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REVIEW

Literature review on virus and host response proteins in COVID-19: pathobiology, management, diagnosis and treatment

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Summary. – Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome caused by a novel strain of coronavirus (SARS-CoV-2) which was declared by WHO as a cause of global pandemic. By human-to-human transmission it caused severe damage to mankind with increased mortality rate worldwide. Coronavirus is a spherical enveloped virus with single stranded positive-sense RNA with a size of ~30 kilobases encoding various structural, non-structural and accessory proteins. The entry of coronavirus into the host cells is mediated by spike proteins. SARS-CoV-2 efficiently replicates in host cell and by evading immune surveillance, like innate and adaptive immune responses, in the host cells ultimately leads to increased virulence and disease outcome. In the current review, we highlighted the molecular insights of SARS-CoV-2 and its infection mechanism in the host cell via host-viral protein interactions based on currently available data up to 16thMay 2020 using various research literature databases. The diagnostics of SARS-CoV-2 is mainly done by RT-qPCR and serological tests. There is no effective treatment for COVID-19, however, few methods like plasma therapy and remdesivir treatment are reported to show promising results in improving patient's health and decreasing mortality rate.

Keywords: SARS-CoV; spike protein; nucleocapsid; COVID-19; interferon

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Abbreviations: ACE2 = angiotensin converting enzyme 2; COVID-19 = coronavirus disease 2019; COX-2 = cyclooxygenase 2; E protein = envelope protein; ER = endoplasmatic reticulum; IFN = interferon; IL = interleukin; M protein = membrane protein; MERS-CoV = Middle East respiratory syndrome coronavirus; N protein = nucleocapsid protein; nsp = non-structural protein; NF-κB = nuclear factor kappa B; NTD = N-terminal domain; RBD = receptor binding domain; rN = recombinant N protein; rS = recombinant S protein; RdRp = RNA-dependent RNA polymerase; RDT = rapid diagnostic test; SARS-CoV(-2) = severe acute respiratory syndrome coronavirus (2); S protein = spike protein; TM = transmembrane; TMPRSS2 = transmembrane protease serine 2; VLP = virus like particles

Introduction

In late 2019, coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China and has extensively spread in China and worldwide. According to the World Health Organisation (WHO) COVID-19 situation report by May 16th, the number of people infected has increased to 4.65 million cases with 312 thousand of deaths globally. In the majority of cases the infection is characterized by a range of symptoms including fever, cough, and general malaise (Chen *et al.*, 2019). The causative agent of this outbreak is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belonging to the family *Coronaviridae* which contains single-stranded positive-sense RNA viruses with a genome size of ~30 kilobases. Among the four common

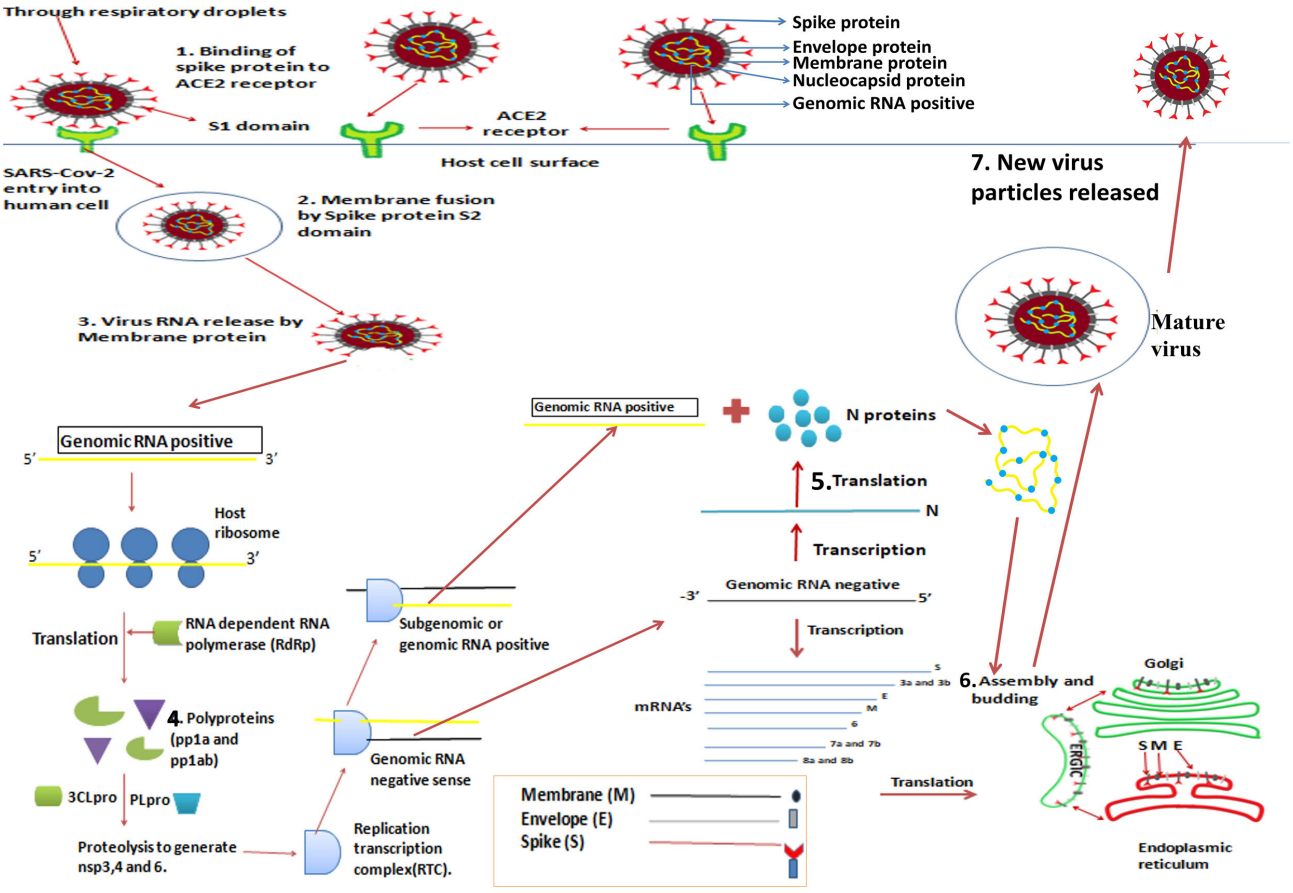

Fig. 1

Virus entry into the host cell and replication (Song *et al.*, 2019, Jiang *et al.*, 2020)

coronavirus genera: alpha, beta, delta, and gamma coronavirus, COVID-19 is caused by beta coronaviruses. Researchers proposed that SARS-CoV-2 has genomic sequence similar to SARS-CoV from 2002–2003 causing respiratory and enteric symptoms (Wrapp *et al.*, 2020). Similar to SARS-CoV, novel SARS-CoV-2 genome encodes several smaller open reading frames (ORFs) such as ORF1ab, ORF3a, ORF6, ORF7a, ORF7b, ORF8 and ORF10. These ORFs are predicted to encode for the replicase polyproteins, the spike (S) glycoprotein, envelope (E), membrane (M), nucleocapsid (N) proteins, accessory proteins, and non-structural proteins (nsp) (Kandeel *et al.*, 2020; Srinivasan *et al.*, 2020). SARS-CoV-2 encodes four structural proteins, spike (S), nucleocapsid (N), envelope (E) and membrane (M) protein, and sixteen non-structural proteins (nsp1-16) (Fig. 1). The structural proteins are responsible for the formation of viral capsid that surrounds and encapsulates the genome (Srinivasan *et al.*, 2020), virus entry into host cells and assembly of the virus (Li, 2016). Some members of coronavirus family encode additional membrane glycoprotein known as hemagglutinin-esterase protein (HE) (Li, 2016; Risco *et al.*, 1998). This review concerns the SARS-CoV-2 proteins and its interaction with host proteins and also discusses possible diagnostics and treatment.

Spike protein (S)

Coronavirus entry into host cells is mediated by spike glycoproteins that in the micrographs create an image resembling solar corona. SARS-CoV-2 spike glycoproteins (S) share 76% aa sequence identity with the SARS-CoV, while receptor binding domain (RBD) in the S glycoprotein molecule share 72% aa identity (Ou *et al.*, 2020). SARS-CoV-2 RBD has a stronger interaction with angiotensin converting enzyme 2 (ACE2) which is present on the surface of many human cells. SARS-CoV-2 affinity is 10- to 20-fold higher than that of SARS-CoV contributing thus to the higher infectivity and transmissibility (Wrapp *et al.*, 2020). Additionally, SARS-CoV-2 S glycoprotein has a

distinct loop with flexible glycyl residues replacing rigid prolyl residues in SARS-CoV (Chen *et al.,* 2020).

S protein of SARS-CoV consists of two functional subunits responsible for: 1.) binding to the host cell receptor (S1 subunit); 2.) fusion of the viral and cellular membranes (S2 subunit). S1 subunit is located within the N-terminal 14–685 aa of S protein, containing N-terminal domain (NTD), receptor binding domain (RBD), and receptor binding motif (RBM). S2 subunit contains fusion peptide (FP), heptad repeat 1 (HR1), heptad repeat 2 (HR2), transmembrane domain (TM), and cytoplasmic domain (CP) active in membrane fusion (Walls *et al*., 2020; Xia *et al.,* 2020). Prior to membrane fusion, the S protein is cleaved and activated to release the fusion peptide to mediate the fusion of envelope and host cell membranes (Gallagher and Buchmeier, 2001). The cleavage of S protein occurs to be between the S1 and S2 domains (S1/S2 site) and within the S2 domain proximal to the fusion peptide (S2' site). This cleavage has been proposed to activate the protein for membrane fusion via extensive irreversible conformational changes and results in the release of the viral genome into the host cell cytoplasm (Ashour *et al.,* 2020). Gui *et al.* (2017) reported that the S1 subunit also contributes to stabilisation of the membrane perfusion state of S2 subunit. By using molecular dynamics simulation of co-crystal structure of ACE2 and SARS-CoV-2 RBD, Zhang *et al.,* reported that in ACE2 receptor peptidase domain '1 helix plays major role in binding to SARS-CoV-2 RBD (Zhang *et al*., 2020). Further, Ou *et al.* (2020) demonstrated that fusion of SARS-CoV-2 S protein with human cell plasma membrane is mainly regulated by endocytosis using enzyme phosphatidylinositol 3-phosphate 5-kinase (PIKfyve), TPC2, TMPRSS2 and cathepsin. Madu *et al.* (2009), stated that by host proteases enzyme cathepsin L or cellular serine protease TMPRSS2, S protein is cleaved at the S2' site (Madu *et al.,* 2009; Hoffmann *et al.,* 2020). This proteolytic cleavage activates the membrane fusion protein in S2 subunit through extensive irreversible conformational changes (Belouzard *et al*., 2009). Using western blot analysis, Walls *et al.* (2020) also identified that in SARS-CoV-2 S protein, an unpredicted furin cleavage site at the S1 and S2 subunit, is cleaved during the process of biosynthesis and this is considered to be a main distinct feature that differentiates it from SARS-CoV. Blocking of furin cleavage site might affect the entry of SARS-CoV-2 into host cells (Belouzard *et al.,* 2009).

