Blood-based biomarkers for diagnosis, prognosis, and severity prediction of COVID-19: Opportunities and challenges

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ABSTRACT

The reasons for high morbidity and mortality with Coronavirus disease (COVID-19) disease remain unanswered with extremes of manifestation and uncertainty of modes of transmission for which biomarkers are urgently needed for early prediction of severity and prompt treatment. We have reviewed publications from PubMed (years 2019–2021) analysing the biochemical, immune-inflammatory, nucleic acid, and cellular biomarkers that predict infection, disease progression in COVID-19 with emphasis on organ-specific damage. Our analysis of 65 biomarkers assessing the impact of SARS-CoV-2 infection on five organs (lung, liver, cardiac, kidney, and neural) reported that increased levels of CRP, TNF-α, ferritin, IL-6, D-dimer, Procalcitonin, Fibrinogen to Albumin Ratio (FAR), and decrease platelet count (PC), lymphocyte count, leukocyte count, and CD4⁺/CD8⁺ ratio shows promising association in the early diagnosis, prediction of prognosis and severity disease and also correlates with cytokine storm a cardinal feature of COVID-19 progression. In the above scenario, this review has put forth the most promising biomarkers for COVID diagnosis and prognosis based on the reported literature. In recent year’s chemically synthesized antibody-like biomolecules, aptamers were also used in the diagnosis of COVID-19 which could be preferably used for diagnosis over antibodies. Biomarkers including increase in free DNA and Fibrinogen-to-Albumin Ratio, CRP, PCT, and Ferritin along with a consequential decrease of CD3⁺ T, CD4⁺ T, CD8⁺ T, NK cells with corresponding increase in CD4⁺/CD8⁺ ratio following SARS CoV-2 infection has been consistently correlated with disease severity. Despite the two waves of COVID-19 pandemic, currently there is no standard clinical practice guideline for evaluating the severity of the devastating pandemic of COVID-19, hence these biomarkers will have immense relevance for the third and subsequent wave of COVID-19 and related pandemic.

Keywords: Aptamer, COVID-19 cellular biomarker and COVID-19, cytokines, inflammation, multi-organ injury, SARS-CoV-2 biomarkers

Introduction

The pandemic of COVID-19 disease primarily affects the pulmonary system caused by the SARS-CoV-2 virus and the patients present with a variety of symptoms from asymptomatic carrier to symptoms indicating affliction of almost all organ systems of the human body, viz. fever, cough, myalgia, dry
cough with or without sputum production.\textsuperscript{[1]} It is important for the clinician to categorize the morbidities into different stages to carry out important investigations and interventions when required. Hence, as the most promising among all noninvasive investigations, the role of biomarkers comes into play here to halt this dreaded pandemic.\textsuperscript{[2]} In the above scenario, we have reviewed biochemical, immune-inflammatory, nucleic acid, and cellular biomarkers that predict infection disease progression in COVID-19 with emphasis on organ-specific damage.

**Clinical perspective**

The reasons for high morbidity and mortality with COVID-19 disease remain unanswered with extremes of manifestation and uncertainty of mode of transmission. COVID-19 pandemic has rapidly spread from an index case reported from mainland China in November 2019 within a span of a few months to the furthest corners of the world affecting 216 countries irrespective of age, gender or ethnicity and affecting all organ systems perplexing the global medical community regarding mode of diagnosis, interventions, and outcomes. The patients present with a variety of symptoms from asymptomatic carrier to symptoms indicating affliction of almost all organ systems of the human body, viz. fever, cough, myalgia, dry cough with or without sputum production, dyspnoea, haemoptysis, diarrhoea, headache, and severe uncontrolled progression acute respiratory distress syndrome (ARDS) followed by multi-organ failure.\textsuperscript{[3]}

There is no reported valid and reliable diagnostic algorithm to demarcate mild or asymptomatic from full-blown cases of COVID-19. Thus, predictable clinical parameters and reliable biomarkers are urgently required for early prediction of disease progression of COVID-19 patients and risk stratification of the patients for optimal resource allocation. The search for a standard biomarker is challenging because the symptoms and progression of the disease varies from person to person in different parts of the world. Reverse transcription polymerase chain reaction (RT-PCR) is the most widely accepted test for the diagnosis of SARS-CoV-2 and can give false negative results even in the presence of florid symptoms in COVID-19 patients. The presence of false negative results in RT-PCR test may be due to the mean incubation period of the virus, which is presumed to be approximately six days. Thus, during the incubation period and at the time of recovery, the RT-PCR test can be negative, yet patients are still infectious at these phases, further necessitating a need for biomarker indication COVID-19 infection.\textsuperscript{[4]}

**Overview**

Among the biochemical biomarkers, higher cytokine levels, viz. interleukins like IL-6, IL-2, IL-4, IL-10, Tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and interferon-inducible protein 10 (IP-10) corroborated with viral load and worse prognosis. In the models for the severity prediction in the spectrum of morbidity, an increase in free DNA and Fibrinogen to Albumin Ratio, CRP, PCT, and Ferritin along with a consequential decrease of CD3⁺T, CD4⁺T, CD8⁺T, NK cells with corresponding increase in CD4/CD8 ratio following SARS CoV-2 infection has been consistently reported. Currently, there is no standard clinical practice guideline for evaluating the severity of a devastating pandemic of COVID-19 disease of almost all countries of the world since the last Dec 2019. Biomarkers are produced during the natural history of a disease as a molecule, genes, or attributes by which any precise patho-physiological process or morbidity can be recognized. There are several biomarkers produced in the body due to different metabolic pathways upon entry of microbes which corresponds to almost all clinical features. Yet, choosing the right one can make a difference between life and death from among the most important general biomarkers, which can help detect the pathophysiology of inflammation as localized, organ-specific, or systemic infection.

