Mechanism involved in the pathogenesis and immune response against SARS-CoV-2 infection

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Abstract SARS CoV-2, a causative agent of human respiratory tract infection, was first identified in late 2019. It is a newly emerging viral disease with unsatisfactory treatments. The virus is highly contagious and has caused pandemic globally. The number of deaths is increasing exponentially, which is an alarming situation for mankind. The detailed mechanism of the pathogenesis and host immune responses to this virus are not fully known. Here we discuss an overview of SARS CoV-2 pathogenicity, its entry and replication mechanism, and host immune response against this deadly pathogen. Understanding these processes will help to lead the development and identification of drug targets and effective therapies.

Keywords SARS-CoV-2 · Pathogenesis · Viral replication · Host immune response

Introduction

The first outbreak of coronavirus pandemic occurred in 2002–2003 by virus from the genus beta coronavirus-Severe Acute Respiratory Syndrome (SARS) and in 2011 by Middle East Respiratory Syndrome (MERS). The most severe outbreak of coronavirus was reported in Wuhan, Hubei, China in late December 2019, leading to a respiratory-related illness called the Corona Virus Disease 2019; COVID-19 caused by SARS-CoV-2 [1]. According to World Health Organization (WHO), till 16 February 2021, the number of infected people worldwide reached approximately 108,822,960, and about 2,403,641 people died globally since the start of the pandemic.

Coronaviruses (CoVs) are enveloped positive sensed, single-stranded RNA (+ssRNA) viruses belonging to the family Coronaviridae with the largest known RNA genome of approximately 29–31 kb [2]. These viruses are broadly divided into 4 groups: alpha, beta, gamma, and delta-CoVs. The causative agent of COVID-19, SARS-CoV-2 is a beta coronavirus. It shows great genome and structural similarity with SARS and MERS CoVs. Major symptoms of COVID-19 include- severe pneumonia, RNAemia, the incidence of ground-glass opacities, and acute cardiac injury similar to the symptoms of SARS-CoV and MERS-CoV infections [3]. Apart from respiratory distress, few COVID-19 patients showed symptoms of neurologic distress like- nausea, vomiting, and headache which is also marked in other coronavirus infections [4]. The studies from Europe and the United States highlighted a new hyper-inflammatory condition of a severe multi-system inflammatory syndrome (MIS-C) associated with COVID-19 in children [5]. Major symptoms include: multiple organ failure, hyper-ferritinemia, and cardiogenic or vasoplegic shock [5]. Furthermore, abnormal blood coagulation parameters due to irregularities of blood flow, vascular injury and abnormalities within the circulating blood leading to systemic coagulopathy in COVID-19 patients [6]. The SARS-CoV-2 genome comprises several structural and non-structural genes crucial for virus replication and infection. Three fourth part of SARS-COV-2 genome codes for open reading frames- ORF1a/b, ORF3a/b, ORF6, ORF7a/b, ORF8, ORF9a/b, and ORF10. The ORF1a/b genes codes for viral replicase polyproteins (pps) pp1a and pp1ab [2]. These pps are further processed to form sixteen...
mature non-structural proteins (Nsps), which play a crucial role in forming the replicase transcriptase complex (RTC). Rest of the genome codes for structural proteins spike (S), envelope (E), membrane (M), nucleocapsid protein (N), and other accessory proteins [2]. All of these proteins are necessary for viral replication and triggering host immune response. The immune response to CoV-2 is a key feature for the recognition and killing of virus-infected cells in the lower respiratory tract. Different studies related to the immune-pathogenesis of SARS-CoV-2 are currently being investigated [7, 8]. To date, enormous attempts have been made for the discovery of different drug candidate molecules as well as production of monoclonal antibodies against SARS-CoV-2 [3]. Furthermore, world-wide different vaccines are being developed using inactivated virus, non-replicating viral vectors, DNA/RNA and protein subtypes etc. [9, 10]. In this review, we have discussed the pathogenesis, including the entry, replication and the role of viral Nsps in immune evasion and viral replication (Table 1) associated with the SARS-CoV-2 infection. In addition, we have also summarized the different drug and vaccine candidates currently being used under clinical trials. A knowledge of mechanism involved in the pathogenesis and immune response triggered by SARS-CoV-2 will provide better insight for the development of effective therapeutics against different CoV infection.

