The Role of Biomarkers in Hospitalized COVID-19 Patients With Systemic Manifestations

Michael Schneider (iD)

University of Queensland Ochsner Clinical School, New Orleans, LA, USA.

ABSTRACT: The following article aims to review COVID-19 biomarkers used in hospital practice. It is apparent that COVID-19 is not simply a pulmonary disease but has systemic manifestations. For this reason, biomarkers must be used in the management of diagnosed patients to provide holistic care. Patients with COVID-19 have been shown to have pulmonary, hepatobiliary, cardiovascular, neurologic, and renal injury, along with coagulopathy and a distinct cytokine storm. Biomarkers can effectively inform clinicians of systemic organ injury due to COVID-19. Furthermore, biomarkers can be used in predictive models for severe COVID-19 in admitted patients. The utility of doing so is to allow for risk stratification and utilization of proper treatment protocols. In addition, COVID-19 biomarkers in the pediatric population are discussed, specifically in predicting Multisystem Inflammatory Syndrome. Ultimately, biomarkers can be used as predictive tools to allow clinicians to identify and adequately manage patients at increased risk for worse outcomes from COVID-19. Both literature review and anecdotal evidence has shown that severe COVID-19 is a systemic disease, and understanding associated biomarkers are crucial for hospitalized patients' proper clinical decision-making. For example, the cytokine storm releases inflammatory markers in different organ systems such as the pulmonary, hepatobiliary, hematological, cardiac, neurological, and renal systems. This review summarizes the latest research of COVID-19 that can help inform healthcare professionals how to better mitigate morbidity and mortality associated with this disease and provides information about certain systemic biomarkers that can be incorporated into hospital practice to provide more comprehensive care for hospitalized COIVD-19 patients.

KEYWORDS: COVID-19, biomarkers, cytokine storm, hospital medicine, risk stratification, acute respiratory distress syndrome by coronavirus 2, laboratory medicine

Introduction

Biomarkers are measured characteristics used in detecting the presence or absence of disease, monitoring changes in the clinical course of an illness, interpreting the response to an intervention or the environment, predicting treatment response, identifying populations at high risk for disease progression, recurrence, or clinical events, identify susceptibility or risk and to determine the likelihood of adverse events.[1-4] Biomarkers have emerged as a valuable diagnostic tool in the management of the SARS-CoV-2 (severe acute respiratory syndrome coronavirus) strain of coronavirus, the causative organism for coronavirus disease 2019 (COVID-19).[5,6] In hospital practice, biomarkers aid in the diagnosis, prognosis prediction, and the development of risk stratification strategies for patients with COVID-19.[7-9]

The current modalities for diagnosing COVID-19 include the immunoassays of SARS-CoV-2 antibodies and real-time reverse-transcription polymerase chain reaction (RT-PCR) using specimens from nasal swabs and blood bronchoalveolar lavage fluid, feces, sputum, and pharyngeal swabs.[10] The diagnostic modalities do not provide information about infection in specific tissues and fail to reveal the presence or extent of the inflammatory response associated with severity. The immunological biomarkers can determine the extent of the immune response and provide prognostic information about a COVID-19 infection.

Although COVID-19 has become known as a respiratory illness that manifests as pneumonia or acute respiratory distress syndrome (ARDS), clinical observation and literature have demonstrated that severe COVID-19 has a wide array of extrapulmonary manifestations.[11] Hence, understanding biomarkers and their relationship to pathophysiologic mechanisms of COVID-19 are vital for clinicians to mitigate patient burden and comorbidity, practice evidence-based medicine, and, importantly, ensure favorable long-term outcomes post-acute care hospitalization COVID-19 patients. The evidence from research has demonstrated that severe COVID-19 is associated with manifestations in multiple organ systems in the body, such as ocular symptoms, reproductive system involvement, skin lesions, vascular abnormalities, hematologic complications, acute renal injury, neurological damage, gastrointestinal complications, liver dysfunction, cardiac manifestations, and mediastinal findings.[12-16] Even though the COVID-19 commonly presents as a respiratory infection, the multisystemic dysfunction underscores the need for biomarkers in multiple organs to assess the prognosis, risk for severe COVID-19 condition, response to intervention, and adverse events. Recent studies have identified efficacious biomarkers indicative of severe organ dysfunction such as serum ferritin, C-reactive protein, d-dimer, and procalcitonin and highlight the role of these biomarkers in determining patients at risk for poor outcomes COVID-19.[17,18] This review aims to provide an updated review for clinicians in hospital practice treating severe COVID-19, with a specific emphasis on understanding the extrapulmonary manifestations in patients of this disease and

providing data on other biomarkers that can be used based on peer-reviewed research to better manage hospitalized COVID-19 patients.

Search criteria

This review utilized various databases to search for information relevant to COVID-19 biomarkers. The databases included peer-reviewed journals from Embase, Cochrane, CINAHL, LitCovid, Scopus, and PubMed. These databases were chosen for their relevance in having suitable materials. The search process was developed from keywords in the research that included "COVID-19," "biomarkers," "cytokine storm," "hospital medicine," "risk stratification," "acute respiratory distress syndrome by coronavirus two," and "laboratory medicine." These were combined to get the best results for papers that fit into the search criteria. Upon getting the search results, the papers were reduced to the most relevant results by employing a filtering method, using an inclusion and exclusion criteria developed. The inclusion criteria involved publications from March 2019 to March 2022, written in English, relevant to the COVID-19 biomarkers, observational studies, narrative reviews, systematic reviews, and meta-analysis related to COVID-19 biomarkers in hospitalized patients. Publications with a cohort size of less than 30 patients and non-peer reviewed literature were excluded.

COVID-19 pathophysiology and biomarkers

The coronavirus is a positive-stranded RNA virus with an envelope and surface spikes of glycoproteins.[19] SARS-CoV-2 cellular tropism is based upon the spike subunit, which interacts with the angiotensin-converting enzyme 2 (ACE2) receptors. ACE2 expression varies in different tissues; however, its presence in smooth muscle cells, lung alveolar epithelial cells, venous endothelial cells,[20] gastrointestinal cells, and liver cells[21] enables SARS-CoV-2 to cause dysfunction in the corresponding organs. Additionally, SARS-CoV-2 is dependent on the host cell serine proteases (TMPRSS2) to modify its spike protein for gaining successful entry into the host cell.[22] Single-cell RNA sequencing (scRNA-seq) datasets identified the expression of ACE2 receptors in esophageal epithelial cells, type II alveolar cells, renal proximal tubule cells of the kidney, and myocardial cells, making these tissues vulnerable to SARS-CoV-2 infections.[32] Consequently, SARS-CoV-2 can infect and damage these tissues and cause multiorgan dysfunction in COVID-19.

