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A SHORT REVIEW OF SARSCoV-2

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ABSTRACT
Almost every area of fast paced life in every country got shattered with growing cases of infections due to nCoronavirus (novel coronavirus) SARSCoV-2 from the end of 2019 and it still continues to be so in the year 2021. Meanwhile many precious lives of common people as well as medical field personnel have been lost to this unexpected and uncontrolled outbreak and still it has not been under complete check. Group of scientists and research institutes are working at their best to understand every feature and factor causing, initiating, spreading and continuing of the pandemic. This review article is an effort to bring together some important facade of the virus causing the entire shuffle. Focus of the article is on molecular aspects and pathogenesis of the virus SARSCov-2 which is important for the medical and scientific research community.

INTRODUCTION

On December 31, 2019, the China Health Authority alerted the World Health Organization (WHO) to several cases of pneumonia of unknown etiology in Wuhan City in Hubei Province in central China. As per the worldmeter information, by the end of 2020 on December 31st, about 83,214,000 infected patients and 1,815,400 deaths due to COVID19 were reported. The origin of the pandemic causing virus is still being discussed in great details as presently unverified reports from the lung cancer patients from Milan in Italy reported to have SARS-CoV-2 receptor-binding domain antibodies during the screening of patients suffering from the lung cancer.[1,2] Although so far the theory of 'Virus originated or discovered first in China' or in other words 'China origin' is widely believed, accepted and discussed.[3, 4, 5] In this article authors review some crucial highlights of this global pandemic and the virus behind the pandemic.

MAIN

For this review, original research articles as well as data published and available from Governments' official websites in recent past and distant past wherever appropriate were reviewed and used. This article reviews following major aspects of SARSCoV-2 widely recognised as COVID19.

1. Chronology of major events before the COVID19 outbreak
2. Classification and phylogeny of SARSCoV-2
3. Molecular aspects of SARSCoV-2
4. Modus operandi of SARSCoV-2
5. Pathogenesis of SARSCoV-2

1. CHRONOLOGY OF COVID19 MAJOR EVENTS BEFORE THE OUTBREAK

As the globe is moving ahead in time, we are still trying to get a clearer image of the events which may help us put the pieces of sporadic information to create a meaningful picture for understanding of COVID19 pandemic outbreak and global spread. Here is chronology of major events before and during the initial months of current pandemic as depicted in Fig.1.

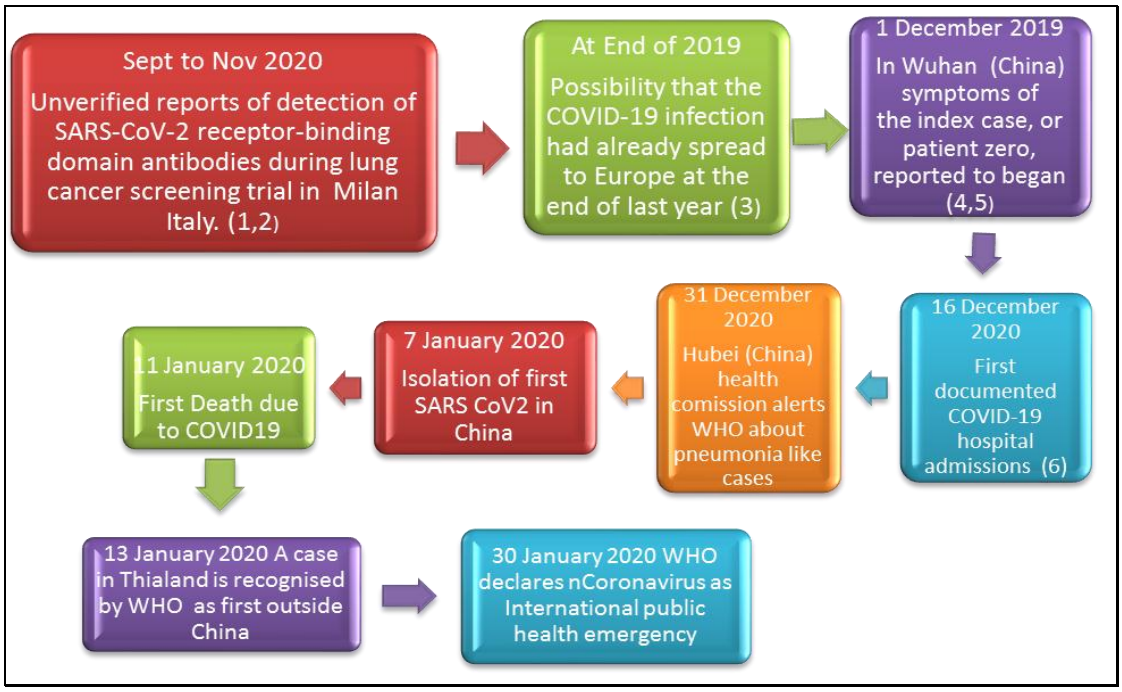

Fig 1: Initial chronological events during development of nCoronavirus COVID19 pandemic.

