takes place at position (S2') in SARS-CoV S protein at which plays a significant role in the membrane fusion and viral infectivity. The unique two-step furin protein activation for the fusion of membrane is progressed in MERS-CoV.[23] After viral entry into cells, the viral genome is released into the cytoplasm and is translated into two polyproteins which splits into structural proteins and they facilitate the process of replication.[7,24] The newly formed glycoprotein is inserted into the membrane of the ER or Golgi complex.[23] The endoplasmic reticulum-Golgi intermediate compartment is a place where virus particle germinates. Lastly, the virus-containing vesicles fuse with the plasma membrane and release the virus outside the cell.[2]

Coronavirus Antigen Presentation During Infection

After the entry of a virus into the cell, the viral antigens are exposed to the antigen-presenting cells, which represent the main part of the anti-viral immunity of a person. T lymphocytes recognize the major histocompatibility complex in humans presented by antigenic peptides, which helps in the interpretation of antigen presentation of SARS-CoV2 and understanding pathogenesis of SARS-CoV2 infection. Unluckily, there is still a scarcity of scientific reports about coronavirus. Major histocompatibility complex (MHC) I molecules are the main entity on which SARS-CoV' antigen presentation depends.[25,26] Earlier studies support that various human leukocytes antigen (HLA) polymorphisms coordinate with the sensitivity of SARS-CoV like HLA-B*4601, HLA-DR B1*1202, HLA-B*0703, and HLA-Cw*0801, though the HLA-Cw1502,,HLA-A*0201, and HLA-DR0301 alleles are related to the coronavirus infection protection.[27-29] MHC II molecules i.e.HLA-DQB1*02:0 and HLA-DRB1*11:01, are related to sensitivity towards infection of MERS-CoV.[30] Apart from, gene polymorphisms of mannose-binding lectin (MBL) related to antigen presentation associated with the threat of SARS-CoV infection. These studies will impart genuine clues for COVID-19 treatment and prevention.[31]

Human Body Response Towards Coronavirus

Antigen stimulates the body' cellular and humoral immunity to conciliate by virus-specific T and B cells. Related to common infections caused by the virus, the Immunoglobulin M (IgM) and Immunoglobulin G (IgG) production showed a typical pattern against the SARS-CoV virus. The SARS-specific Immunoglobulin M antibodies vanish in12 weeks; while the Immunoglobulin G antibodies can stay for a very long time, which designate IgG antibody largely take part in protective roles.[2,32] There are more studies on cell immunity for coronavirus than Humoral responses. The latest research reports indicate that CD4+ and CD8+ T cells in the peripheral blood of COVID-19 patients remarkably reduced, though its status is uncontrolled activation, as revealed by high proportions of CD8 (CD8 39.4%) and HLA-DR (CD4 3.47%) double-positive fractions.[33] Likewise, the acute phase response in indicating severe decrease of CD8+ T and CD4+ T cells in patients related to SARS-CoV.[34] T-cell memory can identify the SARS-CoV infection even after 6 years of SARS recovered patients.[35] The particular CD8+ T cells show resemblance in action on MERS-CoV in rats.[36]

Cytokine Storm in COVID-19

COVID-19 related death is mainly caused by acute respiratory distress syndrome (ARDS) as reports indicate

in Lancet. At the early stage of the outbreak, 41 patients admitted with infection from SARS-CoV-2, out of which 6 died from ARDS.7 SARS-CoV, MERS-CoV, and SARS-CoV-2 infection have a common immune pathological event of ARDS.[33] ARDS includes the large production of immune cells (IFN-α,, IL-1β,IFN-γ, YNF-α,IL-6,IL-18,IL-12, IL-33, TGFβ, etc.) and chemokines (CXCL8, CCL3, CCL5, CXCL9,CCL2, CXCL-10etc) into lungs.[17,37] The cytokine storm is the unregulated systemic inflammatory response which results in the production of the enormous amount of pro-inflammatory cytokines by the effectors cells (such as IFN-α, IL-1β, TNF-α, IFN-γ , TGFβ, IL-6, IL-18,IL-12, and IL-33) and chemokines (such as CXCL8, CXCL9, CXCL10, CCL2, CCL3, CCL5).[37-39] The cytokine storm is one of the principal ways for ARDS. Just like the patients with SARS-CoV patients, the patients with serious MERS-CoV show an elevation in the levels of CCL5, CXCL8, IL-6, IFN- α, CXCL10 in the serum in comparison to the patients with the mild-moderate disease.[40] A violent immune system attack is triggered by the cytokine storm to the body, which results in ARDS, multiple organ failure leading to death in serious cases of SARS-CoV2infection. This is very similar which occurs in infection of MERS-CoV and SARS-CoV.[33]

