Laboratory Biomarkers in the Management of Patients With COVID-19

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ABSTRACT

Objectives: *Laboratory testing and the measurement of appropriate biomarkers play a critical role in managing patients with coronavirus disease 2019 (COVID-19), allowing for disease diagnosis, monitoring progression, prognostication, prediction of treatment response, and risk stratification. We sought to characterize these effects on a more detailed, mechanistic level.*

Methods: *We reviewed the literature and identified a multitude of reports that describe the unique effects of this virus and its devastating consequences to multiple organ systems in COVID-19 patients.*

Results: *There are specific alterations in biomarkers related to coagulation, depopulation of T-cell subtypes, the cytokine storm and inflammation, and kidney and cardiac dysfunction.*

Conclusions: *Laboratory measurement of specific parameters and the use of appropriate prognostic, predictive, and monitoring biomarkers afford clinicians the ability to make informed medical decisions and guide therapy for patients afflicted with this dreaded disease.*

Key Points

- There are unique features of coronavirus disease 2019 (COVID-19) pathology, and several biomarkers have been shown to provide clinical value as they correlate with disease severity and mortality.
- There is an increased thrombosis risk in COVID-19, and the use of D-dimer assays may help to predict in-hospital mortality.
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can have specific effects on the kidney and heart, and specific biomarkers serve as prognostic indicators for disease severity or mortality.

Unique Pathogenicity of COVID-19 and the Role of Laboratory Testing

The novel coronavirus, now termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in the Wuhan province of China in early January 2020 and was declared responsible for a unique respiratory illness described in case reports beginning on December 18, 2019, now termed coronavirus disease 2019 (COVID-19).[1,2] Since then, COVID-19 has rapidly spread to 188 countries/regions with more than 27 million cases identified worldwide at the time of this writing, in addition to greater than 900,000 deaths.[3] Epidemiological and clinical data obtained from the first wave of cases in China quickly identified a relatively high transmissibility relative to other respiratory viruses of the same family. COVID-19 was found to have a mortality rate significantly higher than other respiratory viruses with similar transmissibility. Thus, further characterization of COVID-19, SARS-CoV-2, and the mechanisms by which it was able to produce such severe disease became a focal point of the biomedical community worldwide. From this work, a number of unique clinical characteristics of the virus have been discovered that point toward possible mechanisms for its high mortality rate. These mechanisms also suggest several promising candidate drug targets, as well

as prognostic indicators that would have clinical utility in escalating the care of high-risk patients.

This review will give an overview of these unique features of COVID-19 pathology and their relationship to specific biomarkers and laboratory tests. We will highlight the clinical relevance of these biomarkers and laboratory tests as relating to potential drug targets or as prognostic of disease severity and mortality. In the first section, we describe the growing body of work implicating cytokine release syndrome with COVID-19 and characterizing the precise cytokines that are upregulated in severe or critical cases. The specific upregulation of these cytokines has been proposed as a rationale for several newly emerging therapies that target specific molecules, such as the IL-6 inhibitor tocilizumab. Next, we give an overview of work showing the effect of SARS-CoV-2 on cellular immunity, namely, the depopulation of specific T-cell populations that have been shown to correlate with disease severity. We will then discuss evidence demonstrating the increased thrombosis risk in COVID-19 and summarize data showing the use of D-dimer assays in predicting in-hospital mortality. Finally, we discuss laboratory tests that characterize the effect of SARS-CoV-2 on specific organ systems, such as the kidney and heart. We show how specific laboratory tests that assess the function of these organs may serve as prognostic indicators for COVID-19 severity and mortality or serve in useful monitoring functions to guide therapy.

Cytokine Release Syndrome and Potential Drug Targets

During a normal immune response to pathogenic stimuli, there is a regulated elaboration of inflammatory mediators to ensure effective removal of pathogens. A return to baseline immune activity then normally ensues, after the offending stimulus has been removed. This process involves a coordinated interplay of proinflammatory and anti-inflammatory cytokines to both localize the immune response as well as to extinguish it when appropriate. A cytokine storm, or the dysregulated and excessive release of proinflammatory cytokines, is a systemic inflammatory response that may be triggered by various stimuli, including microbes, iatrogenic interventions such as chimeric antigen receptor T-cell (CAR-T) therapy, and some autoimmune/autoinflammatory conditions.[4-7] Severe respiratory viral infections have been associated with the cytokine storm, involving increased production of the proinflammatory cytokines TNF-α, IL-6, IL-1β, and MCP-1.[8,9] Release of these cytokines has been shown to produce increased vascular permeability, pulmonary edema, acute respiratory distress syndrome, and multiple-organ dysfunction.[8,9]

