Review Article

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Biomarkers in COVID-19: Prospects and Problems

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COVID-19 caused by SARS-CoV-2 was declared as pandemic by WHO. After a havoc caused for a year, there was mutation in the virus resulting in different strains. SARS-CoV-2 attacks ACE2 receptors which are present not only on the lungs, but also in the various other organs causing multiorgan damage. To manifest the disease and to categorize its severity biomarkers are used as diagnostic tool. Biomarkers are biological indicators, indicating normal, diseased and variations in body function. There are three major types of biomarkers; biomarkers of susceptibility, exposure and effect. In COVID-19 viral infection numerous important biomarkers played a role in describing patients' prognosis, predictivity and accurate diagnosis. The inflammatory biomarkers include c-reactive protein, procalcitonin and creatine kinase.Blood biomarkers comprise of lymphocytes, platelet count, D-dimer. Renal damage is measured by the variation in creatinine; liver dysfunction is determined by the elevation of AST, ALT and lactate dehydrogenase. Troponin is considered as cardiac damage biomarker. The c-reactive protein is considered as a major prognostic biomarker. D-dimer predicted the chances of mortality in patients. However, development and validation of relevantbiomarkers and the correlation with clinical features remains a challenging task.

Keywords: COVID-19, SARS-CoV-2, ACE2 receptors, biomarkers.

Introduction

Corona Virus Disease 2019 (COVID-19), was declared aspandemic by World Health Organization on 11th March 2020. The first case was reportedin China on 31st December 2019 and later it spread to different parts of the world **(1)**. It is caused by Severe Acute Respiratory Syndrome (SARS) group of viruses and the causative pathogen is SARS-CoV-2. The symptoms of COVID-19 ranges from being asymptomatic to milder to severe conditions. Milder symptoms, includes fever, dry cough, dyspnoea, myalgia, sore throat and headache, and more severe and emergent manifestation includes confusion, chest pain, hypoxemia, pneumonia and other complications requiring intensive care unit (ICU) admission. These differences can also be caused by variations between countries, in the number of people tested, characteristics of the local healthcare system, the timely actions taken to containthe virus, characterizing the subtypes of thevirus, as well as the socioeconomic conditions, ethnic, geographical, and social determinants of health infrastructure. The following risk factors are associated with COVID-19, such as advanced age, obesity, male gender, heart diseases, diabetes and immunodeficiency, ethnicity/race. COVID-19 is considered as a multi-organ disease and can affect theCardiovascular System (CVS), Central Nervous System (CNS) and Gastrointestinal System (GIT). So appropriate biomarkers for the proper diagnosis, predicting the severity as well as the prognosis of the disease are necessary. Basically, biomarkers are an indicator of normal biological, pathogenic processes or response to any extraneous chemical agents in the living system **(1)**. Biomarkers can reveal the entire spectrum of disease condition from the earliest manifestations to the progression leading to the terminal stage **(2)**.

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SARS-CoV-2

Initially there were six strains of the virus, the spread started with L strain in December, 2019and later in early 2020 there was an emergence of S strain **(3)**. By the mid 2020 there were V and G strains, the G strain was the most widespread and it mutated into GR and GH strains **(4).** Towards the end of the year 2020 there were emergence of potentially damaging and actively spreading variants. Thevariant, B.1.1.7 carried large number of mutations and was considered to be highly infectious. The B.1.351, acquired mutation called E484K that is responsible for the alteration in the shape of a key part of the coronavirus spike protein that helps it to evade the antibodies effective against other variants. The P.1variant has 17 different mutations, including mutations in receptor binding domain of the spike protein **(5).** The second wave of COVID-19 by the strike of B.1.617 strain, commonly called as double mutant strain **(6)**. The recent strike by the new strain, B.1.1.529 named as Omicron is a variant of concern and spread more easily than the original SARS-CoV-2 virus. This variant is hitting various parts of the world including India, triggering it to an experience of third wave of COVID-19. Respiratory cells are most likely to get attacked by coronavirus infection because of the

