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Predicting Disease Severity and Outcome in COVID-19 Patients: A Review of Multiple Biomarkers

Running title: Prognostic Biomarkers in Patients with COVID-19

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Abstract

Context: An abundance of clinical reports focused on specific laboratory parameters have been reported on COVID-19, but a systematic analysis synthesizing these findings has not been performed.

Objective: To review and summarize the current available literature on the predictive role of various biomarkers in COVID-19 patients.

Data Sources: A literature search was performed using databases including PubMed, medRxiv, and bioRxiv. A total of 72 papers were reviewed including 54 peer reviewed papers and 18 non-peer reviewed preprints.

Conclusions: While non-specific, acute phase reactants including CRP, ferritin, SAA, and procalcitonin were reported as sensitive markers of acute COVID-19 disease. Significantly elevated WBC count, marked lymphopenia, decreased CD3, CD4 or CD8 T-lymphocyte counts, high neutrophil count, thrombocytopenia, and markedly elevated inflammatory biomarkers were associated with severe disease and the risk of developing sepsis with rapid progression. Trends observed by serial laboratory measurements during hospitalization including progressive decrease of lymphocyte count, thrombocytopenia, elevated CRP, procalcitonin, increased liver enzymes, decreased renal function, and coagulation derangements were more common in critically ill patient groups and associated with a high incidence of clinical complications. Elevated IL-6 level and markedly increased SAA were most often reported in severely and critically ill patients. Indicators of systemic inflammation such as neutrophil-lymphocyte ratio (NLR), systemic immune-inflammation (SII) index or COVID-19 Severity Score may be utilized to predict disease severity, outcome, and mortality. Interpretation of the data reported in the

studies reviewed here is limited due to study design (mostly retrospective), limited sample size, and a lack of defined clinical criteria.

Key Words: COVID-19; SARS-CoV-2; Acute phase protein (APP); C-reactive protein (CRP); Ferritin; Serum amyloid A (SAA); Lactate dehydrogenase (LDH); Interleukin-6 (IL-6); Procalcitonin; White blood cells (WBC)

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the current pandemic coronavirus disease 2019 (COVID-19). As of July 28, 2020, there have been 16,558,289 confirmed cases including 656,093 deaths worldwide.[1] SARS-CoV-2 is genetically related to the other two members of Betacoronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV).[2]

The majority of individuals with COVID-19 infection (~80%) have been reported to have uncomplicated disease with mild symptoms and only a subset develops severe disease requiring hospitalization.[3,4] The most common initial symptoms in confirmed COVID-19 infected patients were fever, cough, dyspnea, and fatigue.[4–8], with fever reportedly less common than in SARS-CoV (99%) and MERS-CoV (98%).[8] Gastrointestinal symptoms including nausea, vomiting, abdominal pain, and diarrhea, as well as myalgia and headache were among the less commonly reported symptoms.[4–8] Sudden olfactory loss of sensation has also been reported.[9] In addition to nasopharyngeal/oropharyngeal secretions and sputum, SARS-CoV-2 has been detected in other body fluids or secretions including saliva, human breast milk, tears, urine, and semen.[10–13] Sputum, oropharyngeal secretions, and saliva have been suggested as possible alternative samples for molecular testing.[10,11] Based on published data from China, children (0-14 years) were less susceptible to SARS-CoV-2 infection than adults (15-64 years), whereas older individuals (>65 years) were more susceptible.[14]

Previous studies of SARS-CoV patients have demonstrated that initial laboratory analyses with high neutrophil count (>0.7x10³/µL), lymphopenia (<0.8x10³/µL), elevated C-reactive protein (CRP >4.75 mg/dL; to convert to mg/L multiple by 10), and elevated lactate dehydrogenase

(LDH >593 U/L; to convert to µkat/L multiply by 0.0167) were the most important predictors of mortality.[15–17] Hematologic changes were common and included lymphopenia, significantly decreased CD4 and CD8 counts, thrombocytopenia, and occasional leukopenia.[17] Both CD4 and CD8 positive T lymphocytes appeared to play an important role in eliminating virus infected cells and both cell counts were useful in predicting disease severity and clinical outcomes.[18] Increased serum amyloid A (SAA) was also observed and the degree of increase correlated with disease severity.[19] Secondary bacterial or fungal infections, acute renal failure, muscle injury, myocardial infarction and gastrointestinal bleeding were the most common complications associated with SARS-CoV.[16]

The goal of this review was to analyze and summarize the currently available literature on COVID-19 related blood/serum biomarkers because previous studies on SARS-CoV have demonstrated the importance of dynamic changes of various biomarkers and their predictive value in assessing disease severity and outcome. Recently published data on proteomic and metabolomic profiling of COVID-19 sera identified 93 proteins that showed specific modulation in severely ill patients, compared to non-severe and non-COVID-19 sera.[20] The majority of these proteins are associated with the complement system, macrophage function, and platelet degranulation.[20] Alterations in some of these proteins may prove as useful biomarkers which may aid the healthcare provider with rendering the initial diagnosis, predicting the result of SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) testing, staging and risk stratification of presenting patients, patient management, and also understanding the pathogenesis of COVID-19.