COVID-19 Immune Evasion

There are several strategies that are used by SARS-CoV and MERS-CoV to avoid response by the immune system. The pattern recognition receptors present in the body are able to recognize the pathogen-associated molecular patterns (PAMPs), which are the evolutionary conserved microbial structures. MERS-CoV and SARS-CoV synthesize double membrane-bound vesicles that lack PRRs. The viruses replicate within the vesicles and avoid the surveillance of their dsRNA.[41] In the infections caused by SARS-CoV and MERS-CoV, the IFN-α and IFN-β play protective roles, however, the IFN-I is inhibited in the mice infected with the disease.[42-43] The accessory protein 4a directly interacts with the dsRNA and restricts the induction of IFN at the MDA5 activation level. The nuclear transport of IFN regulatory factor 3(IRF3) and IFN- β promoter's activation is inhibited by the membrane proteins, ORF4a, ORF4b, and ORF5 of MERS-CoV.[44,45] Coronavirus also alters the presentation of antigens. For example- there is a down-regulation of the gene expression related to the presentation of antigen.[46] Hence it becomes more important to destruct the immune evasion of SARS-CoV2 for the development of specific drug and treatment. In Figure 1 are presented (A) COVID-19 entry and replication in Human, (B) Coronavirus Antigenic presentation during infection, (C) Cytokine storm in COVID-19, (D) Coronavirus immune evasion.[47]

Diagnosis of COVID-19

The clinical diagnosis is dependent on the auxiliary examinations, clinical manifestations, and epidemiological history. The auxiliary examinations include the blood culture, point of care testing (POCT) of IgM/IgG, enzyme-linked immunosorbent assay (ELISA), CT scan, and nucleic acid detection. The signs and clinical symptoms of the disease are atypical including, fever, cough, dyspnea, and viral pneumonia. This makes the necessity to carry out the auxiliary examinations for the diagnosis of COVID-19.

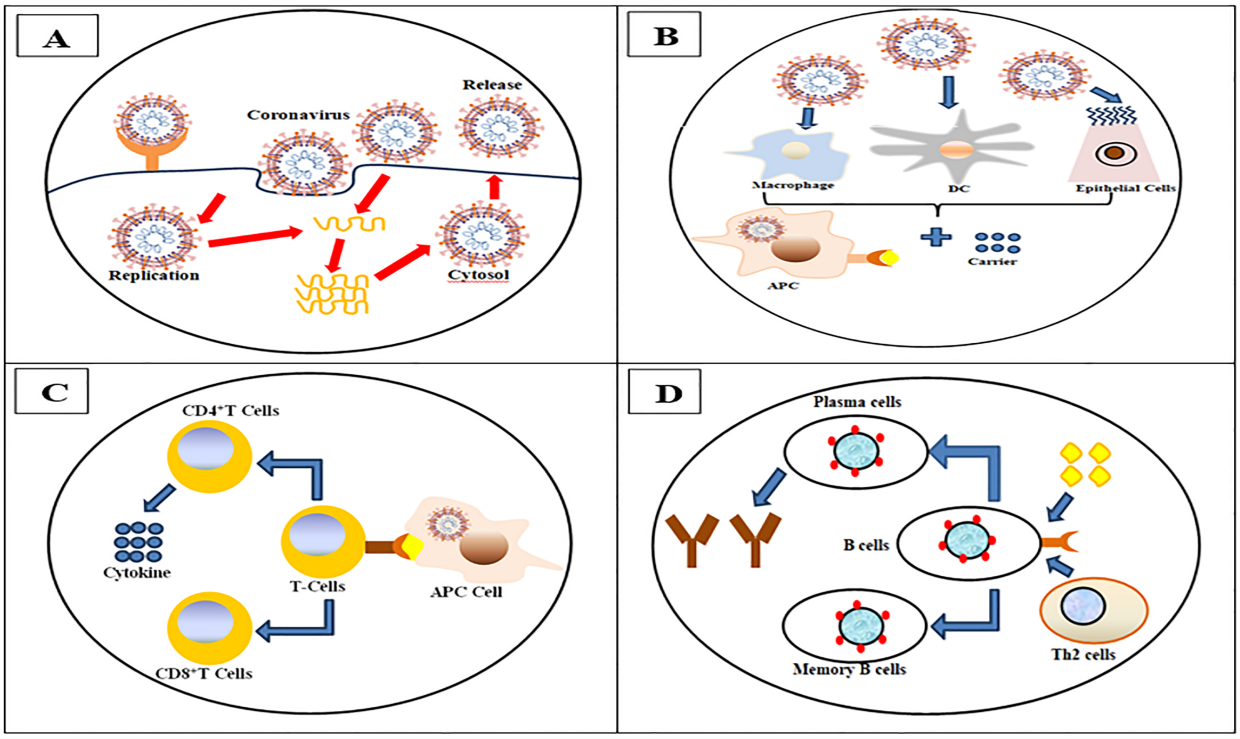

Figure 1. (A) COVID-19 entry and replication in Human, (B) Coronavirus antigenic presentation during infection, (C) Cytokine storm in COVID-19, (D) Coronavirus immune evasion.