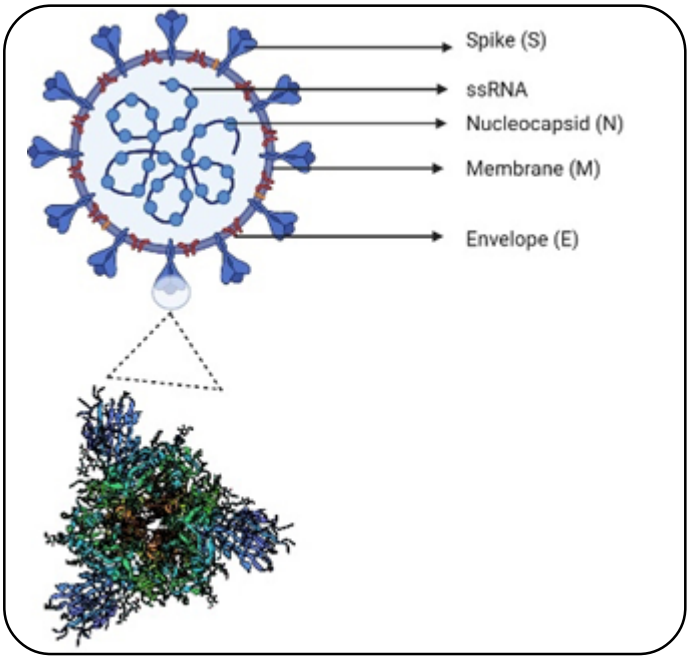

Figure 1: Structure of SARS-CoV-2: The SARS-CoV-2 consists of four main structural proteins; spike (S-) glycoprotein, membrane (M-) glycoprotein, envelope (E-) glycoprotein, and nucleocapsid (N) protein.

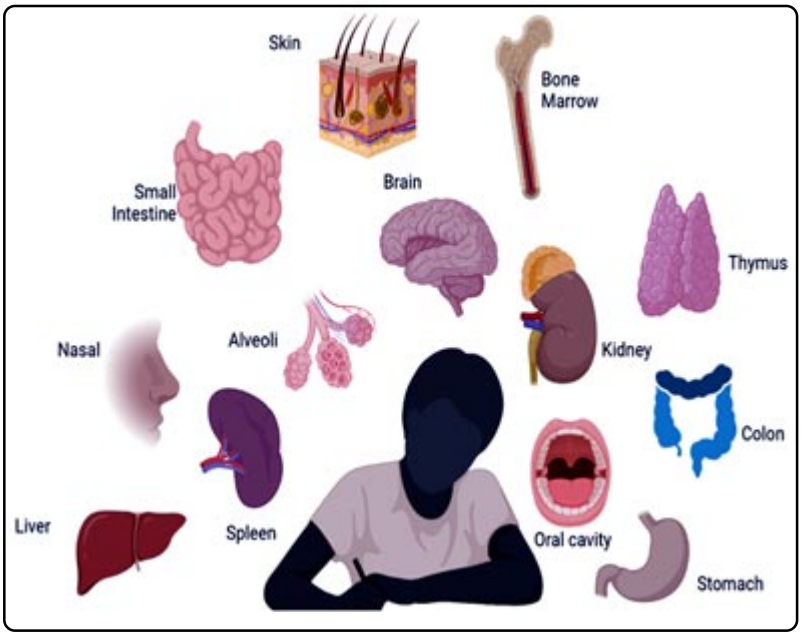

Figure 2: Distribution of ACE2 receptors in human body: ACE2 receptors are widely distributed in the human body particularly in the type II alveolar region. It is also present in lungs, heart, nose, kidney, intestine and brain.