Diagnostics using S protein

As S protein mediates the SARS-CoV-2 entry into host cells, it could be considered as a target for developing virus neutralizing antibodies and a hot candidate for vaccine and other therapeutic designs. Currently to detect IgM and IgG antibodies against SARS-CoV-2 in patient's serum, two enzyme-linked immunosorbent assay (ELISA) kits based on recombinant SARS-CoV-2 S protein (rS) and nucleocapsid protein (rN) are being used. In the same study, Liu *et al.* (2020), suggested that due to high sensitivity, ELISA is preferred for detection of SARS-CoV-2 virus in serum samples. Additionally, the researchers evaluated the diagnostic feasibility of both rN and rS on 214 patients. Their results based on rS ELISA showed 77.1% IgM and 74.3% IgG antibodies. Liu *et al.* (2020) concluded that rS based ELISA has higher sensitivity than rN based ELISA. In another study, Qiang *et al.* (2020) used S protein to identify the infection risk of SARS-CoV-2. They used three algorithms such as amino acid composition, G-gap dipeptide composition and parallel correlation-based pseudo amino acid composition to recognize SARS-CoV-2 pattern and study concluded that seven patterns of human coronaviruses were found, including MERS-CoV, SARS-CoV, and SARS-CoV-2, indicating the S protein as a diagnostic marker for SARS-CoV-2 (Qiang *et al.,* 2020). In recent studies, researchers reported that SARS patients who recovered from viral infection have neutralizing antibody response mostly against S protein. These antibodies can be detected even after 24 months after infection. The experimental vaccine which includes both rS protein and inactivated virus is capable of neutralizing antibodies induction. The increased neutralizing antibody responses against S protein of SARS might give some protection against SARS-CoV-2 infection (Hoffmann *et al*., 2020). Since S protein is main target for neutralizing antibodies induction, it can be targeted for diagnosis and vaccine development. Vaccines such as recombinant subunit vaccine, DNA vaccine, mRNA vaccine containing viral S protein/sequence trigger strong immune response because of neutralizing antibody induction (Yang *et al.,* 2005).

S protein targeted treatment

S protein is one of the major components that determine the virulence of the virus, host range and its infection risk, thereby it can be considered as a main target for designing drugs against SARS-CoV-2. Virus entry into human cell and its fusion is mediated by endocytosis which in turn is regulated by the production of PI(3,5)P2 (phosphatidylinositol-3,5-bisphosphate) *via* phosphatidylinositol 3-phosphate 5-kinase enzyme. The study reported that the inhibition of enzyme phosphatidylinositol 3-phosphate 5-kinase by the drug apilimod, strongly inhibited entry of SARS-CoV-2 mediated by S protein. Therefore it indicates that PI(3,5)P2 pathway is involved in S protein regulation and might be considered as potential drug target for SARS-CoV-2 infection (Ou *et al*., 2020). Zhang *et al.* (2020) reported that blocking the SARS-CoV-2

Table 1. Structural proteins with their functions and drugs

No.	Protein	Function	Drugs
		Structural proteins	
1.	Spike	facilitates entry of the virus into host cells, through formation of protruding homotrimers	apilimod (Ou *et al.*, 2020) mer ACE2 PD peptide fragment (binder), EK1C4 (Xia *et al*., 2020), cathepsin L and TMPRSS2 (camosat mesylate) inhibitors (Uno, 2020), imatinib (Mulgaonkar *et al*., 2020), chloroquine (Ohe *et al.*, 2020)
2.	Nucleocapsid	packaging of viral genome into ribonucleoprotein complex, facilitating self-assembly	vaccines based on N220, N223 and N317 peptide; drugs targeting the protein's oligomerisation and its translocation into the nucleus (Cheung *et al*., 2007)
3.	Envelope	mediates replication of viral particles in host via protein-protein interactions; production of VLPs and viroporin	remdesivir (Wang *et al*., 2020), darunavir (Dong *et al*., 2020), mercaptopurine + melatonin; toremifene + emodin; sirolimus + dactinomycin; epoxyvibsanin C, macaflavanone, luzonoid, grossamide K, blestriarene, macaflavanone F and dolichosterone (Zhou *et al.*, 2020)
4.	Membrane	essential in assembling and releasing VLPs along with E protein; evasion of host's immune response against the virus; induction of apoptosis in host cells	vaccines developed from rabbit antisera obtained from immunization using SARS-CoV, and those that induce CTL activity (Samrat *et al*., 2020)

RBD binding to ACE2 with drugs might inhibit the entry of virus into human cells. In the same study, they proposed that peptide based binders can also be used to inhibit RBD-ACE2 interaction. 23-mer ACE2 PD peptide fragment containing amino acids that are proteinogenic, has been synthesized. Biolayer interferometry showed that 23-mer ACE2 PD peptide particularly linked with the SARS-CoV-2 RBD binds with low nanomolar affinity. This peptide binder could be a new potential drug for COVID-19 treatment as it inhibits the binding of SARS-CoV-2 S protein with hACE2 (Zhang *et al.*, 2020). To develop an effective anti-coronavirus prophylactics and therapeutics, the understanding of molecular mechanisms of viral infection need to be thoroughly studied. Xia *et al.* (2020) developed a SARS-CoV-2 S protein mediated cell-cell fusion assay and reported that SARS-CoV-2 shows efficient fusion capacity with plasma membrane compared to SARS-CoV. They produced lipopeptide EK1C4 derived from EK1 (coronavirus fusion inhibitor of SARS-CoV) and reported that EK1C4 can be considered as a potent protein mediated virus-cell fusion inhibitor also for SARS-CoV-2. EK1C4 also potentially inhibited the infection and replication of SARS-CoV-2 by targeting the HR1 domain in S2 subunit of S protein (Xia *et al*., 2020). Recent evidence suggested that SARS-CoV-2 uses cathepsin L and TMPRSS2 (cellular serine protease) for S protein priming, therefore using cathepsin L and TMPRSS2 inhibitor blocked entry of virus and it might also be considered as treatment option. Hoffmann *et al.* reported that camosat mesylate which is an approved serine protease inhibitor, inhibits TMPRSS2 activity and partially blocks SARS-CoV-2 entry into cells. The complete inhibition is reported to be attained only after both E-64d epoxy succinylpeptide (inhibitor of cathepsin L) and camosat mesylate were administered together (Hoffmann *et al*., 2020). Imatinib which is a BCR-ABL kinase and TMPRSS2 inhibitor was also identified as drug against SARS-CoV-2. TMPRSS2 inhibitor prevents virus entry whereas imatinib blocks the virus-host cell fusion (Dong *et al*., 2020). Chloroquine is another potential antiviral drug which inhibits the endocytosis which is the main process for viral fusion.

Nucleocapsid protein (N)

Coronavirus particles consist of a helical nucleocapsid structure or ribonucleoprotein (RNP), formed by the association between nucleocapsid phosphoproteins and the viral genomic RNA (Table 1). The nucleocapsid protein (N) is one of the four structural proteins of the thirteen major ORFs, expressed from the 3' end of the SARS-CoV-2 genome (Gordon *et al.*, 2020; Tan *et al.*, 2005). N protein of the SARS-CoV-2 shares almost 90% aa similarity with the N protein of the SARS-CoV. Among the four structural proteins, N protein is also one of the most abundant proteins present in the infected host cells (Tan *et al.*, 2005; Ahmed *et al*., 2020; Lee and Lee, 2015). Nucleotide sequence alignment done by Phan *et al.* (2020) showed that there are about 4 missense mutations in the N protein of SARS-CoV-2. Mutations play crucial role in forming helical ribonucleoprotein during packaging of the RNA genome and are involved in regulating viral RNA synthesis during replication, transcription and modulating the metabolism of infected cell (Cong *et al*., 2020).

Coronavirus N proteins contain three characteristic intrinsically disordered regions (IDRs) of N protein; the N-arm or N-terminal RNA-binding domain (NTD), central linker (CL), and the C-tail or C-terminal dimerization domain (CTD). NTD and CTD are the major structural and functional domains of the N protein. The most important function of the N protein NTD is RNA binding, while the CTD is involved in dimerization and the C-tail is necessary for the interaction with M protein and oligomerization which is identified by decreased ability to oligomerize upon removal of 40 aa from the domain of C-tail (Chang *et al.,* 2014). Studies have proven the presence of many phosphorylation sites in the central linker region, owing to the presence of arginine and serine residues (Prajapat *et al.,* 2020). Under normal conditions, the N protein is bound tightly to the viral genome and maintains stability. During the process of infection, N protein dissociates from the genome, which is then exposed and ready for replication (Chang *et al*., 2014).

Function of N protein

One of the major roles of the N protein is the packaging of the viral genome into the RNP complex, thus protecting the genome (McBride *et al*., 2014). Apart from this, it has also been observed in gastroenteritis coronavirus that the C-terminal domain of the M protein directly binds to the N protein and thereby, facilitates greater stability of the viral genome (Escors *et al.,* 2001). The N protein of the SARS-CoV is known to interact with human elongation factor 1-alpha, which in turn, quells the process of protein translation, by inhibiting the F-actin bundling (Zhou *et al*., 2008). In this manner, the virus impedes protein synthesis in the host, while promoting the synthesis of its own proteins (Lee and Lee, 2015). In addition, other studies have shown that the N protein interferes with host cell cycle proliferation by targeting the CDK-cyclin complexes and functions in two ways: 1.) directly, when N protein binds to cyclin D and suppress the activity of CDK4-cyclin D complex and CDK2-cyclin complex; 2.) indirectly, when N protein down-regulates the levels of CDK2 and inhibits its activity and disrupting the balance in the host cell cycle progression (Surjit *et al.,* 2006). Further, in mouse hepatitis virus, N protein acts as an enhancer of mRNA translation (Tahara *et al*., 1994).

