ACTA SCIENTIFIC MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 4 Issue 5 May 2020

Review Article

Diagnostic Strategies for COVID-19

Krishan Karla[1], Shashidhar KN[2] and Krishna S Nallagangula[3]*

[1]*Senior Scientist, Founder and CEO, BioGenex Laboratories, Fremont, California, United States of America*

[2]*Professor and HOD, Co-ordinator, R&D, Vice Principal, Sri Devaraj Urs Medical College, SDUAHER, Kolar, Karnataka, India*

[3]*Molecular Pathology Scientist, BioGenex Life Sciences Pvt Ltd, Adibatla, Hyderabad, Telangana, India*

***Corresponding Author:** Krishna S Nallagangula, Molecular Pathology Scientist, BioGenex Life Sciences Pvt Ltd, Adibatla, Hyderabad, Telangana, India.

Received: March 30, 2020

Published: April 08, 2020

Abstract

COVID-19; single stranded positive RNA (+ssRNA) virus causes common cold to severe diseases viz., Severe Acute Respiratory Syndrome (SARS). At this point of time, prevalence of this pandemic virus increases exponentially and become global health burden. Asymptomatic/paucisymptomatic respiratory failure; characterized by leucopenia, leukocytosis, lymphonia and thrombocytopenia along with elevated levels of hepatic aminotransferases [Aspartate transaminase (AST) and Alanine Transaminase (ALT)] and lactate dehydrogenase (LDH). Detection of viral genome by reverse transcription polymerase chain reaction (RT-PCR) is the World Health Organization (WHO) recommended diagnostic modality. Enhanced inflammatory cytokines level will be observed in serum of severe patients. In the present scenario, therapeutic strategies for this infection are only supportive. Drastic research is needed for structural characterization of virus and pathophysiology of the disease. There is a need for strengthening of research in diagnostic and therapeutic areas to control the prevalence and cure the disease; increases survival rate with decreased mortality. Aggressive social isolation is the best way for progressive reduction of prevalence.

Keywords: Single Stranded Positive RNA (+ssRNA); Aspartate Transaminase (AST); Alanine Transaminase (ALT); Lactate Dehydrogenase (LDH); Reverse Transcription Polymerase Chain Reaction (RT-PCR); World Health Organization (WHO)

Introduction

Coronavirus (Co V); spike like glycoprotein on envelope gives crown like appearance. Orthocoronavirinae of Coronaviridae classified into Alpha, Beta, Delta and Gamma [1]. These viruses can cause respiratory, hepatic and neurological disorders in different animal species including humans. Most of human Co Vs are mild; the out brake of two beta Co V causes Severe Acute Respiratory Syndrome (SARS- Co V) and Middle East Respiratory Syndrome (MERS-Co V). COVID-19 (SARS-Co V or 2019-no V) belongs to Beta coronavirus (beta Co V); with identified seven human Co Vs till date; sensitive to ultraviolet rays and heat and inactivated by lipid solvents [2,3].

Genome of Co V is single strand positive RNA (+ssRNA) from 26 to 32 kb in length with G + C nucleotide base pairs ranging from 32% to 43%; 5' cap structure and 3' poly A tail (Figure 1 and 2) [2,4]. Newly identified SARS-Co V-2 is a single stranded RNA with 29891 nucleotides which translate 9860 amino acids [3,5]. Genomic RNA serves as a template for translation of 1a/1ab poly protein, non-structural proteins (nsps), replication-transcription complex. Discontinuous transcription generates subgenomic RNAs (sgRNAs) with 5'-leader and 3'-terminal sequence; template for production of subgenomic mRNAs. Whole genome and subgenome of Co V encode 16 nsps (Table 1) [3] useful for replication of Co Vs in host [6,7]. Four structural proteins (S, M, E and N) are essential for virion assembly and infection to the host (Figure 3) [2,3]. Spikes on surface, S-protein is responsible for host Angiotensin Converting Enzyme 2 (ACE2) receptor attachment and serine protease TMPRSS2 priming [8,9]. Transmembrane of M-protein binds to nucleocapsid. Viral pathogenesis is by E-protein. Viral replication is enhanced by N-protein with help of nsps. Further research is needed for structural characterization of SARS-Co V [10-14].