Cytokine storm

The immune response to SARS-CoV-2 releases cytokines that can be used as biomarkers to monitor inflammation, determine the risk of organ damage, and guide therapeutic strategy for COVID-19.[24,25] After entry into the host cytoplasm, SARS-CoV-2 releases the RNA genome and forms new viral particles by replication that spread to other cells following the disintegration of the host cell. As the viral particles invade different tissues, an immune response ensues, known as a cytokine storm. The cytokine storm is associated with the activation of leukocytes, platelets, and endothelium that triggers the release of tissue factor and thrombin, fibrin formation, and activation of the coagulation pathway and platelets.[26] Soluble mediators of a cytokine storm include growth factors and cytokines such as interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), and interferon-gamma γ, chemokines such as interleukin-8 (IL-8), monokine induced by gamma (MIG), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP) 1 α and B lymphocyte chemoattractant as well as plasma proteins such as C-reactive protein (CRP), ferritin, and complement.[17,25]

Patients with COVID-19 who are admitted to the intensive care unit (ICU) have been shown to have higher levels of IL-2, IL-7, IL-10, TNF-α, GM-CSF, interferon-γ induced protein 10 kD (IP-10), MCP-1, and MIP-1α.[26] The criteria in Table 1 show the prediction of the development of a cytokine storm and the cutoff values that indicate severe COVID-19 disease.[27] Clinicians should measure the levels of biomarkers in Table 1 at admission and serially during the hospital stay to identify increases above the cutoff values to determine the risk of a cytokine storm in COVID-19 patients.

Pulmonary injury and biomarkers

The lungs are one of the initial sites of infection of SARS-COV-2, and respiratory symptoms are a common manifestation of the disease. Analysis of samples from the respiratory tract identified positive ACE2 expression in 2% of respiratory epithelial cells.[28] As the virus infects the alveoli, the levels of several cytokines and chemokines, including IL-1R, IL-2, IL-6, MCP-1, MIP-1α, and TNF-α, increase with subsequent respiratory failure.[28] Postmortem examination of confirmed COVID-19 cases showed perivascular chronic inflammation with positive lung cultures and scattered small vessel thrombi in most patients with acute pneumonia.[29] These findings support the role of inflammatory compounds such as chemokines and cytokines damaging the perivascular tissue, lungs, and bronchi of COVID-19 patients. Moreover, a comparison of endothelial and epithelial pulmonary injury biomarkers in mechanically ventilated COVID-19 patients with acute respiratory distress syndrome (ARDS) in comparison to those with classical ARDS showed significantly lower levels of receptor of advanced glycation end-products (RAGE) (median value: 60 vs 789 pg/mL) and P-selectin (median value: 96 vs

Table 1. Predictive criteria for COVID-19 storm.

CRITERIA	CUTOFF VALUES	NORMAL RANGE
Ferritin	>250 ng/mL	20-200 ng/mL
C-reactive protein	>4.6 mg/dL	10-1000 mg/L
One variable from each cluster		
Cluster I		
Albumin	<2.8 g/dL	3.5-5.0 g/dL
Lymphocytes (%)	<10.2	18-45%
Neutrophil abs	>11.4 k/mm³	1.5-8.0 k/mm³
Cluster II		
ALT (Alanine aminotransferase)	>60 U/L	7-56 U/L
AST (Aspartate aminotransferase)	>87 U/L	8-48 U/L
D-Dimers	>4930 ng/mL	20-40 ng/mL
LDH (Lactate dehydrogenase)	>416 U/L	45-90 U/L
Troponin	>1.09 ng/mL	0-0.4 ng/mL
Cluster III		
Anion Gap	<6.8 mmol/L	3-10 mEq/L
Chloride	>106 mmol/L	96-106 mmol/L
Potassium	>4.9 mmol/L	3.5-5.1 mmol/L
BUN: Creatinine	>29 ratio	50-110 µmol/L

Predictive criteria for COVID-19 storm.[27]

750 ng/mL) as well as significantly higher levels of angiopoietin-2 (ang-2) (median value: 3909 vs 1045 pg/mL), intracellular adhesion molecule-1 (ICAM-1) (median value: 1093 vs 75.7 ng/mL) vascular cell adhesion molecule-1 (median value VCAM-1) (median value: 1114 vs 739 ng/mL), and E-selectin (median value: 24.9 vs 3.3 ng/dL).[30] The differences in the levels of pulmonary injury biomarkers can be used to differentiate between patients with classical ARDS and those with COVID-related ARDS. Non-survivors of COVID-19 related ARDS had statistically higher plasma levels of Ang-2 (*P*=.04) and ICAM 1 (*P*=.03) after 7 days than survivors.[30] Furthermore, the plasma levels of Ang-2 and ICAM 1 after 7 days can be used to determine the COVID-19 patient's risk for mortality. The criteria in Table 2 can be used to discriminate between lung injury from COVID-19 and classical ARDS.

Measurements of serum biomarkers can also determine prognosis and risk for mortality for patients with COVID-19. Calculations from survival analysis revealed that the probability of dying for patients with an IL-6 cutoff value above 163.4 pg/mL was 91.7%. The likelihood of remaining alive was 42.4%, whereas the probability of dying with a TNF-α cutoff

Table 2. Biomarkers for ARDS-related pulmonary injury in COVID-19.

BIOMARKER	CUTOFF VALUES	RANGE
Ang-2	>2800 pg/mL	25-60 pg/mL
RAGE	<208 pg/mL	188.4-3964.4 pg/mL
VCAM-1	>1312 ng/mL	449-1103 ng/mL
ICAM-1	>1092 ng/mL	100-200 ng/ml

Cutoff levels for endothelial and epithelial pulmonary injury biomarkers in mechanically ventilated COVID-19 patients.[30]

level above 33.91 pg/mL was 75%, and the probability of living was 45.8%.[31] Clinicians can use the cutoff values of 163.4 and 33.91 pg/mL for IL-6 and TNF-α, respectively, to predict the risk of death for patients with COVID-19 infections.

Hepatobiliary injury and biomarkers

Inflammation from the cytokine storm and pneumonia-associated hypoxia have been proposed as triggers for liver damage in

patients with severe COVID-19. After liver damage, the injured hepatocytes release liver biomarkers such as alanine aminotransferase (ALT), bilirubin, and aspartate aminotransferase (AST).[31] Analysis of arterial blood gas tests in patients with COVID-19 revealed that a higher median alveolar-arterial oxygen tension was associated with patients with abnormal liver enzymes than liver enzymes within the normal range.[32] Moreover, evidence from another study showed a more frequent and significant elevation of AST than ALT in patients with severe COVID-19 on admission to the hospital. AST has the highest association with mortality compared to other markers of liver injury.[33]

Liver injury is a significant cause of morbidity in patients with COVID-19 since studies have described hepatocellular injury in 14% to 69% of patients,[30,35,36,38,39]. A study of the role of different biomarkers in predicting COVID-19 severity revealed that most (69%) non-intubated patients showed high levels of AST (median, 46 U/L) and ALT (median, 30 U/L) on admission; however, the AST levels increased to 69 U/L in critically ill patients and 364 U/L I intubated patients.[31] The drastic increase in AST levels as patients become more severely ill and require ventilatory support indicates that serial measurements of AST can be used to determine the prognosis of patients with COVID-19 infection.