expression of ACE2 receptors, which engage the viral S-protein **(Figure 1) (7,8)** and the endothelial cell surface protein TMPRSS2to facilitate the entry of the virus inside the cell **(9,10).** In lungs ACE2is mainly distributed in type II alveolar region. This indicated that the higher the ACE2 or its expression level, the higher the COVID-19 risk.The wide distribution of ACE2 receptors in the body significantly indicates the multifaceted infection of SARS-CoV-2; affecting the cardiovascular, gastrointestinal and even the central nervous systems, hence patients experience different symptoms **(Figure 2)**. A new study carried out in 3D models showed that SARS-CoV-2 could infect organoid cells not only derived from the airway system, but also the gut. The virus enters the host cell via the spike protein, which adheres to the human ACE2 receptor through its receptor binding site **(11,12)**. (**Figure 3)** provides an insight of the entry of virus into the host cell.

Biomarkers and COVID-19

Biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biological process, pathogenic condition or pharmacological response to any therapeutic stimulation **(13)**. It can indicate various disease characteristics including the level and type of exposure to environmental factor, genetic susceptibility, and the genetic responses to environmental changes **(Figure 4)**. Once a biomarker is validated it can be used to assess the disease risk in general population or even to confirm the diagnosis in patients. The severity of the COVID-19 has created the urge of biomarkers; which can detect the development and progression of disease in patients.According to the GlobalBiomarkers Database, a large number of different biomarkers have been utilized for COVID-19 trials for different purposes,but few of them are validated for clinical application, with the risk that the results produced are not reliable and are not of much use for clinicaldecision making.

As per the pathogenesis of the disease after the entry of the virus in the lung cells via ACE2 receptors, there is release of proinflammatory cytokines and inflammation. Initially this inflammation may be seen to have protective role but develops cellular and tissue lesions later **(14)**. Hyperinflammation in systemic region leads to vascular lesions and thereby affects the thrombocytes, cytokines and develop multisystemic lesions. This phenomenon provides the opportunity to develop different biomarkers.As per the variation in disease severity and the possibilities of asymptomatic individuals it remains a challenge to develop validated biomarkers with clinical importance. After 5-14 days of infection there is an immunological response and the person starts to experience symptoms. The neutralizing antibodies arereleased after the responseto the target receptor binding domain of the spike protein of virus. There is an upsurge in the proteins such as azurocidin, cathepsin, ceruloplasmin, gamma-enolase, gap junction delta-2 protein, hemopexin, immunoglobulin heavy constant alpha 1, histone protein, immunoglobulin kappa light chain, immunoglobulin heavy constant mu, myeloblastin, myeloperoxidase, neutrophil elastase, transketolase, transcobalamin-, and vitronectin with decrease in tubulin alpha-1C chain in SARS-CoV-2 infected individuals **(15).** The exacerbated protein response can serve as the biomarkers in COVID-19 infection.

Different Biomarkers of COVID-19

The disease severity of COVID-19 is associated with the presence of inflammation and huge surge of cytokines. This is characterized by anincreased interferon-α, interleukin-2, tumor necrosis factor-α, interferon-inducible protein 10 and macrophage inflammatory protein 1-α. Patients with fatality has shown marked increase in