**Pathogenesis and immune response**

**Entry and translation**

The initial step of CoV infection starts with virus attachment to host cells, followed by entry into the cytoplasm where replication occurs. The entry of enveloped viruses readily occurs at the cell surface by receptor binding or endocytosis mediated internalization. The CoV spike proteins (S) are class I fusion proteins that facilitate viral attachment and fusion of host and viral membranes. It interacts with the host cell receptors angiotensin converting enzyme.

| Nsps     | Function                                                                                                                                                                                                 | References |
|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 1 Nsp1   | Degradation of cellular mRNA                                                                                                                                                                           | [18–20]   |
|          | Blocks host cell translation and innate immune response                                                                                                                                                  |            |
| 2 Nsp2   | Precise function remains unknown                                                                                                                                                                         |            |
| 3 Nsp3   | Large transmembrane proteins with multiple domains- Ubiquitin like domain Ub1 and acidic (AC) domain that interacts with N terminal of polyprotein        | [66–68]   |
|          | An ADP- Ribose-1'-phosphatase (ADRP) activity that promotes expression of cytokines                                                                                                                   |            |
|          | PLpro/deubiquitinase domain associated with viral polyprotein cleavage and blocking host innate immune response                                                                                     |            |
|          | Domains with unknown functions- Ubiquitin like domain 2 Ubl2, nucleic acid binding domain NAB, SARS unique domain SUD and Y domain with unknown function |            |
| 4 Nsp4   | Transmembrane protein responsible for double membrane vesicle formation                                                                                                                                     | [69]      |
| 5 Nsp5   | 3CLpro/Main viral protease responsible for cleavage of viral polyprotein                                                                                                                                     | [70]      |
| 6 Nsp6   | Transmembrane protein                                                                                                                                                                                     | [68]      |
| 7 Nsp7   | Participate in nsp7-nsp8 hexadecamer formation to aids RdRp processivity                                                                                                                                  | [27]      |
| 8 Nsp8   | Hexadecamer formation along with Nsp7, serve as potential primase                                                                                                                                           | [27]      |
| 9 Nsp9   | Single-stranded RNA binding subunit                                                                                                                                                                         | [71, 72]  |
| 10 Nsp10 | Activates Nsp16                                                                                                                                                                                             | [73]      |
| 11 Nsp12 | RdRp                                                                                                                                                                                                   | [24]      |
| 12 Nsp13 | Helicase, liver pathogenesis along with Nsp1 and membrane protein (M), RNA 5'-triphosphatase activity associated with viral RNA capping                                                                 | [25]      |
| 13 Nsp14 | Bifunctional enzyme: 3'-5' exoribonuclease (ExoN) activity                                                                                                                                                  | [26, 29]  |
|          | Guanine-N7- methyltransferase (N7-MTase activities) for capping viral m-RNA                                                                                                                                  |            |
| 14 Nsp15 | Viral endonuclease                                                                                                                                                                                           | [74, 75]  |
| 15 Nsp16 | Evasion of host dsRNA sensors in macrophages                                                                                                                                                              | [73, 76]  |
enzyme-2 (ACE-2) in SARS-CoV and SARS-CoV-2, dipeptidyl peptidase-4 (DPP-4) in MERS-CoV and aminopeptidase N (APN) for HCoV-229E binding purpose [2]. After the binding of S protein with its receptor, the cleavage of viral S protein by host-derived proteases is carried out. The human transmembrane protease serine 2 (TMPRSS2) cleaves the SARS-CoV-2 S protein into two parts—S1 and S2, thereby exposing the receptor-binding domain (RBD) on S1 [2]. The S2 domain is a transmembrane domain comprising of fusion peptide and heptad region, facilitating fusion of viral and cellular membranes by undergoing conformational changes [11]. The presence of both viral S protein and protease TMPRSS2 in the brain, respiratory tract, heart, digestive tract, liver, and other body organs makes them potential targets of SARS-CoV-2 infection [12]. These findings suggest the potential of TMPRSS2 inhibitor- nafamostat mesylate as anti-CoV agent and the in-vitro analysis further supports this idea [13]. The entry of CoVs is a crucial step for anti-viral mechanisms. Drugs such as Umifenovir (Arbidol; Pharmstandard) [14, 15] is under clinical trial (https://clinicaltrials.gov/ct2/show/NCT04260594, https://www.clinicaltrials.gov/ct2/show/NCT04255017, https://clinicaltrials.gov/ct2/show/NCT04286503?term= NCT04286503&draw=2&rank=1) to prevent the entry of SARS-CoV-2.

