Prospective selected biomarkers in COVID-19 diagnosis and treatment

Tahani Tabassum‡,1 [ID], Ahsab Rahman‡,1 [ID], Yusha Araf‡,2 [ID], Md A Ullah3 [ID] & Mohammad J Hosen*,2 [ID]

1Department of Mathematics & Natural Sciences, Biotechnology Program, School of Data & Sciences, Brac University, Dhaka, Bangladesh
2Department of Genetic Engineering & Biotechnology, School of Life Sciences, Shahjalal University of Science & Technology, Sylhet, Bangladesh
3Department of Biotechnology & Genetic Engineering, Faculty of Biological Sciences, Jahangirnagar University, Dhaka, Bangladesh
*Author for correspondence: jakir-gen@sust.edu
‡Authors contributed equally

COVID-19 has become a global health concern, due to the high transmissible nature of its causal agent and lack of proper treatment. Early diagnosis and nonspecific medical supports of the patients appeared to be effective strategy so far to combat the pandemic caused by COVID-19 outbreak. Biomarkers can play pivotal roles in timely and proper diagnosis of COVID-19 patients, as well as for distinguishing them from other pulmonary infections. Besides, biomarkers can help in reducing the rate of mortality and evaluating viral pathogenesis with disease prognosis. This article intends to provide a broader overview of the roles and uses of different biomarkers in the early diagnosis of COVID-19, as well as in the classification of COVID-19 patients into multiple risk groups.

COVID-19 outbreak has become a global public health issue and brought the world to a standstill [1]. Emerging from Wuhan, People's Republic of China, in December 2019, COVID-19 has affected more than 46 million people in 218 countries, and more than one and a half million people have died due to this disease till the date of this writing [2]. COVID-19 is caused by SARS-CoV-2, which is a positive, single-stranded, enveloped RNA virus from the family Coronaviridae [3]. With the help of spike protein (S-protein), SARS-CoV-2 invades the host cell by interacting with the ACE-2 receptor on the host cell membrane [4,5]. Due to its contagious characteristic, it is compulsory to ensure early stratification of SARS-CoV-2-infected patients [6]. The symptom of COVID-19 is heterogeneous, but the common symptoms include fever, dry cough and respiratory distress [7,8]. Many symptoms overlap with those of common flues, making it hard to understand the pathomechanism and diagnosis, as well as the treatment of this disease. Moreover, the lack of sustainable therapeutics is a treatment challenge for COVID-19. The identification of a perfect biomarker set for COVID-19 will enable clinical studies to determine whether a therapeutic has a clinically significant effect on its phenotype [9–12]. In addition to that, biomarkers can play a significant role in the early diagnosis of COVID-19, effectively differentiating it from other pulmonary infections. Pulmonary infections are infections that cause lung inflammation, thereby damaging their larger airways and smaller air sacs. Oftentimes, the overlapping symptoms of COVID-19 with other pulmonary infections such as pneumonia make it difficult to identify COVID-19 patients [13,14]. In this review, we will provide comprehensive insights into the role of different biomarkers for detecting COVID-19 as well as the factors differentiating it from other pulmonary infections.

Pathophysiology of COVID-19

SARS-CoV-2 follows a lytic cycle to replicate itself by employing the metabolic machinery of a cell. It invades a human host cell through five significant steps – attachment, penetration, biosynthesis, maturation and release
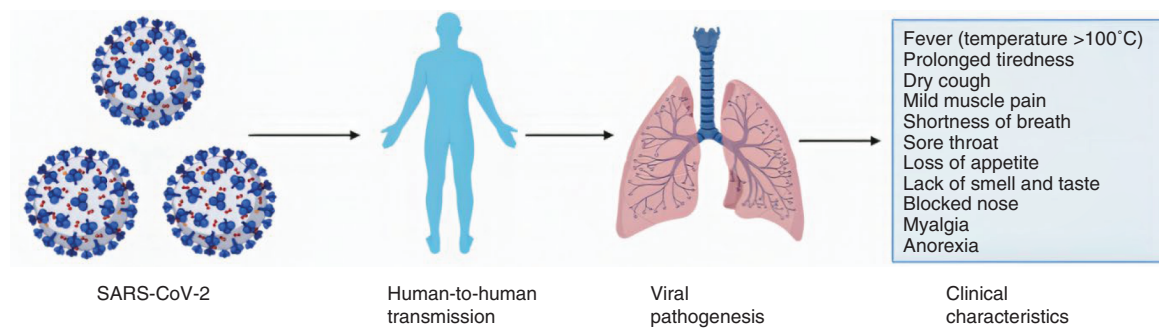
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Figure 1.   Clinical courses of SARS-CoV-2. The virus enters the body and damages the lungs. Symptoms may vary from person to person.