**Clinical presentation of COVID-19**

Conventionally, like all other illnesses, attempts are being made by different research groups in the world to classify the COVID-19 patients for optimum management on the basis of severity of disease demarcated by clinical features of signs, symptoms, radiological and biochemical parameters. On the base of severity, COVID-19 is divided into four variants.

1. Mild: mild symptoms and no pneumonia manifestation.
2. Moderate: Typical chest symptomatic presentation with fever or features of involvement of the respiratory system and imaging expression of pneumonia.
3. Severe: Having any of the three conditions
   a. respiratory distress, respiratory rate ≥30 beats/min
   b. Mean oxygen saturation ≤93% in a resting state
   c. Arterial blood oxygen partial pressure/oxygen concentration ≤300 mmHg
4. Critical: having one of the three conditions
   a. shock incidence
   b. Respiratory failure and requiring mechanical ventilation
   c. Admission to ICU with other organ function failure\textsuperscript{[5]}

**Immuno-inflammatory biomarkers**

Cytokines have been shown to play a crucial role in regulating the inflammatory and immunological responses in COVID-19.\textsuperscript{[6]} The interplay of inflammatory markers is depicted in Figure 1. The proinflammatory cytokines levels are found to be raised in SARS-CoV-2 infection, which leads to inflammation and extensive lung damage.\textsuperscript{[4]} Elevated levels of IL-6, IL-2, IL-4, IL-10, TNF-a, IFN-γ have been observed in COVID-19 patients leading to cytokine storm that has been identified as bad prognostic indicator.\textsuperscript{[8]} The interleukins are held responsible for the differentiation and activation of immune cells and help in the activation of epithelial cells to induce the acute phase of inflammation. They help in recruiting more immune cells at the sites of inflammation following SARS-CoV-2 infection.\textsuperscript{[7]}

**Interleukin-6**

Interleukin-6 levels are increased significantly in severe COVID-19 cases and are closely linked to severe pulmonary inflammation. It is mainly secreted by inflammatory monocytes in the lungs and is synthesized by lung parenchyma, type II
pneumocytes, alveolar macrophages, and lung fibroblasts. IL-6 drives the immune dysregulation and macrophage activation syndrome in critically ill patients with severe respiratory failure by SARS-CoV-2. In SARS-CoV infection, aberrant proinflammatory cytokine secretion of IL-6 leads to the inhibition of T-cell priming ability of dendritic cells. It can promote Th17 cell lineage and function and inhibit the induction of Tregs and CD4 lymphopenia. Serum viral load correlates with elevated IL-6 levels (more than 40 pg/ml) and also with the ARDS severity.[8] IL-6 is also associated with the body temperature of patients, as the initiation of disease body temperature tends to rise along with the levels of IL-6 and falls in the recovery phase.[9] A strong association of high IL-6 levels and critically ill patients were reported, severity of the disease was assessed by the sequential organ failure assessment (SOFA) score, and 6.11 pg/ml was taken as a normal cutoff value for IL-6 showing IL-6 as a promising marker.[10] TNF-α and IL-1β are the main activators of IL-6.

**Interferons**

Interferons produced by virus infected cells provide innate immunity by limiting viral infections.[11] Levels of interferon γ (IFN-γ) and interferon-inducible protein 10 (IP-10) are very high, in COVID-10 patients.[12] The delayed IFN-α/β response is accompanied by some of the accumulation of inflammatory monocyte/macrophages (IMM) resulting in increased levels of monocytes, chemoattractants such as CCL2, CCL7, and CCL12 through IFN-α/β receptor stimulation which further increases the count of IMM and hence enhances the disease severity.[8] There is production of elevated levels of proinflammatory cytokines such as TNF, IL-6, IL-1β, and iNOS by these IMM. Additionally, IFN-α/β or IMM-derived proinflammatory cytokines sensitize T-cells to undergo apoptosis, which further impedes viral clearance.

**Tumor Necrosis Factor – α (TNF-α)**

TNF-α, IL-17A, and IL-8: Th-17 lymphocytes and group 3 innate lymphoid cells (ILC-3) are the main sources of IL-17A and alveolar macrophages and type 1 macrophages located in visceral adipose tissue are the sources of IL-1β, IL-6, IL-15, and TNF-α. Serum concentrations of TNF-α and IL-17A are more elevated in patients with obesity and COVID-19, and consequently, they have a greater probability of developing ARDS and death.[13] IL-17A can act directly on smooth muscle and respiratory epithelium causing bronchial hyperactivity. It also brings about recruitment of neutrophils via IL-8, IL-6, IL-11, GM-CSF, and G-CSF induction. TNF-α brings about the release of matrix metalloproteinase-9 by neutrophils, which stimulates the production of glycosaminoglycans by pulmonary fibroblasts. TNF-α also stimulates the synthesis of collagen by myofibroblasts and fibroblasts. Both TNF-α & IL-17A stimulate the release of IL-8, which is a potent chemotactic agent of neutrophils in the lungs. Tumor necrosis factor-α is responsible for the raise in temperature due to its pyrogenic in nature.[7]

**Immunoglobulins**

Immunoglobulins act as a bridge between innate and acquired immunity. Host immunity is directly correlated with antibody production. For the prediction of immunity of the population and cross-reactivity with other coronaviruses, the virus-specific antibodies levels of IgA, IgM, and IgG are worth to be