Most patients with SARS-CoV-2 infection develop mild symptoms while 15% of patients develop severe disease.[10] Both meta-analyses and structured literature reviews have found comorbid conditions that serve as reliable risk factors for COVID-19 severity and mortality, including diabetes, obesity, hypertension, and chronic lung disease.[11,12] Expression of the angiotensin-converting enzyme 2 (ACE2) receptor, which mediates SARS-CoV-2 entry into cells, is increased in these underlying conditions.[13-18] Together with the general immunosuppression associated with these conditions, increases in ACE2 expression provide a link to the higher risk of SARS-CoV-2 infection and severe disease in this patient population. Despite the prognostic value of comorbidities, there is still significant variability of outcomes in patients with these underlying conditions. In addition, their presence alone provides no information regarding specific pathogenic events of COVID-19. Therefore, such comorbid conditions are less useful for monitoring disease progression or understanding the potential value of specific therapies that target the mechanism of SARS-CoV-2 virulence. The development of the cytokine storm, however, plays a central role in disease pathogenesis of severe COVID-19. Consequently, biomarkers of the cytokine storm may give early warning of severe disease among patients with COVID-19 and prompt more aggressive therapies with specific biomolecular targets. In a study of patients with COVID-19 pneumonia, higher plasma levels of IL-2, IL-7, IL-10, GSCF, IP-10, MCP-1, MIP-1A, and TNF-α were associated with acute respiratory distress syndrome, cardiac injury, and secondary infections necessitating intensive care unit (ICU) admission.[19] Significantly higher levels of IL-2, IL-6, IL-10, and TNF-α were detected at admission in the blood of patients with severe disease.[20] Yang et al[21] found that a combination of IP-10, MCP-3, and IL-1RA levels had a high predictive value for severe disease with receiver operator characteristics (ROC) showing an area under the curve (AUC) of 0.943.

One of the key cytokines that has shown significant promise as a biomarker in various aspects of COVID-19 management is IL-6. IL-6 is a pleiotropic cytokine that plays a seminal role in host defense against infections. IL-6 is secreted by different cell types of the innate and adaptive immune system after encountering microbial antigens.[22] IL-6 then activates the JAK-STAT signaling pathway in effector cells, resulting variably in enhanced survival, proliferation, differentiation, and chemotaxis of T cells, B cells, monocytes, and neutrophils.[22] IL-6, together with IL-1, also induces secretion of C-reactive protein (CRP), an acute phase reactant that activates the classical complement pathway and mediates phagocytosis.[23]

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IL-6 and CRP levels have been shown to be good predictors of severe COVID-19 disease. In a retrospective study of a cohort of admitted patients in two Chinese hospitals, patients who succumbed to disease had significantly higher levels of IL-6 and CRP ($P < .001$) than those who were eventually discharged.[24] In a study comparing patients with mild vs severe disease, Liu et al[25] found a higher likelihood of severe complications in patients with IL-6 of more than 32.1 pg/mL, CRP of more than 41.8 mg/L, or procalcitonin of more than 0.07 ng/mL (hazard ratio [HR], 2.375; 95% confidence interval [CI], 1.058-5.329; $P < .001$ for IL-6; HR, 4.394; 95% CI, 1.924-10.033; $P < .001$ for CRP; HR, 4.908; 95% CI, 1.797-13.402; $P = .002$ for procalcitonin). Using a risk model of combined IL-6, CRP, and hypertension, severe disease could be predicted with an AUC of 0.900, a sensitivity of 100%, and specificity of 66%.[26] Similarly, Gao et al[27] found that a combination of testing for IL-6 and D-dimer, another acute phase reactant, had over 90% sensitivity and specificity in predicting severe disease.

Herold et al[28] found that elevated levels of IL-6 and CRP had high predictive value of need for mechanical ventilation in COVID-19. AUC for IL-6 was 0.97 and 0.90 in evaluation and validation cohorts, respectively, while the AUC for CRP was 0.86 and 0.83 in the same respective cohorts. These authors identified an IL-6 level of more than 35 pg/mL at presentation and a maximal value of more than 80 pg/mL and a CRP of more than 32.5 mg/L at presentation and a maximal value of more than 97 mg/L as the optimum predictive cutoffs. Monitoring of IL-6 together with CRP levels may provide early accurate signals for escalation of management. This would be particularly beneficial for COVID-19 considering the potential for rapid acute deterioration in severe COVID-19.

