How lab diagnostics can support COVID-19 patient management

By Justin E. Jones, PhD

As the coronavirus disease 2019 (COVID-19) pandemic continues to burden the healthcare system with increased demand for bed capacity and personal protective equipment (PPE), as well as a heightened focus on safeguarding the health of other patients and hospital staff and the overall reprioritization of resources, the clinical laboratory has been meeting a parallel set of challenges. The initial reduction in overall testing volume and revenue, driven by fewer primary care visits and reduction of elective surgery, was quickly overtaken by escalating demand for COVID-19 testing and reagents, especially for PCR testing. As overall testing volume continues to return to near normal, new opportunities for labs to contribute to the care of COVID-19 patients are emerging. The scientific community's response to COVID-19 has resulted in a large volume of publications as scientists and clinicians share their experience and glean new insights. For example, it is estimated that hospitalization is required in about 20 percent of diagnosed cases, with 5 percent requiring intensive care unit (ICU) admission and/or respiratory support.[1] Significantly, for COVID-19 patients, median length of stay is well above average: 10.1 days for all admissions and 10.6 days for the intensive care unit (ICU). At the same time, the expanding knowledge base about the clinical course of COVID-19 now includes data correlating levels of biomarkers with patient status to support clinical decision making.[2] To date, the FDA has granted emergency use authorization (EUA) status to two commercial assays for interleukin-6 (IL-6) to assist in identifying severe inflammatory response in COVID-19 patients, adding to the diagnostic tests available to help clinicians make patient care decisions. This article provides an overview of IL-6, procalcitonin (PCT), D-dimer, cardiac troponin, N-terminal pro B-type natriuretic peptide (NT-proBNP) and ferritin and their potential clinical significance in critically ill patients.

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LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Provide an overview of coronavirus disease 2019 (COVID-19) and the underlying pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
2. Recall COVID-19 etiology and its risk factors.
3. Demostrate a working knowledge of the clinical course of COVID-19.
4. Describe key biomarkers and their relevance in COVID-19 clinical decision-making, with focus on IL-6, PCT, D-dimer, cardiac troponin, NT-proBNP and ferritin.

COVID-19 etiology and risk factors

Coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus

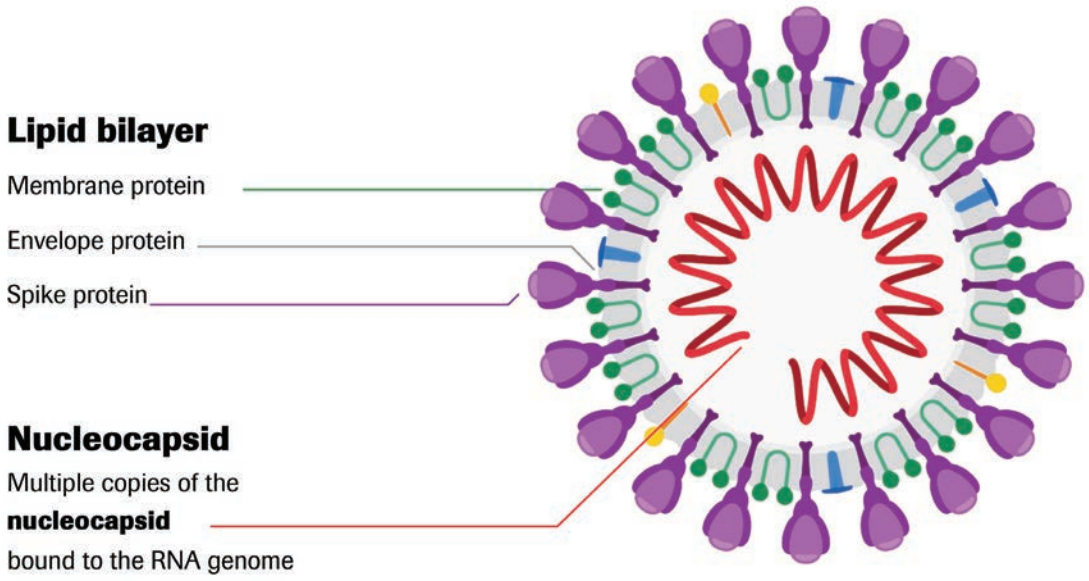

Figure 1. SARS-CoV-2, a large, enveloped RNA virus

2 (SARS-CoV-2), a large, enveloped RNA virus.[3] The lipid bilayer envelope, membrane proteins and nucleocapsid protect the virus when it is outside the host cell. Genetic analyses indicate SARS-CoV-2 originated in bats and is analogous to SARS-CoV, the coronavirus that emerged in 2003–2004 and caused epidemic disease, and Middle East respiratory syndrome coronavirus (MERS-CoV).