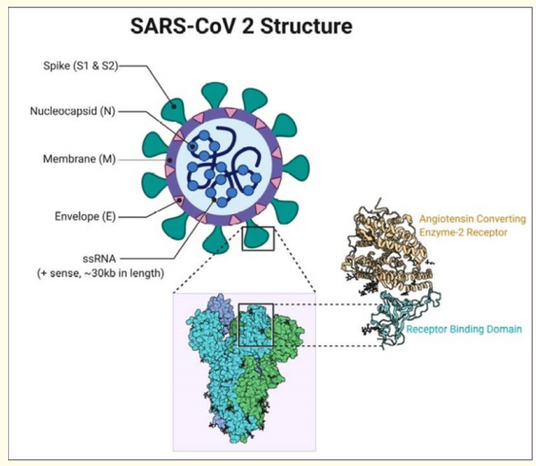

Figure 1: SARS-Co V-2 structure [2].

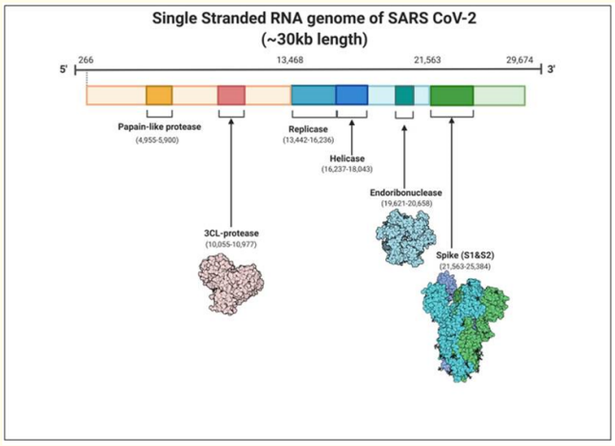

Figure 2: Single stranded RNA genome of SARS-Co V-2 [2].

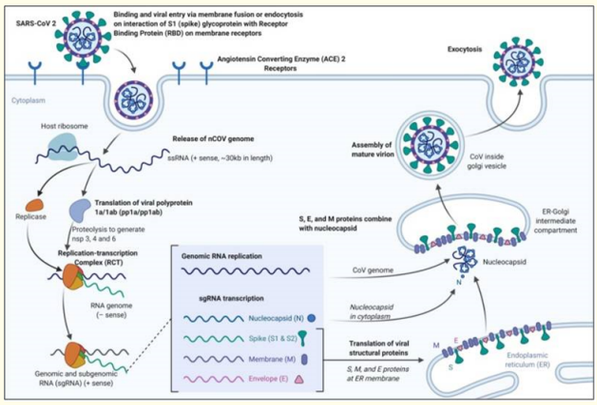

Figure 3: Replication of SARS-Co V-2 in host [2].

nsps	Function
nsp1	Cellular mRNA degradation, inhibiting IFN signaling
nsps2	Unknown
nsps3	PLP, polypeptides cleaving, blocking host innate immune response, promoting cytokine expression
nsps4	DMV formation
nsps5	3CLpro, Mpro, polypeptides cleaving, inhibiting IFN signaling
nsps6	Restricting autophagosome expansion, DMV formation
nsps7	Cofactor with nsp8 and nsp12
nsps8	Cofactor with nsp7 and nsp12, primase
nsps9	Dimerization and RNA binding
nsps10	Scaffold protein for nsp14 and nsp16
nsps11	Unknown
nsps12	Primer dependent RdRp
nsps13	RNA helicase, 5′ triphosphatase
nsps14	Exoribonuclease, N7-Mtase
nsps15	Endoribonuclease, evasion of dsRNA sensors
nsps16	2′-O-MTase; avoiding MDA5 recognition, negatively regulating innate immunity

Table 1: The 16 non-structural proteins of coronaviruses and their functions [3].

Viral infection is responsible for extensive immune reactions in the host; cytokine and chemokine production. Interleukins produced by activated leukocytes are responsible for B lymphocyte differentiation and acute phase proteins production [2]. In-depth research will reveal the pathophysiology of disease which helps to design and develop diagnostic modalities for diagnosis and prognosis and tailored made therapy to control the pandemic disease. Transmission of the Co V is believed to be by respiratory droplets; human-human transmission; frequent source of COVID-19 is symptomatic people. People with no symptoms might transmit the virus [15]. Clinical symptoms includes cold, cough, pneumonia, fever, dyspnea to respiratory failure, septic shock and multi organ dysfunction [2,16]. Significant social isolation is the only preventive mechanism as of now for the prevention of prevalence of the disease.