Table 1. Overlapping symptoms between COVID-19 and other pulmonary diseases.

Disease	Fever	Dry cough	Headache	Dyspnea	Fatigue	Lack of appetite	Ref.
COVID-19	Yes	Yes	Yes	Yes	Yes	Yes	[5,7,8]
Pneumonia	Yes	Yes	No	Yes	Yes	Yes	[13,27]
Chronic bronchitis	No	Yes	No	Yes	Yes	No	[26,28]
COPD	No	Yes	Yes	Yes	Yes	Yes (sometimes)	[25,29]

(Figure 1) [15,16]. There is a molecular key named 'spike protein' (S-protein) in SARS-CoV-2 which provides a route to the virus to enter the cell. Using the S-protein, the virus attacks the host cell by interacting with ACE-2 receptors [17]. There is a furin cleavage site in S-protein, which is responsible for the robust affinity of ACE-2 toward SARS-CoV-2. Furin is present in numerous body organs including the lungs, liver and small intestine, which indicates that the virus can infect multiple organs of the body [18]. Another potential entryway may come via its association with CD147, a transmembrane glycoprotein expressed in high levels in pathogen-infected cells and tumor tissues [19].

Once entered, the viral genome is released, which ultimately leads to the translation of viral polymerase protein. After the translation process, RNA replication takes place. It is then followed with subgenomic transcription and translation of viral structural proteins. S-protein, membrane protein and envelope protein combined with the nucleocapsid. Ultimately, mature virion is formed and exocytosis occurs [20].

A cytokine storm – a physiological phenomenon where the immune system releases excessive level of cytokines – can be observed in COVID-19 following the release of genomic RNA into the cytoplasm. Toll-like receptors, such as TLR-3 and TLR-4, are triggered when double-stranded DNA induces an immune response. While TLR-3 uses a signaling pathway cascade to stimulate type-I interferon, TLR-4 recruits immune cells in the infection site through the activation of pro-inflammatory cytokines [21,22].

A cytokine storm is the result of a sudden acute increase in circulating levels of pro-inflammatory cytokines, including IL-6, IL-1, TNF-α and interferon. This ultimately leads to the influx of several immune cells like macrophages, T cells and neutrophils into the site of infection. Moreover, the elevated cytokine release, specifically the IL-6 stimulation, leads to an increased level of synthesis of prototypic acute phase reactant like C-reactive protein (CRP) from the liver, in the bloodstream. Eventually, all these events lead to different sequalae like destabilization of endothelial cell-to-cell interaction, destruction of capillary and vascular barrier and damage of multiple organs and tissues, which may ultimately lead to the demise of the infected individual [23].

Another potential receptor for viral infection is DC-SIGN, expressed highly in dendritic cells (DCs) and macrophages. However, further studies need to be conducted for it to be confirmed [24].

Differential biomarkers between SARS-CoV-2 & other pulmonary infection patients

A clinical presentation of COVID-19 has several overlapping features with other pulmonary infections including pneumonia, chronic bronchitis and so on (Table 1). Pneumonia patients exhibit symptoms like high fever, cough, fatigue, lack of appetite, etc. [13]. The clinical presentation of chronic obstructive pulmonary disease (COPD) includes cough, dyspnea and extreme tiredness [25]. Similar symptoms can be visualized in chronic bronchitis as

Table 2. Range of biomarkers in mild and severe COVID-19 patients.				
Biomarker	Range in normal human	Range in mild COVID-19	Range in severe COVID-19	Ref.
Procalcitonin (ng/ml)	0.45	0.05 ± 0.05	0.44 ± 0.55	[44,45]
C-reactive protein (mg/l)	<10	33.2	57.9	[46,47]
Serum amyloid A (mg/l)	<10	89.78 ± 54.75	144.29 ± 57.33	[48,49]
IL-6 (pg/ml)	0–16.4	<80	>80	[50,51]
Lymphocyte count (cells/μl)	1000–4800	$1.4 \pm 0.15 \times 10^3$	$0.8 \pm 0.11 \times 10^3$	[52,53]
Platelet count	125×10^9–350×10^9/l	131×10^9–263×10^9/l	165×10^9–263×10^9/l	[54,55]
D-Dimer (mg/l)	<0.25	0.5	2.4	[56,57]
Lactate dehydrogenase (U/l)	140–280	151	248	[58]
Cardiac troponin (ng/ml)	<0.04	0.03–0.09	>0.09	[59,60]
Serum ferritin (ng/ml)	12–300	291.13	1006.16	[61,62]

well [26]. All these pulmonary diseases have signs and symptoms that resemble the signs and symptoms of COVID-19. As a result, oftentimes it becomes difficult to distinguish between SARS-CoV-2-infected patients and other pulmonary disease patients. Biomarkers have proven to be effective in differentiating between these two classes of patients [14,27–29].