N protein targeted diagnostics and therapeutics

High levels of N protein can be detected in the serum of SARS patients via antigen-capture ELISA based assay using monoclonal antibodies against the N protein of SARS-CoV. Liu *et al.* (2020) reported the usage of ELISA based kits that detect IgG and IgM antibodies using recombinant SARS-CoV-2 N protein. The study reported that out of 216 patients tested the success rate of diagnostics was 68.2% (146 patients) with rN-based IgM ELISA kit and of 70.1% (150 patients) with the rN-based IgG ELISA kit. N protein is also reported as a possible target for antiviral drugs, as it due to self-oligomerisation, is less-likely to induce drug-resistant variants. A set of B-cell and T-cell epitopes from the SARS-CoV-2 patients derived from S and N protein, were found to be identical to SARS-CoV, without mutations, hence, facilitating easier immune targeting (Ahmed *et al*., 2020). N220 peptide has been shown to trigger an immune response by activating the cytotoxic T-lymphocytes *in vitro*, due to an innate binding affinity to major histocompatibility complex (MHC) class I molecules, making it possible candidate for vaccine. N223 and N317 peptides, as vaccines, have also been found to induce cytotoxic T-lymphocytes, but aren't as profound as that produced by the N220 peptide (Cheung *et al*., 2007). Leung *et al.* (2004) reported that IgG-mediated response was targeted at N protein in patients infected with SARS virus. Chenavas *et al.* (2013) explored the possible way of targeting the N protein for therapeutic purposes by inhibiting the oligomerization of N protein *via* introducing higher-order oligomers or blocking the exchange domain-binding groove of the N protein.

Envelope proteins (E)

The envelope (E) protein is the smallest protein of SARS-CoV-2 associated with the regulation of the virus life cycle, such as budding, assembly, envelope formation and pathogenesis (Fig. 1) (Ruch and Machamer, 2012). Envelope protein plays a major role in ion channel activity by a protein called viroporin involved in pathogenesis (Gupta *et al*., 2020). E proteins interact with other SARS-CoV-2 structural proteins and host cell proteins (Schoeman and Fielding, 2019). Mostly E proteins are located at the intracellular trafficking sites like Golgi apparatus, ER and mainly in ER-Golgi intermediate compartments where they are involved in assembly of SARS-CoV-2 and budding (Nieto-Torres *et al*., 2011). In recombinant coronaviruses lacking E protein, reduction in virus quantity and crippled maturation of virus has been shown, thereby indicating that E protein plays major role in the virus production and maturation (Nieto-Torres *et al*., 2011) (Fig. 1).

E protein contains 76–109 aa ranging from 8.4 to 12 kDa in size and in virion it is present at a low amount. It has three regions, small 7–12 aa long hydrophilic amino terminus, 25 aa long hydrophobic transmembrane domain (TMD) and remaining majority of amino acids in long hydrophilic carboxyl terminus (Li *et al.,* 2014). TMD

consists of nonpolar amino acids like valine and leucine resulting in strong hydrophobic property of the E protein (Wu *et al.,* 2003). By computational approach, Li *et al.* (2014) predicted the secondary structure of E protein and reported that carboxyl terminus contains conserved proline residue in a binding motif. After the mutation of proline the localization of E protein in Golgi apparatus is disrupted, while replacement of proline mutation leads to re-localization of the protein to plasma membrane (Li *et al.,* 2014; Cohen *et al.,* 2011). Interestingly, envelope amino acids at (N) terminus are negatively charged, whereas carboxyl (C)-terminal tail is positively charged and the middle region remains uncharged, resulting in zero net charge (Schoeman and Fielding, 2019). Kandel *et al.* (2020) calculated nucleotide composition of SARS-CoV-2 E protein by relative synonymous codons usage (RSCU) method and reported that T nucleotides were abundant in higher percentage (34.5% to 40.4%) followed by nucleotide A (21.52% to 25.7%). Nucleotide percentages in the E protein were in the order G<C<A<T (Ahmed *et al*., 2020). When compared to SARS-CoV, SARS-CoV-2 has lesser GC percentage (38.2%) and higher AT percentage (63%).

In recent studies, it has been found that the E protein of SARS-CoV contains a binding motif called PDZ that is located at the carboxyl terminus (Teoh *et al*., 2010). The PDZ domain is a protein-protein interaction module that can bind to the C-terminus of target proteins such as the cellular adapter proteins involved in host-cell processes which are important for viral infection (Schoeman and Fielding, 2019). SARS-CoV-2 has 94.7% genetic similarity with SARS-CoV and is reported to also exhibit the same mechanism in virus maturation and its pathogenesis regulated by E protein (Schoeman and Fielding, 2019). E protein of SARS-CoV-2 has most efficient interaction with membrane protein (M), thus M-E co-expression is sufficient to form and release infectious virus like particles (VLP). Interaction between E and M protein is regulated by C terminal region of both proteins on the ER-Golgi intermediate compartment complex (ERGIC) cytoplasmic site (Schoeman and Fielding, 2019). VLP production is further increased by interaction with N protein. Studies reported that E-N interaction is also mediated by C-terminus of both proteins and when the last residue of C-terminal region is deleted, interaction between the E and N protein was reduced (Schoeman and Fielding, 2019).

E proteins interact with host proteins as they depend on the host protein interaction for its propagation. The first host protein that was found to interact with SARS-CoV E protein was B-cell lymphoma-extra-large (Bcl-xL) which is the anti-apoptotic protein (Yang *et al.,* 2005). Interaction between host and E protein is mediated through the PDZ domain. After binding with host protein, viruses activate host signalling pathways which are responsible for replication, propagation and pathogenesis (Gupta *et al.,* 2020). Further, research found only five host proteins that interact with E proteins, i.e Bcl-xL, syntenin, PALS1, stomatin and sodium/potassium (Na+/K+) ATPase α-1 subunit (Yang *et al.,* 2005).

E protein targeted diagnostics and therapeutics

The presence of SARS-CoV-2 in specimens was confirmed by RT-qPCR and next generation sequencing methods. For rapid detection using RT-qPCR, the probes and primers which target E protein sequence became main diagnostic target for SARS-CoV-2. Since the E protein plays a major role in virus production, maturation and pathogenesis, targeting this protein would inhibit infectious virus particle production. Several studies also reported that in the absence of E protein SARS-CoV-2 becomes attenuated and can be considered as a key biomarker of SARS-CoV-2. Gupta *et al.* (2020) used a computational approach to identify phytochemicals that can be used as inhibitors of SARS-CoV-2 E protein and tested for about 4153 phytochemicals using Drugmint server and CASTp server. They selected 10 phytochemicals based on their binding energies: belachinal, macaflavanone E, vibsanol B 15-epoxyvibsanin C, macaflavanone, luzonoid, grossamide K, blestriarene, macaflavanone F and dolichosterone. Among them, macaflavanone E, belachinal and vibsanol B binds to the E protein of SARS-CoV-2 and decreases the E protein production. The above mentioned three phytochemicals passed the Lipinski's rule of five and ADMET test (absorption, distribution, metabolism, excretion and toxicity) and could be used as potential drugs against SARS-CoV-2 (Gupta *et al.,* 2020). Recently, antiviral drug remdesivir received a lot of attention as it inserts into the viral RNA and limits viral replication and thereby reduces the spread of the SARS-CoV-2 virus. Remdesivir drug also targets E protein and acts by inhibiting the ability of E protein gene to replicate and make viral copies; thereby used as potential drug to inhibit SARS-CoV-2 (Wang *et al*., 2020). Dong *et al.* (2020) also reported that the drug darunavir, a protease inhibitor of HIV, effectively inhibited the viral replication by targeting envelop protein as it plays major role in replication. Zhou *et al.* (2020b) used network proximity analyses in order to understand the interaction between the virus and host proteins and also to identify drug targets with respect to the E protein. They have showed that three drug combinations such as mercaptopurine and melatonin, toremifene and emodin, and sirolimus and dactinomycin act as a potential drugs against SARS-CoV-2. Thus, research studies suggested that E protein can be targeted for both diagnostic and therapeutic applications.

Membrane protein (M)

The membrane (M) protein is a glycoprotein, consisting of around 221 aa, essential for maintaining the shape of the viral envelope (Fang *et al.,* 2007). It is composed of three parts: a short N-terminal glycosylated domain, three transmembrane domains (TM) and a carboxy-terminal domain (Ujike and Taguchi, 2015). The M gene, that codes for the M protein has been found to be composed predominantly of T (29.9%–31.9%), and A nucleotides (24.4%–25.6%). TM domains contain about 80 aa that facilitate the protein's attachment to the membrane. Ma *et al.* (2008) reported that the second (46–68 aa) and third (78–100 aa) segment of TM domain are considered to be primary helices that essentially contribute to membrane penetration. Moreover, the first (14–36 aa) segment for its stabilisation interacts with the other two segments. The second and third TM segments are more efficient in protein transfer from the cytoplasm into the membrane of the ER, in comparison to the first TM segment (Ma *et al.,* 2008). An interesting characteristic of M protein is that when the process of maturation is absent, it starts accumulating in the ER membranes (Rottier *et al.,* 1986).

Functions of M protein

M protein is an important structural protein providing the shape of the viral envelope. It is considered as the central organiser of CoV assembly, interacting with all other virus structural proteins (Masters, 2006). The membrane protein interacts with two stretches of aa, 168–208 aa and 211–254 aa of SARS-CoV N protein. The importance of this interaction is 1.) to restrict the migration of the M protein within the budding compartment or the ERGIC at the same time, 2.) to envelope the nucleocapsid (Narayanan *et al.,* 2000). The envelope assembly is reported to be supported by M-M interactions by forming large complexes. However, it also interacts with the S protein in order to be maintained in the ERGIC, a feature that is unique to coronaviruses (Schoeman and Fielding, 2019). Studies showed that the M protein is also essential for the assembly of viral like particles (VLPs) (Hsieh *et al.,* 2008). Assembly and budding of VLPs is facilitated by the interaction between the M and E proteins which is sufficient for the assembling of viral particles, but can be enhanced by N protein (Narayanan *et al.,* 2000). Deletion mapping data showed that second or third TM segment plays a major role in the interaction of M and E proteins. Both, E and M proteins are essential for the release of VLPs (Schoeman and Fielding, 2019). Fang *et al.* (2007) reports that the carboxyl terminal end of M protein suppresses the activity of nuclear factor kappa B (NF-κB), possibly through direct interaction with IκB kinase (IKKB). The NF-κB is also known to regulate the production of cyclooxygenase-2 (COX-2) which is essential in inducing immune responses. This study also suggests that the SARS-CoV M protein has the ability to cause the down-regulation of COX-2 and evade the host's immune response. Since NF-κB is involved in the body's immune response against a pathogen, its interaction with M protein makes the host much more vulnerable to SARS-CoV infections. Western blot analysis showed that there was a reduction in the translocation of p50 and p65 units of the NF-κB pathway, as a result of M protein expression. Another study suggests that the M protein of SARS-CoV can also tamper with the Akt-mediated cell survival pathway, and induce apoptosis in *Drosophila* (Chan *et al.,* 2007).