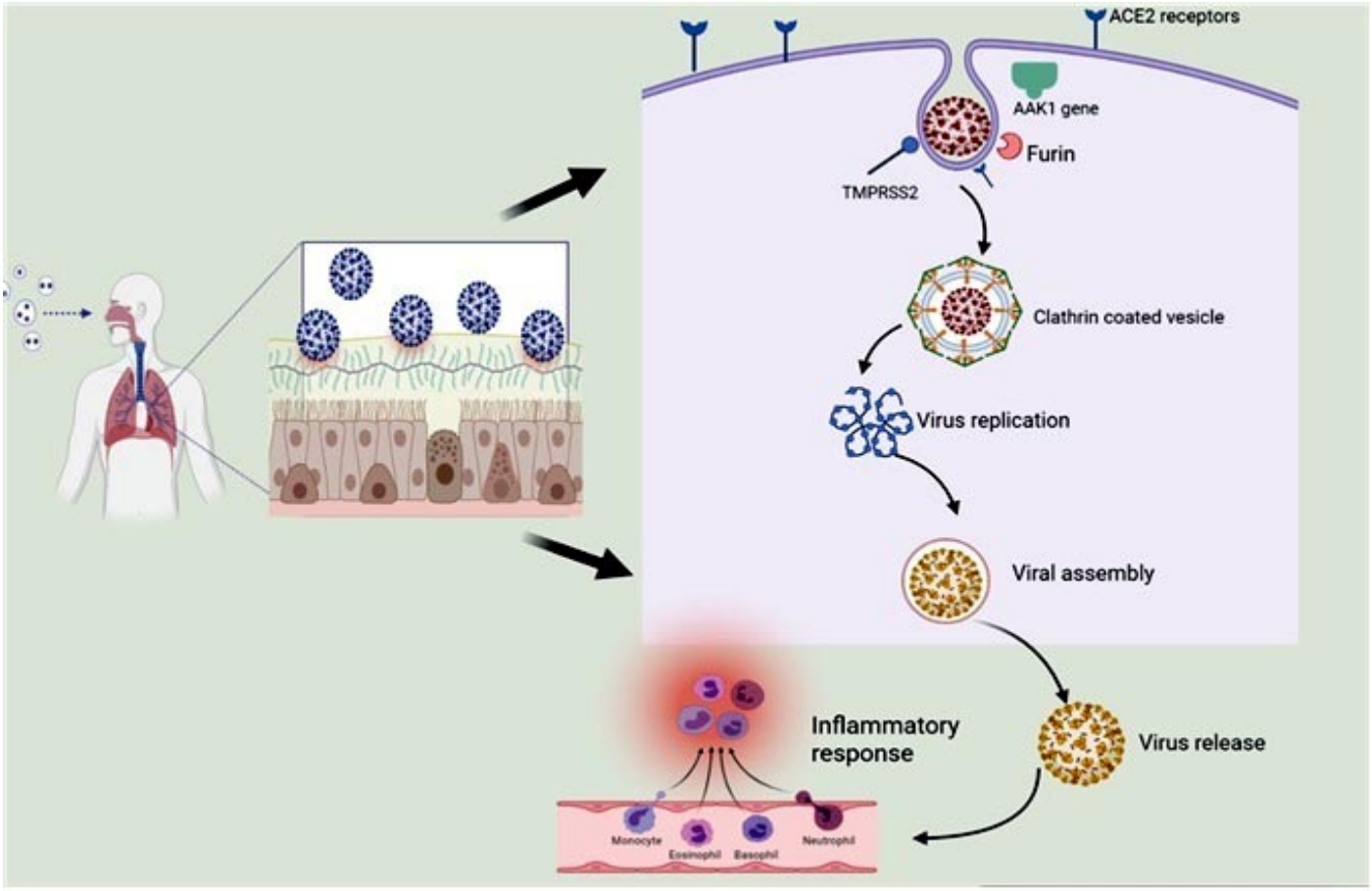

Figure 3: Mechanism of adhesion and entry of SARS-CoV-2: The virus paves its path to the lungs via spike protein which binds to ACE2 receptors with the help of two interacting proteins;furin and TMPRSS2. TMPRSS2 cleaves the viral S glycoprotein and initiate viral activation. Then the virus gets enclosed in clathrin-coated vesicle and replicates inside the cell. Afterreplication the virus releases from the cell to attack other cells and the inflammatory response initiates.