SARS-CoV-2 infection is also associated with mitochondrial swelling, a decrease in glycogen granules, binuclear hepatocytes, endoplasmic reticulum dilatation, and extensive hepatic apoptosis.[33] Concomitant use of medications that increase the risk of liver damage should be considered when assessing liver damage from COVID-19. Longitudinal monitoring of hepatic transaminases is recommended for patients with COVID-19 when administering drugs that can induce liver damage, such as remdesivir, lopinavir, and tocilizumab.[11]

Liver damage and elevated bilirubin are associated with critical illness progression in COVID-19 patients. Levels of AST, a biomarker of liver damage, can be used to determine illness progression and predict the risk of mortality in patients with severe COVID-19. Research findings indicate that AST levels between 40 and 120 U/L are associated with a 4.81-fold increase in all-cause mortality, whereas levels above 120 U/L are associated with the 14.87-fold increase.[34] The AST levels can be used to determine the risk of mortality for COVID-19 mortality in clinical practice because the AST levels are associated with all-cause mortality risk in patients.

Coagulopathy and hematological biomarkers

The severe inflammatory response to SARS-CoV-2 particles is the primary factor that induces hemostatic dysregulation, leading to COVID-related coagulopathy. Damage to the vascular endothelium, uncontrolled coagulation, and tissue damage results from the direct action of the SARS-CoV-2 infection on the epithelial and endothelial cells.[35] COVID-19 patients have increased hypercoagulability, with higher incidences of thrombotic events in intensive care unit (ICU) patients via many mechanisms, such as inflammatory activation of coagulation.[36] Coagulopathy and disseminated intravascular coagulation (DIC) have been identified as associated with a hypercoagulable state and have been associated with mortality in patients with COVID-19.[37] An important clinical consideration is differentiating between COVID-19 associated coagulopathy (CAC) and bacterial-induced sepsis coagulopathy/disseminated intravascular coagulation (SIC/DIC). Consumptive coagulopathy is a typical feature in SIC/DIC; however, consumptive coagulopathy is usually not seen in COVID-19 in its early phase, and the d-dimer levels in COVID-19 patients are typically 5 times higher than the upper limit of normal range.[38] In one study, a d-dimer level on admission greater than 1 μg/mL was associated with an increased risk of hospital mortality.[39] The findings from another study to determine if d-dimer levels could be used to predict mortality in patients with COVID-19 revealed that using the optimum cutoff level of 2.0 μg/mL for d-dimer to predict mortality in hospitalized patients was associated with a sensitivity of 92.3% and specificity of 83.3%.[40] The high sensitivity and specificity of the 2.0 μg/mL cutoff value indicate that clinicians can use a D-dimer cutoff mark of 2.0 μg/mL to identify patients at increased risk of mortality.

Lymphocytes are essential for the body to mount a proper immune response to a novel pathogen such as SARS-CoV-2. In clinical practice, lymphopenia is defined as a decrease below a normal value (often 1.5×10^3 cells/μL) and is thought to be a biomarker for impaired cellular immune response. Among COVID-19 patients, 67% to 90% have lymphopenia.[41] Additionally, a reduction in CD4 T cell and CD8 T cell counts in patients with severe COVID-19 has been demonstrated in the literature.[46] Neutrophils are classically known as the first responders to infection. In hospital practice, lymphocyte levels are often used as a biomarker for detecting infection. COVID-19 patients can have leukocytosis marked by neutrophilia, as well as thrombocytopenia. A study reported that the neutrophil-lymphocyte ratio (NLR) increased in COVID-19 patients with severe disease compared to non-severe diseases with a standard mean difference (SMD) of 2.8.[42]

Several meta-analyses have shown that patients with severe COVID-19 infection have a statistically significant higher neutrophil-lymphocyte ratio (NLR) than patients with non-severe COVID-19,[43] and another meta-analysis showed that an increased NLR is predictive of severe COVID-19 and mortality.[39] The difference in NLR predictive value can be used to identify patients at risk for severe COVID-19. Further, lymphocyte count ≤1100 cells/μL is associated with a 3-fold risk of poor outcome and is characterized by mortality, ARDS, ICU care, and severe COVID-19.[44] Hence, evaluating lymphocyte counts is essential for risk stratification in COVID-19 hospitalized patients.

The levels of several coagulopathies and hematological biomarkers have been shown to have important implications for mortality in COVID-19 patients. For example, non-survivors of COVID-19 in comparison to survivors were shown to have significantly higher d-dimer levels (median value: 1425 vs 385 ng/mL) and elevated prothrombin (PT) levels (median value: 14 vs 12.7 seconds) but lower lymphocyte count (median value: 0.06×10^3 vs $1 \times 10^3/\mu L$) and platelet count (median value: 203×10^3 vs $262 \times 10^3/\mu L$).[52,53] In another study, levels of biomarkers such as fibrinogen, d-dimer, white blood cell (WBC) count, lymphocytes, platelet counts, and prothrombin time were associated with increased severity and mortality of COVID-19 infection, and continuous screening and measurement of these parameters should be done for patients.[45]

Clinicians can also use the trends in WBC, platelets, and lymphocyte counts to determine the prognosis of patients with COVID-19. A retrospective study of patients hospitalized with COVID-19 revealed a significant upward trend of platelets, lymphocytes, and eosinophils in survivors with an inverse relationship between lymphocyte count and disease severity, a 6-fold increase in eosinophil count at midterm. There was an 8-fold rise in the discharge and an increasing trend in platelet levels with a 1.25-fold increase at midterm and a 1.24-fold increase at discharge, whereas the non-survivors maintained low levels of eosinophils and lymphocytes without any significant growth. It developed a decline in platelet levels.[55] The trends in lymphocyte, eosinophil, and platelet counts have important clinical implications because they can be monitored for hospitalized COVID-19 patients to determine their risk for mortality.

German physician Rudolph Ludwig Karl Virchow describe 3 broad groups of factors that contributes to thrombosis and thromboembolic risk, including blood stasis, hypercoagulability, and endothelial. Virchow's explanation can be employed to describe pathophysiology of COVID-19 systemic manifestations. COVID-19 binds to the angiotensin-converting enzyme 2 (ACE-2) enzyme, broadly expressed in many organs, such as intestine, kidney, heart, and lung. Additionally, ACE-2 receptors are expressed on blood vessels endothelium, allowing the virus to enter the bloodstream. The endothelial injury caused by SARS-CoV-2 virus occurs in different organs. According to Mehta et al[68] SARS-CoV-2 once present in the hematic circulation can invade virtually all organs resulting in endothelial injury, myocarditis, multi-organ failure, and systemic vasculitis. The inflamed and injured blood vessels enhance leukocytes, macrophages, and mast cells recruitment, resulting in organ injury from the inflammatory response.[13]

Cardiac injury and biomarkers

Direct invasion of cardiac tissue by the SARS-CoV-2 can lead to cardiac injury in patients with COVID-19. Analysis of scRNA-seq data revealed ACE2 expression in more than 7.5%

of cardiac tissue.[23] The presence of the ACE2 receptor on myocardial tissue is indicative of direct viral infection as a possible mechanism for cardiac injury in COVID-19 patients. In the direct mechanism, SARS-CoV-2 may infect myocardial cells and replicate upon entering the cell, causing necrosis and degeneration with subsequent myocarditis and arrhythmia. Cardiac biomarkers have been found to correlate with cardiac injury and mortality in COVID-19 patients.