The binding of CoVs is followed by the release of the viral genome into the host cell. The replication of the CoV genome begins with the translation of replicase gene that codes for two open reading frames (ORFs)—ORF1a and ORF1b and the translation product of ORF 1a includes Nsps from Nsp1- Nsp11 and for ORF 1b from Nsp12- Nsp16 along with Nsp1-11 altogether forming the RTC. The Nsp 11 becomes Nsp 12, leading to the extension of pp1a from pp11 [16]. After the translation of ORF 1a, the ribosome undergoes ribosomal frameshifting upstream of the ORF 1a termination codon. The ribosome employs an RNA pseudoknot and a slippery sequence (5’UUUAAC3’). In SARS-CoV-2, the efficiency of ribosomal frameshifting between ORF1a and ORF1b is approximately between 45% -70%. This implies that pp1a is expressed approximately 1.4–2.2 times more than pp1lab [17]. The release of all sixteen post-translationally and co-translationally released Nsps (Table 1) is mediated by proteolytic cleavage of pp1a and pp1b. This is carried out by two cysteine proteases -papain-like protease (PLpro) and chymotrypsin-like protease (3CLpro) located at Nsp3 and Nsp5, respectively [2]. The Nsp 1 is released early during translation so that it can inhibit the host translational machinery [18]. The Nsp1 mediated inhibition of host translation machinery occurs by two ways. 1. It interacts with the 40s subunit of the ribosome, preventing canonical mRNA translation [19]. 2. Binding of Nsp1 with the ribosome leads to endonucleolytic cleavage and host mRNA degradation [20]. The proteinase inhibitors target the main protease and pLpro, thereby preventing proteolysis of different pps. Several drugs such as lopinavir and ritonavir are proposed to be used against the SARS-CoV-2 infection [21–23].

The pLpro cleaves Nsp1/2, Nsp2/3, and Nsp3/4 boundaries, and the rest 11 Nsps are cleaved by the main protease [16]. These Nsps assemble to form a RTC in double membrane vesicles (DMVs) responsible for the transcription of sub-genomic RNAs (sgRNAs) along with replication of the entire viral genome. The Nsps consist of crucial transcriptional enzymes, e.g., RNA-dependent RNA polymerase (RdRp) encoded by Nsp12 [24] RNA helicase and RNA 5’-triphosphatase by Nsp13 [25] 3’-5’ exoribonuclease (ExoN) and Guanine-N7- methyltransferase (N7-MTase activities) for capping viral mRNA encoded by Nsp14 [26]. The viral RdRp has a catalytic subunit comprising of Nsp12 and two accessory subunits consisting Nsp7 and Nsp8. These accessory subunits confer the processivity required to replicate the long RNA genome of CoVs [27]. Also, The Nsp14 have an exonuclease domain with 3’-5’ exonuclease activity responsible for maintaining genome stability and excision of error-prone mutagenic nucleotides [28]. The capping machinery of CoVs comprises of Nsp10 acting as a cofactor Nsp13 acting as RNA 5’-triphosphatase followed by Nsp14 and Nsp16 acting as N7-methyltransferase and 2’-O-methyltransferase, respectively [29]. Viral RdRp is among the major drug targets of SARS-CoV-2. Many drugs such as remdesivir [30, 31], favipiravir [32], and ribavirin [22] (https://clinicaltrials.gov/ct2/show/NCT04276688) are being used to target viral RdRp for limiting SARS-CoV-2 infection.