COVID-19 patients had a lower proportion of basophils (BASP), lymphocyte count (LYM) and white blood cell count (WBC) compared with the patients with other pulmonary diseases [13]. The most discriminating biomarkers between these two groups were BASP, prothrombin time activity (PTA), prothrombin time (PT) and international normalized ratio (INR). COVID-19 patients had relatively lower PT and INR and relatively higher PTA. A lower proportion of basophils represent COVID-19 patients, indicating its role in producing T-lymphocyte. Therefore, decreasing basophils may result in the disruption of the adaptive immune system. As a result, it is a crucial biomarker to differentiate between the two groups of patients [30,31].

It was also found that some of the coagulation parameters were different between these two groups as well. COVID-19 patients having relatively lower PT and INR and relatively higher PTA indicate that they were subjected to higher coagulation. Higher coagulation has the potentiality to blunt inflammation, which could explain why clinical inflammation symptoms are not obvious in COVID-19 patients [30,32,33]. So, it is evident that biomarkers can play a salient role in distinguishing COVID-19 patients from other pulmonary disease affected patients.

Differential expression of different biomarkers & their possible mechanism in COVID-19 patients

Procalcitonin

Procalcitonin (PCT) is the precursor of the hormone calcitonin, involved in regulating the levels of calcium and phosphate in the bloodstream [34]. PCT has been beneficial for the diagnosis of severe bacterial infections, as well as for guiding antimicrobial stewardship [35]. PCT is also known as 'hormokine'; either follows a hormonal expression pathway or a cytokine-like expression pathway in response to bacterial infections [36].

The increased concentration of IFN-γ in response to viral infections can inhibit the production of PCT. It is therefore expected that PCT values should remain within the minimum range in patients with noncomplicated COVID-19 infection [37]. Nevertheless, several studies reported an elevated level of PCT among severe COVID-19 patients [38]. Several reasons might be attributable to the increased PCT levels with disease progression. Due to viral-induced lung tissue damage, the normal bacterial flora might become invasive leading to the development of secondary bacterial pneumonia and enhanced PCT secretion. Another reason might be bacterial translocation in response to inflammation and septic illness induced by COVID-19-associated pneumonitis that can significantly increase PCT secretion [39]. Although in mild cases the level of PCT is within the normal range, the levels increase once the disease starts becoming complicated. This substantial increase in the PCT level reflects bacterial co-infection, which can be a significant indicator of disease severity [38]. Several other studies have reported that analysis of PCT levels can be positively associated with the severity of COVID-19 infection (Table 2) [40–45].

C-reactive protein

C-reactive protein (CRP), an acute-phase inflammatory protein synthesized primarily by the liver, plays a crucial role in the innate immunity. CRP is a significant indicator of severity in COVID-19 [63]. In normal conditions, the

concentration of CRP in the blood is less than 10 mg/l [64], but the concentration may increase up to 1000-fold in response to severe bacterial infections and tissue damage [65]. This rapid increase in CRP concentration is observed during the initial 6–8 h and gives the highest peak in around 48 h after the disease onset [64]. However, once the stimuli end, the concentration keeps on decreasing exponentially [66]. Once the conditions are resolved and CRP concentration falls within a range, it can serve as a useful biomarker for estimating disease severity [64].

CRP levels are also reported positively correlated with lung lesion during the early stages of SARS-CoV-2 infection [66]. High CRP levels can also be used for early diagnosis of severe pneumonia in COVID-19 patients [67]. The effect of increased CRP in severe SARS-CoV-2 infections can be linked to the overproduction of inflammatory cytokines. Cytokine storm is a major mechanism of inducing lung damage by the SARS-CoV-2. These inflammatory cytokines and their corresponding tissue damage may evoke the overproduction of CRP and mark worse disease prognosis (Table 2) [46,47,68].

Serum amyloid A

Serum amyloid A or SAA protein, synthesized primarily by the liver, plays an important role in the body's inflammatory responses and lipid metabolism. Amyloid A is also known for its function in central nervous system and its association with multiple neurodegenerative disease has already been postulated. However secreted small amount during the normal conditions of the body, but the SAA levels are elevated during the acute phase of an inflammatory disease due to the stimulated inflammatory mediators including IL-1, IL-6, TNF-α [69]. Thus, SAA can be used as an important and independent biomarker to detect both viral and bacterial infections.