Cardiac biomarker elevations are associated with higher 28-day mortality rates among COVID-19 patients.[56] Biomarkers that were significantly elevated with COVID-19 patients experiencing cardiac manifestations such as myocarditis, acute cor pulmonale, arrhythmias, acute coronary syndrome, cardiogenic shock, and cardiomyopathy included troponin, N-terminal (NT) proB-type natriuretic peptide (BNP), creatine kinase (CK), and CK-myocardial band (MB).[57] The marked elevation of biomarkers in patients with COVID-19 and cardiac manifestations suggests a role of the biomarkers in assessing for cardiac injury. The cutoff values for cardiac injury biomarkers using a mixed-effects Cox model to determine the risk of COVID-19 death are illustrated in Table 3 below.

Cardiac biomarker levels above the cutoff values in Table 3 have been associated with an increased risk of COVID-19 death even though the cutoff values are less than those for heart disease.[57] The lower cutoff value for mortality risk in COVID-19 patients compared to regular cardiac disease highlights the importance of close monitoring of cardiac biomarkers to determine if the values increase patients' risk for COVID-related death.

Neurologic injury and biomarkers

Several mechanisms have been proposed for SARS-CoV-2 invasion of the brain, including direct attack through the cribriform plate and dissemination through systemic circulation.[58] Proposed mechanisms for crossing the blood-brain barrier include transcytosis across pericytes and endothelial cells, direct infection of endothelial or epithelial cells, entering the choroid plexus, or hiding inside leukocytes to access neurologic tissue.[58]

COVID-associated encephalopathy and delirium are thought to be caused by the release of high levels of IL-1, IL-6, and TNF-α in systemic circulation that damage and cross the blood-brain barrier, infiltrating the brain parenchyma; however, direct viral attacks on neurons is considered a plausible cause of seizures.[51] Furthermore, the presence of ACE2 receptors in the nasal, olfactory, and oral mucosa epithelial cells enables SARS-CoV-2 to bind to them, impairing their function and causing COVID-19 symptoms of anosmia and ageusia.[52]

Several neurological biomarkers have been measured at significantly higher levels in COVID-19 patients with Intensive Care Delirium Screening Checklist (ICDSC) scores above 4 in comparison to scores below 4, such as glial fibrillary

Table 3. Cardiac biomarkers and cutoff value for increased risk of mortality.

CARDIAC BIOMARKERS	CUTOFF VALUE	RANGE
High-sensitivity cardiac troponin I (hs-cTnI)	7.12 ng/L	0-14 ng/L
NT-pro) BNP	5.11 pg/mL	0-300 pg/mL
CK-MB	4.86 IU/L	5-25 IU/L
Myoglobin (MYO)	3.56 nmol/L	1.28-3.67 nmol/L

Cardiac biomarkers and cutoff values.[57]

acidic protein (GFAP) (median value: 438 vs 200), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (median: 40.1 vs 16.7), and neurofilament-light chain NF-L (median 77.5 vs 27.7).[53] GFAP, UCH-L1, and NF-L levels released from the nervous neurologic system in response to tissue invasion by SARS-CoV-2 can be used to determine the presence of COVID-19-associated delirium. The evidence from another study assessing cerebrospinal fluid findings of COVID-19 patients with altered mental status revealed a 4-fold increase of neuron-specific enolase (NSE).[54] The high levels of NSE in the CSF of COVID-19 patients suggest that measurements of NSE can be used to monitor the development of altered mental status.

The levels of biomarkers differ between COVID-19 patients with or without neurological manifestations. For example, in one study, patients with CNS symptoms in comparison with patients without CNS symptoms had a lower lymphocyte count (median: 1.0×10^9 vs 1.2×10^9/L), lower platelet count (median: 180.0×10^9 vs 227.0×10^9/L), higher blood urea nitrogen (BUN) levels (median 4.5 vs 4.1 mmol/L) whereas patients with a skeletal muscle injury in comparison to those without skeletal muscle injury had significantly higher levels creatine kinase (median: 400.0 vs 58.5 U/L).[55]

Renal injury and biomarkers

Multisystem organ dysfunction in COVID-19 patients can include acute kidney injury (AKI) from SARS-CoV-2 invasion of renal tissue. AKI develops in patients with comorbid conditions that predispose them to kidney injury, direct viral injury, an increase in inflammatory cytokines, and micro thrombosis.[63,64] COVID-19 patients with cardiovascular disease and/or high blood pressure also have histopathologic changes such as arteriolar hyalinosis and ischemic glomeruli have an increased risk for AKI.[13] Research evidence from one study shows that approximately 4% of kidney proximal tissue cells express ACE2, increasing the risk of renal tissue to SARS-CoV-2 infection.[56] Severe COVID-19 is associated with activation of the renin-angiotensin-aldosterone system, which adds to the effects of the viral invasion of the podocyte to cause proteinuria.[57] AKI develops from the synergistic actions of the severe inflammation from the cytokine storm and direct injury from the SARS-CoV-2 infection.[58]

Renal biomarkers have a significant prognostic value for COVID-19 patients because of the association between chronic kidney disease and severe disease cases. One study found that patients with severe COVID-19 had patients with renal biomarkers such as serum creatinine, blood uric acid (BUA), and BUN levels higher than the normal range on admission had an 11.07, 4.72, and 2.92-fold increased risk of mortality for elevated BUN, serum creatinine, and BUA levels, respectively.[59] In another study, COVID-19 patients with high BUN levels (median value: 4.14, range, 3.33-4.82 mmol/L), positive hematuria, and positive proteinuria on urinalysis at admission had a worse prognosis with ICU admission.[60] These findings indicate that both urinalysis and renal biomarkers have a role in determining the prognosis of hospitalized COVID-19 patients. The elevations in renal biomarkers and associated increased risk for mortality are illustrated in Table 4 below.

The increased risk of mortality with renal biomarkers such as BUN, serum creatinine, and BUA values above normal levels indicates that these biomarkers should be monitored closely in patients with COVID-19 to determine their risk for non-survival.

Role of Biomarkers in Risk Stratification

Diagnosis of COVID-19 is currently based on several tests, including the immunoassays SARS -CoV-2 antibodies and RT-PCR with blood, nasal, bronchoalveolar lavage fluid, sputum, feces, and pharyngeal specimen.[10] However, on admission to the hospital, measurements of biomarkers can provide clinicians with information about the risk of death for patients with COVID-19, severe illness, and complications. The evidence from the literature indicates that biomarkers with a d-dimer level greater than 1 μg/mL are associated with an increased risk of mortality.[62] The evidence from the literature also highlights the value of serial measurements of biomarkers since cutoff values of >250 ng/mL for ferritin, >4.6 mg/dL for C-reactive protein, <2.8 g/dL for albumin, <10.2% for lymphocytes, >60 U/L for ALT, >87 U/L for AST, >4930 ng/mL for d-dimer, >416 U/L for LDH, >416 U/L troponin, <6.8 mmol/L for anion gap, >106 mmol/L for chloride, >4.9 mmol/L for potassium, and >29 ratio for the BUN/creatinine ratio have been associated with severe COVID-19 infection.

Table 4. Renal biomarkers and risk for mortality.