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**Figure 1:** Highjacking the immune system and exaggerated inflammatory response by SARS-CoV-2 infection of alveolar epithelial cells. (a) The SCoV-2 infected cells inhibit the antiviral response through TLR3/7 pathway. (b) The infected cell inhibits viral clearance by immune cells. (c) Excessive upregulation of cytokines causing cytokine storm and cell adhesion molecules. “This figure was prepared using Motifolio software for PowerPoint”
measured. IgA is mainly responsible for mucosal immunity. As this pathogen primarily targets the respiratory system, the levels of IgA can be an effective biomarker. Studies have shown that during 4–25 days of onset of illness, IgA shows the highest sensitivity. In 16–20 days, the median RLU of receptor binding domain-specific IgA reached its peak and then started declining but remained relatively high for 31–41 days of illness. In early disease, the median RLU of receptor binding domain-specific IgG was the lowest but rose at 15 days post-illness, reached its peak for 21–25 days, and remained high for a longer period. The RLU of IgM reached its peak earlier than IgA and IgG, but the levels remained lower than IgA and IgG. It has been observed that the serum IgM and IgG levels in moderate and severe COVID-19 patients were significantly higher than mild cases, while no significant difference was observed between severe and moderate patients. Several studies show a similar periods of rise and fall of immunoglobulins with some variations based on population. Therefore, need is needed for the exact levels and period of rise and fall of immunoglobulins in different populations as the presence of antibodies can also determine the immune population for COVID-19.[14]

Cellular biomarkers

Cellular components are disrupted in all microbial infections to some extent and SARS CoV-2 viral infection is no exception. Lymphocyte and leukocyte counts are normal (despite being on the lower side of normal) during the incubation period (0–14 days) of SARS-CoV-2 infection; thereafter with a surge in inflammatory markers lymphopenia and leukopenia set in. [15] Low lymphocyte and leukocyte count in COVID-19 patients can be attributed the expression of ACE2 receptors on their cell surface. [16] Levels of CD3+, CD4+, CD8+ T cells, and NK cells are significantly decreased in severe cases with a corresponding increase in the ratio of CD4+/CD8+ T cells. Lymphopenia is generally noted in COVID-19 patients with a decrease of CD8+ T cells compared to CD4+ T cells. Lymphopenia is significantly increased in severe cases when compared with the uninfected/apparent healthy cases. Other findings showed that COVID-19 intensive care unit (ICU) patients had over expression of soluble E-selectin (sE-selectin) in circulation in COVID-19 patients. These levels were independent of each other and can be assessed as a single marker; it also states that there are more systemic study that needs to be explored further to determine the elevated levels of ICAM-1, VCAM-1, sP-selectin, sL-selectin, and sE-selectin as standard of care biomarkers for COVID-19 patients. [17]

The lymphopenia (lymphocyte count <1000) in COVID-19 infection can be due to inhibition of cellular immune effective function. However, simultaneously initiation of apoptosis of more cells occurs which leads to an overactive inflammatory response. In COVID-19, the response of both Th1 and Th2 cells is found to be initiated with the severity of disease. It is also found that the CD4+ and CD8+ T cell counts also decrease with the high expression of HLA-DR and CD38. The level of pro-inflammatory markers in the serum including PCT, CRP, ferritin, TNF-α, IL-2R, IL-6, IL-8, IL-10 match the severity of COVID-19 infection, indicating that the magnitude of cytokine storm results in high morbidity and mortality is attributed by immunopathology. IL-2R level increases and lymphoid cells decreases with severity, along with the increase of IL-2R IL-6, IL-8, IL-10, and TNF-α and fall with recovery of COVID-19 disease. [18] CD4+ and CD8+ counts are found to be very low in severely critically ill COVID-19 patients, but the levels of monocyte chemotactic protein-3 (MCP-3), IFN-γ–induced protein 10 (IP-10), hepatocyte growth factor (HGF), monokine-induced IFN-γ, and macrophage inflammatory protein 1 alpha, are significantly high. IP-10 and MCP-3 levels are associated with the severity of disease. These proteins can be assessed to identify severity as higher levels of IP-10 and MCP-3 in week-1 of infection are observed and they decrease during recovery and thereafter. [19]

Cell adhesion molecules (CAMs)

Over expression of endothelial circulating cell adhesion molecules (CAMs) are found in COVID-19 positive cases and was reported to be directly correlated with the severity of the COVID-19 disease. Moreover, these CAMs are known to be an impact on the coagulation regulation. Recently, Tong et al. and Li et al. reported the blood circulating levels of endothelial CAMs in COVID-19 patients. [20,21] These authors reported that vascular CAM-1 (VCAM-1), intercellular CAM-1 (ICAM-1), and platelet endothelial CAM-1 (PECAM-1) were over-expressed in COVID-19 positive cases having mild disease and there were significantly increased in severe cases when compared with the uninfected/apparent healthy cases. Other findings showed that COVID-19 intensive care unit (ICU) patients had over expression of soluble E-selectin (sE-selectin) in circulation in hospitalized. [22] Since CAM overexpression is also related with other autoimmune and inflammatory diseases, so it is still not fully understood the overexpression of CAM and the better biomarkers for COVID-19 positive cases than other biomarkers available for COVID-19. Thus, there are more systemic study that needs to be explored further to determine the elevated levels of ICAM-1, VCAM-1, sP-selectin, sL-selectin, and sE-selectin as standard of care biomarkers for COVID-19 patients.

There are limited review articles in the literature that assess the role of laboratory biomarkers in predicting disease severity and outcome.