M protein targeted diagnostics and therapeutics

The diagnostic and therapeutic aspects of the M protein aren't fully understood and are yet to be explored. However, in the study conducted by Pang *et al.* (2004) rabbit antisera was prepared using the complete SARS-CoV M protein. Under *in vivo* conditions, the neutralisation titre of the obtained antisera was observed to be more than 1:128, thus hinting at the possibility of the development of an efficient vaccine from M protein (Okada *et al.,* 2006). In an experiment conducted by Okada *et al.* (2006) cDNA of the M protein from three strains of SARS-CoV was cloned into pcDNA vectors and expressed in *E. coli* as well as eukaryotic systems. Following this, the vector with the cloned cDNA fragment was introduced into mice and the neutralising antibodies obtained were produced and assayed. In addition to this, the system's immune response was studied using human alveolar epithelial cells or T7 cells, by enabling them to express the M protein antigen (Okada *et al.,* 2006). Wang and Liu *et al.* (2016) suggested the role of the M protein as a cytosolic pathogen-associated molecular pattern (PAMP) and inducer of the interferon-B production that is independent of TNF receptor associated factor 3 (TRAF3). Some of the drugs targeting structural proteins are listed in the Table 1.

Non-structural proteins (nsp)

After receptor recognition, the nucleocapsid with viral genome is released into the cytoplasm of the host cells. The terminal 5' two-thirds of the RNA genome contain two open reading frames (ORF 1a/ORF1b) which produce two viral replicase polyproteins (PP1a and PP1ab) that are auto-proteolytically cleaved into 16 non-structural proteins (nsp1-16) which self-assemble into the viral replication and transcription complex (RTC). The RTC consists of multiple enzymes, including the papain-like protease (nsp3), the main protease (nsp5), the nsp7-nsp8

primase complex, the primary RNA-dependent RNA polymerase (nsp12), a helicase/triphosphatase (nsp13), an exoribonuclease (nsp14), an endonuclease (nsp15), and N7- and 2'O-methyltransferases (nsp 10 and 16) (Gordon *et al.,* 2020). In general, these 16 non- structural proteins are joined together and contain RNA-dependent RNA polymerase enzyme (RdRP) which is responsible for replication/transcription of viral RNA (Risco *et al.,* 1998). Mostly SARS-CoV-2 proteins are processed by main protease (Mpro) and papain-like protease to yield non-structural proteins (Kandeel *et al.,* 2020). The Mpros of the SARS-CoV and the SARS-CoV-2 share an identity of 96% aa. Mpro which is encoded by ORF1 has 11 cleavage sites (Hilgenfeld, 2014; Thiel *et al*., 2003). Additionally, papain like protease (PLP) is responsible for processing non-structural proteins (nsps) 1 to 3 (Hilgenfeld, 2014). Kandeel *et al.* (2020) reported nucleotide composition of two important non-structural proteins including Mpro (nsp5) and RdRP (nsp12). In RdRP of SARS-CoV-2, T and A nucleotides are abundantly present and RdRP contains more pyrimidines than purines while Mpro has low G3s frequencies (nucleotides at the third position of codon) (Kandeel *et al*., 2020).

Nsp1 (cellular saboteur) plays major role in the interaction between the virus and innate immune response and protects the virus from antiviral proteins. In recent studies, the mutation in nsp1 showed to modulate the process of replication and pathogenesis (Dhama *et al.,* 2020). The nsp2 is known as mystery protein for which the function is not yet known, but it regulates the function of nsp1 and nsp3. The nsp3 (untagging and cutting) is a large protein that has many conserved sites that are responsible for PLP activity and is also involved in the synthesis and processing of viral RNA (Graham *et al*., 2008). Nsp3 has two roles, the first one is cutting the other viral proteins and the second one is untagging the antiviral proteins from the viral surface, thereby reducing the ability of the host cell to fight against the virus. The proteins nsp4 (double membrane vesicles maker) and nsp6 (double membrane vesicle factory) are involved in formation of double membrane vesicles filled with the fluids around the ER region where new virions are constructed. Nsp4, a membrane protein with numerous hydrophobic amino acids along with nsp3 and 6 proteins are involved in viral replication in cytoplasm (Sakai *et al.,* 2017). Protein nsp5 called protein scissors is involved in cutting the loose viral proteins similarly to nsp3. Nsp7 and nsp8 proteins are called copy assistants and help nsp12 to synthesize new copies of RNA in SARS-CoV-2. Protein nsp9 is involved in forming tiny channels inside the nucleus of infected cell, however only few information of the tiny channel function is available. Nsp10 called genetic camouflage protein works with nsp16 to protect the viral genes from the attack of antiviral proteins synthesized in the healthy human cell (Sakai *et al*., 2017). Nsp12 known as copy machine contains RdRp (RNA-dependent RNA polymerase) which is responsible for developing new copies of the viral genome (Zhou *et al*., 2020a). Protein nsp13 (unwinding RNA protein) is responsible for unwinding the viral RNA. Protein nsp14 (viral proof-reader protein) is responsible for cutting the wrong nucleotides which were added during genome replication by nsp12 (Eckerle *et al.,* 2010). Researchers reported that nsp15 (cleaning up protein) might clean the remaining or leftover RNA to protect it from the antiviral defences. Protein nsp16 works together with nsp10 to protect the viral genes. Over all, the different non-structural proteins are also reported to possess prominent role in the replication and pathogenesis of SARS-CoV-2 infection.

Therapeutic applications targeting non-structural proteins

Non-structural proteins are widely used for developing antiviral drugs for SARS-CoV-2. It was reported that nucleoside analogues such as favipiravir, ribavirin, remdesivir generally interact with the nucleotide synthesis of virus and inhibit the replication of viral genome by targeting the RdRp in nsp12 (Dong *et al.,* 2020; Ahn *et al.,* 2020). These drugs are also used in the combination with other antiviral proteins specifically with interferon and thereby reduce viral synthesis and replication and could be considered as the most promising agents for treating patients with SARS-CoV-2. However, safety and efficacy of remdesivir and favipiravir are not yet confirmed in clinical trials of SARS-CoV-2 patients. Additionally, researchers reported that protease inhibitors such as lopinavir and ritonavir can be considered as potential antiviral drugs against SARS-CoV-2, as they can reduce the replication of SARS-CoV-2 viral genes by targeting and interacting with the enzymes that are associated with cleavage of viral proteins, however the efficacy of this protease inhibitor in SARS-CoV-2 is still questionable (Ahn *et al.,* 2020). Structure-based selection of drugs was performed recently to identify protease inhibitors of SARS-CoV-2 and identified the drugs with better inhibition potency and binding capacity: indinavir, tipranavir, atazanavir, ritonavir, darunavir, amprenavir, cefixime, cefditoren, clarithromycin, erythromycin and azithromycin. Ohe *et al.,* (2020) also used similar structure-based selection of drugs to identify best protease inhibitors such as clarithromycin, erythromycin and azithromycin which possess better antiviral effects thanks to its anti-inflammatory and immunomodulatory effects. Clinical studies have reported that hydroxychloroquine combined with azithromycin could be effective against COVID-19 (Ohe *et al.,* 2020). Non-structural proteins were also considered for developing

Table 2. Non-Structural proteins with their functions and drugs

No.	Protein	Function	Drugs
		Non-structural proteins	
1.	nsp1	confers protection from host's immune response, mediates pathogenesis	remdesivir, favipiravir, ribavirin (de Lima Menezes and da Silva, 2020)
2.	nsp2	presumed to regulate the functions of nsp1 and nsp3	protease inhibitors such as lopinavir and ritonavir (Mahdi *et al.*, 2020)
3.	nsp3	contains multiple domains, including one for papain-like protease, synthesis and processing of viral RNA, untags antiviral proteins from viral surface	
4.	nsp4	double membrane vesicles formation to facilitate generation of copies of the virus, along with nsp6	macrolides (MAC) such as clarithromycin, erythromycin and azithromycin (Ohe *et al.*, 2020)
5.	nsp5 (or) main protease (M^{pro})	essential in cleavage of pp1a and pp1ab polyproteins	hydroxychloroquine combined with azithromycin (Gautret *et al.*, 2020)
6.	nsp6	double membrane vesicles formation to facilitate generation of copies of the virus, along with nsp4	
7.	nsp7	also known as copy assistants; assist nsp12 in generation of viral RNA	
8.	nsp8		
9.	nsp9	generates tiny channels in the nucleus of an infected host cell	
10.	nsp10 (or) genetic camouflage	protects the virus from antiviral proteins attack along with nsp16	
11.	nsp11	no function reported	
12.	nsp12 (or) RNA-dependent RNA polymerase enzyme (RdRP) (Hillen *et al.*, 2020)	replication/transcription of viral RNA	
13.	nsp13 (or) helicase	unwinding of viral RNA	
14.	nsp14	proof-reading the activity of nsp12	
15.	nsp15	presumed to clean any leftover RNA to avoid host immune response	
16.	nsp16	obscuring viral genes from host antiviral responses	

vaccines. It was reported, that live attenuated SARS vaccine designed using reverse genetic strategies to inhibit the nsp14's exonuclease activity and thereby reduce the virus expression (Dhama *et al.*, 2020). Prajapat *et al.* (2020) reported the usage of lopinavir-ritonavir combination, in inhibiting viral proteases. Apart from this, both lopanivir and retinovir bind to the same target site of Mpro and inhibit the protease (Liu *et al.*, 2020). Analysis by Lung *et al.* using molecular docking methods, revealed that the active site of the SARS-CoV-2 RdRp was able to facilitate the docking of the aflavin, making it a candidate for antiviral drug (Lung *et al.*, 2020). Another study reported that compounds like IDX-184 and setrobuvir can bind tightly to the active site of the RdRp, although their efficiency of inhibiting its activity is unknown (Lung *et al.*, 2020). Some of the drug targets of non-structural proteins are listed in the Table 2.