RENAL BIOMARKER LEVELS ASSOCIATED WITH INCREASED MORTALITY RISK	NORMAL LEVELS OF BIOMARKER (MG/DL)	HAZARD RATIO (MG/DL)
BUN—elevated above normal value	7-21	11.07
Serum creatinine—elevated above normal value	0.84-1.21	4.42
BUA—elevated above normal value	2.4-7.0	2.92

Renal markers and increased risk for mortality.[66]

Several categories of biomarkers have been investigated for their role in risk stratification for COVID-19 patients, including cardiac biomarkers such as troponin, coagulation biomarkers such as d-dimer, and ABO blood groups.[7,63,64]

Additionally, biomarkers such as CRP, IL-6, LDH, d-dimer, BUN, creatinine, and cardiac troponin have been seen in patients with severe COVID-19 infections.[7] Early measurements of CRP, LDH, IL-6, d-dimer, creatinine BUN, and cardiac troponin followed by serial measurements should be obtained in patients with COVID-19 to identify severe disease early in the hospitalization. Cutoff levels of IL-6 >80 pg/mL and CRP level >97 mg/L have been validated in the literature to predict respiratory failure risk in patients with COVID-19 infections. Clinicians should measure the levels of IL-6 and CRP and provide ventilatory support for those with levels above the cutoff point to improve their outcomes.[65]

Changes in the levels of coagulation factors in COVID-19 suggest that they can be used to guide clinicians in determining the risk for severe COID-19 infection and mortality. The findings of a meta-analysis also showed that platelet count had no statistically significant association with disease severity; however, prolonged PT was associated with an increased risk for an ICU admission and mortality and a decrease in antithrombin as well as an increase in fibrin degradation products (FDP) could indicate worsening disease.[66] The association between the above-mentioned coagulation parameters and disease severity suggests that the coagulation biomarkers' measurement early during hospitalization and serial measurements can be used to determine the risk for worsening COVID-19 disease.

Biomarkers from damaged cardiac tissues can also be used in risk stratification since findings from a meta-analysis have shown that cardiac injury develops in 19% of patients with COVID-19, 36% of patients with severe disease, and 48% non-survivors.[67] The presence of cardiac injury in different categories of patients with COVID-19, including non-severe and severe cases and non-survivors, underscores the value of cardiac biomarkers from cardiac injury in risk stratification. Moreover, several cardiac complications such as acute cardiac injury, arrythmias, and heart failure have been identified as risk factors for COVID-related death.[13] This role of cardiac complications in increasing the risk of mortality from COVID-19 infection indicates that the cardiac biomarkers should not be used in isolation in risk stratification. The cardiac biomarkers should be used in conjunction with other aspects of the clinical picture, such as cardiac complications that increase the risk of mortality.

Special considerations for risk stratification for cardiac outcomes in patients with COVID-19 should include ABO blood group types. An assessment of the association between the ABO blood group types and several cardiovascular outcomes, including major arterial and venous thrombosis, major adverse cardiovascular events, and all-cause mortality, revealed 2.5-fold higher odds of major adverse cardiac events in patients with blood group A versus O and reduced odds of major cardiac events in patients with COVID-19 who had the blood group O.[69] The reduced risk of adverse cardiac events for blood group O and increased risk for blood group A can determine the risk of cardiovascular complications in COVID-19 patients.

Role of Biomarkers in Treatment

Low molecular weight heparin (LMWH) has been identified as a potential treatment for severe COVID-19 infections through its actions in reducing the levels of IL-6 and mitigating the cytokine storm. One cohort study found that treatment with LMWH had multiple effects on biomarkers in patients with COVID-19, including a significant reduction in IL-6 levels and d-dimer and FDP levels.[70] Furthermore, these results illustrate the role of medications such as LMWH in mitigating the effects of IL-6 involved in the cytokine storm.

Cytokine inhibitors

Early treatment with anti-inflammatory agents has improved respiratory functions for COVID-19 patients. A retrospective cohort study with SARS-CoV-2 RNA-positive patients revealed that anti-cytokine agents such as tocilizumab and anakinra were effective in treating COVID-19 patients when provided early in the cytokine storm. The study showed that 42.3% of patients treated with tocilizumab and 63.4% of patients treated with anakinra responded favorably to the treatment by being extubated or never intubated. Also, accounting for differences in disease severity at treatment initiation showed that the advantage of using anakinra over tocilizumab was not statistically significant. Furthermore, the study revealed that concomitant treatment with corticosteroids may have resulted

in a better response with anakinra treatment.[71] These findings illustrate the value of using medications based on biomarkers such as the anti-cytokine tocilizumab and anakinra to improve outcomes for patients with COVID-19. Clinicians can measure the levels of cytokines such as IL-6 to identify the early stages of the cytokine storm and administer anti-cytokine medications to improve patient outcomes.

Cytokine inhibitors have decreased mortality in patients with severe COVID-19, highlighting their role in preventing COVID-19 deaths.[71] An observational study was conducted to confirm the optimal timing of IL-6 receptor inhibitor (IL-6ri) administration for 255 COVID-19 patients. During the IL-6ri administration, patients were divided into 2 groups; the stage IIB group required less than 45% of inspired oxygen (FiO_2), and the stage III group required more than 45% of FiO_2.[72] The findings from the study revealed that patients in stage IIB treated with IL-6ri had lower mortality, were more likely to be discharged and were less likely to be intubated.[73] These results highlight the role of biomarkers in the design of pharmaceutical interventions for treating COVID-19 infections. Furthermore, the lower mortality, reduced likelihood of intubation, and increased likelihood of discharge in patients with COVID-19 in the stage IIB group underscore the importance of early treatment with IL-6ri. Early and serial measurements of biomarkers such as IL-6 will enable clinicians to provide early treatment.

Although immunomodulatory agents have shown promise for treating severe COVID-19, other clinical trials have demonstrated that tocilizumab administration did not significantly reduce ICU admission or mortality rate in a cohort of randomized patients with COVID-19 pneumonia.[74] Investigators have proposed that IL-6 blockade can disrupt the immune response against SARS-CoV-2 infection, suggesting that clinicians should be aware of the potential risks of using immunomodulatory agents such as tocilizumab.[75] Immunomodulatory agents such as hydroxychloroquine and interferon were studied, and hydroxychloroquine showed statistically non-significant differences in multiple clinical trials for treating COVID-19, which suggests the need for further research into targeting the severe inflammatory response and cytokine storm caused by SARS-CoV-2.[76] A diagnostic workup must be completed to evaluate the severity of COVID-19 in patients before initiating immunomodulatory therapy. Nevertheless, with so many biomarkers identified for multisystemic involvement in COVID-19, future medications can be designed to target the different organ dysfunctions associated with COVID-19 to decrease morbidity and mortality.

COVID-19 in the Pediatric Population

Multisystem inflammatory syndrome in children

Multisystem inflammatory syndrome in children (MIS-C) is an uncommon presentation of COVID-19 in the pediatric population characterized by a hyperinflammatory syndrome and

Table 5. MIS-C abnormal levels of biomarkers in children.