Biochemical markers

The levels of CRP, D-dimer, ferritin, and IL-6 levels in COVID-19 patients are found to be highly raised in severe cases. These levels were independent of each other and can be assessed as a single marker; it also states that there are disturbed coagulatory state in severely ill patients and detected by the elevated levels of D-dimer >1 µg/l. [23] Increased serum procalcitonin and ferritin levels were reported in patients with...
COVID-19 infection, whereas decreased time for different blood clotting parameters reported in severe cases. Lymphopenia along with increased levels of lactate dehydrogenase (LDH), ALT/AST ratio, prothrombin time, creatinine, D-dimer, CPK, peripheral absolute monocyte count (ANC), absolute neutrophil count (ANC) levels are associated with severity of COVID-19 infection which can also be useful for the diagnosis. Other studies also showed similar association; further, there is also a rise in the level of fibrin/fibrinogen degradation products (FDP), CRP, creatinine, and cardiac troponin.

The correlation between FAR and PC in COVID-19 patients, which is a marker for inflammation and its value increases when there is any infection or malignancy. The level of FAR is high in severe cases compared to mild COVID-19; the level of FAR tend to decline to gradually return to normal as the patient recovers. It was also noted that there is a correlation between the cytokine storm and increased level of FAR. The normal level for FAR is 155–109/L; the patients having this plasma level are less likely to have severe disease. It was also observed that platelet count also decreased in severe disease due to hyper activity of fibrinolysis causing increase platelet consumption. Elevated levels of procalcitonin precursor of calcitonin can be seen in cases of sepsis and organ dysfunction. Procalcitonin is associated with bacterial co-infection and severe disease, so its levels will be elevated in patients with severe COVID-19 infection and can be a useful marker for early indicators of severity in COVID-19 patients.

**C-reactive protein**

C-reactive protein (CRP) is used as conventional biomarker for inflammation. CRP is an acute-phase protein having half-life of 19 hours synthesized by the liver and enters in the blood within 6–10 hours of any tissue damage which can be due to viral infection, tissue destruction, and bacterial infection. It is a highly sensitive biomarker of inflammation and viral infection. It activates the complement system and promotes phagocytosis. The level of CRP in the serum is much higher in severe COVID-19 infected cases as compared with the milder and moderate disease. The level of CRP rises with the severity of disease and decreased in the recovery period, the level of CRP remained high in the deceased patients. Thus, CRP can be used as a prognostic marker and to monitor disease progression. According to various metanalysis reports, a serum CRP level of ≥10 mg/L is considered as the cut-off value for indicating inflammation. CRP may not only be used as a prognostic marker but also to monitor the disease improvement. CRP value is also helpful in predicting the need for invasive ventilation. It is helpful in predicting the impending respiratory failure with high accuracy and guides physicians to start early treatment and benefits the patient.

**D-dimer**

D-dimer is formed in blood as a degradation of a stabilized fibrin product (thrombus) which is plasmin mediated and produced after activation of coagulation and fibrinolysis in the alveoli and lung parenchyma due to lung injury. As lungs have a large number of ACE receptors, increasing lung injury by COVID-19 hence releases D-dimer which subsequently enters the blood stream. The level of D-dimer were found to increase in the COVID-19 patients and to tend to increase as the severity of the disease increases. It is mainly used as diagnostic criteria of disseminated intravascular coagulation (DIC). In the ICU, the incidence of venous thromboembolism (VTE) is a frequent complication in patients with critical illnesses. It is found that 25% of ICU patients with severe COVID-19 have VTE. In COVID-19 patient, coagulation profile changes rapidly within a short period of time, so follow-up is required every 2–3 days. COVID-19 patients can present with hypercoagulable state due to complement release syndrome, which is caused by overactivation of the immune system. IL-6 acts as a key factor for the triggering of immune response and leads to coagulation disorders. The cutoff for D-dimer is considered 0.5 mg/L by assessing the levels of D-dimer anticoagulation therapy like low-molecular-weight heparin (LMWH) can be started early in patients with higher D-dimer levels (>3.0 µg/mL) to prevent the damage due to coagulation disorder.

**Ferritin**

It is an intracellular protein which stores iron and has a major role in the pathogenesis of inflammatory diseases like cancer, infection, and neurodegeneration. High level of circulating ferritin are found in various diseases like catastrophic anti-phospholipid syndrome (CAPS), macrophage activation syndrome (MAS), adult-onset still's disease (AOSD) and septic shock, the combination of these diseases is a pathogenomic feature of “hyperferritinemic syndromes” and increases in many viral diseases like dengue fever, influenza H5N1 and hepatitis B and C. It is an acute phase protein having 24-subunit which is made up of heavy subunit “H” and light subunit “L” surrounding an iron core containing 4000–4500 iron atoms. In COVID-19 cases, it can be used as an independent risk factor for assessing the severity in patients. It is found that the levels of ferritin are significantly higher in critical patients than moderate and severe patients. The levels of ferritin are 3–4 times higher in critical and deceased patients than the mildly to moderately ill. In an severe case, the average concentration (800 g/L) is 1.5–5.3 times higher than that in less severe cases, and the level of ferritin found in nonsurvivors is 1400 g/L, which is 3–4 times higher than those in survivors. Therefore by assessing the level of serum ferritin, the prognosis of the patient can be efficiently monitored. According to the sensitivity of ferritin and CRP (83.3% vs. 91.7%), serum ferritin combined with CRP can predict better prognosis of disease, especially in ICU wards.