Several studies have reported that SAA can be used as an independent predictive biomarker of COVID-19 [70,71]. An increased level of SAA has been found to be associated with the severity of COVID-19 infection [70]. SAA levels were found increased in severe patients and the expression was significantly higher in acute phase infection compared with the convalescent phase infection [72].

The precise mechanism for the elevated release of this acute-phase protein is still unclear, but could be influenced by the increased production and secretion of inflammatory mediators, a characteristic phenomenon of SARS-CoV-2 infection [7]. SAA was also found to be more sensitive and efficient in detecting minor inflammatory stimuli in several viral infections than CRP (Table 2) [48,49,73]. Based on such observations, it is clear that SAA can be a vital biomarker to predict severe outcomes of COVID-19. Further evidence needs to be accumulated to better understand the correlation between SAA levels and disease severity in COVID-19 patients.

Interleukin-6

IL-6 is one of the most important cytokines that is produced in response to infections and tissue damages and plays a crucial role in the host immune defense mechanism. It is mostly synthesized during the initial stage of inflammation and then moves to the liver to rapidly induce the secretion of a huge range of acute-phase proteins [74]. As a major mediator of the inflammatory response, it has been found to play a pivotal role in the pathophysiology of COVID-19-induced lung damage [75]. Besides, levels of IL-6 are reported to be linked with the increased need for incubation and mechanical ventilation of COVID-19 patients [76,77].

Multiple studies have reported a significant difference in the IL-6 levels between complicated and non-complicated COVID-19 cases. It was reported that IL-6 levels were around three-times higher in the severe COVID-19 patients than the non-severe patients [64,66]. Higher IL-6 levels could also be linked to higher mortality rates [77]. Thus, IL-6 can be a good prognosticator for combined end-point progression to detect severe COVID-19 infection and in-hospital mortality rate detection [75].

Acute respiratory distress syndrome (ARDS) generates a cytokine storm by eliciting the overproduction of many inflammatory mediators including IL-6, and contributes to the mortality and severity of a COVID infection. In COVID-19 patients, the overproduction of IL-6 was found to be linked with complicated outcomes, such as hepatic, renal, cardiac and lung injuries [78]. At present, several IL-6 blocking agents are being used to treat COVID-19 patients and are displaying positive outcomes by reducing disease severity (Table 2) [50,51,79]. It is suggested that further investigation be initiated, regarding the reliability of IL-6 as an independent and vital biomarker to detect COVID-19 disease severity.

Lymphocyte count

Lymphocytopenia (ALC = <1000 cells/µl blood) is a common systemic manifestation of many immunodeficiencies and viral infections [80]. Previously, SARS-CoV and MERS-CoV had demonstrated lymphocytopenia as a vital

feature of the viral infection [81]. A low lymphocyte count is rarely observed in COVID-19-infected children, but frequently observed in the elderly infected population [82].

A recent study reported that the majority of the COVID-19 patients had an abnormally low count of lymphocytes [83]. On the contrary, a higher $CD4^+$ and $CD8^+$ cell count was seen to contribute to a reduction in the severity of the disease [83]. Another study also reported that the average absolute lymphocyte (ALC) count was significantly lower in COVID-19 patients who needed ICU admission than in the patients who did not [84]. A systemic review and meta-analysis report compared the lymphocyte count of the severe patient group with the nonsevere group and concluded that lymphocytopenia is associated with a threefold increase in the risk of disease severity in SARS-CoV-2 infection (Table 2) [52,53,85].

Several factors may be responsible for the lymphocytopenia in severe COVID-19 patients, including inflammatory cytokine storm, direct viral attack on lymphocytes, increased level of pro-inflammatory cytokines, increased serum levels of IL-6 and interference with T-cell proliferation by downregulation of several gene expressions by the virus [82]. All these observations suggest that lymphocytopenia can be a vital biomarker to predict COVID-19 disease severity. An investigation regarding the underlying cause of lymphocytopenia and its reliability as a biomarker to predict disease severity should be further explored.

Platelet count

Thrombocytopenia, a condition characterized by abnormally low levels of platelets, is a common effect of many viral infections. A study in a different cohort of COVID-19 patients reported that 36.2–55% of the patients had thrombocytopenia and the abnormalities were even worse in severe patients [42,86]. As a result of the reduced level of platelets, different cardiovascular events are anticipated such as venous and arterial thrombosis, of which there is already evidence in COVID-19 patients. These aggregated events can eventually lead to the multiorgan failure and death of the infected individual [87,88]. A meta-analysis showed thrombocytopenia is associated with a threefold increased risk of severe infection; platelet count was significantly lower in severe patients and even lower in patients who died of COVID-19 [89]. Another case report showed that the platelet count of a 57-year-old declined significantly during the initial stages of hospital admission, but increased in response to treatments during the latter stages [90]. These evidences suggest that platelet counts can be important biomarkers to detect disease progression and severity in SARS-CoV-2 infections.