ferritin. To predict the disease severity biomarkers used are neutrophils, lymphocytes, C-reactive proteinan indicator ofinflammatory condition, and D-dimer an indicatorofblood clotting status **(14)**. In adults, neutrophils, lymphocytes, C-reactive protein, and D-dimer are used to predict the disease severity. Lymphocyte count, C-reactive protein, lactate dehydrogenase can be used as biomarkers for predicting the susceptibility of mortality. In severely affected patients there was a marked increase in IL-6, IL-2, IL-4, IL-10, TNF-á; whereas IFN-ã remained within the normal limits **(16,17,18)**. Lymphopenia is also considered as the marker of disease severity **(19,21)**. As SARS-CoV-2 affects cardiovascular system, which induced hypotension and tachycardia,elevated levels of cardiac biomarkers such as troponin I and BNP can provide evidence forthe involvement of CVS **(15)**. The association between inflammation and thrombosis involves the endothelial cell activation and the platelet activation as well as the proinflammatory mediators. Even though routine biochemical assays do not provide complete descriptive knowledge about the disease severity, they appear to give the likelihood of the current status and theprogression of the disease.Protein biomarkers enhance the evidence of the extent of infection and other

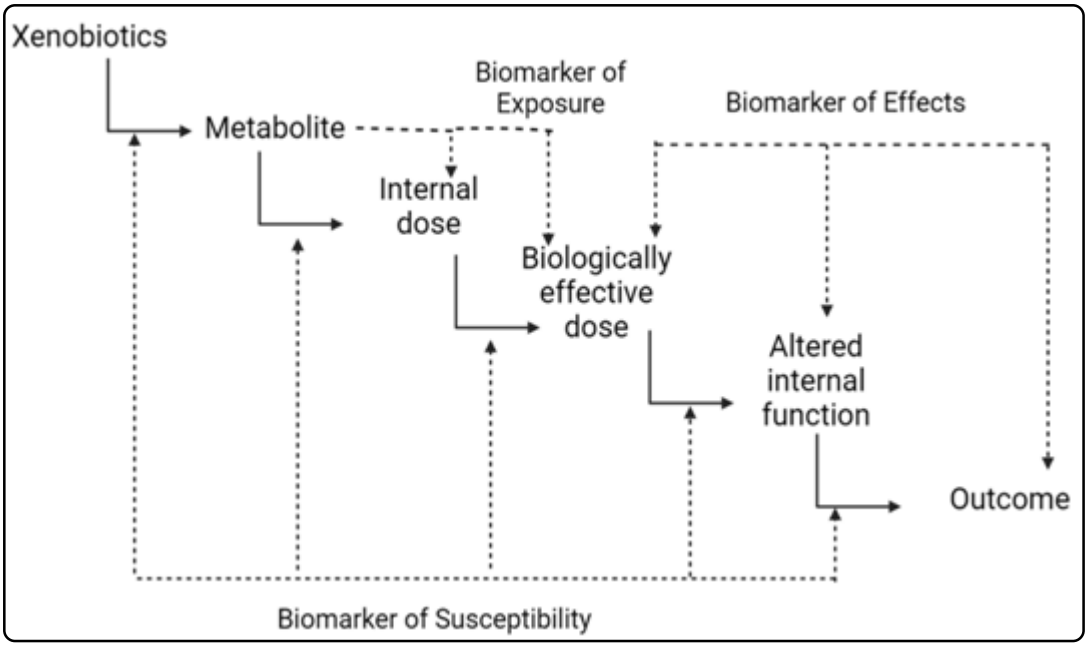

Figure 4: Flowchart of different classes of biomarkers: The three major types of biomarkers are biomarkers of susceptibility (environmental factors interact with individuals), biomarkers of exposure (an external chemical, xenobiotics, or metabolite interact with the cells), and biomarkers of effects (represent the measurable changes of adversity due to exposure of external chemicals).

immunological parameters such as IgG, IgM that canprovide a wider view about the possibility of chronic effects and the treatment choices during the acute phase of infection **(2,21).**

Lymphocyte count

It has been observed that there is a decrease in lymphocytes count in Covid 19 patients **(21).** This decrease in lymphocyte count can facilitate the increase in neutrophil tolymphocyte ratio, being considered as one of the prominent biomarkers for distinguishing between the severe and non-severe patients. However, it has been observed that during the course of treatment, the ratio could be disrupted and the exact state of the disease would not be established.