The significant role of IL-6 in pathogenesis of the cytokine storm, as well as its value in predicting severity of disease, makes it an ideal therapeutic target in management of severe disease. Tocilizumab, an anti–IL-6 receptor monoclonal antibody that is approved by the US Food and Drug Administration (FDA) for rheumatoid arthritis and the cytokine release syndrome during CAR-T therapy, has shown promise as supportive therapy in management of COVID-19 in preliminary studies.[29,30] The Randomised Evaluation of COVID-19 Therapy (RECOVERY) trial at the University of Oxford has begun testing of tocilizumab in over 6,000 hospitalized patients in the United Kingdom.[31] The RECOVERY trial is conducting clinical trials for "off-label" use of five drugs that have already been approved to treat other diseases and therefore deemed safe in the treatment of those diseases. These drugs include dexamethasone (indicated for numerous autoimmune, endocrine, respiratory, and other disorders), hydroxychloroquine (indicated for rheumatic conditions such as rheumatoid arthritis and systemic lupus erythematosus), lopinavir-ritonavir (approved for treatment of human immunodeficiency virus), azithromycin (an antibiotic used to treat infections of the respiratory and gastrointestinal tracts), and tocilizumab (an anti–IL-6 agent used to treat several forms of arthritis). On June 16, 2020, the RECOVERY trial announced results of its dexamethasone trial, showing that the anti-inflammatory drug dexamethasone reduced 28-day mortality by 35% in ventilated patients with COVID-19 and 20% in patients requiring oxygen supplementation.[32] Dexamethasone treatment has been shown to suppress IL-6 levels in multiple patient settings, including community-acquired pneumonia.[33,34] The role of IL-6 in predicting response to dexamethasone has yet to be tested but could represent a useful marker in assessing which patients may benefit from dexamethasone therapy. Elevated IL-6 levels may serve as an early warning sign of cytokine release syndrome, prior to the need of mechanical ventilation. We therefore encourage investigation into whether dexamethasone treatment of non–ventilator-dependent patients with increased levels of IL-6 significantly reduces the number of patients who need mechanical ventilation.

Several of the cytokines found to be elevated in patients with severe COVID-19, such as IL-1β and TNF-α, are known to promote a TH17 response in a subset of CD4+ T cells. The TH17 subset of T cells in turn produces additional cytokines and chemokines, such as IL-17A, IL-17F, and IL-22, leading to attraction of predominantly neutrophil infiltrates and matrix degradation.[19,35,36] A variety of new drugs targeting different effectors of the TH17 pathway, including IL-17A (secukinumab and ixekizumab) and IL-12/23p40 (ustekinumab), have been approved by the FDA for use in the treatment of psoriasis or psoriatic arthritis.[36] In addition, the JAK2 inhibitor fedratinib has been shown to suppress the production of several TH17 signature cytokines. Further study is needed to assess whether the cytokines associated with the TH17 pathway may be used as early markers for severe COVID-19 disease. If so, these FDA-approved drugs may represent candidates in preventing or minimizing cytokine release syndrome through the TH17 pathway in the setting of COVID-19 disease.[36]

Downregulation of Adaptive Cellular Immunity in COVID-19

In addition to a marked upregulation in specific inflammatory cytokines, early studies of patients in China

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reported characteristic changes in immune cell populations.[37-39] Lymphopenia has been reported as a common finding associated with other coronaviruses causing severe respiratory syndromes, such as severe acute respiratory syndrome (SARS),[40] and the Middle East respiratory syndrome (MERS) virus has been demonstrated to infect and induce apoptosis of T cells.[41] Early reports of patients with COVID-19 confirmed marked lymphopenia in the most severe patients.[37-39] Lymphocytes are crucial players in the adaptive immune system. They are involved in a variety of roles, including activation, regulation, and effector responses in both cellular and antibody-mediated immunity.