Multiple factors may influence the abnormally reduced number of platelet in COVID-19 patients. It has been suggested that SARS-CoV-2 can enter the bone marrow and inhibit the process of hematopoiesis through certain receptors resulting in a decreased platelet count in patients [91]. Some studies suggest that the cytokine storm-generated during the COVID-19 infection may damage the hematopoietic progenitor stem cells in the bone marrow that reduces the primary production of platelets resulting in abnormal hematopoiesis and a low platelet count [92]. Enhanced immune response due to viral infection may also be a reason for the extensive destruction of platelets [91]. Moreover, damage to the lung tissues and endothelial cells caused by SARS-CoV-2 may trigger microthrombin formation and platelet accumulation, which may increase platelet consumption at the site of injury [54,55,86]. Since several reports suggesting a correlation between platelet count and disease severity in COVID-19 exists, further evidence should be collected to evaluate the importance of low platelet count as a biomarker of worse COVID-19 disease prognosis (Table 2).

D-Dimer

D-Dimer, a fibrin degradation product, is an indicator of hypercoagulation state, secondary fibrinolysis, the abnormal process of coagulation and thrombosis [93–95]. D-Dimer assays are commonly used in clinical practices to indicate an increased risk of abnormal blood clotting and for the diagnosis of deep-vein thrombosis or pulmonary embolism. On analysis of patients with severe community-acquired pneumonia, it was found out that an increased D-Dimer level is related to a higher risk for complications and higher mortality rate [96,97].

Several reports have suggested an elevated concentration of D-Dimer is associated with COVID-19 severity. Studies reported that an elevated level of D-Dimer in severe COVID-19 patients compared with mild cases (Table 2) [98], but its level was significantly elevated in ICU patients compared with non-ICU patients [7]. Similarly, in a Chinese COVID-19 cohort showed that D-Dimer levels >2.0 mg/l during hospital admission were the only variable that could be linked to the increased risk of mortality [99].

D-Dimer may serve as an indicator for severe viral infection as a viral infection is likely to develop into sepsis and induce coagulation dysfunction, which in turn can serve as a common representation of severe disease progression.

Moreover, the cytokine storm generated during a COVID-19 infection can recruit several inflammatory and proinflammatory mediators to create an imbalance in the coagulation process and fibrinolysis, ultimately elevating the level of D-Dimer in patients [100]. In a cohort of hospitalized COVID-19 patients, it was reported that anticoagulant treatment could reduce the risk of mortality, especially in patients receiving mechanical ventilation [56,57,100]. Since evidence suggesting a correlation of elevated D-Dimer levels to COVID-19 infection severity has been found, it can be used as an efficient prognostic biomarker for predicting worse disease outcome and also be targeted to reduce mortality in severe patients.

Lactate dehydrogenase

Lactate dehydrogenase (LDH) is an intracellular glycolytic enzyme implicated in the conversion of lactate to pyruvate and is present in higher amounts in almost all the body cells. It is considered as an inflammatory marker and may serve as a common indicator of acute or severe tissue damage [101]. It has also been considered to be the biomarker of cardiovascular event like myocardial ischemia and a recent study suggests an association with coronary artery disease [102]. Evidence also suggests that serum LDH levels can serve as a nonspecific indicator of cellular death and is associated with poor prognosis in malignancy [103,104]. LDH was considered to be one of the biomarkers strongly associated with ARDS mortality [105,106]. Elevated LDH levels were also found in patients with SARS-CoV and MERS-CoV infection [107].

Elevated serum LDH levels have been attributed to increased disease severity and a higher risk of in-hospital COVID-19 mortality. A recent pooled analysis showed a >sixfold increase in odds of developing severe COVID-19 infection outcomes and a >16-fold increase in odds of mortality in patients with high levels of LDH (Table 2) [108–110]. A positive correlation of increased LDH levels with the CRP and cardiac troponin I (cTnI) level and a negative correlation with lymphocyte count in severe COVID-19 patients were also reported [109].

Cytokine-mediated lung tissue damage, which is a primary feature of severe SARS-CoV-2 infection, may be responsible for the elevated release of LDH [111]. The greater amounts of LDH released in circulation as the severe form of interstitial pneumonia may evolve into ARDS, which is one major hallmark of COVID-19 infection [108]. Moreover, normalizing the LDH titer has been successful in predicting treatment success in several cases and should be further investigated [58,112].