MATERIALS AND METHODS

Databases including PubMed, medRxiv and bioRxiv were searched for peer reviewed and non-peer reviewed papers. Search terminology included COVID-19 search terms (COVID-19, SARS-CoV-2, 2019-ncov) and laboratory parameters: white blood cell count (WBC), lymphocyte count, platelet/thrombocyte count, coagulation, prothrombin time (PT), D-dimer, fibrinogen, LDH, ferritin, CRP, SAA, procalcitonin, and interleukin-6 (IL-6).

A total of seventy-two papers including fifty-four peer reviewed papers and eighteen non-peer reviewed preprints with data including hematological, coagulation, biochemical, or inflammatory parameters and their role and predictive value in mild, severe, and critically ill patients were reviewed. Within the total reviewed papers, forty-three cohort studies and eleven systematic reviews (including meta-analysis) were extracted. Where applicable, statistical significance was represented in the literature by minimum p-values of $P \leq .05$. Relevant data were collected by two independent reviewers.

RESULTS

Clinical Characteristics And Risk Factors Associated with SARS-CoV-2 Infection

The majority of studies analyzed were performed on COVID-19 affected patients in China while the minority were from Italy, UK, Iran, Brazil, and USA. In some studies, the clinical staging of patients was generally categorized into four groups based on treatment guidelines issued by the Chinese National Health Committee.[21] Patients with uncomplicated illness demonstrated mild

clinical symptoms without radiologic findings of pneumonia while those with mild disease had fever, respiratory symptoms, and findings consistent with pneumonia on chest imaging studies. Severe disease was defined clinically as respiratory distress with respiratory rate ≥ 30/min, pulse oximeter oxygen saturation ≤ 93% at rest, and oxygenation index (artery partial pressure of oxygen/inspired oxygen fraction, PaO2/FiO2) ≤ 300 mmHg. Critically ill disease was defined as respiratory failure requiring mechanical ventilation, shock, or other organ failure requiring intensive care unit (ICU) monitoring.

Zhang et al. analyzed the clinical characteristics of 140 patients with an overall median age of 57 years.[6] No gender predominance was observed between severe and non-severe illness. Initial laboratory results on admission revealed normal WBC with decreased lymphocyte and eosinophil counts in most patients. Serial follow-up testing (starting at > 3 days) demonstrated further decreases in lymphocyte and eosinophil counts and these changes were positively correlated with disease severity. Significantly higher levels of D-dimer (>0.4 µg/mL; to convert to nmol/L multiply by 5.476), CRP (4.76 mg/dL), and procalcitonin (0.01 µg/dL) were associated with a severe disease course. Older age, multiple comorbidities, and prominent laboratory abnormalities were also associated with severe disease course. Allergic diseases, asthma, and chronic obstructive pulmonary disease (COPD) were not risk factors for SARS-CoV-2 infection in this study.

Other studies have demonstrated an association of severity, disease progression and adverse outcome with older individuals (> 60 years) with comorbidities who were predominantly males.[22–25] Hypertension [5,22,23,26], diabetes mellitus [5, 22,23,26], cardiovascular disease [5,22,23,26], and high body mass index (BMI)[24] were among the most commonly reported risk factors.

Inconsistent results were reported for COPD and asthma as risk factors.[6,24,27] Two studies analyzing the correlation between ABO blood group and clinical outcome suggested that blood group A individuals as well as those with rhesus antigen positivity were more susceptible to SARS-CoV-2 infection compared to blood groups O or AB.[28,29] However, blood groups are not among the risk factors for either disease severity or complications. The leading cause of death in SARS-CoV-2 infection were acute respiratory distress and respiratory failure, but systemic involvement with end organ damage including sepsis, thrombotic or hemorrhagic events, cardiac failure, liver failure, and renal failure also contributed to death.[5,22,23,30]

Concomitant Blood Test as Predictor of SARS-CoV-2 RT-PCR Results

 A few studies examined the presence of changes in blood tests found concurrently with initial positive SARS-CoV-2 RT-PCR results.[25,26] The majority of molecular diagnostic tests have utilized the RT-PCR targeting different SARS-CoV-2 genomic regions, including the ORF1b or ORF8 regions, and the nucleocapsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope (E) genes.[31]