2. CLASSIFICATION AND PHYLOGENY OF SARSCOV-2

Pathogen

The virus classification and origin:

SARS-CoV-2 is a member of the Family: Coronaviridae and Order: Nidovirales

The family Coronaviridae consists of two subfamilies: *viz.* - Coronavirinae

and - Torovirinae

Members of the subfamily Coronavirinae are subdivided into four

Genera (a to d below):

(a) Alphacoronavirus contains :

- Human coronavirus (HCoV) - 229E
- HCoV-NL63

(b) Betacoronavirus includes:

- HCoV-OC43
- (SARS-HCoV); Severe Acute Respiratory Syndrome human coronavirus
- HCoV-HKU1
- (MERS-CoV); Middle Eastern Respiratory Syndrome Coronavirus
- SARS-CoV-2

(c) Gammacoronavirus includes viruses of whales and birds

(d) Deltacoronavirus includes viruses isolated from pigs and birds.[6]

Belonging to Betacoronavirus, (b above) SARS-CoV-2 (Fig.2) is an enveloped RNA virus which possesses and positive-sense single- stranded RNA (+ssRNA)[7] currently termed as a novel human-infecting Betacoronavirus.[8] Previously scientists were known people getting infected with other human coronaviruses *viz.* 229E, NL63, OC43, and HKU1.[9] SARS-CoV-2 genome analysis provides indication of the virus being closely related to two bat-derived SARS-like coronaviruses with almost 88% identity. The sample compared for this study was collected in 2018 in eastern China (bat-SL-CoVZC45 and bat-SL-CoVZXC21) and it was found to be genetically distinct from SARS-CoV (with about 79% similarity) and MERS-CoV in its comparative analysis.[8] A study of genome sequences of SARS-CoV-2, RaTG13, and SARS-CoV[10] reported that this virus is better linked with BatCoV RaTG13, which are known to be bat coronavirus, detected in *Rhinolophus affinis* from Yunnan Province, with 96.2% overall genome sequence identity.[11] There were no evidence/s of recombination events reported in the genome of SARS-CoV-2 from other viruses originating from bats *viz* BatCoV RaTG13, SARS-CoV and SARSr-CoVs.[11] Although, these studies propose that bats might be the original host of this virus. However, a study is needed to elucidate whether any intermediate hosts have facilitated the transmission of the virus to humans.[10,11] and now unconfirmed reports (as per WHO) of detection of SARS-CoV-2 receptor-binding domain antibodies during lung cancer screening trial in Milan Italy.[1,2] If verified, it may significantly alter present understanding of the current pandemic.

3. MOLECULAR ASPECTS OF SARSCOV-2

There are close to ~ 30000 nucleotides forming the genome of coronavirus, which encodes four structural proteins, *viz* Nucleocapsid (N), Membrane (M), Spike (S), and Envelop (E) protein (Fig 3) in addition to several non-structural proteins (nsp) Structurally there is a protein shell capsid inside which, a nuclear capsid (N-protein) is attached to the single positive strand of RNA that the virus contains (Fig.2). This structural arrangement allows the virus to seize the targeted human

cells and continue their propagation by the processes of replication. During the fundamental processes of transcription and replication the N protein functions to coat the viral RNA genome. The most abundant protein is M-protein which is found on the viral surface and possibly plays a central role as an organizer for the coronavirus assembly. During the viral entry into cell, S-protein (which is integrated over the surface of the virus), mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell.[12] The E-protein is a small membrane protein composed ~ 76 to 109 amino-acid and minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell and virus host cell interaction.[13] A lipid envelop encapsulates the genetic material. Hemagglutinin-esterase dimer (HE) have been located on the surface of the viral. The HE protein may be involved in virus entry, is not required for replication, but appears to be important for infection of the natural host-cell.[14] In general, the spike protein of coronavirus is divided into the S1 and S2 domain, in which S1 is responsible for receptor binding and S2 domain is responsible for cell membrane fusion.[8](Figure 2)

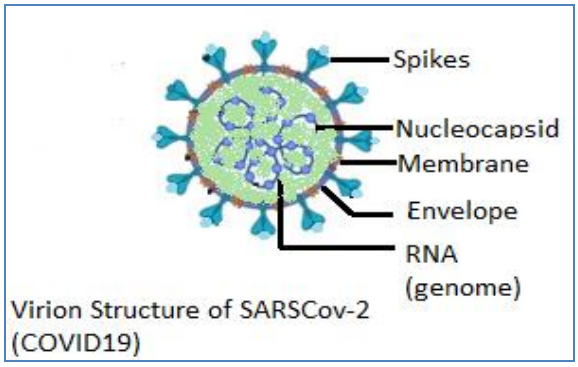

Fig 2: Virion Structure.

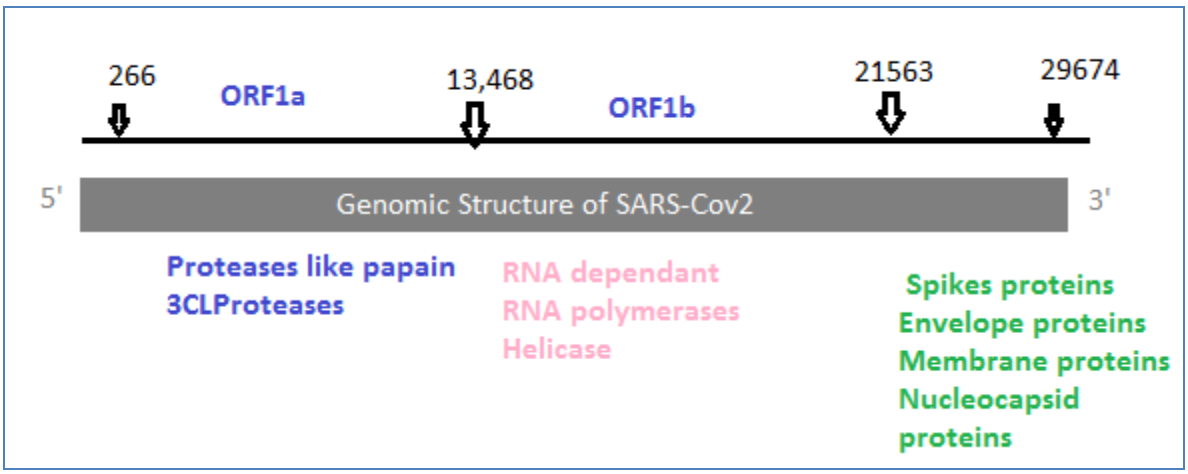

Fig. 3: Genome of coronavirus, the number of sequence above which encodes four structural proteins, *viz* Nucleocapsid (N), Membrane (M), Spike (S), and Envelop (E) protein in addition to several non-structural proteins (nsp) or accessory proteins.[15]