Host proteins in response to SARS-CoV-2

The SARS-CoV-2 viral genome is 75 to 80% identical to the SARS-CoV and has similar pathogenic mechanisms to SARS-CoV and MERS-CoV (Prompetchara *et al.*, 2020). Studies have shown that the increased release of cytokines by host immune system in response to the viral infection and/or secondary infections can result in a cytokine storm and sepsis symptoms, leading to death in 28% of sever COVID-19 cases. Additionally, uncontrolled inflammation causes multi-organ damage leading to organ failure, especially of the cardiac, hepatic and renal systems (Tay *et al.*, 2020). The entry of the virus into the host cell is triggered by the recognition of virus-specific components *via* pattern recognition receptors (PRRs) including Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) that distinguish the viral genome from the host genome mainly by DNA, single-stranded RNA (ssRNA), double stranded RNA (dsRNA) and surface gly-

coproteins (Lee *et al*., 2019). The proteins like melanoma differentiation-associated protein 5 (MDA5), laboratory of genetics and physiology 2 (LGP2), and oligomerization domain-containing protein-2 bind to viral RNA in the cell cytosol *via* RNA binding motifs, followed by interaction of signalling domain with downstream proteins and resulting in the activation of signalling events to counter the viral infections (Jensen and Thomsen, 2012). Virus infection triggers the secretion of IFNs and cytokines to enhance innate immune responses *via* autocrine and paracrine mechanisms and induce expression of interferon stimulated genes (ISGs) that inhibit viral replication inside the host cell. The secreted cytokines and chemokines are also critical for inducing effective adaptive and memory immune responses. In most mild cases, type I IFN is highly effective in inhibiting viral replication during early short periods of viremia. BALB/c mice infected with SARS reported to show enhanced viral replication accompanied by a delayed IFN-I response, which in turn promotes the accumulation of pathogenic inflammatory monocyte-macrophages, resulting in elevated lung cytokine/chemokine levels, vascular leakage, and impaired virus-specific T cell responses (Channappanavar *et al.,* 2016). IFN stimulates immune responses by activation of JAK/STAT pathway which forms complex with interferon regulatory factor (IRF) and is transported to the nucleus to stimulate the expression of over 300 IFN-stimulated genes (ISGs) that inhibit viral replication (Teijaro, 2016). Baricitinib, another inhibitor of cytokine-release, is a Janus kinase inhibitor (anti-JAK) and it seems to act though its affinity for AP2-associated protein AAK1, thereby reducing SARS-CoV-2 endocytosis (Richardson *et al*., 2020)

Interferon-inducible transmembrane proteins (IFITMs) are key ISGs induced by interferon and interfere with virus entry. It has been reported that interferon-induced transmembrane protein-3 genetic variant rs12252-C is associated with disease severity in COVID-19 (Zhang *et al.,* 2013). However, in severe forms of viral replication, the ability of type I IFN to inhibit viral replication is overwhelmed by excess release of pro-inflammatory cytokines such as IFN-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-α, TGFβ, and chemokines CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10 from immune effector cells and cause hyper inflammation (Cavalcanti *et al*., 2012). Importantly in response to SARS-CoV-2, NHBE cells elicited strong chemotactic and inflammatory response, indicated by the expression of CCL20, CXCL1, IL-1B, IL-6, CXCL3, CXCL5, CXCL6, CXCL2, CXCL16, and TNF-α (Blanco-Melo *et al*., 2020). The post-mortem studies of COVID-19 patients suggested that COVID-19 caused an inflammatory response in the lower airway and led to lung injury (Eketunde *et al.,* 2020). In COVID-19 patients, few cytokines and chemokines were observed in the plasma including IL-1, IL-2, IL-4, IL-7, IL-10, IL-12, IL-13, IL-17, GCSF, macrophage colony-stimulating factor (MCSF), MCP-1, hepatocyte growth factor (HGF), IFN-γ and TNF-α (Blanco-Melo *et al*., 2020). Interferon-induced with helicase C domain 1 (IFIH1) gene encodes a cytoplasmic receptor critical for viral RNA sensing, and belongs to a family of helicases. Loss of IFIH1 and Z-DNA-binding protein provided an evolutionary advantage by reducing inflammation-induced damage to host tissues and thereby contributes to a switch from resistance to the tolerance of viral infections (Fischer *et al*., 2020).

The role of NF-κB in interferon response during viral infection is well reported. The NF-κB family of proteins are significant for both innate and adaptive immunity determination. NF-κB activity has been demonstrated to be important for the inflammatory response and pathological condition induced by respiratory viruses such as SARS-CoV (Fischer *et al.,* 2020) and influenza A virus (Oslund and Baumgarth, 2011). The SARS-CoV nsp1, nsp3a, nsp7a, spike, and nucleocapsid proteins promote NF-κB activation and thus also contribute to pathogenesis. Plasmin, a key player in fibrinolysis was reported to enhance the virulence and pathogenicity of SARS-CoV-2. The worsening of clinical outcomes of COVID-19 patients with hypertension, diabetes, chronic obstructive pulmonary disease, and kidney dysfunction is severe and may lead to death (Hong-Long *et al*., 2020). Prostaglandin-endoperoxide synthase 2 (PTGS2) or COX-2 is an inducible pro-inflammatory enzyme. COX-2 is an immediate-early response gene, which upon induction generates mainly hyperalgesic and pro-inflammatory prostaglandins at sites of inflammation. Some studies have shown that two structural proteins from the SARS-CoV induced the expression of COX-2 *in vitro* (Liu *et al.,* 2007). Excessive prostaglandin E2 (PGE2) accumulation in the urine of COVID-19 patients which is mediated by COX-2 was the key pathological symptom of COVID-19. It was suggested that celebrex adjuvant treatment may be helpful for the therapy of COVID-19 as it reduced the PGE2 levels and promotes recovery of ordinary or severe COVID-19 (Hong *et al.,* 2020).

The complement is a major component of innate immunity that functions to recognize and eliminate invading pathogens. In a SARS-CoV-infected mice study, it was reported that complement activation results in immune-mediated damage of lungs in C3 deficient mice and suggests that inhibition of the complement pathway might be an effective therapeutic target (Gralinski and Menachery, 2020). The lung biopsy samples from patients with severe COVID-19 showed complement activation, characterized by C3a generation and C3-fragment deposition together with increase in the levels of C5a in the serum of COVID-19 patients (Gao *et al.,* 2020). Better recovery of patients treated with an antibody blocking complement

component C5a suggested a potential benefit of targeting complement in patients with COVID-19 with severe lung injury (Gao *et al*., 2020).

Sample collection and diagnostics of COVID-19

The WHO recommends collecting samples from both the upper and lower respiratory tracts. This can be achieved through sputum, bronchoalveolar lavage, or endotracheal aspirate and also mouth/nose swabs. These samples are then assessed for viral RNA extraction followed by PCR. If tested positive, it is recommended to repeat and confirm the test. Collecting the correct specimen at the right time from patients is very important for laboratory testing of the COVID-19 diagnostics. The main sample collection sites for detection are from upper and lower respiratory sources including, nasal, nasopharyngeal (NP), throat, sputum and bronchial fluid (De Virgilio *et al*., 2020). It was reported during COVID-19 outbreak in Wuhan of China that only 32% of oropharyngeal swabs were successful in detecting SARS-CoV-2 RNA in patients, however other studies have shown that saliva is a promising agent and exhibits higher sensitivity for COVID-19 detection than nasopharyngeal swabs. Saliva collection is also less invasive and can be self-administered providing less exposure to health workers. Collected swab specimens should be placed in a universal viral medium for better results. The highly sensitive detection of SARS-CoV, MERS-CoV, and SARS-CoV-2 is possible also from both upper and lower respiratory samples like sputum and bronchoalveolar lavage fluid (BALF) (De Virgilio *et al*., 2020). The collection of BALF *via* bronchoscopy is difficult and requires technically skilled health workers and also risk of biosafety is higher as it may spread through aerosols. RNA detection of SARS-CoV and MERS-CoV can also be done from stool, urine and blood specimen's (Chen *et al*., 2020b). Similar to SARS-CoV RNA detection after onset symptoms within 7–10 days, SARS-CoV-2 is also expected to be detected within 5–14 days after the illness (He *et al*., 2020).

WHO declared health emergency for COVID-19 on January 30 and thereafter, many different companies started developing diagnostic kits and many are still in the development stage for rapid detection kits. In response to the COVID-19 emergency many *in vitro* diagnostics entered the market and many are still in research and development. Most of the kits detect the COVID-19 antigens or antibodies in a so-called "Rapid Diagnostic Test" (RDT) design. Antigen based RDT kits detect the presence of a protein of the virus in body fluids like blood or serum and mostly in secretions of the upper respiratory tract like bronchiolar lavage fluid. The antibody-based detection RDT kits can detect antibodies produced by B-lymphocytes of the virus infected patients during the infection period. Antibodies are mostly detected in the blood during the post infection period of around 5–10 days after the onset of illness (He *et al*., 2020). These tests are qualitative tests and do not give any information about virus quantity. The advantages of RDTs are its possibility to be performed on spot, they are small stand-alone tests that are simple to use outside the hospital, by minimally trained staff, and provide test results within 15–30 min.

Real-time reverse transcription PCR (RT-qPCR)

The most important method for COVID-19 diagnostics is nucleic acid detection by real time reverse transcription polymerase chain reaction (RT-qPCR). It is considered as the 'gold standard' for the detection of viruses and involves the reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the RdRp, E and N genes (Choudhary *et al*., 2020, Kageyama *et al*., 2003) by real-time PCR (qPCR). It has been performed on nasopharyngeal and oropharyngeal swabs in symptomatic people, in individuals who are in close contact with SARS-CoV-2 positive people and also among health care workers, in potentially exposed category individuals (policemen, military) (Sanduzzi and Zamparelli, 2020). RT-qPCR helps efficiently to confirm a viral infection in suspected patients within 2 h. Studies conducted in COVID-19 patients reported that RT-qPCR is positive even at a low level of viral load until day 13 in lower respiratory tract specimens and day 14 in upper respiratory specimens (Tahamtan and Ardebili, 2020). However, sample collection, transportation, and kit performance affect the total positive rate of RT-qPCR for throat swab samples and was reported to be about 30% to 60% at initial presentation (Yang *et al*., 2020). Most of the kits were checked for the cross-reactivity with other respiratory viruses with samples positive for other viruses like influenza A, influenza C, SARS-CoV and MERS-CoV with RT-qPCR results. The RT-qPCR kits also contain a specific positive control, which is *in vitro* transcribed RNA derived from SARS-CoV-2 or recombinant plasmid DNA of RdRp and E gene. For the countries like USA, Brazil, Italy, UK and other countries with rapid raise in the number of SARS-COV-2 infections, it is strongly suggested to use RT-qPCR/SARS-CoV-2 NAT. More personal should be trained and prepared for RT-qPCR detection under a Biological safety cabinet level 4.