A retrospective, single-center study on patients with COVID-19 (n = 40) treated in Wuhan in January 2020 demonstrated that multiple populations of T cells were markedly reduced relative to matched controls in the general population. CD8+ T cells, the subset of T cells that specifically target virus-infected host cells, showed particularly marked reduction.[38] Neutrophil populations, which are the central effectors of the innate immune system, were conversely upregulated in patients with COVID-19, whereas B-cell populations, involved primarily in the antibody-mediated immune response, showed no significant change.[38] Interestingly, decreases in both total lymphocyte population and CD8+ T-cell population appeared to correlate strongly with disease severity. Authors defined severe disease based on respiratory symptoms: either respiratory rate more than 30 breaths/min, a decrease in O_2 saturation below 93%, or partial pressure of oxygen/fraction of inspired oxygen ratio (PaO_2/FiO_2) less than 300 mm Hg. When decreased lymphocyte populations were combined with increased neutrophil counts, the resulting T-cell/neutrophil ratio (NLR) and CD8+ T-cell/neutrophil ratio (N8R) were able to predict mild vs severe disease with odds ratios of 143 and 63.5, respectively. NLR and N8R were able to predict mild vs severe disease with high specificity and sensitivity in this population, with AUCs of 93.1 and 94.4, respectively. Of particular prognostic value in this study was that CD3+ and CD8+ T-cell populations were significantly lower in severe patients within 3 days of symptom onset, reaching their average nadir by days 4 to 6.[38] Whether these early changes were due to SARS-CoV-2 infection or to preexisting immunosuppression, screening of blood counts early in the course of disease may help triage the care of patients with COVID-19 before decline in respiratory status. Another single-center study of a larger patient population (n = 463) based in Wuhan also compared total, CD3+, CD4+, and CD8+ T-cell populations in patients with mild or severe disease, defined identically to the study above. Significant decreases in each T-cell population were found in the severe disease group.[42]

One major limitation of these studies is their criteria for severe disease, which had low stringency in the setting of COVID-19, especially considering patients who have underlying respiratory conditions such as asthma or chronic obstructive pulmonary disease (COPD) and may have reduced O_2 saturation of PaO_2/FiO_2 ratios at baseline. A more stringent definition of severe COVID-19 disease would comprise patients requiring ICU care, whereas mild to moderate disease would be classified as non-ICU patients. One such single-center retrospective study of patients with COVID-19 (n = 103) and noninfected controls in Wuhan between January and February compared T-cell subpopulations in patients admitted to a hospital with or without need of ICU.[37] These authors found a significant decrease in CD3+, CD4+, and CD8+ T-cell populations when comparing all patients with COVID-19 to uninfected controls, as well as when comparing ICU to non-ICU patients with COVID-19. They also noted that CD4+ and CD8+ T-cell counts (AUC = 0.888) were roughly equivalent as predictors of disease severity relative to the other T-cell subpopulations (AUC = 0.867 and 0.862, respectively). Combining CD8+ with CD4+ counts marginally improved the prognostic value of COVID-19 disease severity (AUC = 0.881).

While these two studies assessed different categories of disease severity, a third group also looked at the association of T-cell subpopulations and COVID-19 patient mortality in a group of patients (n = 187) in a single hospital in Wuhan from late December 2019 through March 1, 2020.[43] This study compared T-cell subpopulations across two dimensions: (1) disease severity, defined as mildly ill (n = 80), severely ill (n = 45), or critically ill (n = 62), as well as (2) disease mortality, by comparing overall survivors (n = 117) and nonsurvivors (n = 28) during the study interval (n = 145 patients with an outcome). Severe COVID-19 illness was defined as a decline in respiratory function, as described in Liu et al,[38] and critically ill patients having respiratory failure, shock, or other need for ICU admission. The authors found that overall lymphocyte count, in addition to CD3+, CD4+, and CD8+ subpopulations, decreased in a stepwise manner with an increase in disease severity, as well as B-cell and natural killer (NK)–cell populations. The authors also found that these same populations were significantly decreased in the survivor relative to the nonsurvivor group. Subsequently, they used ROC curves to establish cutoff values for each cell population that could be used as thresholds to predict in-hospital mortality. They found that overall T-cell count less than 500 (odds ratio [OR], 11.61), CD3+ T-cell count less than 200 (OR, 29.33), CD4+ T-cell count less than 100 (OR, 30.54), CD8+ T-cell count less than 100 (OR, 10.88), and B-cell count less than 50 (OR, 6.50) were the optimal thresholds to predict in-hospital mortality.[43]