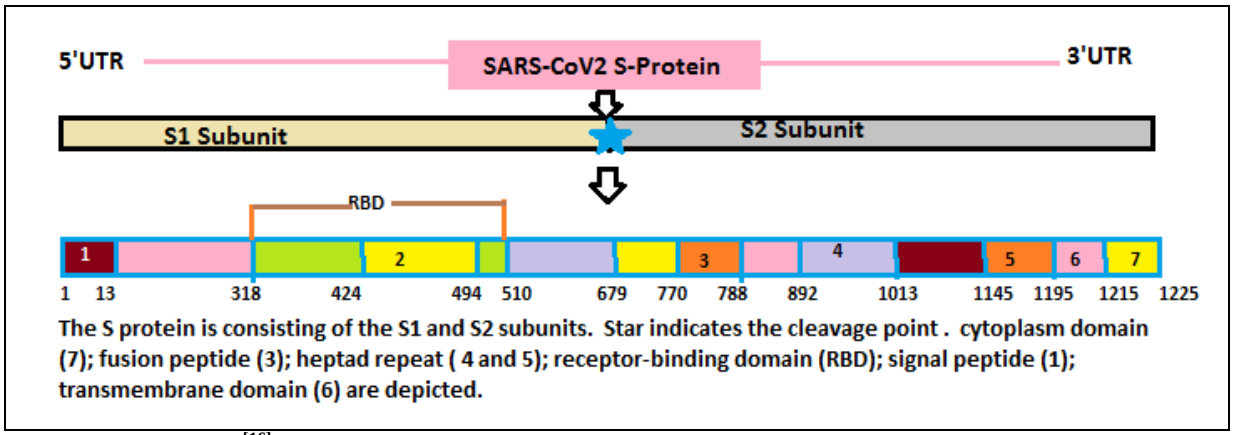

Fig. 4: Recreated from[16] The viral surface proteins, spike, envelope and membrane, are embedded in a lipid bilayer. The single-stranded positive-sense viral RNA is associated with the nucleocapsid protein.