SARS-CoV-2 is transmitted person-to-person via respiratory secretions and indirectly through contaminated surfaces. Infection begins with SARS-CoV-2 spike engagement with host angiotensin-converting enzyme 2 (ACE2) receptors, followed by a series of conformational changes that result in fusion between the viral envelope and host cell membrane. An incubation period of up to 14 days follows. Clinical presentation varies from asymptomatic infection to mild illness, pneumonia or fatal disease. Risk factors for disease progression include older age, male sex, obesity, comorbidities (hypertension, diabetes, cardiovascular disease, chronic respiratory disease, cancer) and history of smoking.[4]

Disease progression

Figure 2 summarizes a three-stage classification system for COVID-19 illness, with three grades of increasing severity that correspond with distinct clinical findings, response to therapy and clinical outcomes.[5]

Stage 1: Early infection involves an incubation period that varies from 2 to 14 days, with a median of 5 days. During this period, the virus multiplies and establishes residence in the host, primarily focusing on the respiratory system. Symptoms are mild and often nonspecific, such as malaise, fever and dry cough. For the 75 percent of diagnosed patients in whom the virus remains limited to this stage of COVID-19, prognosis and recovery are excellent.

Stage 2: The pulmonary phase can be viewed as part of the viral response phase, in which the virus itself causes symptoms by infecting cells and lysing those cells during proliferation. Viral multiplication and localized inflammation in the lungs are the norm. Patients may develop viral pneumonia, with cough, fever and possible hypoxia,