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While limited as small, single-center studies in the Wuhan district, these three studies suggest that CD8+ T-cell counts in peripheral blood, as well as CD4+ T-cell and neutrophil count, may improve decision making in the context of hospital and ICU admission of patients with COVID-19. The conclusions of these studies should be further assessed for this purpose by larger, multicenter studies combining different patient populations. The upregulation of innate immune responses, signified by increased neutrophil count, along with downregulation of T-cell populations, suggests that severity of COVID-19 might relate closely to a given patient's ability to efficiently mount an adaptive immune response.[38] While much attention has focused on antibody-mediated responses to SARS-CoV-2, the findings reviewed here indicate that cellular immune responses represented by CD4+ and CD8+ T-cell populations also play a central role in the antiviral response. Further study is required to determine the degree to which SARS-CoV-2 can actively deplete or suppress T-cell populations or if the lymphopenias observed in these studies merely represent risk factors for COVID-19 severity and mortality. Distinguishing between these possibilities will be crucial to gauge the potential for COVID-19 treatments that specifically target the processes by which SARS-CoV-2 is able to deplete lymphocyte subpopulations.

Increased Thrombotic Risk and D-Dimer Assays

Dating back to initial reports from Wuhan, derangements in coagulation parameters have been associated with COVID-19. In particular, reports from two independent hospitals found that D-dimer levels were increased in 26% (n = 99) and 36% (n = 138), respectively.[44,45] The D-dimer level measures fibrin degradation products that occur due to thrombolysis, suggesting the presence of thrombosis and thrombolysis. The larger of these two studies compared 5 nonsurvivors with 28 survivors, to find that D-dimer levels were five- to sixfold higher in nonsurvivors, with a mean level over 1,000 mg/L.[45] When comparing ICU (n = 36) with non-ICU patients (n = 102), this study also found a significant increase in D-dimer levels in patients requiring ICU care (414 vs 166 mg/dL). In contrast, there was no significant change in prothrombin time (PT) or partial thromboplastin time (PTT), suggesting that COVID-19 increased thrombosis but did not lead to overt coagulopathy. However, a larger study that combined 191 patients from two hospitals in Wuhan found that not only elevated D-dimer levels (>1,000 mg/L) but also increased PT and PTT (defined as 3- and 5-second increases, respectively) were associated with mortality.[46] Yet the presence of a high incidence of secondary infection in nonsurvivors in the latter study raised the possibility that COVID-19 infection may be producing such coagulopathy in patients with severe infections indirectly through a predisposition for secondary infections.

A subsequent retrospective study compared D-dimer levels with mortality in 343 COVID-19–positive patients at Wuhan Asia General hospital from January to March 2020. ROCs were used to assess the optimal D-dimer cutoff to divide surviving from nonsurviving patients. It was found that a cutoff of more than 2,000 mg/L was able to predict in-hospital mortality with a sensitivity and specificity of 92.3% and 83.3%, respectively.[47] Thus, D-dimer levels alone may be a useful prognostic indicator in the hospital setting, allowing for more efficient screening of patients on admission or early in the hospital course for those who are at a greater risk of thrombosis.

A more recent multicenter prospective cohort study conducted in four ICUs at a French hospital found a significantly higher rate of thrombotic complications in COVID-19–positive patients (n = 150) with acute respiratory distress syndrome (ARDS) than patients with ARDS without COVID-19 infection (11.7 vs 2.1%, $P < .008$).[48] In line with studies conducted in Wuhan, most (>95%) of these severe or critically ill patients had elevated D-dimer levels. Interestingly, 87.7% of patients also were found to be positive for lupus anticoagulant. This study also assessed whether these patients met criteria for disseminated intravascular coagulation (DIC) as established by the Japanese Association for Acute Medicine (JAAM) and International Society for Thrombosis and Hemostasis (ISTH). Only 2.7% of the 150 patients met JAAM-DIC criteria, and no patients met ISTH-DIC criteria. This was attributed to the large proportion of this patient population with no abnormality in PT (72%) or platelet count (80%), despite larger aberrations in D-dimer and fibrinogen levels.[48] From these data, authors propose that higher coagulation targets may be warranted for COVID-19–positive patients due to higher-than-expected rates of thrombosis, even in cases of ARDS. Indeed, patients with COVID-19 with ARDS had an approximately threefold higher (18%) incidence of thromboembolic events overall and more than a 12-fold increase in rates of pulmonary embolism in this ICU patient population.

A multicenter cohort study looking at COVID-19 ICU patients in the Netherlands found a far higher incidence of thromboembolism, 49%, after adjusting down for competing causes of death.[49] The use of autopsy findings to determine cause of death in 12 patients with COVID-19 has also shown both a high incidence of venous thromboembolism (7/12) and pulmonary embolism

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as cause of death in one-third of the patients (4/12).[50] This represents strong preliminary data that the D-dimer may serve as a useful monitoring tool for pulmonary embolism in patients with COVID-19, particularly in the ICU setting, to guide anticoagulation or thrombolytic therapy. Additional assessment of the time course of D-dimer elevation during the hospitalization of patients with COVID-19 could be used to determine whether D-dimer assays could be used to guide prophylactic anticoagulation or antiplatelet therapy.