Serological test

By using the ELISA method, it is possible to detect the presence of antibodies to coronavirus in a whole blood or serum sample. These tests detect IgM and IgG antibodies

Table 3. Some of the commercial kits available for the detections of COVID-19

Name of kit	Type of test	Test time	Application	Samples
In Bios Smart detect SARS-CoV-2	RT-qPCR	4 h	diagnosis (detection)	nasal and nasopharyngeal swabs
Gnomegen COVID-19 RT-digital PCR detection kit	RT-qPCR	3–4 h	diagnosis (*in vitro* detection)	nasal and nasopharyngeal swabs
Logix Smart Coronavirus 2019 (COVID-19) kit	RT-qPCR	4 h	diagnosis (*in vitro* detection)	upper and lower respiratory tract specimens and also serum
Cellex qSARS-CoV-2 IgG/IgM rapid test	immuno-assays to detect IgG & IgM antibodies	20 min	diagnosis (detection)	blood samples

produced during the viral infection. Tests can determine whether a patient has previously been infected (IgG) with coronavirus and also, they will give positive results during infection (IgM). Currently, serological tests are not provided by most of the countries as a part of routine diagnostics. These tests are less likely to detect the early stage of infection, however they are widely used to check how large population was exposed to coronavirus (Jacofsky *et al.,* 2020). If the tests are to be used in diagnostics, they always should be supported by RT-qPCR.

Rapid antigen tests

Rapid antigen tests usually based on S protein detection provide the advantage of fast, efficient and low-cost diagnostic method of human coronavirus. The actual principle behind this assay is its fluorescence based immunochromatographic method for detecting S protein of SARS-CoV-2 in COVID-19 patients by using nasopharyngeal swabs for rapid, early and simple method for diagnostics. The commercial diagnostic kits present in the market for COVID-19 detection (FDA approved) are shown in Table 3.

CT scan

Some of the published reports have revealed higher sensitivity of chest CT in early detection of SARS-CoV-2 positive cases other than diagnostic tests. The importance of CT scan was recommended because of the false-positive tests reported in many cases. In a recent COVID-19 testing report on 51 patients, around 70% of patients showed positive results after RT-qPCR on swabs whereas, abnormal CT scan findings compatible with viral pneumonia was seen in almost all the patients (98%) (Ai *et al.,* 2020). CT scans are reported to be more sensitive than PCR. The reasons for low sensitivity of PCR may include insensitive nucleic acid detection methods, viral load, handling errors and instrumental error. There are now over seven different SARS-CoV-2 nucleic acid PCR tests (van Kasteren *et al.*, 2020). In respiratory swab method it has been shown that upper respiratory tract samples have their peak viral loads 3 days after onset of symptoms, and that nasal, rather than throat samples have the highest viral loads (van Kasteren *et al.,* 2020). On contrary, reports suggested that safety measures using CT to study COVID-19 patients are necessary to be with proper cleaning protocols and health-care professionals. Additionally, CT scanners could become a source for infection to other patients who has to undergo CT scanning (Hope *et al.,* 2020).

Management and treatment for COVID-19

Isolation is the best and most effective measure to avoid COVID-19. Till date no specific treatment or specific antiviral medication or vaccine is currently available, however, many countries all over the world using different strategies like a combination of antiviral and antibiotic drugs are used to treat patients. The treatment of COVID-19 mainly includes symptomatic care and oxygen therapy. Patients with mild viral infections require early oxygen therapy, nutritional supplements and antibacterial therapy. The patients with complications like severe pneumonia or acute respiratory distress syndrome (ARDS) may require mechanical ventilation, glucocorticoid therapy and chemotherapy. Currently, the drug repurposing approach is being used for the rapid identification of therapeutic strategies against COVID-19. The viral proteins such as S, N, E and M proteins are considered for the identification of potential inhibitors by virtual screening-based approaches. Our team also suggested possible repurposable drugs for COVID-19 treatment based on upregulated proviral factors by the host-transcriptome-based drug-repurposing approach. Such *in silico* studies can generate potential drug candidates for wet lab and clinical level validation experiments. Many countries also recommended avoiding unnecessary administration of antibiotics. Patients with a history of vascular disease, respiratory failure, gastrointestinal

problems and multiple organ dysfunction require intensive care unit with full supply of oxygen via mechanical ventilation or high-flow nasal oxygen ventilation. Here we discussed ongoing important clinical studies of treatment for COVID-19.

Interferon therapy

In this type of treatment, a class of cytokines called type 1 interferons (IFN-I) are used to treat virus infected patients. They are secreted mainly by dendritic and NK cells upon recognition of viral components by pattern recognition receptors. IFN-I is the first line of defence in the form of cytokines produced during a viral infection. IFNs interfere with replication of virus inside the host cells and slow the cell metabolism and promote the secretion of cytokines and activation of the adaptive immunity in the infected host. IFN-I treatment has been extensively studied during SARS-CoV breakout in 2004 in numerous experiments both *in vitro* and *in vivo* but failed to significantly improve the recovery of the patients (Sallard *et al*., 2020). Daniel *et al.* (2020) in recent study from USA, showed some interesting results on IFN-I, where the post-mortem lung samples of COVID-19 patients were compared with lung samples of healthy individuals. The transcriptional profiling of the genes was significantly induced in response to SARS-CoV-2 however no IFN-I or IFN-III RNA was detected by sequencing. Interestingly, serum sample analysis consistently showed absence of both IFN-β and the IFN-γ family, however analyses of cytokines and chemokines showed a significant increase in circulating IL-6, IL-1β, IL1RA, CCL2, CCL8 CXCL2, CXCL8, CXCL9, and CXCL16 levels (Blanco-Melo *et al.,* 2020, Major *et al.,* 2020). The sequence similarity of SARS-CoV and SARS-CoV-2 is high and presents similar pathology consequences. Many preclinical trails testing IFN therapy against SARS-CoV-2 are carrying on over the world.

Plasma therapy

During the Spanish flu pandemic in 1918, plasma isolated from the survivors contributed to a 50% reduction in deaths among severely ill patients. Plasma-derived therapy was used to treat patients during outbreaks of SARS-CoV, MERS-CoV, and Ebola. The main principle behind this is that antibodies developed by recovered patients boost the immune system of virus infected patients with no adverse side effects. Collected plasma injected into the infected patients, provides "passive immunity" until the patient's body produces enough antibodies to fight against the viral infection. During the SARS outbreak, data show a shorter hospital stay and lower mortality in patients treated with plasma than those who were not treated with plasma. Study of *in vivo* model also showed that the effects of plasma antibodies in viral clearance and blocking reinfection (Rajendran *et al*., 2020). During the COVID-19 outbreak, many countries like USA, China, India and other countries have implemented the therapy after obtaining permission from their respective agencies. In India, states like Delhi, Maharashtra and Tamil Nadu implemented the plasma therapy. Most of the countries to the best of available data, are implementing plasma therapy to treat COVID-19 patients (Duan *et al.,* 2020; Ahn *et al.,* 2020b).

Hydroxychloroquine

Chloroquine (CQ) and hydroxychloroquine that are commercial drugs used to treat malaria, were recently proposed as a treatment that could reduce the coronaviral infection, however with only limited data and information available for use as prophylaxis to treat COVID-19. Although there is limited data on chloroquine and hydroxychloroquine in viral studies, *in vitro* studies show that both can inhibit SARS-CoV-2 transmission *via* alkalinisation of the intracellular phagolysosomes. These drugs prevent virion fusion and uncoating thus, delay the viral infection (Duan *et al*., 2020). The cell culture studies also showed that chloroquine works by inhibiting the entry of SARS-CoV-2 S protein and increases the acidity of endosomes inside the cell. Hydroxychloroquine, in particular, has a variety of side effects and can in rare cases affect heart – increases QT interval in the ECG and may cause cardiac arrhythmias and high doses are toxic. The possible mechanism of CQ is by suppression of T cell proliferation, Th1 cell differentiation and also suppression of chronic inflammatory reactions. CQ also activates the p53-induced transcription of p21 by unknown mechanism.

Remdesivir

Remdesivir is an antiviral drug developed to treat Ebola virus by American drug company Gilead Sciences. Similar to hydroxychloroquine, this drug was also suggested to treat COVID-19 patients. This drug is a nucleotide analogue that works by inhibiting the RdRp (Pruijssers *et al*., 2020). A group of researchers from North Carolina studied both *in vitro* and *in vivo* inhibition of SARS-CoV and MERS-CoV by remdesivir. Ongoing clinical studies suggest that remdesivir (GS5734) can be used for prophylaxis and more clinical studies are under observation (Pruijssers *et al*., 2020; Cao *et al*., 2020). The remdesivir was given emergency use authorisation by the US Food and Drug Administration (FDA) on May 1, under emergency circumstances only. The leading manufacturer of remde-

sivir Gilead Sciences Inc. has done two clinical trials, and results are expected to be released by June 2020.

ACE inhibitors

Angiotensin-converting enzyme 2 (ACE2) has been shown to be a co-receptor for viral entry for SARS-CoV-2. It is widely expressed in kidney, heart, and recently ACE2 was reported in type II alveolar cells in the lungs. Researchers expected that ACEIs (angiotensin-converting enzyme inhibitors) and ARBs (angiotensin receptor blockers) would affect the severity and mortality of COVID-19 patients. One of the suggestions was that ACEIs could directly inhibit ACE2 in lung tissue and could act as a carboxypeptidase, however expected inhibition of ACE2 by the clinically recommended ACEIs has failed. The SARS-CoV worsened lung injury was improved by the treatment with ARB during the SARS outbreak in 2003. There are limited data and evidence to suggest that treatment with ACEIs or ARBs can control the severity of pulmonary injury by SARS-CoV-2 in COVID-19 patients with hypertension (Verdecchia *et al.*, 2020).

Conclusion

The recent COVID-19 pandemic has become the greatest public health crisis caused by a novel strain of coronavirus SARS-CoV-2. Infected patients showed mild symptoms as fever, cough, body pain while severe symptoms include chest pain and difficulty in breathing which leads to mortality with an estimation of about 312,000 deaths among 4.65 million infected cases according to WHO. The entry of the virus into the host cell is triggered by a receptor and virus evades the host's immune response by suppressing the host innate and adaptive immune response proteins. Rapidly increasing infection rate of virus can be decreased by deep understanding and analyses of mechanisms in viral protein interaction with host, viral genetics and its replication, host immune response signalling and improved therapeutic strategy. Various diagnostic methods including RT-qPCR, rapid test kits and also treatment protocols like plasma therapy, remdesivir treatment and also vaccine development could improve patients' survival. Major countries of the world are in race for the vaccine development. Moreover many vaccines are in clinical trial stage and many of the phase II results are expected in the end of June 2020.