BIOMARKER	NORMAL RANGE	MEDIAN ABNORMAL VALUE IN MIS-C
C-reactive protein	<6.0 mg/L	149 mg/L
Erythrocyte sedimentation rate	<13 mm/h	50 mm/h
D-dimer	<500 ng/mL	2523 ng/mL
Ferritin	20-300 mg/L	539 mg/L
Lactate dehydrogenase	110-210 U/L	359 U/L
hsTnT	<10 ng/L	32 ng/L
NT-proBNP	<500 pg/mL	2121 pg/mL

Abnormal levels of biomarkers in children with MIS-C.[83]

multiorgan involvement that clinically presents as prolonged fever, abdominal pain, shock, and cardiac dysfunction occurring after a SARS-CoV-2 infection.[77] MIS-C develops in children with positive SARS-COV-2 serology or a history of contact with a person having COVID-19 infection seronegative patients, usually 2 to 4 weeks after the infection.[78] Lymphocyte and myeloid cells are activated and move toward the periphery, causing drastic increases of inflammatory mediators in the peripheral blood.[77] The inflammatory state is evidenced by elevated levels of inflammatory biomarkers such as CRP, neutrophilia and ferritin, and troponin and NT-proBNP.[79] An evaluation of peripheral immune profiles of patients with MIS-C showed an antibody response with low levels of circulating IgM and elevated IgG with a predominance of IgG1 and low levels of IgG3 and effective neutralization of the SARS-COV-2 virus similar to serology from convalescent adults with COVID-19.[80] The laboratory results of children with MIS-C have demonstrated markedly elevated biomarkers, including C-reactive protein, erythrocyte sedimentation rate, D-dimer, ferritin, lactate dehydrogenase, hsTnT, and NT-proBNP (Table 5).[81] In the literature, a standard therapeutic approach for MIS-C is immunomodulatory drugs that mitigate the inflammatory response and inhibit cytokines such as intravenous immune globulin, interleukin-6 inhibitors, and glucocorticoids.[82]

Biomarkers in the pediatric population

Elevated levels of several biomarkers have been reported in children with MIS-C, with one study reporting CRP (median value: 229 mg/L), neutrophilia (13×10^9/L) as well as ferritin (610 μg/L) as well as high levels of Troponin and NT-proBNP concentrations in 68% in 83% of the children, respectively.[79] Children with MIS-C and severe cardiac complications also demonstrate elevated levels of cardiac biomarkers that monitor the development of complications. Severe MIS-C with cardiac abnormalities such as reduced left ventricular ejection fraction

Table 6. Biomarker levels for severe acute COVID-19, MIS-C, and Kawasaki disease.

BIOMARKER	SEVERE ACUTE COVID-19	MIS-C	KAWASAKI
CRP mg/dL Median value	0.1	22.8	11.3
Ferritin ng/mL Median value	58	550	186
Albumin g/dL Median value	4.3	29	3.7

Comparison of biomarker levels for severe acute COVID-19, MIS-C, and Kawasaki disease.[84]

(LVEF) on echocardiogram has been associated with significant elevations of cardiac biomarkers, including a hs-TNT > 100 pg/L and or NT-proBNP > 5000 ng/mL that correlate with the severity of echocardiographic abnormalities.[81] Early and serial measurements of hs-TNT and NT-proBNP should be performed by clinicians to assess the risk of developing cardiac complications.

Biomarker profile for severe COVID-19 in children

A prospective, multicenter, observational study of 79 COVID-19 patients admitted to a pediatric ICU in Brazil showed that MIS-C pediatric patients had higher inflammatory markers with CRP > 3 mg/dL in 50% of non-MIS-C patients versus > 10 mg/dL in 50% of the MIS-C patients and increased pro-BNP in 86% of MIS-C patients with a median value of 5829 pg/mL.[77] These findings must be interpreted with caution because only 10 participants were in the MIS-C group. However, these preliminary findings suggest that clinicians can monitor pro-BNP and CRP to identify children with COVID-19 developing MIS-C.

Severe acute COVID-19, MIS-C, and Kawasaki disease have some common characteristics such as hyperinflammation; however, there are differences in the 3 syndromes illustrated in the biomarker profile illustrated in Table 6 below.

Early measurement of CRP, ferritin, and albumin and serial measurements should be taken in children with COVD-19 infections to assess their risk for developing MIS-C. The comparatively high values in patients should raise the clinician's index of suspicion about MIS-C.

Conclusion

The present study reviews the role of biomarkers in COVID-19 diagnosis in hospital settings. Evidently, severe COVID-19 is a systemic disease, and understanding associated biomarkers are crucial for proper clinical decision-making in hospitalized patients. The cytokine storm in COVID-19 releases inflammatory markers in different organ systems such as the pulmonary, hepatobiliary, hematological, cardiac, neurological, and renal systems. ICU patients diagnosed with COVID-19 have higher IL-2, IL-7, IL-10, TNF-α, GM-CSF, interferon γ induced protein 10 kD (IP-10), MCP-1, and MIP-1α, and measurements of the biomarker levels can be used to determine the clinical severity of COVID-19 in hospitalized patients. Cut-off values of > 2800 pg/mL for Ang-2, < 208 pg/mL for RAGE, > 1312 ng/mL for VCAM-1, and > 1092 ng/mL for ICAM-1 have been identified for ARDS-related pulmonary injury in patients with COVID-19. AST levels can determine the risk of mortality since the evidence shows a 4.81 and 14.87 increased risk of mortality with AST levels between 40 and 120 U/L and greater than 120 U/L, respectively. The levels of hs-cTnI, NT-pro BNP, MYO, and CK-MB were shown to be cardiac biomarkers that can be used to predict mortality in COVID-19 patients. Elevations of renal biomarkers such as BUN, BUA, and serum creatinine above the normal range have been associated with increased mortality risk in COVID-19 patients. Clinician knowledge of COVID-19 biomarkers in the hospital will allow for better patient care, risk stratification, and effective management plans.

This review makes it clear that treating severe COVID-19, clinicians must employ a systemic approach toward treatment, a treatment strategy commonly seen in critical care units. Timely measurement of the biomarkers mentioned in this review will allow clinicians to gain a better understanding of the expected clinical course and escalate care for undertreated patients. The strength of this review is that it provides the latest information on both commonly used, and potential biomarkers for systemic manifestations organized by organ system. It is crucial that hospitals continue to update their COVID-19 management protocols based on the latest research available, as this is a field of rapid advancement.

One of the key limitations of the present study is that it does not to provide sufficient summaries of the included studies. It did not report on the settings of test use, the test's expected role, study design characteristics, and participants' demographics. Additionally, a meta-analysis could not be conducted due to inadequate sample size for the data of many of the biomarkers and large heterogeneity of biomarkers discussed. Additionally, much of the research reported relied on observational studies, rather than strictly randomized controlled trials or meta-analyses. Lastly, not all the biomarkers discussed may have the potential to be incorporated in hospital practice due to resource limitations within the healthcare system. However, this review does highlight the latest research results of COVID-19 biomarkers that medical professionals can use to better care for hospitalized COVID-19 patients.[47-50,61]

Authors' Note

The manuscript has not been previously published and is not currently under consideration elsewhere, that is at any other journal or publisher.

Author Contribution

MS performed literature search, wrote, drafted, and edited the paper.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Ethics Approval

Institutional Review Board (IRB) approval was not sought for the present study since no human subjects were used in this narrative review.