**Nucleic acid-based biomarkers**

Free DNA is produced mainly by dying cells like lymphocytes, injured lung cells, alveolar macrophages, and other immune cells which lead to damage of vascular endothelial cells and activation of various tissue cells, thereby initiating the production of a large number of cytokines that progresses in a vicious cycle.
leading to the cytokine storm. Due to cytokine storm, the permeability of blood vessels increases, which is augmented by damage of vascular endothelium by free DNA. The damage to the endothelium augments the ARDS which is a major cause of death in COVID-19 patients. 8-oxodG is the product of free DNA, which is responsible for the damage of lung vessels and contributes to the production of ARDS. Free DNA in inflammatory exudation of alveoli and bronchioles due to its sticky nature forms a mucus plugs by binding to fibrin and cell debris. The tissues and organs which have abundant blood supply like kidney, liver, brain, and heart are more prone to such damage. As free DNA circulates along with cytokines, it further damages the vascular endothelium which leads to accumulation of fluid leading to edema of these organs and eventually leads to multiorgan dysfunction and high mortality. It has been observed that more plasma levels of free DNA are found in critically ill patients with SARS-CoV-2 infection compared to less severe cases. This has been demarcated as a useful prognostic marker for assessing and managing the severity of disease[^9].

The growing threat due to COVID-19 and its variants has caused a pandemic and many losses to the whole world. Globally, different laboratory biomarkers are linked as a sign of disease severity, progression, and outcome. Disturbed cell counts, like anemia, polycythemia, leukopenia, and leukocytosis with neutrophil predominance and decreased platelet count are reported and found direct associations with disease severity and poor outcome in hospitalised patients. Moreover raised liver enzymes and total bilirubin levels were identified in severe and crucial patients[^8]. Increased inflammatory reaction of the body as shown by laboratory findings of many interleukins and C-reactive proteins (CRPs) are well reported and accepted parameters for active COVID-19 disease. Moreover high coagulation biomarkers such as fibrinogen and prothrombin time are found in seriously and crucially ill patients with COVID-19.

Both virus-specific factors and host inflammatory responses have been implicated in determining disease severity and clinical outcome. It has been proposed that an ineffective early innate antiviral response followed by impaired adaptive immune responses and hyperinflammation may lead to micro-thrombosis and tissue injury, resulting in acute respiratory distress syndrome (ARDS) and multiorgan failure, and death[^7,8].

Abnormalities in blood levels of several pro- and/or anti-inflammatory cytokines, chemokines, and other mediators have been associated with worse outcomes. Elevated IL-6 levels were shown to correlate with an increased risk of death. Furthermore, patients requiring ICU admission exhibit higher plasma levels of granulocyte colony-stimulating factor (G-CSF), CXCL10/IP-10, MCP-1/CCL2, IL-2, IL-7, IL-10, MIP-1α/CCL3, and TNF-α[^9]. Improved understanding of the immunopathogenesis of COVID-19 may allow for the identification of personalized prognostic markers and the design of personalized, targeted therapeutic interventions. However, most published studies to date have focused on a restricted panel of inflammatory mediators in relatively small patient cohorts with brief follow-up. In addition, limited information exists related to how the levels of biomarkers may be affected by confounding factors, such as the presence and nature of comorbidities, various therapies (particularly immunomodulatory medications), and the timing of sampling relative to the onset of infection and recovery/death. Immune and inflammatory-based biomarkers such as Type I and II interferons (IFNs), NF-κB, CCL2, IL-15, soluble ST2 [sST2], NGAL, sTNFRSF1A, ferritin, IL-6, S100A9, MMP-9, IL-2, sVEGFR1, IL-10, TNF-α, IFN-γ, IL-1β, IL-4, IL-8, and IL-18. In addition, there are some reports on immune cell migration, differentiation, and activation Chemokine (C-X-C motif) ligand 9 (CXCL9) and human lactoferin (hLF)[^10]-[^12]. In addition, differences (hypo- and hyper-levels) of electrolyte levels were reported for sodium, potassium, and calcium values were reported in critically ill patients with severe disease outcomes.

### Chemically synthesized aptamers for the diagnosis of COVID-19

Aptamers have many advantages over antibodies. Aptamers are nucleic acid (DNA, RNA) based chemically synthesised while antibodies are protein-based biological material and needs months to develop. Aptamer has a wide range, including protein targets, while antibodies have a limited target composed of amino acids. Aptamers have 3D structure recognition binding while antibodies have peptide sequence recognition. The size of aptamers is very less (~20 kDa) while antibodies are very big (~150 kDa), so difficulty in crossing cell walls and has less penetration/internalisation capacity. Aptamers can be stored at room temperature while antibody need to store in cold temperature. Aptamers, also known as chemically synthesised and bio-compatible, low/no immunogenicity chemical antibodies with better applications than antibodies on the base of their structure, sizes, and compatibilities with hosts were explored for the diagnosis of various diseases including COVID-19. Aptamers are short single-stranded nucleic acids with high-levels of binding affinity, specificity, and sensitivity to other biomolecules. Moreover, aptamers have several therapeutic applications including but not limited to carriers for targeted delivery of other therapeutic agents to cells or tissues, agonists to stimulate cellular signalling pathways of target receptors, and antagonists to inhibit protein–protein interactions. These aptamers could be used to target infected cells/ organs and drugs can be delivered specifically by bioconjugations.

There are many viruses’ specific aptamers have been identified and used for viral diagnosis and therapeutics. Aptamers for double-stranded DNA viruses such as Herpes Simplex Virus (HSV). Aptamers for Positive Sense Single-Stranded RNA Hepatitis C Virus (HCV), Zika Virus (ZIKV), Dengue Virus (DENV), Japanese Encephalitis Virus (JEV), Tick-Borne Encephalitis Virus (TBEV), and Norovirus (NoV).