Cardiac troponin

Cardiac troponins are a group of cardiac regulatory proteins that control muscular contraction and may serve as an indicator to detect heart muscle injury. Cardiac troponin is still the only standard biomarker for the detection of acute myocardial infarction. The onset of myocardial infarction is demonstrated by an elevated level of cardiac troponin [113]. However, this protein is often associated with viral infection-induced sepsis and the incidence of acute respiratory infections as a marker of disease severity [114–116]. Previous observations regarding the SARS-CoV and MERS-CoV outbreaks provided enough evidence of myocardial injury and myocarditis [117].

Cardiac troponin I (cTnI) was found to be marginally increased in the mild severe patients and significantly higher in severe COVID-19 patients [118]. A considerably high cTnI level was also reported in COVID-19 patients suffered from cardiac injury [119]. Furthermore, one out of five hospitalized COVID-19 patients were reported to have significantly elevated levels of cardiac troponin and these patients were more likely to require invasive or noninvasive ventilation [120]. The mortality rate in patients with myocardial injury was found to be tenfold enhanced compared to patients without myocardial injury [120].

Several mechanisms may explain this phenomenon of elevated cardiac troponin in severe COVID-19 patients. ACE-2 is highly expressed in the pericytes of the human heart tissues, which allows SARS-CoV-2 to directly enter the heart cells and cause severe damage [121]. The virus can induce direct virus-mediated lysis of cardiomyocytes and ventricular dysfunction by inducing a strong T-cell response [122,123]. SARS-CoV-2-induced myocarditis has been reported to be responsible for up to 7% of COVID-19-related deaths [124]. Early measurement of cardiac troponin levels could facilitate the understanding of the systemic consequence of the disease and interpret the requirement of inotropes, vasopressors, diuretics and other additional therapeutic implications in patients with cardiac dysfunction [59,60,125]. All these shreds of evidence suggest that cardiac troponin levels can be a vital biomarker in predicting COVID-19 severity, especially in people with a history of cardiovascular disease, however, further investigation to understand the underlying mechanisms should be undertaken in order to ascertain its validity as a biomarker for detecting severe disease outcomes (Table 2).

Serum ferritin

Ferritin, an iron-storing protein, is a key mediator of immune dysfunction. Serum ferritin is an indicator of inflammation, released by the damaged cells, but whether the presence of this protein in serum is usual or not has yet to be ascertained [126]. Researchers found increased levels of serum ferritin in different lung diseases such as idiopathic pulmonary fibrosis [127], and reflecting its potential elevated release from damaged lung cells after infection with respiratory viruses. Increased level of serum ferritin has been reported in disease severity as well as fatal outcomes in COVID-19 patients (Table 2) [128–131]. Although hyper-ferritinemia was observed in severe cases of COVID-19, the concentration of ferritin was within the normal range for the nonsevere disease cases [132].

Higher ferritin levels in COVID-19 are not exclusively driven by SARS-CoV-2 infection; it may also occur due to other inflammatory diseases or several inflammatory cytokines such as IL-6 [133]. Moreover, some COVID-19 patients may have higher ferritin levels due to the pre-existence of comorbid conditions such as bacterial infections, autoimmune diseases, pre-existing neoplasm [61,62,134]. Further exploration of the reliability of serum ferritin levels as a biomarker for the prediction of COVID-19 disease prognosis should be undertaken.

Acute kidney injury

Although the SARS-CoV-2 primarily damages the lung tissues and generates symptoms of acute respiratory failure, it has been found to enter the bloodstream and accumulate in several other organs causing secondary organ damage [135]. Among the secondary organs, the incidence and clinical characteristics of acute kidney injury (AKI) have been reported in many laboratory-confirmed COVID-19 patients [136]. A recent study conducted on 116 patients found an incidence of AKI in 18.1% of the patients and the majority were in critical condition, as well as had a higher prevalence of combined shock. Whereas, among the non-AKI patients the incidence of combined shock was significantly lower, and only a quarter of the patients were critical [136]. A similar phenomenon was also observed during the SARS-CoV outbreak, where patients with acute renal impairment in addition to the viral infection had a higher mortality rate in contrast to patients affected by the virus, but no renal impairment [137].

Various degrees of renal dysfunction have been reported in COVID-19 patients, characterized by increased levels of blood urea nitrogen (BUN) and serum creatinine [138]. A study reported that 27% of the COVID-19 patients showed elevated BUN levels, and of those, two-thirds of the patients with elevated BUN levels and serum creatinine levels over 200 µmol/l had died [139]. Another study also reported that more than 40% of the hospitalized patients had evidence of kidney disease and were associated with higher cases of in-hospital mortality [135].