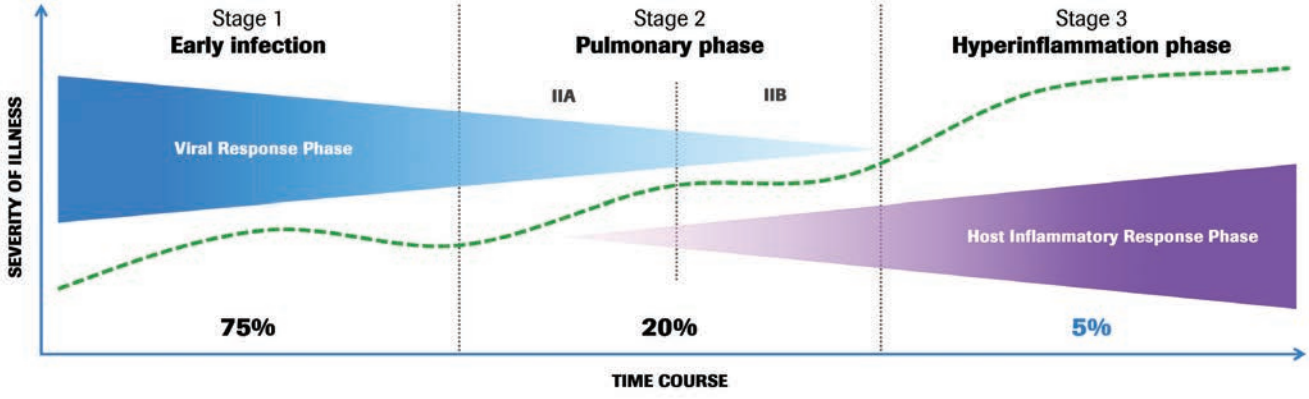

Figure 2. COVID-19 illness exhibits three stages of increasing severity

	Stage 1—Early infection	Stage 2—Pulmonary phase	Stage 3—Hyperinflammation phase
Tests	SARS-CoV-2 PCR TB PCR RSV PCR Influenza A/B PCR SARS-CoV-2 Antigen	PCT*, IL-6*, Lactate, CRP*, Ferritin*, AST*, Albumin*, LDH*, Urea*, Glucose, Creatinine*, D-Dimer*, Influenza A/B PCR, RSV PCR, TB PCR	PCT*, IL-6*, Lactate, CRP*, ALT*, Albumin*, LDH*, Bilirubin*, Ferritin*, Urea*, Glucose, Creatinine*, Cystatin C, D-Dimer*, Troponin*, NT-proBNP*, Influenza A/B PCR, RSV PCR
Comments	Mild, nonspecific changes in biomarkers; reduction in lymphocyte count (CBC) as immunological response to virus	Mild to moderate changes in biomarkers including PCT, CRP and Ferritin	Significant elevations in CRP, Ferritin, IL-6, PCT (if bacterial co-infection is present and sepsis develops) and Lactate (marker of tissue hypoperfusion; indicates widespread organ damage)

Table 1. Commonly used laboratory tests by COVID-19 clinical staging
*Listed in IFCC Interim Guidelines[2]

defined as PaO2/FiO2 <300 mm Hg.[5] This group of patients, who represent about 20 percent of diagnosed cases, will likely require hospitalization. The subgroup (IIB), characterized by hypoxia, will likely progress to requiring mechanical ventilation and Stage. [3]

Stage 3: The hyperinflammation phase represents about 5 percent of diagnosed cases, characterized by the body responding to the infection and manifesting as an extrapulmonary systemic hyperinflammation syndrome. Clinical symptoms include acute respiratory distress syndrome (ARDS), heart failure, septic shock, coagulopathy, acute cardiac injury, acute kidney injury and secondary infections, all with worse outcomes.[5,6] Inflammatory cytokines and biomarkers, such as IL-6, ferritin and D-dimer, are significantly elevated in these patients, as are troponin and N-terminal pro B-type natriuretic peptide (NT-proBNP).[5] This article will focus on biomarkers and laboratory testing for this group of critically ill patients.

Laboratory diagnostics in COVID-19 patient management

Laboratory testing protocols vary by institution. Commonly used tests by COVID-19 clinical staging are summarized in **Table 1**. Notably, many of the tests are markers of complications with poor survival, as summarized in **Table 2.**

Unique challenges in critical care testing

Critical care marks a turning point in disease progress. More than ever, clinicians are looking for answers to make life-saving decisions, decisions that can also impact health system resource allocation and prioritization. Test results are often needed urgently. Just as urgently, clinicians need to understand test results and how they translate into patient care decisions, especially as accelerated publication schedules continue to offer new data that can change clinical practice. The following is an overview of key biomarkers that can impact patient care decisions, with the goal of helping the clinical laboratory inform and educate their clinical care counterparts within the healthcare system.

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a marker of severe inflammatory response in COVID-19 patients and the abnormal release of circulating cytokines such as interferons, interleukins and tumor necrosis factors, cell-signaling molecules that play a key role in the immune response.[6] Overproduction of early response proinflammatory cytokines results in a "cytokine storm" that leads to severe downstream effects with multi-organ involvement, including development and progression of acute respiratory distress syndrome (ARDS). Elevated circulating concentrations of IL-6, a key mediator of inflammation, can serve as an early alarm signal of SARS-CoV-2 infection-triggered hyperinflammation, helping clinicians identify this process in severely ill COVID-19 patients.[7] Aside from assisting in the identification of hyperinflammation in COVID-19 patients, IL-6 may predict respiratory failure in hospitalized, symptomatic COVID-19 patients and thus provide an objective data point to assist clinicians in mechanical ventilation resource allocation. One study found that after IL-6 of 35 pg/mL was reached, the median time to mechanical ventilation was 2 days.[8] Because IL-6 is released early during a severe infection, it can help physicians to identify severely ill COVID-19 patients in a timely manner.