These results motivated another group to perform a meta-analysis of 22 studies involving 4,489 patients with COVID-19 in China, looking directly for parameters associated with DIC.[51] DIC represents a syndrome wherein widespread clotting is triggered by an initiating event, such as sepsis, and may lead to multiorgan failure. The depletion of platelets and coagulation factors secondary to widespread thromboses may also lead to bleeding. This meta-analysis looked at multiple parameters associated with DIC, including D-dimer, PT, PTT, platelet, and fibrinogen levels. The authors found no significant increase in these markers in patients with COVID-19 overall but observed small but significant increases in D-dimer levels (0.443 µg/mL; 95% CI, 0.228-0.658) and prolonged PT (0.654 seconds; 95% CI, 0.360-0.948) in patients with severe relative to mild disease. Interestingly, when comparing survivors with nonsurvivors, there were more marked increases in D-dimer (5.914 µg/mL; 95% CI, 3.559-8.269) and PT (1.228 seconds; 95% CI, 0.601-1.855), as well as a decrease in platelet count (–38.4 10^9/L; 95% CI, –55.8 to –20.941). There was an overall 6.2% incidence of DIC in the meta-analysis and a 26-fold increase in DIC incidence among nonsurvivors compared with survivors, leading these authors to conclude the close monitoring of coagulation and fibrinolysis markers (including thrombomodulin, thrombin-antithrombin complex, and plasminogen activator inhibitor 1) related to DIC is supported by these findings to achieve early intervention.[51]

However, increased rates of thrombotic events do not appear to lead to frank DIC in most patients, and COVID-19 with ARDS did not have a significantly increased rate of hemorrhagic complications relative to noninfected patients with ARDS, suggesting that increased thrombosis does not lead to coagulopathy in most cases.[48] The finding of elevated lupus anticoagulant in patients with COVID-19 suggests that therapies targeting such autoantibodies—such as plasma transfusion, especially from convalescent patients with COVID-19, and intravenous immunoglobulin—may also be efficacious at reducing the risk of thrombotic events.

Indeed, more recent work has suggested that D-dimer might provide a useful test to distinguish patients with COVID-19 who would benefit from more intensive anticoagulation therapy from those in whom the risks would outweigh a benefit. A retrospective study of 449 patients with COVID-19 at a single hospital in Japan showed that heparin did not significantly improve 28-day mortality in the full patient population, but heparin treatment achieved a 37.4% decrease in mortality among those patients with a D-dimer level less than 3.0 µg/mL, sixfold the upper threshold of normal.[52] Interestingly, heparin is also known to bind to IL-6,[53] and sequestration of IL-6 by heparin may make it a particularly useful anticoagulant therapy in the setting of COVID-19 infection, although further study into this mechanism is needed. Based on these findings, the European Society of Cardiology has recommended a specific algorithm for anticoagulation in patients with COVID-19 based on thromboembolic risk based on respiratory status, D-dimer, fibrinogen, and CRP levels.[46,52,54] While hospitalized patients at high risk for thromboembolism may benefit from full-length heparin or aggressive low-molecular-weight heparin therapy, this algorithm recommends increasing standard thromboembolic prophylaxis with low-molecular-weight heparin based on D-dimer levels.[54]

Renal Function Assays and COVID-19

While respiratory failure remains the predominant clinical finding in COVID-19, ACE2 receptors known to bind and facilitate SARS-CoV-2 cell entry have been found in almost every other organ in patients with COVID-19.[55] Of particular note, this study found that ACE2 receptors are found in particularly high concentrations in the small intestine, testis, kidney, heart, and thyroid. High rates of acute kidney injury (AKI) known to occur in patients with SARS (6.7%) prompted investigation into the degree to which renal injury and renal failure contribute to COVID mortality.[56,57] Initial reports of patients from Wuhan, China, in January 2020 showed a strong association between acute elevation of creatinine and COVID-19 patient mortality.[45]

A recent meta-analysis of nine studies looking at AKI in hospitalized patients with COVID-19 found a pooled incidence rate of 3% (95% CI, 1%-7%) in hospitalized patients (n = 2,702), although the pooled incidence of ICU patients (n = 122) was more than sixfold higher, at 19% (95% CI, 9%-31%).[58] This suggests an association between disease severity and renal injury, although whether the relationship between the two variables is direct or indirect cannot be assessed without more high-resolution