Nucleic acid detection technology

The two principal nucleic acid detection technologies used in the diagnosis are high-throughput screening and real-time quantitative polymerase chain reaction. The authorities approved identification methods for SARS-CoV2 are high throughput sequencing of the whole genome and the blood culture.[1] The high throughput sequencing finds less application in clinical diagnosis as a consequence of its high cost and reliability on the equipment. This makes RT-qPCR the most efficient and common way for clinical diagnosis. Through RT-qPCR pathogenic viruses can be detected in the blood and respiratory secretions.[47]

Soon after the outbreak of SARS-CoV2 in Wuhan, China, several companies launched the detection kits based on the RT-qPCR for the clinical diagnosis. The Chinese Center for Disease Control and Prevention (China CDC) suggests the use of probes and primers in the region of N gene and ORF1ab by RT-qPCR. When the patient is positive for both the target regions, then only the patient is said to have the laboratory-confirmed infection.[48] Chu et al[48] mentioned two different RT-qPCR assays to detect ORF1b and N region of the genome of virus. In some other studies, the positive rate of SARS-CoV2 was found to be 91.7%, in the self-collected saliva sample of the patient by using RT-qPCR (non-probes SYBR based fluorescence signal). It can be used to conclude that the saliva can be treated as a specimen for the monitoring and diagnosis.[49] High specificity and sensitivity have been shown by RT-qPCR for SARS-CoV and MERS-CoV.[50] In another study, five patients were initially found to be negative for SARS-CoV2 in the results of RT-qPCR but presented positive chest CT findings. However, repeated swab test confirmed the presence of SARS-CoV2 infection.[51] The sensitivity of RT-qPCR depends on the type of sample, the number of collected clinical specimens.[52] So, it is important to enhance the detection rate of RT-qPCR. RT-qPCR has some other problems such as difficult nucleic acid detection operation, longer waiting time, biological safety hazards.

CT scans and RT-qPCR

Though the specificity of RT-qPCR is high, the false-negative rate cannot be surpassed. As a consequence of more sensitivity, clinicians have proposed CT scans as a necessary auxiliary diagnostic method. A combination of chest CT and RT-qPCR might be helpful in diagnosing if the result of RT-qPCR is negative.[53] For preliminary diagnosis and evaluation of disease severity, the high-resolution CT is essential.[54] There have been various studies that have analyzed the images of chest CT of COVID-19 patients.[55-56] The CT image of lung show S ground glass bilateral pulmonary parenchyma and pulmonary opacities with peripheral lung distribution and rounded morphology. In patients with MERS-CoV and SARS-CoV, the involvement of lung with peripheral predominance was seen and the chest CT depicted the progression of the disease with consolidation and ground-glass opacities.[57-58] As per these studies, CT scans have an important diagnostic value for COVID-19. Just like other diagnostic methods, this also comes with some shortcomings such as inefficient in distinguishing different types of hysteresis and pneumonia of abnormal CT imaging.

Taking the flaws of CT scans and nucleic acid detection into consideration, the clinical laboratories need to develop some immunological detection kits targeting viral antigens and antibodies. Nowadays, ELISA kits and POCT of IgM/IgG are available. These have been shown to have better detection rates as compared to nucleic acid detection and CT scans, however, published articles are still missing in favor of these. The sensitivity of SARS-CoV S-based IgG ELISA (58.9%) is lesser than that of SARS-CoV N-based IgG ELISA (94.7%)[49], however, the sensitivity of SARS-CoV2 IgG/IgM needs to be further studied. So, other specific and sensitive auxiliary methods need to be developed on an urgent basis for the diagnosis of COVID-19.

Current Treatment Strategies for COVID-19

Similar to MERS-CoV andSARS-CoV, there is no proven antiviral agent for COVID-19. Still, supporting treatment such as oxygen therapy, the use of broad-spectrum antibiotics and conservation fluid management are the main management strategy.[17] Based on the research on genomic organization[6] and molecular mechanisms of coronavirus infection,[59,60] various potential therapeutic targets are repurposed to existing antiviral agents.