**RNA replication**

The CoV replication is initiated by synthesizing an inter- mediated negative sensed mRNA, serving as a template for new positive sensed genomic RNA [33]. The key feature of CoVs is discontinuous replication forming nested 3’ and 5’ co-terminal sgRNAs [33, 34]. This discontinuous replication was first proposed by Sawicki and Sawicki [33]. During the synthesis of intermediate negative sensed single-stranded RNA (−ssRNA), the RTC gets interrupted when it encounters transcription regulatory sequences (TRSs) called TRS body, located at 3’ end of one-third of the viral genome. The synthesis of −ssRNA is reinitiated at the TRS adjacent to a leader sequence (TRS-L) located near the 5’ end of the genome [33, 34]. The CoV genome’s discontinuous replication involves interaction between TRSs of −ssRNA (TRS body) and TRS of +ssRNA (TRS-L). After the re-initiation of RNA synthesis, the negative strand copy of TRS-L is added to the −ssRNA to complete
its synthesis. This results in a set of sgRNAs, that are utilized as the template for transcription of +sgRNAs which are translated to structural and non-structural proteins [33, 34]. The presence of replication organelles is a characteristic feature of CoV replication. They provide adequate macromolecule concentrations required for RNA synthesis and prevent viral intermediates’ exposure to host pattern recognition receptors (PRRs) [2, 35]. The formation of RTC occurs on specialized compartments at ER membranes formed by deploying the protein-synthesis, packaging, and distribution machinery of the endoplasmic reticulum and Golgi complex complex [35]. After the transcription of genomic and sub-genomic mRNAs, translation of structural proteins like S, M and E takes place, which are first transported to the endoplasmic reticulum and later into an intermediate compartment of endoplasmic reticulum–Golgi intermediate compartment (ERGIC) (Fig. 1) [36]. The hijacked host proteins [36] and viral proteins (M, N, Nsp3, 4 and 6) [37] are used in this process that deploys the lipid-modifying enzymes and different cellular pathways. The CoV interferes with the host cell secretory pathway to transport and deliver the cargo virus protein at the final packaging site and budding after forming a complete virus particle [35–37].

**Immune response**

There is an active innate immune response followed by COVID-19 infection, which is evident from increased levels of C-reactive protein (CRP) and serum amyloid A (SAA) [38]. After entering the host, the viral RNAs are detected by class PRRs including Toll like receptors (TLR), Nod like receptors (NLR) and RIG like receptors (RLR) [39]. This activates the nuclear factor-κB (NF-κB) and interferon regulatory factor (IRF)-3/7 signaling leading to transcription of several pro-inflammatory cytokines like tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-17, and interferon (IFN)-γ. These cytokines are known to play a pivotal role in viral clearance [40]. An increase in levels of IL1-β, IL7, IL8, IL9, IL10, fibroblast growth factors (FGF2), granulocyte-colony stimulating factor (GCSF), granulocyte–macrophage colony stimulating factor (GMCSF), IFN-γ, interferon gamma-induced protein 10 (IP-10), monocytes chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet derived growth factor subunit B (PDGFB), TNF-α, and vascular endothelial growth factor A (VEGFA) causes hypercytokinemia, a hallmark of COVID-19 infection. Out of all the cytokines the concentration of certain cytokines like IL-1 β, IL-6, IL-7, IL-10, IP-10, and TNF-α determines the severity of infection (Fig. 1) [41] and contribute to severe inflammatory response named as cytokine storm.

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**Fig. 1 Overview of immune-pathogenesis in SARS-CoV-2**
which leads to severe conditions, such as acute respiratory distress syndrome (ARDS), disseminated intravascular coagulation or multiple organ failure. The increase in cytokine is followed by a rise in several chemokines working as monocytes and neutrophil recruiting mediators which includes – chemokine C-X-C motif ligand (CXCL)-8, CXCL1, CXCL-2, CXCL-10, C–C motif chemokine ligand (CCL)-2, and CCL-7 for recruitment of neutrophils.