Diagnostic strategies

Differential diagnosis of COVID-19 must include a wide range of investigations from infectious to non-infectious respiratory disorders. Altered levels of total white blood cells with decreased platelet count along with elevated levels of hepatic aminotransferases [Aspartate transaminase (AST) and Alanine Transaminase (ALT)] and lactate dehydrogenase (LDH) and C-reactive protein (CRP) are the indictors of the infection [2]. World Health Organization (WHO) recommended diagnostic modality is detection of viral genome [envelope (E), RNA-dependant RNA polymerase genes and N gene which encodes viral nucleocapsid phosphoprotein] in clinical samples by reverse transcription polymerase chain reaction (RT-PCR) [17,18]. Abnormalities in hematological investigations are the evidence for infection. Altered levels in coagulation profile [prothrombin time (PT) and D-dimer] might be observed [2]. Serological identification of IgG/IgM antibodies gives additional information about infection [19]. Estimation of cytokine storm (cytokine and chemokine) might give characterization of acute phase of the illness. Biochemical tests for liver and renal along with electrolytes and coagulation profile might give indication for Multi Organ Dysfunction (MOD). Radiological investigations viz., chest computerized tomography (CT) might be useful to rule out pneumonia [20,21].

Identification of viral gene sequence

Due to high sensitivity and specificity, analysis of viral specific genes by real time polymerase chain reaction (RT-PCR) is highly recommended [17]. Study conducted by Wnag., *et al*. used real time RT-PCR/next generation sequencing for identification of COVID-19 in respiratory specimens; 5′-TCAGAATGCCAATCTCCCCAAC-3′ as forward prime, 5′-AAAGGTCCACCCCGATACATTGA-3′ as reverse prime and 5′-CY5-CTAGTTACACTAGCCATCCTTACTGC-3′BHQ1 as probe. Amplification was achieved at 50°C for 15 minutes, 95°C for 3 minutes followed by 45 cycles of 95°C for 15s and 60°C for 30s [20]. Based on gene sequence present at Gene Bank, primers should be designed for comprehensive analysis and detection of viral genes in clinical samples. Comparison should be done for amplified sequence with the help of NCBI BLAST [19,22].

In-depth research is needed which gives detailed replication of COVID-19 RNA will elaborate viral life cycle proteins in host cell. COVID-19 will enter the host cell by the viral S-proteins to cellular receptor (ACE2) and priming by host serine protease TMPRSS2 [9]. In the present scenario, there is a need for extensive research for the development of highly sensitive and specific probes for S-protein as well as ACE2 receptor mRNA and TMPRSS2 for visualization by *in situ* Hybridization (ISH).

Serological investigations

COVID-19 viral exposure initiated antibodies in host can be identified qualitatively which gives evidence for viral infection. Early stage antibody will be IgM followed by IgG during disease progression. Chromatographic immunoassay for qualitative detection for these IgM and IgG specific for nucleocapsid protein of COVID-19 has been developed in human blood, plasma and serum. Zhou., et al. in their study developed qualitative Enzyme Linked Immuno Sorbent Assay (ELISA) by using nucleocapsid (N) protein from bat SARSr-Co V Rp3 as antigen for IgG and IgM. Compared to RT-PCR nucleic acid analysis, these serological investigations are faster and easier; early detection of viral exposure might not effective by this diagnostic modality and care should be taken for cross reactivity. Detailed proteomic research about viral replication is needed to design simple qualitative western blot detection of COVID-19 infection [19,23].

Estimation of cytokine storm

Excessive immune reactions in the host against viral infection by production of cytokines and chemokines are a complex mechanism. Monitoring of cytokine storm gives evaluation of disease progression. Elevated levels of inflammatory cytokines correlate with disease severity. High levels of IL-1β, Interferon- γ (IFN-γ), C-X-C motif chemokine 10/Interferon- γ induced protein (Mol. Wt. 10 kda) (CXCL10/IP-10) along with proinflammatory factors viz., interleukins (IL-2, IL-7 and IL-10), chemokine (C-C motif) ligand 2/Monocyte Chemoattractant Protein 1 (CCl2/MCP-1), Vascular Endothelial Growth Factor (VEGF) and Tumor Necrosis Factor- α (TNF-α) were seen in COVID-19 infection. Activated leukocyte derived IL-6 levels will be enhanced during inflammation and infection. Estimations of this cytokine storm might give evidence for disease progression; multiple cytokine quantitative ELISA assay panel might be useful for tailored made therapy and drug monitoring [2,20,23].

Social isolation is the only way to stop prevalence of the COVID-19 infection as of now. Personal hygiene, use of lipid solvents viz., 75% ether, ethanol etc. and personal protective equipment (PPE) usage might reduce the chance of transmission of diseases. Till date, neither specific anti-viral therapy nor vaccine is available to cure and prevent the COVID-19 infection. Symptomatic treatment with oxygen therapy is the only therapeutic intervention for patients with infection. Mechanical ventilation plays a vital role for those with respiratory failure with support of hemodynamic for the management of septic shock [2]. Drugs which inhibit binding of S-protein to ACE2 receptors and TMPRSS2 may be useful to prevent viral entry into host cell [9]. Inhibitors of RNA polymerase might be effective; controls replication of RNA in host cell. There is urgency for the development of vaccine to prevent the COVID-19 infection. Extensive research is needed for the usage of inactivated or attenuated whole virus or structural and functional proteins viz., S-protein as immunogens for vaccine development. Even though no clinical evidence to cure and prevent COVID-19 infection by stem cell therapy, research in the direction of Mesenchymal Stem Cell (MSC) may give successful clinical outcome; MSC reported to cure acute lung injury caused by H5N1 and H9N2 influenza viruses [23].