4. MODUS OPERANDI OF SARSCOV-2

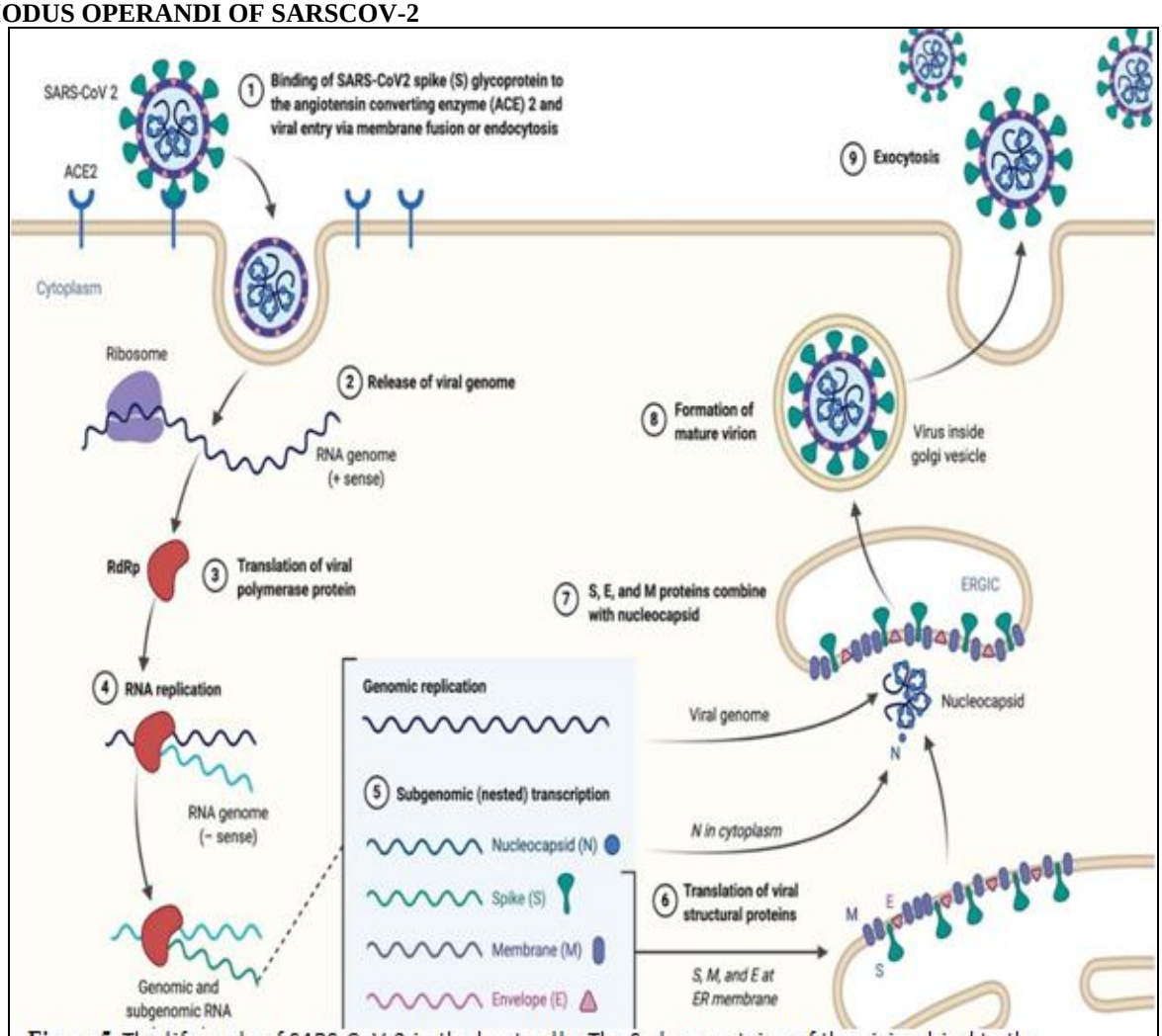

Figure 5. The life cycle of SARS-CoV-2 in the host cells. The S glycoproteins of the virion bind to the cellular receptor angiotensin-converting enzyme 2 (ACE2) and enters target cells through an endosomal pathway. Following the entry of the virus into the host cell, the viral RNA is unveiled in the cytoplasm. ORF1a and ORF1ab are translated to produce pp1a and pp1ab polyproteins, which are cleaved by the proteases of the RTC. During replication, RTC drives the production full length (−) RNA copies of the genome and used as templates for full-length (+) RNA genomes. During transcription, a nested set of sub-genomic RNAs (sgRNAs), is produced in a manner of discontinuous transcription (fragmented transcription). Even though these sgRNAs may have several open reading frames (ORFs), only the closest ORF (to the 5′ end) will be translated. Following the production of SARS-CoV-2 structural proteins, nucleocapsids are assembled in the cytoplasm and followed by budding into the lumen of the endoplasmic reticulum (ER)–Golgi intermediate compartment. Virions are then released from the infected cell through exocytosis (figure created with biorender.com)[17].

Fig. 5 (from[15]) is reproduced for depicting modus operandi of viral entry and replication as well as the RNA packing in human cell the SARSCoV-2 (Figure and description is reproduced and authors do not have copyright.

Angiotensin converting enzyme 2 (ACE2) receptars are well known and found on the surface of many human cells, including certain lung cells. These receptors provide gateway for the viral entry in human cells. The spike (S) protein of virus plays a crucial role while intruding host cell by attaching itself to these ACE2 receptors. Together with host cell factors (such as the cell surface serine protease TMPRSS2), viral uptake and fusion at the cellular or endosomal membrane is promoted.[17] As human cells possess mechanisms of endocytosis as a default pathway of cellular protection from viruses and bacteria[18], a proteolytic cleavage of the coronavirus spike (S) protein by host proteases *viz.* trypsin and furin as consequence of this process is inevitable. This particular cleavage occurs in two sites located at the edge between the S1 and S2 subunits (S1/S2 site) (depicted in Fig. 4). After ingesting the virus (endocytosis)[18], endosomes release the virus inside

cytoplasm for the uncoating of viral nucleocapsid (N) by proteasomes which typically can hydrolyse endogenous proteins. Hoever they are also capable of cleaving exogenous proteins like SARS nucleocapsid protein.[19]. Ultimately, the ssRNA forming the entire viral genetic material is freed into the cytoplasm. Processes like the replication and transcription begin from here onwards and are mediated by the molecular complex machinery referred as replication/transcription complex (RTC). Non-structural proteins are responsible for encoding RTC complex in the viral genome. The RTC is believed to induce double-membrane structures in the cytoplasm of the infected cell.[20] Translation of positive RNA occurs to generate replicase proteins from open reading frame 1a/b (ORF 1a/b) (see Figure 3). These proteins use the genome as a template to generate full-length negative sense RNAs, which subsequently serve as templates in generating addition full-length genomes. Also the structural viral proteins, namely M, S and E corresponding to membrane, spike and envelope proteins are synthesized in the host cell cytoplasm and introduced into the endoplasmic reticulum (ER) (Figure 5), and transfer to endoplasmic reticulum-Golgi intermediate compartment (ERGIC) occurs as a part of this cellular process.[21] Additionally, nucleocapsids are formed from the encapsidation of replicated genomes by N protein (in host cell cytoplasm), and eventually these coalesce within the ERGIC membrane. This is required for the to self-assembly of new virions. Ultimately, smooth walled vesicles are utilized to export the novel virions from infected cells to the cell membrane for secretion, which is known as exocytosis. This event in due course enables virion to infect other cells. Between the intervening time, the enhanced stress of viral replication process on ER pushes the cell death.[22]

5. PATHOGENESIS OF COVID-19

Coronavirudae family are long known to cause disease in both humans and animals. Four human coronaviruses *viz.* 229E, NL63, OC43 and HKU1) are recognised for characteristically infecting upper respiratory tract. Symptoms caused by these above four types of coronaviruses are rather inconsiderable.[23]Conversely, other three types of coronaviruses namely severe acute respiratory syndrome coronavirus (SARS- CoV), Middle East respiratory syndrome coronavirus (MERS- CoV) and SARS- CoV-2 are capable of replicating themselves in the lower respiratory tract as are basis of causing pneumonia. In severely infected cases or if left untreated pneumonia can be fatal.[24]

The current pandemic is caused by SARS- CoV-2 which belongs to the betacoronavirus genus. Its closest relative among human coronaviruses is SARS- CoV, with 79% genetic similarity.[24]