Virally targeted inhibitors

One of the most promising antiviral drugs used against a broad range of RNA viruses, Remdesivir, an adenosine analog is used for targeting the RNA dependent RNA polymerase and blocking the synthesis of viral RNA. It is used for SARS/MERS-CoV infections in cultured cells,[61] mice,[62] and nonhuman primate models.[63-64] The intravenous administration of remdesivir by the Washington Department of Health led to the foundation that it provides protection from SARS-CoV2 infection.[65] In vitro, the combinations of chloroquine and remdesivir have been found to inhibit SARS-CoV2.[66] Hence it can be concluded that the other nucleoside analogs such as ribavirin, galidesivir, andfavipiravir may be clinically effective against SARS-CoV2.[59,67] Essential roles are played by chymotrypsin-like protease (3C-like protease, 3CLpro) and papain-like proteasein the replication of coronavirus. These can inhibit the host immune response.[68] Some other pleasing choices to counter the SARS-CoV2 are 3CLpro inhibitors, such as cinanserin[69] flavonoids,[70] and diarylheptanoids.[71] As a receptor of coronaviruses, the entry into the cell is mediated by ACE2. Hence, inhibiting the binding of the S protein with ACE2 can be a potential way against SARS-CoV2 infection.[12]

Antibody and plasma therapy

It has been revealed in studies that several convalescent patients have donated plasma against SARS-CoV2 just like

SARS-CoV[72] and MERS-CoV.[73] It has shown promising results in acute and severe SARS-CoV2 patients. One of the straight forward paths to neutralize SARS-CoV is through the production of recombinant human monoclonal antibody. The CR3022 is a coronavirus-specific human monoclonal antibody that can bind to the receptor-binding domain of SARS-CoV2.[74] Other alternatives for the treatment of SARS-CoV2 are monoclonal antibodies such as CR3014 and m396.[75]

Vaccines

For reducing the transmission, viral shedding, and severity of diseases, effective vaccines against SARS-CoV vaccines are required. This would help in controlling the coronavirus outbreaks. Several types of vaccines such as protein vaccines, subunit vaccines, recombinant DNA, inactivated virus, live-attenuated virus, and viral vectors are being studied against SARS-CoV and MERS-CoV.[76] However, these vaccines would require years to develop. Presently, there are several potential targets for SARS-CoV, however, more clinical and laboratory evidence needs to be explored. Researchers of WHO and Chinese scientists are working together to launch clinical trials. In those clinical trials, some new drugs such as stem cells and HIV drugs are being testified.

Future Directions to Restrict the Spread of the Disease

The current COVID-19 outbreak can be controlled by reducing the person-person contact extensively. The most susceptible population of our society like children, healthcare providers, and elderly people must be given special attention to protecting from the transmission of COVID-19. A general guideline for the interested person, researchers, medical staff and, health care providers has been published in 2019-nCOV.[77] The possible death in elderly people in the early outbreak of COVID-19 occurred due to their weak immune system, which allows the faster spread of viral infection.[78,79] The personal cleaning of face, body, and hand with decontaminating reagents on a regular basis should be provided by public services and facilities.[80] One needs to keep physical contact with contaminated and wet objects in mind. Special care is needed when dealing with feces and urine samples, as these can serve as one of the routes of transmission.[80-81] To prevent the further spread of the disease, china and several other countries including the US have implemented control measures involving travel screenings.[82] The potential transmitting routes and subclinical infections need to be taken into account for monitoring the epidemiological changes in COVID-19. Apart from these, the evolution, adaptation, and spread of virus between the humans and intermediates also need to be taken into account for monitoring epidemiological changes. There are several questions that need consideration, such as the number of people tested, the proportion of positive patients and whether the rate remains standstill or changes with time, and the list goes on. Another question that remains unanswered is the lack of pediatric cases either because of a lack of data or because of a lack of susceptibility. Some other questions which are related to pediatric cases are the number of cases that turned serious and the numbers of cases where the test was positive, however, symptoms were not developed. The answers to these basic questions would build a framework on which detailed and specific public health measures can be framed.

Conclusion

In conclusion, the interaction between the immune system and virus relies on the occurrence and development of SARS-CoV-2. The viral titer, viral load, mutation, viral type and viability of virus invitrodecide the viral factors.A person's immune system factor comprises HLA genes, neuroendocrine-immune regulation, nutritional status, physical status, gender and, age. All these factors provide whether an individual is contaminated with infection of virus, severity, and duration of the disease and reinfection. Aprecise diagnosis facilitates the control of the spread of disease, in the early stages of a pandemic. It is vital to develop safe, fast, accurate, and simple technologies to detect SARS-CoV-2. Undoubtedly, clinicians deliberately intercede in a couple of factors to develop a treatment, beneficial to human health which can help a patient to recover fast. Nonetheless, it must be conceded that medical intervention can achieve a 100% therapeutic response.

Acknowledgements

I am highly obliged to Prof. Arun Kumar Mishra, Department of Chemistry, IFTM University, Moradabad, for his valuable suggestions and guidance.

Conflict of Interest

The authors declare they have no conflict of interest.

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