One possible explanation of the high incidence of AKI in COVID-19 patients may be related to the mechanism of SARS-CoV-2 entrance in cells. Renal tubular epithelial cells have a high expression of ACE-2 protein, the receptor that binds to and facilitates viral entrance in cells [140]. The higher concentration of inflammatory mediators in response to a cytokine storm can also result in renal impairment [141]. Another possible explanation can be the high prevalence and history of chronic kidney diseases in COVID-19 patients at the time of hospital admission [142]. Dysfunction of the endothelial system, coagulopathy and activation of the complement pathway may also contribute to the development of AKI in a subset of COVID-19 patients [143]. Since AKI has been strongly associated with increased COVID-19 mortality and morbidity, it can serve as an important biomarker for the prediction of disease severity and as a potential therapeutic target to reduce COVID-19-related deaths.

Others

Besides these, a number of other biomarkers can be considered for the diagnosis of COVID-19. Different types of viral protein-coding genes can be used in the appropriate diagnosis of COVID-19 patients. In fact, the most commonly used reverse transcriptase polymerase chain reaction (RT-PCR) technology utilizes the polymerization reaction of different genes of SARS-CoV-2 involved in the expression of structural and nonstructural proteins. These genes are predominantly involved in the expression of spike protein (S), membrane protein (M), nucleocapsid protein (N), RNA-dependent RNA polymerase (RdRp) and so on. Use of nasopharyngeal or oropharyngeal swab of infected patients is enough to extract and amplify the above-mentioned viral genes when using RT-PCR technology for COVID-19 diagnosis [144,145]. Loss of smell and taste have been characterized as a potential early symptom of COVID-19 infection [146]. In a cohort of Iranian hospitalized COVID-19 patients, >98% of patients had an impaired sense of smell characterized by psychophysical olfactory testing [147]. It is suggested that SARS-CoV-2 targets the olfactory and gustatory system and may be involved in the peripheral damage of the olfactory receptor cells in the nasal neuroepithelium [148]. Intra-nasal administration of SARS-CoV-2 in a transgenic mice model has

demonstrated that the virus can penetrate the brain through the olfactory bulb [149]. Since the high prevalence of smell dysfunction has been reported in several cases, it can serve as a vital biomarker for COVID-19 screening.

Vitamin D is an important regulator of host inflammatory and immune response. Recent studies showed that a decreased level of 25 hydroxyvitamin D or 25(OH)D has been associated with the severity of COVID-19. It has been reported that hospitalized COVID-19 patients with vitamin D deficiency experienced a higher risk of developing ARDS compared with patients with normal vitamin D levels [150]. Healthy vitamin D levels can turn down the cytokine storm generated by the virus and reduce the overproduction of pro-inflammatory cytokines [150]. However, in a recent MR study, the results indicated no linear causal relationship of 25(OH)D concentration with coronavirus disease severity and susceptibility [151]. Further investigation paying more attention to the randomization control trials must be done to better evaluate the association between vitamin D levels and COVID-19 disease prognosis.

Serum IgA, IgG and IgM immunoglobulins that are produced by the host immune system in response to SARS-CoV-2 infection could serve as a potential biomarker in COVID-19 diagnosis. A recent study demonstrated that IgA levels reached the peak value during the initial 16–20 days after disease onset and then began to decline but continues to remain at relatively high value for a considerable period, IgG level was initially low but started to rise at 15 days after disease onset and stayed at a relatively high value for a considerable period, and IgM levels peaked at early stage, but the reading was comparatively lower than IgA and IgG [152]. Overall, IgM and IgG levels were found to be elevated in both moderate and severe COVID-19 patients compared with the mild cases, whereas IgA levels were only found to be elevated in severe cases of the infection [152]. Another study also showed increased serum IgG and IgM antibodies in severe COVID-19 patients compared with mild cases [153]. These shreds of evidence suggest that serological anti-SARS-CoV-2 antibody tests can serve as a potential biomarker to predict disease severity.

Some epidemiological studies suggest that one of the major predictors of COVID-19 severity is age, pre-existing disease conditions and habits associated with an unhealthy lifestyle [154]. So, biological age is more reliable in predicting worse COVID-19 prognosis than chronological age [154]. Glycan clock is one of the biomarkers that can be used for the prediction of biological age and can predict the prevalence and susceptibility of COVID-19 infection [155]. Besides, glycan also serves as a principal regulator of host immune responses [155]. Based on such findings, it is suggested that biological age may serve as an important biomarker in COVID-19 severity.