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data of the time course of AKI relative to clinical status and mechanisms by which SARS-CoV-2 could produce renal injury. With regard to overall rates of AKI, eight of nine studies included in this meta-analysis were conducted in China, in the Wuhan province, and rates of AKI have been shown to vary from 0.5% to 29% in other patient populations with varying rates of underlying kidney disease, severity of disease, and comorbidities.[59]

A recent multicenter retrospective cohort study spanning all COVID-19–positive patients (n = 5,449) hospitalized in 13 hospitals in the New York Metropolitan from March 1, 2020, to April 5, 2020, sought to characterize rates of AKI in this broad patient population encompassing both academic and community hospitals.[59] They found a markedly higher rate of AKI (36.6%) compared with the other studies discussed so far. AKI is also categorized into three stages based on comparison of the peak serum creatinine relative to baseline: stage 1, 1.5-fold or more to less than 2-fold increase; stage 2, 2-fold or more to less than 3-fold increase; and stage 3, 3.0 mg/dL or more.[56,59] When these authors categorized AKI by stage, they found markedly high rates of stages 2 and 3, which comprised 22.4% and 31.1% of total AKI, respectively. Of note, this patient population had a markedly high rate of comorbidities associated with kidney disease, including hypertension (55.7%) and diabetes (33.0%). Indeed, these authors found that diabetes, hypertension, and cardiovascular disease were the comorbid conditions most closely associated with risk of AKI (adjusted OR, 1.76, 1.48, and 1.25, respectively), while mechanical ventilation and vasoactive medications (pressors and inotropes) were the treatment factors most closely associated with AKI (OR, 10.7 and 4.53, respectively). Therefore, both preexisting conditions in a given patient population must be considered carefully when considering the prevalence of AKI and therefore its use as a predictor of clinical outcome. In addition, this study draws a strong association between respiratory failure and AKI. In combination with their finding that that AKI rates peaked on the first day after admission, this suggests that monitoring for AKI early in hospital admissions could greatly aid decision making as to overall clinical status.

A larger prospective cohort study based out of a tertiary teaching hospital in Wuhan province looked more closely at renal function in all 701 COVID-19–positive patients admitted in a 2-week period from January 28, 2020, to February 11, 2020.[56] Of note, this study included 600 patients with normal baseline serum creatinine levels, as defined as their level on admission. As one might expect, those patients with elevated creatinine had a significantly higher rate of AKI, defined as a 0.3-mg/dL increase in serum creatinine in 48 hours, or a 50% increase from baseline within 7 days. The overall rate of AKI was 5.1% for patients included in the study, but those with normal baseline creatinine had an incidence of only 4%, while those with creatinine elevated at baseline (>104 µmol/L and >84 µmol/L for men and women, respectively) had an AKI incidence of 11.9. Elevated creatinine at baseline also correlated with a 2.5-fold higher risk of death in this patient population (13.2% vs 33.7%), suggesting it is a significant risk factor for mortality in the presence of COVID-19 infection.

When adjustments were made for factors associated with mortality, such as age, sex, disease severity lymphocyte count, and other comorbidities, stage 2 to 3 AKIs were both associated with significantly increased risk of death (hazard ratio [HR], 3.53 and 4.72, respectively; $P < .001$), and an increase in serum creatinine concentration over 1.5 mg/dL was associated with more than a threefold increase in mortality risk (HR, 3.09; $P < .001$).[56] Several other markers of kidney injury were also associated with significantly increased risk of mortality. Proteinuria showed a dose-dependent increase in mortality risk, with 1+ proteinuria conferring a 2.47-fold risk and 2 to 3+ proteinuria a 6.80-fold risk ($P < .001$). Hematuria showed an even stronger dose-dependent increase in mortality risk, with 1+ hematuria conferring a 3.05-fold risk and 2 to 3+ hematuria an 8.89-fold risk ($P < .001$). Both elevated serum creatinine and serum urea nitrogen on admission were associated with significantly increased risk of mortality, but elevated serum urea nitrogen (defined based on average for sex and age in this patient population)[56] had more than twice the level of associated risk compared with AKI (HR, 2.04 vs 4.20). Therefore, both serum creatinine and serum urea nitrogen concentrations on admission may serve as useful additions to guidelines for assessment of disease severity and level of care for patients with COVID-19, especially those who otherwise do not meet criteria for severe disease based on respiratory symptoms. Furthermore, close monitoring of serum creatinine is warranted in the setting of COVID-19 infection due to the high risk for severe AKI, so that appropriate renal replacement therapies can be administered without delay, as AKI has been shown here to be an independent risk factor for mortality.