ORCID iD

Michael Schneider [iD] https://orcid.org/0000-0002-5198-7322

REFERENCES

1. Califf RM. Biomarker definitions and their applications. *Exp Biol Med*. 2018;243:213-221.
2. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. *Crit Rev Clin Lab Sci*. 2020;57:389-399.
3. Keddie S, Ziff O, Chou MKL, et al. Laboratory biomarkers associated with COVID-19 severity and management. *Clin Immunol*. 2020;221:108614.
4. Apostolopoulos ID, Aznaouridis SI, Tzani MA. Extracting possibly representative COVID-19 biomarkers from X-ray images with deep learning approach and image data related to pulmonary diseases. *J Med Biol Eng*. 2020;40:462-469.
5. Callaghan E. Diagnostic accuracy and reliability remain key in SARS-CoV-2 testing. *MLO: Med Lab Obs*. 2020;52:18-21.
6. Malik P, Patel U, Mehta D, et al. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. *Evid Based Med*. 2021;26: 107-108.
7. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 - a systematic review. *Life Sci*. 2020;254:117788.
8. Luo L, Xu M, Du M, et al. Early coagulation tests predict risk stratification and prognosis of COVID-19. *Aging*. 2020;12:15918-15937.
9. Mahajan K, Chand Negi P, Ganju N, Asotra S. Cardiac biomarker-based risk stratification algorithm in patients with severe COVID-19. *Diabetes Metab Syndr*. 2020;14:929-931.
10. Alpdagtas S, Ilhan E, Uysal E, Sengor M, Ustundag CB, Gunduz O. Evaluation of current diagnostic methods for COVID-19. *APL Bioeng*. 2020; 4:041506.
11. Gupta A, Madhavan MV, Sehgal K, et al. Extrapulmonary manifestations of COVID-19. *Nat Med*. 2020;26:1017-1032.
12. Behzad S, Aghaghazvini L, Radmard AR, Gholamrezanezhad A. Extrapulmonary manifestations of COVID-19: radiologic and clinical overview. *Clin Imaging*. 2020;66:35-41.
13. Qin JJ, Cheng X, Zhou F, et al. Redefining cardiac biomarkers in predicting mortality of inpatients with COVID-19. *Hypertension*. 2020;76:1104-1112.
14. Lauc G, Sinclair D. Biomarkers of biological age as predictors of COVID-19 disease severity. *Aging*. 2020;12:6490-6491.
15. Dujardin RWG, Hilderink BN, Haksteen WE, et al. Biomarkers for the prediction of venous thromboembolism in critically ill COVID-19 patients. *Thromb Res*. 2020;196:308-312.
16. Churchill TW, Bertrand PB, Bernard S, et al. Echocardiographic features of COVID-19 illness and association with cardiac biomarkers. *J Am Soc Echocardiogr*. 2020;33:1053-1054.
17. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. *Ther Adv Respir Dis*. 2020;14:1753466620937175.
18. Chaudhary R, Garg J, Houghton DE, et al. Thrombo-inflammatory biomarkers in COVID-19: systematic review and meta-analysis of 17,052 patients. *Mayo Clin Proc Innov Qual Outcomes*. 2021;5:388-402.
19. Mittal A, Manjunath K, Ranjan RK, Kaushik S, Kumar S, Verma V. COVID-19 pandemic: insights into structure, function, and hACE2 receptor recognition by SARS-CoV-2. *PLoS Pathog*. 2020;16:e1008762.
20. Babapoor-Farrokhran S, Gill D, Walker J, Rasekhi RT, Bozorgnia B, Amanullah A. Myocardial injury and COVID-19: possible mechanisms. *Life Sci*. 2020;253:117723.
21. Galanopoulos M, Gkeros F, Doukatas A, et al. COVID-19 pandemic: pathophysiology and manifestations from the gastrointestinal tract. *World J Gastroenterol*. 2020;26:4579-4588.
22. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181:271-280.e8.
23. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med*. 2020;14:185-192.
24. Soy M, Keser G, Atagündüz P, Tabak F, Atagündüz I, Kayhan S. Cytokine storm in COVID-19: Pathogenesis and overview of anti-inflammatory agents used in treatment. *Clin Rheumatol*. 2020;39:2085-2094.
25. Fajgenbaum DC, June CH. Cytokine storm. *N Engl J Med*. 2020;383:2255-2273.
26. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395:497-506.
27. Caricchio R, Gallucci M, Dass C, et al. Preliminary predictive criteria for COVID-19 cytokine storm. *Ann Rheum Dis*. 2021;80:88-95.
28. Jøntvedt Jørgensen M, Holter JC, Christensen EE, et al. Increased interleukin-6 and macrophage chemoattractant protein-1 are associated with respiratory failure in COVID-19. *Sci Rep*. 2020;10:21697.
29. Roden AC, Bois MC, Johnson TF, et al. The spectrum of histopathologic findings in lungs of patients with fatal Coronavirus disease 2019 (COVID-19) infection. *Arch Pathol Lab Med*. 2021;145:11-21.
30. Spadaro S, Fogagnolo A, Campo G, et al. Markers of endothelial and epithelial pulmonary injury in mechanically ventilated COVID-19 ICU patients. *Crit Care*. 2021;25:74.
31. Mandel M, Harari G, Gurevich M, Achiron A. Cytokine prediction of mortality in COVID19 patients. *Cytokine*. 2020;134:155190.
32. Velavan TP, Meyer CG. Mild versus severe COVID-19: laboratory markers. *Int J Infect Dis*. 2020;95:304-307.
33. Wang Y, Liu S, Liu H, et al. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol*. 2020;73: 807-816.
34. Lei F, Liu Y, Zhou F, et al. Longitudinal association between markers of liver injury and mortality in COVID-19 in China. *Hepatology*. 2020;72:389-398.
35. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708-1720.
36. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. 2020;395:1054-1062.
37. Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with covid-19. *J Thromb Haemost*. 2020;18:1324-1329.
38. Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. *JAMA*. 2020;323:1574-1581.
39. Tjendra Y, Al Mana AF, Espejo AP, et al. Predicting disease severity and outcome in COVID-19 patients: a review of multiple biomarkers. *Arch Pathol Lab Med*. 2020;144:1465-1474.
40. Bhatraju PK, Ghassemieh BJ, Nichols M, et al. Covid-19 in critically ill patients in the Seattle region - case series. *N Engl J Med*. 2020;382:2012-2022.
41. Nogueira SÁR, Oliveira SCS, Carvalho AFM, et al. Renal changes and acute kidney injury in covid-19: a systematic review. *Rev Assoc Med Bras*. 2020;66Suppl 2:112-117.
42. Colling ME, Kanthi Y. COVID-19-associated coagulopathy: an exploration of mechanisms. *Vasc Med*. 2020;25:471-478.
43. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost*. 2020;18:844-847.
44. Iba T, Levy JH, Connors JM, Warkentin TE, Thachil J, Levi M. The unique characteristics of COVID-19 coagulopathy. *Crit Care*. 2020;24:360.
45. Ziadi A, Hachimi A, Admou B, et al. Lymphopenia in critically ill COVID-19 patients: a predictor factor of severity and mortality. *Int J Lab Hematol*. 2021;43:e38-e40.
46. Cavalcante-Silva LHA, Carvalho DCM, Lima ÉA, et al. Neutrophils and COVID-19: the road so far. *Int Immunopharmacol*. 2021;90:107233.
47. Chan AS, Rout A. Use of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in COVID-19. *J Clin Med Res*. 2020;12:448-453.