### Aptamers for coronavirus (CoV)

Coronavirus (CoV) is a positive-sense single-stranded RNA virus with the biggest genome size among RNA viruses. These viruses have mainly four structural proteins such as membrane (M), envelope (E),
spike (S), and nucleocapsid (N), responsible for enabling host-cell interactions and their functions after penetration to the host cells. In nature, there are majorly seven human viruses known as severe acute respiratory syndrome, MERS-CoV, HCoV-229E, SARS-CoV-2, HCoV-OC43, SARS-CoV, HCoV-NL63, and HCoV-HKU1 where SARS-CoV is a major class.\(^{43}\)

### Aptamers for SARS-CoV

Recently, RNA aptamers against the SARS-CoV NTPTase/Helicase (nsP10) have been developed.\(^{44}\) These RNA aptamers have been shown to efficiently inhibit the double-stranded DNA unwinding activity of the helicase by up to 85\%, however, aptamers showed only a slight effect on ATPase activity.\(^{44}\) In another study, DNA aptamers against the SARS-CoV Helicase were also developed.\(^{43}\) Later, Ahn et al.\(^{44}\) also developed a DNA aptamer-based SARS-CoV for its diagnosis using an RNA aptamer against the SARS-CoV nucleocapsid (N) protein by using nanorarrayaptamer chips and chemiluminescence immunosorbent assay. The assay could detect the N protein at concentrations as low as 2 pg/mL and even lower detectable concentrations up to 0.1 pg/mL.\(^{47}\)

### Aptamers for SARS-CoV2

For tackling the ongoing pandemic, Song et al.\(^{48}\) developed DNA aptamers against the SARS-CoV-2 receptor-binding domain (RBD) of the S protein. They isolated two aptamers with partial identical binding sites for the human SARS-CoV-2 receptor angiotensin-converting enzyme 2 (ACE2) on the SARS-CoV-2 RBD, and the binding is through hydrogen bonding. These aptamers could help in the diagnosis and treatment of SARS-CoV-2. Zhang et al.\(^{49}\) developed DNA aptamers against the SARS-CoV-2 nucleocapsid (N) protein. They found that four pairs of aptamers could bind to the N protein through a sandwich-type interaction. Using aptamers, they created an ELISA assay and colloidal gold immunochromatographic strips with the ability to detect the N protein at levels of tens of pM. Recently, thiolated aptamers with silver nanoparticles was used to detect spike protein binding by measuring the Raman spectrum of the aptasensor\(^{46}\) which can measure the slightest changes in molecule conformation\(^{46}\) from a very small sample volume (10 mL). More recently, aptamers with very high specificity and affinity towards viral N or S protein were explored and SARS-CoV-2 infection could be detected in saliva samples\(^{49}\) [Table 1]. In the near future, new development might lead to ready-to-use diagnostic kits for fast in-house monitoring of infection.

### Mitochondrial DNA

Mitochondrion is a major source of energy and regulates the energy need of the cell depending upon its needs. Defect in mitochondria is associated with various disorders such as cardiovascular diseases, diabetes, cancer, and aging. Mitochondria contribute the major source of reactive oxygen species (ROS) to a cell and increase its intracellular oxidative stress. TNF-α increases the generation of ROS in mitochondria. IL-6 and IL-10 change the activity of the electron transport chain, and thus modulating mitochondrial ROS generation. Inflammatory mediators like TNF-α and IL-6 hamper the mitochondrial oxidative phosphorylation and associated ATP production, which increases the production of ROS that leads to altered mitochondrial dynamics, mitochondrial membrane permeabilization, and results in apoptosis. Due to excessive destruction of mitochondria by ROS, mitochondrial DNA is released into the cytosol and circulation, this leads to the production of proinflammatory cytokines IL-1β and IL-6 which further causes the cytokine storm.\(^{52}\) Increased levels of circulating mitochondrial DNA (MT-DNA) can be assessed to know the severity of disease. The levels of MT-DNA found to be more in the ICU patients.\(^{53}\) For normal platelet function like metabolism and aerobic respiration, mitochondria is essential because its dysfunction leads to apoptosis, and apoptotic platelets may induce clotting ≥50-fold faster than normal platelets which contributes to enhanced thrombosis.\(^{54}\) There is increased production of inflammatory cytokines like CXCL-8 and IL-6 by the dysfunctional mitochondrial cells of human alveolar epithelial cells, which causes further alveolar tissue damage and forms a vicious cycle. Therefore the high level of MT-DNA is associated with severe acute lung injury and by assessing the level of MT-DNA, we can check the severity of disease.\(^{53}\)