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References

Ahmed SF, Quadeer AA, McKay MR., Viruses 12, 254, 2020. https://doi.org/10.3390/v12030254

Ahn DG, Shin HJ, Kim MH, Lee S, Kim HS, Myoung J, Kim BT, Kim SJ., J. Microbiol. Biotechnol. 30, 313-324, 2020a. https://doi.org/10.4014/jmb.2003.03011

Ahn JY, Sohn Y, Lee SH, Cho Y, Hyun JH, Baek YJ, Jeong SJ, Kim JH, Ku N., Yeom JS, Roh J, Ahn MY, Chin BS, Kim YS, Lee H, Yong D, Kim HO, Kim S, Choi JY., J. Korean. Med. Sci. 13, e149, 2020b.

Belouzard S, Chu VC, Whittaker GR., Proc. Natl. Acad. Sci. USA 106, 5871-5876, 2009. https://doi.org/10.1073/pnas.0809524106

Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR., Cell 181, 1036-1045 e9, 2020. https://doi.org/10.1016/j.cell.2020.04.026

Cao YC, Deng QX, Dai SX., Travel Med. Inf. Dis. 101647, 2020. https://doi.org/10.1016/j.tmaid.2020.101647

Cavalcanti YV, Brelaz MC, Neves JK, Ferraz JC, Pereira VR., Pulm. Med. 2012, 745483, 2020. https://doi.org/10.1155/2012/745483

Chan CM, Ma CW, Chan WY, Chan HY., Arch. Biochem. Biophysics. 459, 197-207, 2007. https://doi.org/10.1016/j.abb.2007.01.012

Chang CK, Hou MH, Chang CF, Hsiao CD, Huang TH., Antiviral Res. 103, 39-50, 2020. https://doi.org/10.1016/j.antiviral.2013.12.009

Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, Meyerholz DK, Perlman S., Cell Host Microbe 19, 181-193, 2020. https://doi.org/10.1016/j.chom.2016.01.007

Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu, Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L., Lancet 395, 507-513, 2020. https://doi.org/10.1016/S0140-6736(20)30211-7

Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, Yang Y, Liu B, Wang W, Wei C, Yang J, Ye G, Cheng Z., J. Med. Virol. 92, 833-840, 2020. https://doi.org/10.1002/jmv.25825

Chen Y, Guo Y, Pan Y, Zhao ZJ., Biochem. Biophys. Res. Commun. 525, 135-40, 2020. https://doi.org/10.1016/j.bbrc.2020.02.071

Chenavas S, Crepin T, Delmas B, Ruigrok RW, Slama-Schwok A., Future Microbiol. 8, 1537-1545, 2013. https://doi.org/10.2217/fmb.13.128

Cheung YK, Cheng SC, Sin FW, Chan KT, Xie Y., Vaccine 25, 6070-6077, 2007. https://doi.org/10.1016/j.vaccine.2007.05.025

Choudhary ML, Vipat V, Jadhav S, Basu A, Cherian S, Abraham P, Varsha AP., Indian J. Med. Res. 151, 251-254, 2020.

Cohen JR, Lin LD, Machamer CE., J. Virol. 85, 5794-5803, 2011. https://doi.org/10.1128/JVI.00060-11

Cong Y, Ulasli M, Schepers H, Mauthe M, V'Kovski P, Kriegenburg F, Thiel V, de Haan CAM, Reggiori F., J. Virol. 94, e01925-19, 2020. https://doi.org/10.1128/JVI.01925-19

de Lima Menezes G, da Silva RA., J. Biomol. Struct. Dyn. 1-11, 2020. https://doi.org/10.1080/07391102.2020.1792992

de Virgilio A, Costantino A, Mercante G, Spriano G., Oral Oncol. 106, 104802, 2020. https://doi.org/10.1016/j.oraloncology.2020.104802

deDiego ML, Nieto-Torres JL, Regla-Nava JA, Jimenez-Guardeno JM, Fernandez-Delgado R, Fett C, Castano-Rodriguez C, Perlman S, Enjuanes L., J. Virol. 88, 913-924, 2014. https://doi.org/10.1128/JVI.02576-13

Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, Chaicumpa W., Hum. Vaccin. Immunother. 16, 1232-1238, 2020. https://doi.org/10.1080/21645515.2020.1735227

Dong L, Hu S, Gao J., Drug Discov. Ther. 14, 58-60, 2020. https://doi.org/10.5582/ddt.2020.01012

Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al., Proc. Natl. Acad. Sci. USA 117, 9490-9496, 2020. https://doi.org/10.1073/pnas.2007408117

Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, et al., PLoS Pathog. 6, e1000896, 2010. https://doi.org/10.1371/journal.ppat.1000896

Eketunde AO, Mellacheruvu SP, Oreoluwa P. Cureus 12, e9438, 2020.

Escors D, Ortego J, Laude H, Enjuanes L., J. Virol. 75, 1312-1324, 2001. https://doi.org/10.1128/JVI.75.3.1312-1324.2001

Fang X, Gao J, Zheng H, Li B, Kong L, Zhang Y, Wang W., Zeng Y., Ye L., J. Med. Virol. 79, 1431-1439, 2007. https://doi.org/10.1002/jmv.20953

Fischer H, Tschachler E, Eckhart L., Front. Immunol. 11, 939, 2020. https://doi.org/10.3389/fimmu.2020.00939

Gallagher TM, Buchmeier MJ., Virology 279, 371-374, 2001. https://doi.org/10.1006/viro.2000.0757

Gao T, Hu M, Zhang X, Li H, Zhu L, Liu H, et al., medRxiv 2020.03.29.20041962, 2020.

Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al., Int. J. Antimicrob. Agents 56, 105949, 2020. https://doi.org/10.1016/j.ijantimicag.2020.105949

Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al., Nature 583, 459-468, 2020.

Graham RL, Sparks JS, Eckerle LD, Sims AC, Denison MR., Virus Res. 133, 88-100, 2008. https://doi.org/10.1016/j.virusres.2007.02.017

Gralinski LE, Menachery VD., Viruses 12, 135, 2020. https://doi.org/10.3390/v12020135

Gui M, Song W, Zhou H, Xu J, Chen S, Xiang Y, Wang X., Cell Res. 27, 119-29, 2017. https://doi.org/10.1038/cr.2016.152

Gupta MK, Vemula S, Donde R, Gouda G, Behera L, Vadde R., J. Biomol. Struct. Dyn. 1-11, 2020. https://doi.org/10.1080/07391102.2020.1751300

Hashem AM, Alghamdi BS, Algaissi AA, Alshehri FS, Bukhari A, Alfaleh MA, Memish ZA., Travel Med. Infect. Dis. 35, 101735, 2020. https://doi.org/10.1016/j.tmaid.2020.101735

He X, Lau EHY, Wu P, Deng X, Wang J, Hao X et al., Nat. Med. 26, 672-675, 2020. https://doi.org/10.1038/s41591-020-0869-5

Hilgenfeld R., FEBS J. 281, 4085-4096, 2014. https://doi.org/10.1111/febs.12936

Hillen HS, Kokic G, Farnung L, Dienemann C, Tegunov D, Cramer P., Nature 584, 154-156, 2020. https://doi.org/10.1038/s41586-020-2368-8

Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S et al., Cell 181, 271-280 e8, 2020. https://doi.org/10.1016/j.cell.2020.02.052

Hong W, Chen Y, You K, Tan S, Wu F, Tao J et al., Front. Pharmacol. 11, 561674, 2020. https://doi.org/10.3389/fphar.2020.561674

Honke N, Shaabani N, Merches K, Gassa A, Kraft A, Ehrhardt K et al., Eur. J. Immunol. 46, 372-380, 2016. https://doi.org/10.1002/eji.201545765

Hope MD, Raptis CA, Shah A, Hammer MM, Henry TS, six signatories, Lancet 395, 1189-1190, 2020. https://doi.org/10.1016/S0140-6736(20)30728-5

Hsieh YC, Li HC, Chen SC, Lo SY., J.Biomed. Sci. 15, 707-717, 2008. https://doi.org/10.1007/s11373-008-9278-3

Jacofsky D, Jacofsky EM, Jacofsky M., J. Arthroplasty, 35, S74-S81, 2020. https://doi.org/10.1016/j.arth.2020.04.055

Jensen S, Thomsen AR., J. Virol. 86, 2900-2910, 2012. https://doi.org/10.1128/JVI.05738-11

Jiang S, Hillyer C, Du L., Trends Immunol. 41, 355-359, 2020. https://doi.org/10.1016/j.it.2020.03.007

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushi S, Hoshino FB, et al., J. Clin. Microbiol. 41, 1548-1557, 2003. https://doi.org/10.1128/JCM.41.4.1548-1557.2003

Kandeel M, Ibrahim A, Fayez M, Al-Nazawi M., J. Med. Virol. 92, 660-666, 2020. https://doi.org/10.1002/jmv.25754

Lee HC, Chathuranga K, Lee JS., Exp. Mol. Med. 51, 1-13, 2019. https://doi.org/10.1038/s12276-019-0299-y

Lee S, Lee C., Virus Res. 208, 136-145, 2015. https://doi.org/10.1016/j.virusres.2015.06.013

Leung DT, Tam FC, Ma CH, Chan PK, Cheung JL, Niu H, Tam JSL, Lim PL., J. Infect. Dis. 190, 379-386, 2004. https://doi.org/10.1086/422040

Li F., Annu. Rev. Virol. 3, 237-261, 2016. https://doi.org/10.1146/annurev-virology-110615-042301

Li Y, Surya W, Claudine S, Torres J., J. Biol. Chem. 289, 12535-12549, 2014. https://doi.org/10.1074/jbc.M114.560094

Liu M, Yang Y, Gu C, Yue Y, Wu KK, Wu J, Zhu Y., FASEB J. 21, 1586-1596, 2007. https://doi.org/10.1096/fj.06-6589com

Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, Wang Q, Tan Li, Wu W, Tang S., Xiong Z, Zheng S., J. Clin. Microbiol. 58:e00461-20, 2020. https://doi.org/10.1128/JCM.00461-20

Liu X, Wang XJ., J. Genet. Genomics 47, 119-121, 2020. https://doi.org/10.1016/j.jgg.2020.02.001

Lung J, Lin YS, Yang YH, Chou YL, Shu LH, Cheng YC, Liu HT, Wu C-Y., J. Med. Virol. 92, 693-697, 2020. https://doi.org/10.1002/jmv.25761

Ma HC, Fang CP, Hsieh YC, Chen SC, Li HC, Lo SY., J. Biomed. Sci. 15, 301-310, 2008. https://doi.org/10.1007/s11373-008-9235-1

Madu IG, Roth SL, Belouzard S, Whittaker GR., J. Virol. 83, 7411-7421, 2009. https://doi.org/10.1128/JVI.00079-09

Mahdi M, Mótyán JA, Szojka ZI, Golda M, Miczi M, Tőzsér J., Virol. J. 17, 190, 2020. https://doi.org/10.1186/s12985-020-01457-0

Major J, Crotta S, Llorian M, McCabe TM, Gad HH, Prienstnall SL, Hartmann R, Wack A., Science 369, 712-717, 2020. https://doi.org/10.1126/science.abc2061

Masters PS., Adv. Virus Res. 66, 193-292, 2006. https://doi.org/10.1016/S0065-3527(06)66005-3

McBride R, van Zyl M, Fielding BC., Viruses 6, 2991-3018, 2014. https://doi.org/10.3390/v6082991

Mulgaonkar N, Wang H, Mallawarachchi S, Fernando S, Martina B, Ruzek D., bioRxiv 158196, 2020.