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**Table 2** Different vaccine candidates against COVID-19

| Type | Vaccine | Organization | Clinical Phase | Registry index |
|---|---|---|---|---|
| Inactivated | CoronaVac/PiCoVacc | Sinovac | 3 | NCT04456595 | NCT04582344 | ChiCTR2000034780 |
| | Inactivated COVID-19 vaccine (Vero cells) | Wuhan Institute of Biological Products/Sinopharm | 3 | NCT04569881 | ChiCTR2000034780 |
| | BBIBP-CorV | Beijing Institute of Biological Products/Sinopharm | 3 | NCT0470609 | | |
| | Inactivated SARS-CoV-2 Vaccine | Institute of Medical Biology, Chinese Academy of Medical Sciences | 1/2 | NCT04530357 | |
| | QazCovid-in® | Research Institute for Biological safety Problems, Republic of Kazakhstan | 1/2 | NCT04530357 | |
| Non-Replicating Viral Vector | Ad5-nCoV | Cansino Biological Inc./Beijing Institute of Biotechnology | 3 | NCT04526990 | NCT04540419 | |
| | Gam-COVID-Vac | Gamaleya Research Institute | 3 | NCT04530396 | NCT04564716 | |
| | Ad26.COV2-S | Janssen Pharmaceutical Companies (Johnson&Johnson) | 3 | NCT04505722 | |
| | DelNS1-2019-nCoV-RBD-Opt1 | Beijing Wantai Biological Pharmacy/Xiamen University | 1 | ChiCTR2000037782 | |
| mRNA | mRNA-1273 | Moderna/NIAID | 3 | NCT04470427 | |
| | BNT162b2 and BNT162b1 | BioNTech/Fosun Pharma/Pfizer | 3 | NCT04368728 | |
| | CVnCoV | Curevac | 1/2 | NCT04515147 | |
| saRNA | ARCT-021 | Arcturus/Duke-NUS | 2 | NCT04480957 | |
| | LPN-nCoVsaRNA | Imperial College London | 1 | ISRCTN17072692 | |
| DNA | INO-4800 | Inovio Pharmaceuticals/International Vaccine Institute | 1/2 | NCT04447781 | |
| | AG0301-COVID19 and AG0302-COVID19 | Osaka University/AnGes/Takara Bio | 1/2 | NCT0463472 | NCT04527081 | |
| | nCov Vaccine | Cadila Healthcare Limited | 1/2 | CTRI/2020/07/026,352 | |
| | GX-19 | Genexine Consortium | 1/2 | NCT04445389 | |
| Protein Subunit | SARS-CoV-2 rS/Matrix-M1 Adjuvant (NVX-CoV2373) | Novavax | 3 | 2020-004,123-16 | NCT04533399 | |
| | Recombinant new coronavirus vaccine (CHO cell) | Anhui Zhigei Longcom Biopharmaceutical/ Institute of Microbiology, Chinese Academy of Sciences | 2 | NCT04466085 | |
| | KBP-COVID-19 / KBP-201 | Kentucky Bioprocessing, Inc | 1/2 | NCT04473690 | |
| | SARS-CoV-2 vaccine formulation 1/2 | Sanofi Pasteur/GSK | 1/2 | NCT04537208 | |
| | EpiVacCorona | FBRI SRC VB VEC OR, Rospotrebnadzor, Kolosovo | 1/2 | NCT04527575 | |
| | SCB-2019 | Clover Biopharmaceuticals Inc./GSK/Dynavax | 1 | NCT04405908 | |
| | COVAX-19 | Xavitec Pty Ltd/Medtox | 1 | NCT04453852 | |
| | SARS-CoV-2 Sclamp vaccine | University of Queensland/CSL/Seqirus | 1 | ACTRN12620000674932p ISRCTN51232965 | |
| | MVC-COV1901 | Medigen Vaccine Biologics Corporation/NIAID/ Dynavax | 1 | NCT04487210 | |
The precise molecular pathways involved in immune evasion of SARS-CoV-2 are yet to be explored; these mechanisms in SARS and MERS have been well established and are conserved. Thereby implying that SARS-CoV-2 also employs a similar mechanism to evade host immune response. The viral structural proteins N and M and non-structural proteins Nsp1, Nsp3b, Nsp4a, Nsp4b, Nsp15 aids the evasion of host immune response by the virus [53]. The SARS and MERS CoVs replicate within a double membrane vesicle that protects their RNA genome to get recognized by the cytosolic RIG and endosomal TLR PRRs [2, 35]. The induction of type I IFN response and anti-viral activity require TLR signaling, but SARS PLpro antagonizes the TLR pathway [54]. The PLpro of SARS-CoV-2 and SARS are 76% conserved, indicating that this mechanism is active in SARS-CoV-2 as well. Also, during viral replication the viral N protein is involved in SUMOylation of human hUbc9 protein [55] and also interacts with TRIM25, preventing the ubiquitination required for activation of RIG-I [56]. The Nsp1 of SARS induces IFN-β mRNA degradation [57], while ORF6 prevents IFN response by preventing the nuclear localization of IRF3 and STAT1 [58]. The CoVs ORF4a protein also acts as an IFN antagonist [59], and ORF4b, ORF5, and M protein prevent IRF3 translocation into the nucleus [60].