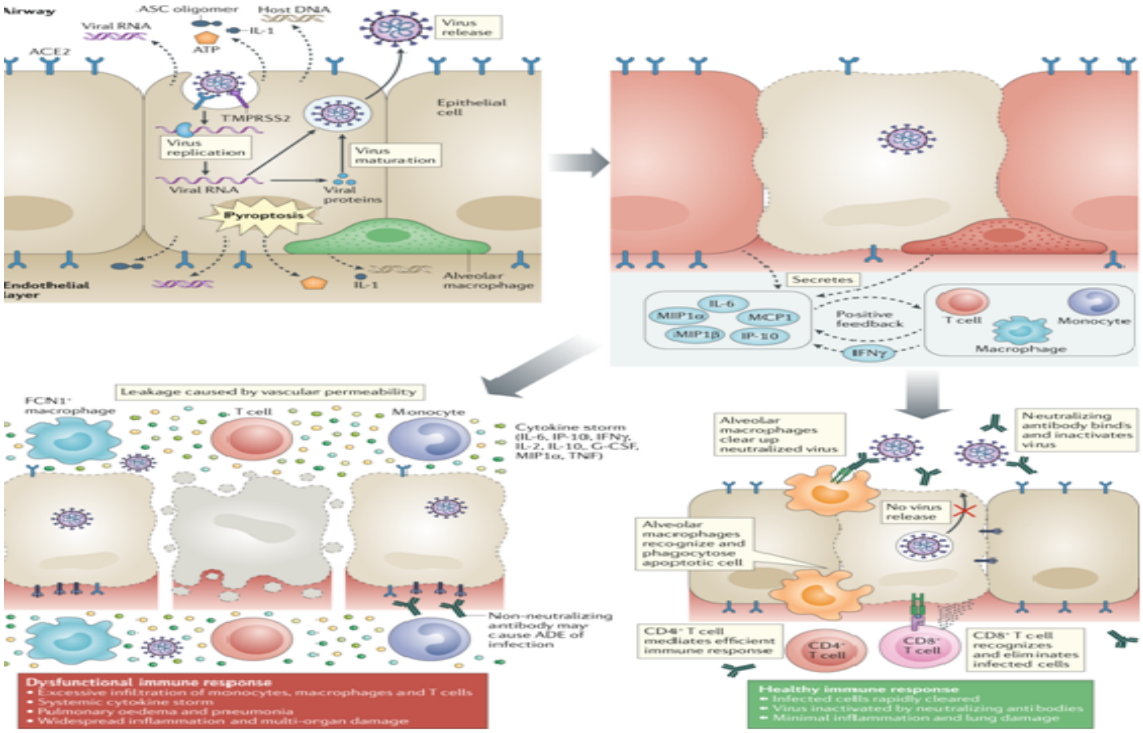

Fig. 6 - Chronology of events during SARS-CoV-2 infection. When severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) infects cells expressing the surface receptors angiotensin- converting enzyme 2 (ACE2) and TMPRSS2, the active replication and release of the virus cause the host cell to undergo pyroptosis and release damageassociated molecular patterns, including ATP, nucleic acids and ASC oligomers. These are recognized by neighbouring epithelial cells, endothelial cells and alveolar macrophages, triggering the generation of pro- inflammatory cytokines and chemokines (including IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β and MCP1).These proteins attract monocytes, macrophages and T cells to the site of infection, promoting further inflammation (with the addition of IFNγ produced by T cells) and establishing a pro-inflammatory feedback loop. In a defective immune response (left side) this may lead to further accumulation of immune cells in the lungs, causing overproduction of pro- inflammatory cytokines, which eventually damages the lung infrastructure. The resulting cytokine storm circulates to other organs, leading to multi- organ damage. In addition, non-neutralizing antibodies produced by B cells may enhance SARS- CoV-2 infection through antibody- dependent enhancement (ADE), further exacerbating organ damage. Alternatively , in a healthy immune response (right side), the initial inflammation attracts virus- specific T cells to the site of infection, where they can eliminate the infected cells before the virus spreads. Neutralizing antibodies in these individuals can block viral infection, and alveolar macrophages recognize neutralized viruses and apoptotic cells and clear them by phagocytosis. Altogether, these processes lead to clearance of the virus and minimal lung damage, resulting in recovery. G- CSF, granulocyte colony- stimulating factor; TNF, tumour necrosis factor.

(This figure and description is reproduced as it is from[29] Authors of this article do not have copyright].

SARS- CoV-2 and related respiratory coronaviruses are predominantly transmitted by means of respiratory droplets. A possibility of fecal–oral transmission route is often suspected but still remains unverified. Upon the onset of infection, approximately 4 to 5 days of median period occurs before symptoms commence.[26, 27] Data shows that 97.5% of patients who exhibit symptoms develope them within 11.5 days.[28, 29] At the instant of hospital admission, COVID-19 infected patients typically complain of fever and dry cough; while less commonly, patients also experience difficulty in breathing, muscle and/or joint pain, headache/dizziness, diarrhoea, nausea and the coughing up of blood.[30, 31]

In a duration of 5–6 days of symptom onset, SARS-CoV-2 viral load reaches its peak. This duration is significantly short when compared with that of another related virus SARS- CoV, where viral load peaks at about 10 days after symptom onset.[32, 33] Severe COVID-19 cases progress to acute respiratory distress syndrome (ARDS), on average around 8–9 days after symptom onset.[34, 35]

The SARS- CoV-2 infection pathophysiology intimately resembles that of SARS- CoV infection, featuring aggressive inflammatory responses strongly implicated

in the resulting damage to the airways.[36] Therefore, disease severity in patients is due to not only the viral infection but also the host response.[37]

Breathing difficulty characterizing ARDS in severe COVID-19 is accompanied by low blood oxygen level.[38] As a consequence, some patients may yield secondary bacterial and fungal infections.[37] ARDS may lead directly to respiratory failure, turning to be a cause of death in 70% of fatal COVID-19 cases. In addition, the vast release of cytokines by the immune system in response to the viral infection and/or secondary infections can result in a cytokine storm and symptoms of sepsis that are the cause of death in 28% of fatal COVID-19 cases. In these cases, uncontrolled inflammation inflicts multi- organ damage leading to organ failure, especially of the cardiac, hepatic and renal systems.[38] (Fig. 6).