48. Zeng F, Li L, Zeng J, et al. Can we predict the severity of coronavirus disease 2019 with a routine blood test? *Pol Arch Intern Med*. 2020;130: 400-406.
49. Ghahramani S, Tabrizi R, Lankarani KB, et al. Laboratory features of severe vs. non-severe COVID-19 patients in Asian populations: a systematic review and meta-analysis. *Eur J Med Res*. 2020;25:30.
50. Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. *J Med Virol*. 2020;92:1733-1734.
51. Simadibrata DM, Calvin J, Wijaya AD, Ibrahim NAA. Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: a meta-analysis. *Am J Emerg Med*. 2021;42:60-69.
52. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): systematic review and meta-analysis. *J Intensive Care*. 2020;8:36.
53. Martín-Rojas RM, Pérez-Rus G, Delgado-Pinos VE, et al. COVID-19 coagulopathy: an in-depth analysis of the coagulation system. *Eur J Haematol*. 2020;105:741-750.
54. Elshazli RM, Toraih EA, Elgaml A, et al. Diagnostic and prognostic value of hematological and immunological markers in COVID-19 infection: a meta-analysis of 6320 patients. *PLoS One*. 2020;15:e0238160.
55. Chen R, Sang L, Jiang M, et al. Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. *J Allergy Clin Immunol*. 2020;146:89-100.
56. Shafi AMA, Shaikh SA, Shirke MM, Iddawela S, Harky A. Cardiac manifestations in COVID-19 patients-a systematic review. *J Card Surg*. 2020;35: 1988-2008.
57. Maramattom BV, Bhattacharjee S. Neurological complications with COVID-19: a contemporaneous review. *Ann Indian Acad Neurol*. 2020;23:468-476.
58. Fotuhi M, Mian A, Meysami S, Raji CA. Neurobiology of COVID-19. *J Alzheimers Dis*. 2020;76:3-19.
59. Cooper J, Stukas S, Hoiland RL, et al. Quantification of neurological blood-based biomarkers in critically ill patients with coronavirus disease 2019. *Crit Care Explor*. 2020;2:e0238.
60. Toklu H, Ganti L, Crimi E, Cintron C, Hagan J, Serrano E. Cerebrospinal fluid findings and hypernatremia in COVID-19 patients with altered mental status. *Int J Emerg Med*. 2020;13:63.
61. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in Wuhan, China. *JAMA Neurol*. 2020;77: 683-690.
62. Adapa S, Chenna A, Balla M, et al. COVID-19 pandemic causing acute kidney injury and impact on patients with chronic kidney disease and renal transplantation. *J Clin Med Res*. 2020;12:352-361.
63. Aloisio E, Chibireva M, Serafini L, et al. A comprehensive appraisal of laboratory biochemistry tests as major predictors of COVID-19 severity. *Arch Pathol Lab Med*. 2020;144:1457-1464.
64. Gabarre P, Dumas G, Dupont T, Darmon M, Azoulay E, Zafrani L. Acute kidney injury in critically ill patients with COVID-19. *Intensive Care Med*. 2020;46:1339-1348.
65. Liu YM, Xie J, Chen MM, et al. Kidney function indicators predict adverse outcomes of COVID-19. *Med (N Y)*. 2021;2:38-48.e2.
66. Zhou H, Zhang Z, Fan H, et al. Urinalysis, but not blood biochemistry, detects the early renal-impairment in patients with COVID-19. *BioRxiv*. Published online April 16, 2020. doi:10.2139/ssrn.3569863
67. Nauffal V, Achanta A, Goldhaber SZ, Piazza G. Association of ABO blood group type with cardiovascular events in COVID-19. *J Thromb Thrombolysis*. 2021;51:584-586.
68. Mehta JL, Calcaterra G, Bassareo PP. COVID-19, thromboembolic risk, and Virchow's triad: lesson from the past. *Clin Cardiol*. 2020;43:1362-1367.
69. Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol*. 2020;146:128-136.e4.
70. Huang Z, Huang P, Du B, et al. Prevalence and clinical outcomes of cardiac injury in patients with COVID-19: a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis*. 2021;31:2-13.
71. Martins-Filho PR, Barreto-Filho JAS, Santos VS. Myocardial injury biomarkers and cardiac complications associated with mortality in patients with COVID-19. *Arq Bras Cardiol*. 2020;115:273-277.
72. Shi C, Wang C, Wang H, et al. The potential of low molecular weight heparin to mitigate cytokine storm in severe COVID-19 patients: a retrospective cohort study. *Clin Transl Sci*. 2020;13:1087-1095.
73. Langer-Gould A, Smith JB, Gonzales EG, et al. Early identification of COVID-19 cytokine storm and treatment with anakinra or tocilizumab. *Int J Infect Dis*. 2020;99:291-297.
74. Sinha P, Mostaghim A, Bielick CG, et al. Early administration of interleukin-6 inhibitors for patients with severe COVID-19 disease is associated with decreased intubation, reduced mortality, and increased discharge. *Int J Infect Dis*. 2020;99: 28-33.
75. Colaneri M, Bogliolo L, Valsecchi P, et al. Tocilizumab for treatment of severe COVID-19 patients: preliminary results from SMAtteo COvid19 REgistry (SMACORE). *Microorganisms*. 2020;8:695.
76. Tsai A, Diawara O, Nahass RG, Brunetti L. Impact of tocilizumab administration on mortality in severe COVID-19. *Sci Rep*. 2020;10:19131.
77. Kashour Z, Riaz M, Garbati MA, et al. Efficacy of chloroquine or hydroxychloroquine in COVID-19 patients: a systematic review and meta-analysis. *J Antimicrob Chemother*. 2021;76:30-42.
78. Prata-Barbosa A, Lima-Setta F, Santos GRD, et al. Pediatric patients with COVID-19 admitted to intensive care units in Brazil: a prospective multicenter study. *J Pediatr*. 2020;96:582-592.
79. Kabeerdoss J, Pilania RK, Karkhele R, Kumar TS, Danda D, Singh S. Severe COVID-19, multisystem inflammatory syndrome in children, and Kawasaki disease: immunological mechanisms, clinical manifestations and management. *Rheumatol Int*. 2021;41:19-32.
80. Whittaker E, Bamford A, Kenny J, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA*. 2020;324:259-269.
81. Gruber C, Patel R, Trachman R, et al. Mapping systemic inflammation and antibody responses in multisystem inflammatory syndrome in children (MIS-C). *Cell*. 2020;183:982-995.e14.
82. Kelly MS, Valle CW, Fernandes ND, Cummings BM, Lahoud-Rahme M, Chiu JS. Multisystem inflammatory syndrome in children: cardiac biomarker profiles and echocardiographic findings in the acute and recovery phases. *J Am Soc Echocardiogr*. 2020;33:1288-1290.
83. Dufort EM, Koumans EH, Chow EJ, et al. Multisystem inflammatory syndrome in children in New York State. *N Engl J Med*. 2020;383:347-358.
84. Mueller C, Giannitsis E, Jaffe AS, et al. Cardiovascular biomarkers in patients with COVID-19. *Eur Heart J Acute Cardiovasc Care*. 2021;10:310-319.