Conclusion

Rapidly growing pandemic COVID-19 became fatal infection globally. As of now no effective treatment and vaccine, the best precaution to stop the prevalence of infection is to control the source. Early diagnosis might be useful for isolation of infected patients and provide supporting treatment. Sensitive and specific RT-PCR is the best diagnostic modality recommended by WHO for diagnosis. Serological qualitative identification of IgG and IgM might provide confirmation for infection. Continues monitoring of cytokine storm, renal, hepatic and vital biochemical parameters will give evidence for drug response. Present scenario demands aggressive research in the field of companion diagnostics to design, develop and implement accurate, sensitive and specific biomarkers for diagnosis and prognosis of infection. There is a need for in-depth research which is evidence based for drug discovery and vaccine development.

Bibliography

1. Chan JF., *et al*. "Interspecies transmission and emergence of novel viruses: lessons from bats and birds". *Trends in Microbiology* 10 (2013): 544-555.

2. Cascella M., *et al*. "Features, evaluation and treatment coronavirus (COVID-19)" (2020).

3. Chen Y., *et al*. "Emerging coronavirus: Genome structure, replication and pathogenesis". *Journal of Medical Virology* 92.4 (2020): 418-423.

4. Malik YS., *et al.* "Emerging novel coronavirus (2019-n Co V) - current scenario, evolutionary perspective based on genome analysis and recent developments". *Veterinary Quarterly* 40.1 (2020): 68-76.

5. Chan JF., *et al.* "Genomic characterization of the 2019 novel human pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan". *Emerging Microbes and Infections* 9.1 (2020): 221-236.

6. Sawicki SG., *et al.* "A contemporary view of coronavirus transcription". *Journal of Virology* 81.1 (2007): 20-29.

7. Periman S., *et al.* "Coronaviruses post-SARS: update on replication and pathogenesis". *Nature Reviews Microbiology* 7.6 (2009): 439-450.

8. Beniac DR., *et al.* "Architecture of the SARS coronavirus perfusion spike". *Nature Structural and Molecular Biology* 13.8 (2006): 751-752.

9. Hoffmann M., *et al.* "SARS-Co V-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor". *Cell* 181 (2020): 1-10.

10. Nal B. "Differential maturation and sub cellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E". *Journal of General Virology* 86.5 (2005): 1423-1434.

11. Neuman BW., *et al.* "A structural analysis of M protein in coronavirus assembly and morphology". *Journal of Structural Biology* 174.1 (2011): 11-22.

12. Nieto-Torres JL., *et al.* "Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis". *PLOS Pathogens* 10.5 (2014): e1004077.

13. Fehr AR., *et al*. "Coronavirus: an overview of their replication and pathogenesis". *Methods in Molecular Biology* 1282 (2015): 1-23.

14. Hurst KR., *et al.* "Identification of in vivo-interacting domains of the murine coronavirus nucleocapsid protein". *Journal of Virology* 83.14 (2009): 7221-7234.

15. Li Q., *et al.* "Early transmission dynamics in Wuhan, China of novel coronavirus-infected Pnemonia". *The New England Journal of Medicine* (2020).

16. Wu Z., *et al.* "Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in china: Summary of a report of 72314 cases from the Chinese centre for disease control and prevention". *Journal of the American Medical Association* (2020).

17. World Health Organization. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance (2020).

18. Sah R., *et al.* "Complete genome sequence of a 2019 novel coronavirus (SARS-Co B-2) strain isolated in Nepal". *Microbiology Resource Announcements* 9 (2020): e00169-20.

19. Zhou., *et al.* "A pneumonia outbreak associated with a new coronavirus of probable bat origin". *Nature* 579 (2020).

20. Huang C., *et al.* "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China". *The Lancet* (2020).

21. Kogan A., *et al.* "Acute respiratory distress syndrome following cardiac surgery: Comparison of the American – European consensus conference definitions versus the Berlin definition". *Respiration* 97.6 (2019): 518-524.

22. Poon LL., *et al.* "Identification of a novel coronavirus in bats". *Journal of Viral* 79 (2005): 2001-2009.

23. Tools to support new coronavirus research.