Inflammatory immunopathogenesis

Infection by SARS- CoV-2 and eventual damage of lung cells onsets a local immune response, involving the immune cells like macrophages and monocytes. These cells acting as respondent to the infection, release cytokines and prime adaptive T and B cell immune responses. In majority cases, this process itself is able to ultimately resolve the infection. Though, in some cases, an inappropriate immune response occurs, causing significant lung and even systemic pathology. Cytopathic viruses which also include SARS- CoV-2.[39], bring death and injury of virus- infected epithelial cells in airway, and tissues as an unavoidable result of the viral replication cycle.[40] The kind of virus-linked pyroptosis observed in patients with SARSCoV is also observed in patients infected with SARSCov-2.[41] In these infected patients the epithelial cells in the airway- which is vital for survival of the patient- cause virus- linked pyroptosis with associated vascular leakage.[40] Cytopathic viruses are capable to elicit the lytic and highly inflammatory form of programmed cell death known as Pyroptosis.[42] Pyroptosis is a likely trigger for the succeeding inflammatory response.[43] Interleukin (IL)-1β, an important cytokine released during pyroptosis. Levels of Interleukin (IL)-1β are elevated during SARS- CoV-2 infection.[44] Alveolar epithelial cells and alveolar macrophages use diverse pattern- recognition receptors (PRRs), to detect the released pathogen- associated molecular patterns (PAMPs), like the viral RNA, and damage- associated molecular patterns (DAMPs), including ATP, DNA and apoptosis-associated speck-like protein with a caspase-recruitment domain oligomers (ASC). A local inflammation wave ensues, which involves elevated secretion of the pro-inflammatory cytokines and chemokines IL-6, interferon –γ (IFNγ), monocyte chemoattractant protein-1 (MCP1) and induced protein-10 (IP-10) into the bloodstream of infected patients.[7,40] These cytokines are indicators of a T helper 1 (TH1) cell- polarized response Similar observations are also reported for SARS- CoV and MERS- CoV.[45] Secretion of such cytokines and chemokines attract immune cells, notably monocytes and T lymphocytes, except that of neutrophils, from the blood into the infected site.[46,47] Pulmonary recruitment of immune cells from the blood and the infiltration of lymphocytes into the airways may explain the lymphopenia and increased neutrophil–lymphocyte ratio seen in around 80% of patients with SARS- CoV-2 infection.[48, 49]

In majority of infected individuals, these immune cells and their cascade response can clear the infection in the lung. Later the immune response recedes and patients recover. However, in some patients, as discussed above, a dysfunctional immune response occurs, which triggers a cytokine storm causing widespread lung inflammation. It was observed that patients with severe COVID-19, requiring intensive care in hospitals, exhibited higher blood plasma levels of IL-2, IL-7, IL-10, granulocyte colony- stimulating factor (G- CSF), IP-10, MCP1, macrophage inflammatory protein 1α (MIP1α) and tumor necrosis factor (TNF).[7] Characteristically IL-6 levels in these patients continued to increase over time. It is observed that these levels are comparatively more elevated in non- survivors than survivors.[50] A highly inflammatory monocyte- derived FCN1+ macrophage population exists in the bronchoalveolar lavage fluid of patients with severe COVID-19 patients. Such FCN1+ macrophage population is not observed in patients having mild COVID-19.[51] Also, patients with severe disease show a significantly higher percentage of CD14+CD16+ inflammatory monocytes in peripheral blood than patients with mild Disease.[52] These cells secrete inflammatory cytokines that contribute to the cytokine storm, including MCP1, IP-10 and MIP1α.

When inflammatory cell infiltration continues without inhibition it can lead to damage in the lung through excessive secretion of proteases and reactive oxygen species. These events occur in addition to the direct cellular damage resulting from the virus. In summation, these events result in diffuse alveolar damage, including desquamation (shedding of outermost membrane or layer) of alveolar cells and additionally hyaline membrane formation and pulmonary oedema. This restricts the in breathing causing low blood oxygen levels. The lungs consequently also becomes more susceptible to secondary infections.[46,47]

In addition to local damage, cytokine storm also has ripple effects across the body. Elevated levels of cytokines such as TNF can cause septic shock and multi-organ failure. These may result in myocardial damage and circulatory failure observed in some patients Older people (those aged over 60 years) and people with co-morbidities are more likely to develop such a dysfunctional immune response that causes pathology and also fails to successfully eradicate the pathogen.[53]

CONCLUDING REMARKS

This pathogen and nomenclatured as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group[54] and the disease termed as coronavirus disease 2019 (COVID-19) by the WHO has infections and deaths continuing all over the world with varying but alarming rate of mortality ad so WHO declared the SARS-CoV-2 outbreak as a Public Health Emergency of International Concern (PHEIC).[55] Although the major organ involved in COVID-19 is the lungs, the heart, kidneys, genitals, and liver are also damaged.[11, 56, 57] A recent retrospective study found that the proportion of patients with severe COVID-19 who develop acute respiratory distress syndrome (ARDS), acute kidney injury, abnormal hepatic function, and cardiac injury are 67.3, 28.9, 28.9, and 23.1%, respectively, and the 28-day mortality rate is 61.5%.[58] Due to the unique work nature of the intensive care unit (ICU), COVID-19 poses an immense challenge to medical staff in the ICU, as not only does it require an increase in manpower and materials but there is also a risk of infection.[59] In such situation, the focus on prevention and cure both are equally important till the vaccination is available to whole populations.