Platelet count

It is a simple, cheap and rapidly adopted biomarker for COVID-19 patients.Studies have reported that low platelet count has been associated with an increased risk of severity and mortality for COVID-19 patients. The patients with significant lower platelet count and elevated immature platelet fraction (IPF) during treatment had longer average hospitalization stay. Lung tissue damage and pulmonary endothelial cells may activate platelets, resulting in the aggregation and formation of microthrombi and thereby increase the platelet consumption. Study indicated thatthrombocytopenia was more prominent in non survivors than survivors during the time of admission of patients **(21,23).**

C-Reactive Protein (CRP)

The elevated levels of CRP (>10 mg/l) are seen in the infected patients. Studies have shown that CRP is the first biomarker that changes during the perturbations in the physiologicalcondition. Hence, CRP can be used to predict the COVID 19 disease severity **(21).** CRP, can alsobe used to detect the hyper inflammation condition. However, the Erythrocyte Sedimentation Rate (ESR) islower than CRP during the early phase of severe cases and hence can be used as a sensitive biomarker for the disease prediction **(24)**.

Procalcitonin (PCT)

Procalcitoninis another important and crucial biomarker for predicting the disease severity. It is a glycoprotein produced in the C- cells of the thyroid gland. Under normal condition, the level (0.1 ng/ml) is undetectable and increased(>0.5 ng/ml) during COVID-19 infection. During severe infection (bacterial, parasitic, and fungal) with systemic manifestations, PCT level may rise to over 100 ng/ml, produced mostly by extra-thyroid tissue.Although PCT value may be helpful initially in the determination of the severity of illness, but it is not a reliable prognostic indicator **(25)**.It may be influenced by preexisting comorbid conditions, such as chronic kidney disease and congestive heart failure.The baseline values observed in these cases generally very high **(26)**.

Creatine kinase (CK)

Patients infected with SARS-CoV-2 is seen to have increased creatine kinase. Elevation in CK is an indication of muscle damage and hence serve as a useful biomarker. The mechanism of this elevation and damage to muscle is not well understood, however during viral infection there is viral mediated invasion and damage to the myocytes. Further, this process can also be arisingdue to thehyper inflammation caused by anincreased cytokine storm. The viral antibodies get deposited in the muscles which can damage to the myocyte **(23).**

There are various other metabolic biomarkers which exists during the infection with SARS-CoV-2 virus.

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

AST and ALT are the markers of hepatotoxicity, and the underlying cause of liver damage due to COVID-19 is not well understood. The SARS-CoV-2 virus attacks the ACE2 receptors present on the liver and bind to cholangiocytes and perturb the liver function. The inflammatory response due to lymphocytes and macrophages initiates cytokine storm during viral infection, and also affects the pulmonary function. The clinical relevance of abnormal liver function test is limited and hence utilization of AST, ALT as biomarkers for SARS-CoV-2 infection is unclear **(27)**.

Creatinine

The abnormality in the kidney function results in an increase of creatinine and Blood Urea Nitrogen (BUN). An increase in biomarkers of kidney function is seen in patients suffering from COVID-19. The hypothesis postulated is that there is a spread and build-up of virus in the kidney resulting in renal necrosis **(23).** Creatininelevel wassignificantly increased during the mortality and therefore it can be a relevant prognostic biomarker.