### Biomarkers specific to organ-related injuries in COVID-19 patients

Multiorgan injury owing to SCoV-2 has been observed in several studies. COVID-19 mainly affects the lungs, but it also infects other organs like kidney, muscles, lung, and liver, etc. This leads to the decrease/elevation of certain enzymes/markers in the circulation by assessing the level of these markers we can assess the condition of the patient and locate the organ affected. It is crucial to analyse the specific markers for patients presenting with comorbidities of diseases of heart, kidney, lungs, and liver. Such candidate biomarkers are listed in Table 2. Highly deflected cardiac markers are observed in COVID-19 patients, as the myocardium also presents a high number of ACE-2 receptors, on which the SARS-CoV-2 can attack.\(^{34}\) Cardiac injury, defined by raised serum cardiac troponin I (cTnl) levels, in COVID-19 patients were associated with a more than 50\% mortality rate. Furthermore, heart failure was prevalent in 23\% of patients presenting with COVID-19, which was also

| Table 1: Aptamers for the SARS-CoV-2 diagnostic |
|-----------------------------------------------|
| **Name (DNA)** | **KD/KM** | **Length (nt)** | **Limit of Detection/IC$_{50}$** | **Target** | **Reference** |
|----------------|-----------|----------------|-------------------------------|-----------|--------------|
| Aptamer 1-3    | N.A.; (88-57) | 10 ng/mL.(ELAA) | SARS-CoV-2 N protein | [51]      |
| CoV2-RBD-1C    | 5.8±0.8 nM; (51) | N.A. | SARS-CoV-2 S protein | [48]      |
| CoV2-RBD-4C    | 19.9±2.6 nM; (67) | N.A. | SARS-CoV-2 S protein | [48]      |

N.A.: information not available
There are multiple probable mechanisms discussed earlier, the SARS-CoV-2 virus not only affects the lungs but other organs like kidney. The prevalence of involvement of the kidney is depending upon age, facility of treatment, comorbid conditions, and any previous history of kidney disease. The pathogenesis of kidney damage is multifactorial in origin, it damages the renal system by having a direct cytotoxic effect on renal cells by acting on angiotensin-converting enzyme II (ACE2) and TRMPS as a cell entry receptor, cytokine storm can be one of the contributing factor along with the damage by immune complexes, cytotoxic drugs, super impose infections in prolonged hospitalized patients are also one of the contributing factors for renal injury. The features of renal damage can be described as proteinuria, hematuria, reduced GFR, raised creatinine, and BUN. We have found that there is a rise in creatinine level in a great number of COVID-19 cases along with proteinuria, hematuria, and reduced GFR.

### Kidney injury related biomarkers

In addition to the above, a clear association between COVID-19 and liver injury. The cause of this liver injury in severe cases can be due to the direct effect of SARS-CoV-2 virus and due to the extensive treatment in severe cases which leads to compromise of liver function.

### Neural injury related biomarkers

Neurotropic viruses are those which can invade the nervous tissue and cause infection of immune-functioning macrophages, microglia, or astrocytes in the CNS. Many such viral infections cause structural and functional damage to the nervous system. Encephalitis, toxic encephalopathy, demyelinating lesions all are the results of such systemic or localised viral CNS infection. Studies have already showed that the crown-shaped SARS-CoV-2 virus is demonstrable in the cerebrospinal fluid of the patients. The role of blood-brain barrier in preventing it from accessing the neural tissue is still not clear and needs to be explored more. Recently, a study posted in medRxiv has reported neurological manifestations in COVID-19 that involved 214 patients, of which 78 (36.4%) patients had neurologic manifestations which affirms the rationale behind the neurotropic potential in the COVID-19 virus. Past studies in SARS-CoV-2 affected patients showed that, during the early or later phases of infection, the dissemination of the virus in the systemic circulation or across the cribiform plate of ethmoid bone can lead to cerebral involvement. Only cerebral involvement alone could be a potential cause of cerebral edema in COVID-19, causing death in patients, much before systemic homeostatic dysregulation sets in. Access of the virus through the transcribral route could have been explained as the reason behind hyposmia and acute respiratory failure. There are multiple probable mechanisms through which COVID-19 could affect the CNS, such as:

- Direct viral injury through ACE2 receptor
- Injury from cytokine storm
- Injury to host immune response following acute infection
- Indirect injury although systemic disease.

These mechanisms might lead to the minimization of lysosomal activity, following which protein aggregation in neurons may cause

table: | Organs | Biomarkers | Prognostic potential | References |
|-------|------------|--------------------|------------|
| Heart | cTn (cardiac troponin) | +++ | [56] |
|       | BNP        | ++   | [56] |
|       | CK-MB      | +    | [56] |
| Liver | Decrease serum albumin | +   | [57,58] |
|       | AST and ALT elevation | + | [57,58] |
|       | Total bilirubin, ALP | + | [57,58] |
|       | Prothrombin time (PT) | | |
|       | Lactate dehydrogenase (LDH) | +++ | [59] |
| Kidney | Hyponatremia/ hypernatremia, hyperkalemia | + | [60] |
|       | Creatinine and BUN | +++ | [59] |

Mild: (+) Moderate: (+++) Severe: (+++)
neurodegenerative diseases. These might lead to liberation of the following markers in blood, such as neurofilament light chain protein (NFL), a marker of intra-axonal neuronal injury, and glial fibrillary acidic protein (GFAP), a marker of astrocytic activation/injury. Neurofilament, an axonal structural protein, is released in neuroaxonal damage during neurodegeneration. NFL can be detected in the cerebrospinal fluid (CSF) and is a sensitive biomarker of neurodegenerative processes. Similarly, GFAP is a type III intermediate filament forming part of the cytoskeleton of mature astrocytes and other glial cells. GFAP is not found outside the CNS and could be used as marker of astroglial injury.

Future Perspective

Symptomatic COVID-19 patients with fever and/or respiratory symptoms can be screened for eosinophil count and C-reactive protein (CRP). With eosinopenia (<0.02 x 10⁹/L) and high CRP (≥4 mg/L) levels, patients are more likely to have corona infection. The combined sensitivity and specificity of both these tests’ is 67.9% and 78.2%, respectively, and have Likelihood ratio (LR+) more than 3. Therefore, this can be supplemented with leucopenia and lymphopenia and test for COVID-19 infection confirmed by RT–PCR and can be piloted as a candidate screening tool for COVID-19 suspects and contacts.