ORF3b of SARS-CoV-2 also acts as an IFN antagonist, which is more efficient than SARS ORF3b [61]. Also, ORF3b is a common antigen recognized by the antigen presenting cells during COVID-19, acting as an immune-dominant epitope [61]. In addition, ORF6, Nsp13, Nsp14, and Nsp15 of SARS-CoV-2 are suggested to inhibit nuclear localization of IRF3, thereby serving as IFN antagonists [62]. The SARS-CoV-2 M protein inhibits the production of type I and III IFNs by interacting with the RIG-I/MDA-5-MAVS signaling pathway [63]. Altogether these findings suggest the active involvement of different Nsps of CoV in avoiding immune recognition, antagonizing the anti-viral immune response, and promoting viral persistence.

Discussion

The coronaviruses have been a threat to humanity for the past several years, right from the first outbreak of SARS followed by MERS and most recently the SARS-CoV-2, the causative virus for the COVID-19 pandemic. These viruses possess a high rate of mutation, recombine and cross the species barrier causing severe menace. The window of time provided due to the incubation period increases the risk of transmission of COVID-19. The display of fewer symptoms along with asymptomatic patients contributing to a higher transmission rate. The significant events that can be used against COVID-19 include viral entry and replication, crossing the species barrier, and upholding long-term cellular and humoral immune response. Currently, different vaccines are being developed across the world using inactivated virus, non-replicating viral vector, DNA, mRNA and protein sub-units [9, 10] (Table 2). In the Indian sub-continent ‘COVAXIN/
BBV154’ [64] and ‘COVISHIELD/ChAdOx1 nCoV-19’ [65] are already being administered to a large set of the population. In addition, different drugs targeting the viral entry, viral enzymes such as protease and RdRp are also under clinical trials. Umifenovir (Arbidol; Pharmstandard) targeting the viral entry [14, 15], lopinavir and ritonavir targeting viral protease [21–23], and remdesivir [30, 31], favipiravir [32], and ribavirin [22] (https://clinicaltrials.gov/ct2/show/NCT04276688) targeting viral RdRp are being tested to limit COVID-19. The non-structural and accessory proteins of coronaviruses also remain unspecified. Further knowledge in the working mode of these proteins is required to better understand SARS CoV-2 and other coronaviruses. Finally, the unique replication behavior of these viruses comprising continuous and discontinuous transcription requires more study. In addition to vaccines and drugs, there are different monoclonal antibodies such as tocilizumab (Actemra) and sarilumab that are being used to counter the symptoms of COVID-19. The administration of tocilizumab, a monoclonal antibody, inhibits the human interleukin-6 receptor (IL-6R) ligand binding, which is a major pro-inflammatory cytokine involved in CoV-induced cytokine storm. It is recently approved in China to decrease lung difficulties in COVID-19 patients [3]. Sarilumab monoclonal antibody is being used to encounter IL-6 mediated inflammation (https://clinicaltrials.gov/ct2/show/NCT04327388, http://www.news.sanofi.us/2020-03-16-Sanofi-and-Regeneron-begin-global-Kevzara-R-sarilumab-clinical-trial-program-in-patients-with-severe-COVID-19).

The best indication of disease severity is directly correlated with the host immune response regulation, which is not fully explored. Further research on various aspects of innate and adaptive immune response right from serum proteins, antigen presentation, and cytokine response to the clinical progression of COVID-19 is needed. Finally, a detailed mechanism using different animal models and ex vivo studies will pave the way to a better understanding of SARS-CoV-2 biology and immunopathology, and will be crucial for the development of effective immunotherapeutics against COVID-19.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Consent for publication All the authors have provided their consent for the publication of his article.

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