Cardiomyocyte Injury, COVID-19, and Relevant Laboratory Tests

Based on ACE2 receptor density, one might also suspect the heart as another organ to be directly affected by SARS-CoV-2 infection.[55] One retrospective cohort

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study comparing ICU with non-ICU hospitalized patients in Wuhan province found 6.4% of patients with COVID-19 had an elevated troponin I on admission, a hypersensitive marker for cardiac myocyte injury, but this increased to 11% among ICU patients.[45] When these authors looked at acute cardiac injury as an outcome, defined as elevation in troponin I or diagnostic imaging findings, they found a similar 7% overall incidence in patients with COVID-19, but this was increased to 22% among ICU patients ($P < .001$). Another retrospective single-center study also in Wuhan compared troponin I levels, as a sign of cardiomyocyte injury, in hospitalized patients with COVID-19 categorized as either survivors or nonsurvivors.[46] This patient population had a remarkably more than twofold higher incidence of elevated troponin I on admission (17%), with a striking 46% elevation in patients who subsequently died during the hospitalization ($P < .0001$). When acute cardiac injury was assessed as an outcome in this patient population and defined as in the previous study, a similar proportion of overall patients met these criteria (17%), but a striking 59% of nonsurviving patients met these criteria.[46]

As one might expect, troponin I was found to be strongly associated with mortality in patients with COVID-19 in this latter study (OR, 80.07). Another nonspecific marker of cardiomyocyte injury, lactate dehydrogenase (LDH), was also significantly elevated in most (67%) patients with COVID-19 on admission, and nearly all (98%) of those who did not survive had elevated LDH.[46] LDH was also strongly associated with mortality in this patient population (OR, 45.43). Authors of this study also compared troponin I and LDH levels from illness onset, finding that LDH levels only differed significantly between survivors and nonsurvivors late in the disease course (after day 19). Highly sensitive troponin I showed significant differences as early as day 7, exceeding the 99th percentile threshold value (28 pg/mL) on average by day 13 in nonsurvivors, while survivors remained well below this threshold throughout.[46] Based on the low troponin I levels throughout the hospital course of surviving patients in this study, this assay could be a useful screening tool for prediction of mortality if used on admission or early in the hospital course, although a follow-up study to determine the optimal cutoff value to maximize sensitivity and specificity is recommended.

In addition, the etiology of the cardiomyocyte injury, whether SARS-CoV-2 has a direct effect through cardiomyocyte infection or indirectly leads to ischemia and infarct through thromboembolic events or another mechanism of injury, is unclear. For both cardiac and renal injury, further research into whether SARS-CoV-2 acts directly or indirectly may yield valuable information as to what therapies are most appropriate in the setting of screening assays for reduced kidney function or cardiomyocyte injury.

Conclusion

In conclusion, we have presented herein a detailed review of the multiplicity of pathologic features that characterize severe disease in patients with COVID-19. These include the cytokine release syndrome, downregulation of adaptive cellular immunity, increased thrombotic risk, lung and acute kidney dysfunction, and cardiomyocyte injury. In the context of these unique pathologic features of COVID-19, we detail several types of biomarkers whose levels are altered and can inform clinical decision making in the management of patients with COVID-19. Some biomarkers are prognostic: they can be measured early in disease presentation and can portend a good or bad prognosis. They help to identify patients who will have a more severe course of disease and should be triaged to more aggressive therapy early on, while others can be spared from these harsher treatments along with their debilitating consequences. Other biomarkers can be used prospectively for monitoring: they can be used in serial measurements to help determine whether a patient is responding to a particular therapy or, alternatively, requires a different course of treatment; many of the organ-specific test analytes are of this category. Other biomarkers are predictive: they can allow us to identify patients who potentially respond to a particular therapy, such as IL-6 for tocilizumab. These markers detect signaling molecules resulting from the activation of specific pathways or can themselves serve as direct pharmacologic targets. A deeper understanding of the clinical utility of the various biomarkers and how they can be used in concert is critical for successfully treating patients. Improving patient outcomes will require earlier detection of these issues, targeted treatments, and appropriate triage of patients, particularly those who are susceptible to the most severe course of this disease.

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