Narayanan K, Maeda A, Maeda J, Makino S., J. Virol. 74, 8127-8134, 2020. https://doi.org/10.1128/JVI.74.17.8127-8134.2000

Nieto-Torres JL, Dediego ML, Alvarez E, Jimenez-Guardeno JM, Regla-Nava JA, Llorente M, Kremer L, Shuo SS, Enjuanes L., Virology 415, 69-82, 2011. https://doi.org/10.1016/j.virol.2011.03.029

Ohe M, Shida H, Jodo S, Kusunoki Y, Seki M, Furuya K, Goudarzi H., Biosci. Trends 14, 159-160, 2020. https://doi.org/10.5582/bst.2020.03058

Okada M, Takemoto Y, Okuno Y, Hashimoto S, Fukunaga Y, Tanaka T, et al., Adv. Exp. Med. Biol. 581, 561-566, 2006. https://doi.org/10.1007/978-0-387-33012-9_102

Oslund KL, Baumgarth N., Future Virol. 6, 951-962, 2011. https://doi.org/10.2217/fvl.11.63

Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, et al., Nat. Commun. 11, 1620, 2020. https://doi.org/10.1038/s41467-020-15562-9

Phan T., Infect. Genet. Evol. 81, 104260, 2020. https://doi.org/10.1016/j.meegid.2020.104260

Pang H, Lin, Y, Han X, Xu Y, Jiang F, Wu D., J. Gen. Virol. 85, 3109-3113, 2004. https://doi.org/10.1099/vir.0.80111-0

Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H, et al., Indian J. Pharmacol. 52, 56-65, 2020. https://doi.org/10.4103/ijp.IJP_115_20

Prompetchara E, Ketloy C, Palaga T., Asian Pac. J. Allergy Immunol. 38, 1-9, 2020.

Pruijssers AJ, George AS, Schäfer A, Leist SR, Gralinksi LE, Dinnon KH et al., Cell Reports 32, 107940, 2020. https://doi.org/10.1016/j.celrep.2020.107940

Qiang XL, Xu P, Fang G, Liu WB, Kou Z., Infect. Dis. Poverty 9, 33, 2020. https://doi.org/10.1186/s40249-020-00649-8

Rajendran K, Krishnasamy N, Rangarajan J, Rathinam J, Natarajan M, Ramachandran A., J. Med. Virol. 92, 1475-1483, 2020. https://doi.org/10.1002/jmv.25961

Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, Rawling M, Savory E, Stebbing J., Lancet 395, e30-e31, 2020. https://doi.org/10.1016/S0140-6736(20)30304-4

Risco C, Anton IM, Muntion M, Gonzalez JM, Carrascosa JL, Enjuanes L., Adv. Exp. Med. Biol. 440, 341-346, 1998. https://doi.org/10.1007/978-1-4615-5331-1_44

Rottier PJ, Welling GW, Welling-Wester S, Niesters HG, Lenstra JA, Van der Zeijst BA., Biochemistry 25, 1335-1339, 1986. https://doi.org/10.1021/bi00354a022

Ruch TR, Machamer CE., Viruses 4, 363-382, 2012. https://doi.org/10.3390/v4030363

Sakai Y, Kawachi K, Terada Y, Omori H, Matsuura Y, Kamitani W., Virology 510, 165-174, 2017. https://doi.org/10.1016/j.virol.2017.07.019

Sallard E, Lescure FX, Yazdanpanah Y, Mentre F, Peiffer-Smadja N., Antiviral Res. 178, 104791, 2020. https://doi.org/10.1016/j.antiviral.2020.104791

Samrat SK, Tharappel AM, Li Z, Li H., Virus Res. 288, 198141, 2020. https://doi.org/10.1016/j.virusres.2020.198141

Sanduzzi A, Zamparelli SS., Int. J. Environ. Res. Public Health 17, 3289, 2020. https://doi.org/10.3390/ijerph17093289

Schoeman D, Fielding BC., Virol. J. 16, 69, 2019. https://doi.org/10.1186/s12985-019-1182-0

Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R., J. Adv. Res. 24, 91-98, 2020. https://doi.org/10.1016/j.jare.2020.03.005

Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Q C., Viruses 11, 59, 2019. https://doi.org/10.3390/v11010059

Srinivasan S, Cui H, Gao Z, Liu M, Lu S, Mkandawire W, Narykov O, Sun M, Korkin D., Viruses. 12, 360, 2020. https://doi.org/10.3390/v12040360

Surjit M, Liu B, Chow VT, Lal SK., J. Biol. Chem. 281, 10669-10681, 2006. https://doi.org/10.1074/jbc.M509233200

Taccone FS, Gorham J, Vincent JL., Lancet Respir. Med. 8, 539-541, 2020. https://doi.org/10.1016/S2213-2600(20)30172-7

Tahamtan A, Ardebili A., Expert Rev. Mol. Diagn. 20, 453-454, 2020. https://doi.org/10.1080/14737159.2020.1757437

Tahara SM, Dietlin TA, Bergmann CC, Nelson GW, Kyuwa S, Anthony RP, Stohlman SA., Virology 202, 621-630, 1994. https://doi.org/10.1006/viro.1994.1383

Tan YJ, Lim SG, Hong W., Antiviral Res. 65, 69-78, 2005. https://doi.org/10.1016/j.antiviral.2004.10.001

Tay MZ, Poh CM, Renia L, MacAry PA, Ng LFP., Nat. Rev. Immunol. 20, 363-374, 2020. https://doi.org/10.1038/s41577-020-0311-8

Teijaro JR., Eur. J. Immunol. 46, 300-302, 2016. https://doi.org/10.1002/eji.201546224

Teoh KT, Siu YL, Chan WL, Schluter MA, Liu CJ, Peiris JS, Bruzzone R, Margolis B, Nal B., Mol. Biol. Cell 21, 3838-3852, 2010. https://doi.org/10.1091/mbc.e10-04-0338

Thiel V, Ivanov KA, Putics A, Hertzig T, Schelle B, Bayer S, Weißbrich B, Snijder E, Rabenau H, Doerr HW, Gorbalenya AE, Ziebhur J., J. Gen. Virol. 84, 2305-2315, 2003. https://doi.org/10.1099/vir.0.19424-0

Ujike M, Taguchi F., Viruses 7, 1700-1725, 2015. https://doi.org/10.3390/v7041700

Uno Y., Intern. Emerg. Med. 15, 1577-1578, 2020. https://doi.org/10.1007/s11739-020-02345-9

van Kasteren PB, van der Veer B, van den Brink S, Wijsman L, de Jonge J, van den Brandt A, Molenkamp R., Reusken CBEM, Meijer A., J. Clin. Virol. 128, 104412, 2020. https://doi.org/10.1016/j.jcv.2020.104412

Verdecchia P, Reboldi G, Cavallini C, Mazzotta G, Angeli F., G. Ital. Cardiol. (Rome) 21, 321-327, 2020.

Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D., Cell 181, 281-292 e6, 2020. https://doi.org/10.1016/j.cell.2020.02.058

Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Shi Z, Hu Z, Zhonh W, Xiao G., Cell Res. 30, 269-271, 2020. https://doi.org/10.1038/s41422-020-0282-0

Wang Y, Liu L., mBio. 7, e01872-15, 2016. https://doi.org/10.1128/mBio.00792-16

Wilk CM., Nat. Rev. Immunol. 20, 350, 2020. https://doi.org/10.1038/s41577-020-0314-5

Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS., Science 367, 1260-1263, 2020. https://doi.org/10.1126/science.abb2507

Wu Q, Zhang Y, Lu H, Wang J, He X, Liu Y et al., Genomics Proteomics Bioinformatics 1, 131-144, 2003. https://doi.org/10.1016/S1672-0229(03)01017-9

Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P et al., New England J. Med. 383, 1283-1286, 2020.

Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S et al., Cell Res. 30, 343-355, 2020. https://doi.org/10.1038/s41422-020-0305-x

Yang Y, Xiong Z, Zhang S, Yan Y, Nguyen J, Ng B, Lu H, Brendese J, Yang F, Wang H, Yang XF., Biochem. J. 392, 135-143, 2005. https://doi.org/10.1042/BJ20050698

Yang Y, Yang M, Shen C, Wang F, Yuan J, Li J et al., medRxiv. 20021493, 2020.

Zarghi A, Arfaei S., Iranian J. Pharm. Res. 10, 655-683, 2011.

Zhang G, Pomplun S, Loftis AR, Loas A, Pentelute BL., bioRxiv 999318, 2020.

Zhang T, Cui X, Zhao X, Wang J, Zheng J, Zheng G, Guo W, Cai C, He S, Xu Y., J. Med. Virol. 92, 909-914, 2020. https://doi.org/10.1002/jmv.25795

Zhang YH, Zhao Y, Li N, Peng YC, Giannoulatou E, Jin RH et al., Nat. Commun. 4, 1418, 2013. https://doi.org/10.1038/ncomms2433

Zhou B, Liu J, Wang Q, Liu X, Li X, Li P, Ma Q, Cao C., J. Virol. 82, 6962-6971, 2008. https://doi.org/10.1128/JVI.00133-08

Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W et al., Nature. 579, 270-273, 2020a. https://doi.org/10.1038/s41586-020-2012-7

Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F., Cell Discov. 6, 14, 2020b. https://doi.org/10.1038/s41421-020-0153-3