REFERENCES

1. Vagnoni, Giselda (16 November 2020). "Researchers find coronavirus was circulating in Italy earlier than thought". *Reuters*. Retrieved 9 December 2020.
2. Apolone, Giovanni; Montomoli, Emanuele; Manenti, Alessandro; Boeri, Mattia; Sabia, Federica; Hyseni, Inesa; Mazzini, Livia; Martinuzzi, Donata; Cantone, Laura; Milanese, Gianluca; Sestini, Stefano; Suatoni, Paola; Marchianò, Alfonso; Bollati, Valentina; Sozzi, Gabriella; Pastorino, Ugo (11 November 2020). "Unexpected detection of SARS-CoV-2 antibodies in the prepandemic period in Italy". *Tumori Journal*. doi:10.1177/0300891620974755.
3. Platto, S.; Xue, T.; Carafoli, E. (September 2020). "COVID19: an announced pandemic". *Cell Death Dis*, 11(799). doi:10.1038/s41419-020-02995-9.
4. Duarte, Fernando (24 February 2020). "Who is 'patient zero' in the coronavirus outbreak?". BBC Future. BBC. Archived from the original on 26 February 2020 – via bbc.com.
5. ^ *Wang, Yiqing (18 February 2020). "Mohubuqing de "linghao bingren" yu xinguan bingdu laiyuan zhengyi" 模糊不清的"零号病人"与新冠病毒来源争议 [The ambiguity over "patient zero" and the debate on the origins of the novel coronavirus]. BBC Chinese (in Chinese). BBC. Archived from the original on 18 February 2020. Retrieved 1 May 2020 – via bbc.com.*
6. Burrell C, Howard C, Murphy F. Fenner and White's medical virology. 5th ed. United States: Academic Press; 2016.
7. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist oninanimate surfaces? A systematic review. BMC Infect Dis, 2006; 6: 130.
8. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins andreceptor binding. Lancet, 2020.
9. https://www.cdc.gov/coronavirus/types.html
10. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreakassociated with a new coronavirus of probable bat origin. Nature 2020.
11. Fan ZY, Chen LP, Li J, Tian C, Zhang YJ, Huang SP. Clinical features of COVID-19 related liver damage. medRxiv, (2020). doi: 10.1101/2020.02.26.200269717.
12. Kirchdoerfer, R. N., Cottrell, C. A., Wang, N., Pallesen, J., Yassine, H. M.,Turner, H. L., Corbett, K. S., Graham, B. S., McLellan, J. S., & Ward, A. B.(2016). Pre-fusion structure of a human coronavirus spike protein. Nature, 531(7592): 118–121. https://doi.org/10.1038/nature17200.
13. Gupta, M. K., Vemula, S., Donde, R., Gouda, G., Behera, L., & Vadde, R. (2020). In silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. Journal of Biomolecular Structure and Dynamics. https://doi.org/10.1080/07391102.2020.1751300
14. Lissenberg, A., Vrolijk, M. M., van Vliet, A. L. W., Langereis, M. A., de Groot-Mijnes, J. D. F., Rottier, P. J. M., & de Groot, R. J. (2005). Luxury at a Cost? Recombinant mouse hepatitis viruses expressing the accessory hemagglutinin esterase protein display reduced fitness in vitro.Journal of Virology, 79(24): 15054–15063. https://doi.org/10.1128/JVI.79.24.15054-15063.2005.
15. Chen, Y.; Liu, Q.; Guo, D. Emerging coronaviruses: Genome structure, replication, and pathogenesis. J. Med. Virol, 2020; 92: 418–423. [CrossRef] [PubMed]
16. Subramanian Boopathi, Adolfo B. Poma & Ponmalai Kolandaivel; Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS https://doi.org/10.1080/07391102.2020.1758788.
17. V'kovski, P., Kratzel, A., Steiner, S. *et al.* Coronavirus biology and replication: implications for SARS-CoV-2. *Nat Rev Microbiol* (2020). https://doi.org/10.1038/s41579-020-00468-6 21.
18. Simmons, G., Gosalia, D. N., Rennekamp, A. J., Reeves, J. D., Diamond,S. L., & Bates, P. (2005). Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. Proceedings of the National Academy of Sciences, 102(33): 11876–11881. https://doi.org/10.1073/pnas.0505577102.