Thrombocytopenia observed in the initial phase of the disease can be due to apoptosis or damage of cytoplasmic function of lymphocytes; yet, there is increase in neutrophil count which may be due to bacterial super infection. Patients having these features along with eosinopenia can be a sign of high viral load and poor prognosis.

Conclusion

Our review of studies suggests that high-quality laboratory reports for biomarkers in COVID-19 infection varied significantly in the selection of patients and their ethnicity; collection, transport, and processing of biological samples; protocols for measurement of biomarkers, and other factors limited the external validity as well as the generalizability of all these findings in the published literature. We have reviewed biochemical, immune-inflammatory, nucleic acid, and cellular biomarkers that predict infection, disease progression in COVID-19 with emphasis on organ-specific damage. Our analysis of 65 biomarkers assessing the impact of SCoV-2 infection on five organs (lung, liver, cardiac, kidney, and neural) reported that increased levels of CRP, TNF-α, ferritin, IL-6, D-dimer, Procalcitonin, FAR, and decrease PC, lymphocyte count, leukocyte count, and CD4⁺/CD8⁺ ratio shows promising association in the prediction of early prognosis and severity disease and also correlates with cytokine storm a cardinal feature of COVID-19 progression. Apart from these biomarkers, there is rise in the levels of immunoglobins like IgA, IgM, and IgG with due course of time of infection. As COVID-19 mainly affects the lungs, but it also infects and affects other organs such as kidney, muscles, lung, and liver. This leads to the decrease/elevation of certain enzymes/markers in the circulation by assessing the level of these markers we can assess the condition of the patient and locate the organ affected. This leads to the decrease/elevation of certain enzymes/markers in the circulation by assessing the level of these markers we can assess the condition of the patient and locate the organ affected. The organ-specific markers include elevation of cTn (cardiac troponin) for heart damage, lactate dehydrogenase (LDH), AST, and ALT in the involvement of liver damage, decreased serum albumin and increased creatinine and BUN in case of kidney disease, and neurofilament light chain protein (NFL), and glial fibrillary acidic protein (GFAP), in case of neural injury in COVID-19 patients. Future multi-centric studies with standard treatment guidelines, consistent protocol for collection of samples, biomarker measurement, and validation with high sensitivity and specificity are indispensable to classify biomarkers of different stages of SARS-CoV-2 infection as well as the disease in different levels of clinical set-up during this pandemic and thereafter. There is a dire need of cellular markers for asymptomatic contact and patients in the incubation period for screening and diagnosis at the earliest when active intervention will have the best possible outcomes. It is warranted to categorize the COVID-19 disease from the incubation period to the multiple spectrums of manifestations emerging from SARS-CoV-2 infection of different human organ systems. These range from carrier stage as symptomatic although mild clinical features to full-blown cases needing intensive care intervention. Hence, there is a need of application of the suitable biomarker with a high index of suspicion for assessing severity in the early phase of the pathogenesis and proper evaluation of COVID-19 patients. Many of these biomarkers and their products have been explored for therapeutic applications for COVID-19 treatment. In addition, with the advancement of nanotechnological based, targeted delivery systems will be explored to kill the virus alone and normal cells can be spared for future nanomedicine-based treatments and therapeutics.

In this comprehensive review, we summarise the important biomarkers that are consistently associated with mortality across analyses. These biomarkers may provide novel and important insights into immunopathogenesis and correlate ions with severity of the disease.

Summary

In this study we have reviewed 65 biomarkers from 75 publications from PubMed. These biomarkers including biochemicals, immune-inflammatory, nucleic acid, and cellular molecules that predict infection, disease progression and organ specific damage in COVID-19 and organ-specific damage. We report that increased levels of CRP, TNF-α, ferritin, IL-6, D-dimer, Procalcitonin, FAR, and decreased, lymphocyte count, leukocyte count and CD4⁺/CD8⁺ ratio shows promising association in the prediction of early prognosis and disease severity in lung, liver, cardiac, kidney, and brain. These biomarkers also correlate with cytokine storm a cardinal feature of COVID-19. These biomarkers may serve the dire need of markers for asymptomatic contact and patients in the incubation period for screening and
diagnosis at the earliest when active intervention will have the best possible outcomes. Owing to the second, third and probable subsequent waves of the COVID-19 pandemic, it is warranted to categorize the COVID-19 disease from the incubation period to the multiple spectrums of manifestations emerging from SARS-CoV-2 infection in different human organ systems. These range from carrier stage as symptomatic although mild clinical features to full-blown cases needing intensive care intervention. There is a need of application of the suitable biomarker with a high index of suspicion for assessing severity in the early phase of the pathogenesis and proper evaluation of COVID-19 patients. We are hopeful that the biomarkers mentioned above will be explored for therapeutic applications for COVID-19 treatment using nano-technology-based target delivery systems. In this comprehensive review, we summarise the relevant biomarkers that are consistently associated with disease severity. These biomarkers may provide novel and important insights into immunopathogenesis and correlate with severity of the disease.

The review describes twelve routine biochemical and immunological investigations that are promising for early diagnosis and prediction of prognosis of COVID-19. These investigations can also help in predicting disease severity of COVID-19 in five organs. Utilization of these investigations is warranted in COVID-19 pandemic in the current third wave and subsequent waves of the pandemic as there is currently there is no standard guideline for predicting the severity of the COVID-19 pandemic.

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Conflicts of interest
There are no conflicts of interest.

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