D- dimer

D-dimer is the product of cross-linked fibrin that is present in blood during blood clotting **(28)**. Patients suffering from severe corona virus infection is seen with increased D-dimer level (36-43%) and other coagulation factors **(14).** The elevated D-dimer level indicates an increased coagulation and fibrinolysis condition **(21).** This biomarker more prominently increased in patients with COVID-19 than in the patients suffering only from pneumonia. Along with CRP, D-dimer is an important biomarker of inflammation. The non-survivors have demonstrated with an increase in D-dimer level and hence considered as a useful prognostic biomarker. Anticoagulant regimens display a decreases in D-dimer level, suggesting a better outcome in COVID-19 patients **(23,29).**

Lactate dehydrogenase (LDH)

LDH level increased during cell necrosis, and served as an indicator oflungs damage due to SARS-CoV-2. The LDH levels are high in ICU patients than in non-ICU,which can infer that lactate dehydrogenase can beused as a predictive biomarkerin COVID-19 infection **(21).**

Troponin-I

The COVID-19 infected patients have seen with an increased risk of cardiovascular disorders. High-sensitivity

cardiac troponin I (hs-TnI) is a biomarker of disease progression and mortality. Troponin I levels are significantly high in severe COVID-19 patients **(14,21).** This can be used as a biomarker of cardiac damage in COVID-19 infection.

Emerging Clinical Biomarkers in COVID-19

There are recent advancements in the biomarkers of COVID-19 and it is emerging day-by-day. The miRNAs are the key players in several biological processes that regulate the differentiation, development and activation of immune cells in both innate and adaptive immunity. The miRNAs have the potential to be used as diagnostic and therapeutic biomarkers. However, discovery and validation are essential for improving the diagnosis of infection and the clinical monitoring in COVID-19, before these are established as a valid biomarker **(30)**. Decreased serum level of sphingosine-1-phosphate: a novel predictor of clinical severity in COVID-19 can help to evaluate the severity/mortality of COVID 19 patients **(31)**. Several lines of evidences signal a probable link between the gut microbiota and the host's immune response in COVID-19. Although still in a nascent stage, efforts are being made to establish a link between the complex immunological crosstalk and the microbiome's potential as a biomarker and therapeutic target in COVID-19 infection **(32)**.

Challenges in Biomarker Development

Developing different categories of biomarkers is a challenging task and an incredible effort have been made in developing them. From the beginning of the development such as selection and isolation of body sample, use of various techniques to validate the same and finally the approval by the regulatory authorities hasits own difficulty and challenges **(33)**. To ascertain the usefulness of the biomarkers listed in this review as indicators of disease progression, and whether they definitively rise in COVID-19 requires further data information as well as validation **(34)**. The collection of the sample and its processing as well as the storage has to adopt a uniform procedure. Disease heterogenicity, the severity of individuals towards a particular disease, the genetic factors, previous history of disease prevalence, co-infections, hormone related variability are the important aspects to be considered, when biomarkers are used in clinicalrisk assessment process **(34,36)**. Focusing on the technical limitations; lengthy experimental procedures, lack of uniformity in the data acquisition, reproducibility of results should be taken into consideration when biomarkers are developed. Currently, biomarker discovery is based on using the omics and high throughput technologies **(37,38)**. Universally approved guidelines are not available due to compromise in quality control standards **(39,40,41)**.

In summary, as the vaccine efficacy for COVID-19 treatment is very limited, rapid and an early diagnosis is imperative in providing timely health measures as well as to reduce the risk of health complications. Biomarkers played an important role in providing progression, diagnosis, and the predictability of disease occurrence. Biomarkers also provides with plethora of information regarding the severity of disease condition, which enable the healthcare workers to provide a right medical treatment at an earliest possible time.

Although the biomarkers play a crucial role in the detection, prognosis and severity of the disease, the patients after recovery may experience reinfection of the disease and this would require more specific biomarker, as general inflammatory biomarkers will not play a role in this case. The rates of evolution and mutations in coronaviruses are very high, which makes it difficult to identify and develop a specific biomarker against them.Hence, there is a need of more specific and validated biomarkers which will predict the recurrence, severity, and specificity of the disease. Finally, the application and combination of all these biomarkers in detection, diagnostics, treatment and prevention will be the ultimate weapon to win the war against COVID-19.

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