	Total (n=191)	Non-survivor (n=54)	Survivor (n=137)	P value
Sepsis	112 (59%)	54 (100%)	58 (42%)	<0·0001
Respiratory failure	103 (54%)	53 (98%)	50 (36%)	<0·0001
ARDS	59 (31%)	50 (93%)	9 (7%)	<0·0001
Heart failure	44 (23%)	28 (52%)	16 (12%)	<0·0001
Septic shock	38 (20%)	38 (70%)	0	<0·0001
Coagulopathy	37 (19%)	27 (50%)	10 (7%)	<0·0001
Acute cardiac injury	33 (17%)	32 (59%)	1 (1%)	<0·0001
Acute kidney injury	28 (15%)	27 (50%)	1 (1%)	<0·0001
Secondary infection	28 (15%)	27 (50%)	1 (1%)	<0·0001

Table 2. COVID-19 complications
See reference 6

Procalcitonin (PCT)

Procalcitonin (PCT) aids in measuring the inflammatory response to bacteria, enabling the differentiation of bacterial infections from nonbacterial causes of lower respiratory tract infections. PCT levels rise rapidly, generally within 3 to 6 hours after a bacterial infection. In COVID-19 patients, increased PCT is associated with higher risk of severe COVID-19 and may reflect bacterial co-infection.[9] Increase in PCT concentration correlates with the severity of the bacterial infection, with significant elevated levels being associated with patients at risk of developing severe sepsis or septic shock, both of which have been identified as COVID-19 complications.[11] (See **Table 2.**) Sustained elevated PCT is associated with patients with ARDS.[10] For COVID-19 patients with respiratory failure requiring

mechanical ventilation, empiric antimicrobial treatment may be initiated.[12] Conversely, a decrease in PCT concentration signals relief of infection. In this context, PCT testing can guide de-escalation of antimicrobial therapy (e.g., shortening the duration of therapy or discontinuing empiric antibiotics) and support its optimal use — the core elements of antimicrobial stewardship.[13]

D-dimer

D-dimer is one of several fragments produced during the degradation of a fibrin clot by plasmin. Viral infections elicit a systemic inflammatory response and cause an imbalance between procoagulant and anticoagulant homeostatic mechanisms. The dysregulation of the coagulation cascade and the subsequent formation of intra-alveolar or systemic fibrin clots are prominent findings in coronavirus infections associated with severe respiratory diseases such as SARS-CoV-1 and MERS-CoV. The most common pattern of coagulopathy observed in patients hospitalized with COVID-19 is characterized by elevations in D-dimer and fibrinogen levels. As shown in Table 2, coagulopathy has been identified as a complication in COVID-19 patients. Another study of 560 hospitalized COVID-19 patients showed that 69.4 percent of the patients who were in the ICU, were on mechanical ventilation, or died had elevated D-dimer levels.[14]

Cardiac troponin and N-terminal pro B-type natriuretic peptide (NT-proBNP)

Cardiac troponin and N-terminal pro B-type natriuretic peptide (NT-proBNP) are relevant in COVID-19 because myocardial injury is prevalent among patients hospitalized with COVID-19, and patients with cardiovascular disease (CVD) are more likely to have myocardial injury than patients without CVD.[15] One study showed that patients with underlying CVD accounted for 4.2 percent of COVID-19 cases but 18.3 percent of COVID-19 deaths.[16] For critically ill COVID-19 patients, the utility of biomarkers such as cardiac troponin and NT-proBNP lies also in their high negative predictive value. When the values are low and stable, they provide the confidence needed to triage patients away from imaging or further work-up for cardiac-related issues.

Ferritin

Ferritin is an indicator of severe inflammation in COVID-19 patients and is associated with worse clinical outcomes.[2,17] Several studies showed that serum ferritin level correlates with severity of disease. In COVID-19 patients who died, ferritin levels were high upon hospital admission and throughout the hospital stay.[18]

The clinical laboratory's role

"While the trajectory of this outbreak is impossible to predict, effective response requires prompt action from the standpoint of classic public health strategies to the timely development and implementation of effective countermeasures."[3]

As the COVID-19 pandemic continues, clinical laboratories can take this call to action to heart by sharing our knowledge and expertise with colleagues -- nurses, respiratory therapists, phlebotomists, administrators, clinicians — and begin a dialogue about the role of lab diagnostics in supporting COVID-19 patient care decisions, with the oal of improving patient outcomes.

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TEST QUESTIONS

Circles must be filled in, or test will not be graded. Shade circles like this: ● Not like this: ✗

1. Hospitalization is required in about ____ percent of diagnosed cases of COVID-19, with 5 percent requiring intensive care unit (ICU) admission and/or respiratory support.
- A.10
- C.25
- B.20
- D.30

2. For COVID-19 patients, median length of stay is well above average: ____ days for all admissions and ____ days for the ICU.
- A. 10.1;10.6
- C. 9; 9.4
- B. 10.1; 11.5
- D. 9; 10.