19. Wang, Q., Li, C., Zhang, Q., Wang, T., Li, J., Guan, W., Yu, J., Liang, M., & Li, D. (2010). Interactions of SARS Coronavirus Nucleocapsid Protein with the host cell proteasome subunit p42. Virology Journal, 7(1): 99–98. https://doi.org/10.1186/1743-422X-7-99.
20. Van Hemert, M. J., Van Den Worm, S. H. E., Knoops, K., Mommaas, A. M., Gorbalenya, A. E., & Snijder, E. J. (2008). SARS-coronavirus replication/ transcription complexes are membrane-protected and need a host factor for activity in vitro. PLoS Pathogens, 4(5): e1000054. https://doi.org/10.1371/journal.ppat.1000054.
21. Masters, P. S. (2006). The molecular biology of coronaviruses. Advances in Virus Research, 65(06): 193–292. https://doi.org/10.1016/S0065-3527(06)66005-3.
22. Song, H. C., Seo, M.-Y., Stadler, K., Yoo, B. J., Choo, Q.-L., Coates, S. R.,Uematsu, Y., Harada, T., Greer, C. E., Polo, J. M., Pileri, P., Eickmann, M., Rappuoli, R., Abrignani, S., Houghton, M., & Han, J. H. (2004) Synthesis and characterization of a native, oligomeric form of recombinant severe acute respiratory syndrome coronavirus spike glycoprotein. Journal of Virology, 78(19): 10328–10335. https://doi.org/10.1128/JVI.78.19.10328-10335.2004.
23. Fehr, A. R. & Perlman, S. Coronaviruses: an overview of their replication and pathogenesis. *Methods. Mol. Biol*, 2015; 1282, 1–23.
24. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome- related coronavirus: classifying 2019- nCoV and naming it SARS- CoV-2. *Nat. Microbiol*, 2020; *5:* 536–544.
25. Matthew, Z .T. et al. The trinity of COVID-19: immunity, inflammation and intervention. Nature Reviews Immunology, June 2020; 20: 363–374.
26. Guan, W. J. et al. Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* https://doi. org/10.1056/NEJMoa2002032 (2020).
27. Pung, R. et al. Investigation of three clusters of COVID-19 in Singapore: implications for surveillance and response measures. *Lancet*, 2020; 395: 1039–1046.
28. Lauer, S. A. et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann.Intern. Med.* https://doi.org/10.7326/m20-0504 (2020).
29. Li, Q. et al. Early transmission dynamics in Wuhan,China, of Novel Coronavirus- infected pneumonia.*N. Engl. J. Med*, 2020; 382: 1199–1207.
30. Chan, J. F. et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person- to- person transmission: a study of a family cluster. *Lancet*, 2020; 395: 514–523.
31. Phan, L. T. et al. Importation and human- to- human transmission of a novel coronavirus in Vietnam. *N. Engl. J. Med*, 2020; 382: 872–874.
32. Pan, Y., Zhang, D., Yang, P., Poon, L. L. M. & Wang, Q.Viral load of SARS- CoV-2 in clinical samples. *Lancet Infect. Dis.* https://doi.org/10.1016/s1473- 3099(20)30113-4 (2020).
33. Peiris, J. S. et al. Clinical progression and viral load in a community outbreak of coronavirus- associated SARS pneumonia: a prospective study. *Lancet*, 2003; 361: 1767–1772.
34. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 2020; 395: 497–506.
35. Wang, D. et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirusinfected pneumonia in Wuhan, China. *JAMA*, 2020; 323: 1061–1069.
36. Wong, C. K. et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin. Exp. Immunol*, 2004; 136; 95–103.
37. Chen, N. et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*, 2020; 395: 507–513.
38. Zhang, B. et al. Clinical characteristics of 82 death cases with COVID-19. Preprint at *medRxiv* https://doi.org/10.1101/2020.02.26.20028191 (2020).
39. Park, W. B. et al. Virus isolation from the first patient with SARS- CoV-2 in Korea. *J. Korean Med. Sci*, 2020; 35: e84.
40. Zhang, H. et al. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. *Ann. Intern. Med.* https://doi.org/ 10.7326/m20-0533 (2020).
41. Chen, I. Y., Moriyama, M., Chang, M. F. & Ichinohe, T. Severe acute respiratory syndrome coronavirus viroporin 3a activates the NLRP3 inflammasome. *Front. Microbiol*, 2019; 10: 50.
42. Fink, S. L. & Cookson, B. T. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. *Infect. Immun*, 2005; 73: 1907–1916.
43. Yang, M. Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV infection. *SSRN* https://doi. org/10.2139/ssrn.3527420 (2020).
44. Huang, C. et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 2020; 395: 497–506.
45. Huang, K. J. et al. An interferon- gamma- related cytokine storm in SARS patients. *J. Med. Virol*, 2005; 75: 185–194.
46. Xu, Z. et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med*, 2020; 8: 420–422.
47. Tian, S. et al. Pulmonary pathology of early phase 2019 novel coronavirus (COVID-19) pneumonia in two patients with lung cancer. *J. Thorac. Oncol.* https://doi. org/10.1016/j.jtho.2020.02.010 (2020).

48. Guan, W. J. et al. Clinical characteristics of coronavirus disease 2019 in China. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2002032 (2020).
49. Qin, C. et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin. Infect.Dis.* https://doi.org/10.1093/cid/ciaa248 (2020).
50. Zhou, F. et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*, 2020; 395: 1054–1062.
51. Liao, M. et al. The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single- cell RNA sequencing. Preprint at *medRxiv* https://doi.org/ 10.1101/2020.02.23.20026690 (2020).
52. Zhou, Y. et al. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. *Natl Sci. Rev.* https://doi.org/10.1093/nsr/nwaa041 (2020).
53. Ruan, Q., Yang, K., Wang, W., Jiang, L. & Song, J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* https://doi.org/10.1007/ s00134-020-05991- x (2020).
54. Gorbalenya AEA. Severe acute respiratory syndrome-related coronavirus: thespecies and its viruses – a statement of the Coronavirus Study Group. BioRxiv2020, http://dx.doi.org/10.1101/2020.02.07.937862.
55. Burki TK. Coronavirus in China. Lancet Respir Med 2020.
56. Ma L, XieW, Li D, Shi L,Mao Y, Xiong Y, et al. Effect of SARS-CoV-2 infection upon male gonadal function: a single center-based study. medRxiv. (2020). doi: 10.1101/2020.03.21.20037267.
57. Li X,Wang LW, Yang SN, Yang F, Xiang LK, Zhu JL. Clinical characteristics of 25 death cases infected with COVID-19 pneumonia: a retrospective review of medical records in a single medical center, Wuhan, China. medRxiv. (2020). doi: 10.1101/2020.02.19.20025239.
58. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia inWuhan, China: a singlecentered, retrospective, observational study. Lancet Respir Med, 2020; 8: e26. doi: 10.1016/S2213-2600(20)30079-5.
59. Arabi YM, Murthy S, Webb S. COVID-19: a novel coronavirus and a novel challenge for critical care. Intensive Care Med. (2020) 1–4. doi: 10.1007/s00134-020-05955-1.