Conclusion

Due to the contagious nature of the virus and lack of any specific approved treatment, it becomes important to ensure early stratification of the infected patients to reduce the risk of transmission among susceptible groups as well as to allocate optimal resources to the patients who are in severe condition. Biomarkers can play a significant role by facilitating timely diagnosis, risk monitoring and directing the optimal treatment of COVID-19 patients. Based on the aforementioned promising evidences, it is apparent that biomarkers are essential to optimize the COVID-19 prognosis and hereby to minimize the mortality rates. However, individual variation among patients in biomarker levels may interrupt the optimal interpretation of disease prognosis. Therefore, further studies must be conducted to evaluate and identify best potential biomarker for COVID-19 diagnosis.

Future perspective

The severe global crisis generated by the highly contagious SARS-CoV-2 has affected over 103 million people and has resulted in around 2.24 million deaths worldwide. The spread of the virus is so swift that the general population, healthcare professionals and regulatory authorities from different countries have employed vastly different, but effective strategies to battle against the virus. In this situation, early diagnosis and effective management of the infected population are critical. Biomarkers can play a key role in this regard by ensuring the efficient diagnosis as well as guiding improvements in the clinical management of patients with COVID-19. More significantly, the use of biomarkers can aid the development and approval of biological agents and therapeutics in addition to evaluating COVID-19 pathogenesis and accessing disease prognosis. Although research is still in its early stages, the characteristic features of different biomarkers during the disease progression could help categorizing and understanding the underlying disease pathomechanism. Moreover, the identification of efficient biomarkers can also assist in predicting future complications or severity of the disease. In addition, the identification of integral biomarkers of the SARS-CoV-2 may facilitate a much more robust response against future respiratory viral outbreaks with the potential of creating global pandemics. The accumulated knowledge regarding SARS-CoV-2 infection biomarkers might be utilized to generate a much more efficient resource allocation in tackling future outbreaks and pandemics and more research works are warranted in this regard.

Executive summary

- Biomarkers can play a significant role in the early diagnosis and differentiation of SARS-CoV-2 infection from other pulmonary infections exhibiting almost similar symptoms.
- SARS-CoV-2 invades a human host cell through five significant steps – attachment, penetration, biosynthesis, maturation and release.
- Clinical presentation of chronic obstructive pulmonary disease (COPD) and SARS-CoV-2 infected patients are quite similar which makes it difficult to distinguish the symptoms. Biomarkers have proven to be effective in differentiating between these two classes of patients. The most determining biomarkers between these two groups are BASP, PTA, PT and INR. Some of the coagulation parameters were different between these two groups as well.
- PCT values were expected to remain within the minimum range in patients with noncomplicated COVID-19 infection as the increased concentration of IFN-γ can inhibit PCT production. Nevertheless, PCT levels were reported to be increased among severe COVID-19 patients, which may be attributed to viral-induced lung tissue damage and bacterial translocation, resulting in bacterial coinfection.
- Under normal conditions, the concentration of CRP in the blood is less than 10 mg/l, but the concentration may increase up to 1000-fold, serving as a significant indicator of COVID-19 infection severity.
- SAA levels are significantly increased in severe COVID-19 patients. Studies suggest that SAA can be used as an independent predictive biomarker of COVID-19. The expression level is significantly higher in acute phase infection compared with the convalescent phase infection.
- IL-6 levels are around three-times higher in the severe COVID-19 patients than the nonsevere patients and can also be linked to higher mortality rates.
- Lymphocytopenia is associated with a threefold increased risk of disease severity in SARS-CoV-2 infection. The average ALC count is reported to be significantly lower in COVID-19 patients requiring ICU admission compared with patients who do not require ICU admission.
- Thrombocytopenia can be linked to different cardiovascular events like venous and arterial thrombosis that are highly prevalent as comorbid conditions in COVID-19 patients. Besides, the lower levels of platelets and associated events can eventually lead to multiorgan failure and death of the infected individual.
- Elevated D-Dimer levels might serve as an indicator for severe COVID-19 infection as the cytokine storm-generated during the viral pathogenesis can recruit several inflammatory mediators to create an imbalance of the coagulation process and fibrinolysis resulting in enhanced D-Dimer secretion.
- A recent pooled analysis showed a >sixfold increase in odds of developing severe COVID-19 infection outcomes and a >16-fold increase in odds of mortality in patients with elevated levels of LDH suggesting LDH as an effective biomarker to mark disease severity. Besides, this biomarker can also be used as a predictor of comorbid cardiovascular events in COVID-19 patients.
- High cardiac troponin levels are reported in COVID-19 patients, especially those suffering from cardiac injury or having previous history of cardiovascular diseases.
- Apart from these, major biomarkers like serum ferritin, AKI indicators, serum immunoglobulins, vitamin D levels, biological age, and loss of smell and taste can also be considered as crucial biomarkers in detecting and accessing COVID-19 disease severity.

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