3. The ____, membrane proteins and____ protect the virus when it is outside the host cell.
- A. spike protein; envelope protein
- B. spike protein; nucleocapsid
- C. lipid bilayer envelope, spike protein
- D. lipid bilayer envelope; nucleocapsid

4. Which commerical assay has the FDA granted emergency use authorization (EUA) status to, which would assist in identifying severe inflammatory response in COVID-19 patients?
- A. HbA1c
- B. interleukin-6 (IL-6)
- C. PD-L1
- D. HER2

5. SARS-CoV-2 is transmitted person-to-person via respiratory secretions and indirectly through ____.
- A. environmental reservoirs
- B. insect bites
- C. contaminated surfaces
- D. food and drinking water

6. An incubation period of up to ____ days follows infection with SARS-CoV-2.
- A. 7
- C. 10
- B .9
- D. 14

7. The three phases of severity of COVID-19 illness are:
- A. early infection, pulmonary response, and hyperinflammation.
- B. early infection, pulmonary response, and death.
- C. early infection, hyperinflammation, and death.
- D. pulmonary response, hyperinflammation, and death.

8. During early infection, the virus multiplies and establishes residence in the host, primarily focusing on the ____system.
- A. nervous
- C. respiratory
- B. cardiovascular
- D. endocrine

9. During the pulmonary response, patients may develop viral pneumonia, with cough, fever and possible ____.
- A. hypoxia
- B. low blood pressure
- C. hyperpituitarism
- D. gastric distress

10. During the hyperinflammation phase, inflammatory cytokines and biomarkers, such as IL-6, ferritin and D-dimer, are significantly lowered.
- A. True
- B. False

11. Diagnostic tests given to patients with COVID-19 include:
- A. ferritin, PCT, and IL-6.
- B. rheumatoid factor, IL-6, and LDH.
- C. antinuclear antibodies, HbA1c, and ferritin.
- D. LDH, antinuclear antibodies, and PCT.

12. Severe medical complications of COVID-19 include:
- A. heart failure, sepsis, and wound infection.
- B. septic shock, secondary infection, and ARDS.
- C. ARDS, hemorrhage,and secondary infection.
- D. wound infection, acute kidney injury, and ARDS.

13. Interleukin-6 (IL-6) is a marker of severe _____ in COVID-19 patients.
- A. inflammatory response
- C. hypothyroidism
- D. hypotension
- B. depression

14. Aside from assisting in the identification of hyperinflammation in COVID-19 patients, IL-6 may predict _____ failure in hospitalized, symptomatic COVID-19 patients and thus provide an objective data point to assist clinicians in _____ resource allocation.
- A. respiratory; mechanical ventilation
- B. heart; ventricular assist device
- C. kidney; dialysis
- D. liver; transplant

15. PCT levels rise rapidly, generally within ____ to _____ hours after a bacterial infection.
- A. 2; 4
- C. 4; 7
- B. 3; 6
- D. 3; 8

16. The ____ in PCT concentration correlates with the severity of the bacterial infection, with significant _____ levels being associated with patients at risk of developing severe sepsis or septic shock.
- A. increase; elevated
- B. decrease; lowered
- C. increase; lowered
- D. decrease; elevated

17. Viral infections elicit a systemic inflammatory response and cause an imbalance between procoagulant and anticoagulant homeostatic mechanisms.
- A. True
- B. False

18. The most common pattern of coagulopathy observed in patients hospitalized with COVID-19 is characterized by elevations in D-dimer and _____ levels.
- A. fibrinogen
- C. creatinine
- B. LDL
- D HbA1c

19. For critically ill COVID-19 patients, the utility of biomarkers such as _____ and NT-proBNP lies also in their high negative predictive value.
- A. CK-MB
- B. myoglobin
- C. cardiac troponin
- D. creatinine kinase

20. Ferritin is an indicator of severe_____ in COVID-19 patients and is associated with worse clinical outcomes.
- A hypotension.
- B. inflammation
- C. hypertension
- D. hyperglycemia

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