An early study was performed on patients admitted to the emergency room in Italy.[25] Retrospective analysis was used to compare the results of routine blood tests in COVID-19 positive (n=105) and negative (n=102) patients where infection status was confirmed by molecular testing. On admission, samples from the RT-PCR positive group, which were predominantly male, showed significant differences ($P < .05$) in levels for WBC count (6.47 ± 2.61 x $10^3/\mu L$), CRP (8.71 ± 8.12 mg/dL), aspartate aminotransferase (AST; 56.2 ± 40.8 U/L; to convert to $\mu kat/L$ multiply by 0.0167), alanine aminotransferase (ALT; 47.9 ± 40.9 U/L; to convert to $\mu kat/L$ multiply by 0.0167), and lactate dehydrogenase (LDH) (388.0 ± 154.5 U/L).

No association was found between positive status and platelet count. The study demonstrated that routine blood tests had similar detection rates compared to RT-PCR. A positive predictive value (PPV) and negative predictive value (NPV) of 83.3% and 90.6%, respectively, were reported.

A similar study evaluated the accuracy of laboratory parameters in predicting cases with positive RT-PCR for COVID-19.[26] Based on the result of RT-PCR testing, patients were classified into positive (n=70) and negative groups (n=130). Mean patient age was 41.3 ± 14.6 years. No gender predominance was observed. Significantly lower WBC count (4.04 ± 1 x $10^3/ \mu L$), increased neutrophil count, hypoalbuminemia (2.9 ± 0.8 g/dL; to convert to g/L multiply by 10) as well as elevated levels of CRP, LDH (465.2 ± 100.2 U/L), AST (32.1 ± 8.01 U/L), and ALT (37.8 ± 7.9 U/L) were observed in the RT-PCR positive group compared with the negative group. Overall, neutrophil counts, CRP, LDH, and ALT had a good predictive value (Area Under the Curve (AUC) > 0.8) and were proposed as useful markers to predict the result of molecular testing.

Hematological Parameter Trends Including Lymphopenia, Increased Neutrophil Count, Increased Neutrophil-Lymphocyte Ratio, And Thrombocytopenia

Several clinical pathology studies have reported changes in hematological parameters (Table 1). A study of the viral genomes has suggested two major viral genetic variation of SARS-CoV-2, Clade I and Clade II.[32] Both strains showed similar virulence and clinical outcomes.[32] Decreased lymphocyte count on admission, especially CD4 and CD8 positive T lymphocyte, was the most important predictor of disease progression and outcome along with age.[32]

A large multi-centric, retrospective study including 548 confirmed COVID-19 patients demonstrated significantly different hematologic and immunologic parameters on admission and at endpoint between survivors and non-survivors.[33] The overall median age was 56 years with significantly older age for non-survivors. On admission, marked lymphopenia, decreased eosinophil count, increased neutrophil count, and thrombocytopenia were predominantly seen in severe and critical cases, and in non-survivors. High neutrophil-lymphocyte ratio (NLR) as inflammatory biomarker and indicator of systemic inflammation was also observed among those groups. Low CD3, CD4, and CD8 counts were seen in severe cases. During hospitalization, the longitudinal variation in levels of lymphocytes, eosinophils, and platelets showed an increasing trend in survivors and a downward trend in non-survivors. Additionally, non-survivors showed an upward trend of neutrophil counts.

The dynamic of hematologic parameters and coagulation factors were compared between mild and severe groups in one retrospective study.[34] The mean age of the cohorts (n=75) was 46.6 ± 14 years. On admission, significantly severe lymphopenia in addition to higher NLR, D-dimer, and fibrinogen levels was observed in the severe group compared to the mild group ($P < .05$). Serial measurements of NLR (on days 1, 4, and 14) and D-dimer levels (on days 1, 7, and 14) differed significantly between the two groups. Other study suggested that elevated NLR significantly associated with disease severity and was independent biomarkers for poor clinical outcomes.[35]

In summary, leukocytosis characterized by neutrophilia, lymphopenia with low CD3, CD4, and CD8 subset counts and/or percentages, and increased NLR were associated with severe disease in several studies (Table 1). Lymphopenia and clinically severe disease were the main risk factors contributing to longer hospitalization.[36] Thrombocytopenia was more likely to occur in severe or critically ill and fatal cases (Table 1). Other laboratory findings including decreased WBC count[25,26], decreased neutrophil count[25], decreased eosinophil count[3,6,25,33,37,38], and variable monocyte count[25,36,39] have been described among COVID-19 patients.

Coagulation Profile Derangements Including Prolonged Prothrombin Time, Increased D-Dimer, And Fibrinogen/ Fibrin Degradation Products

The major coagulation indices including PT, INR, D-dimer, or fibrinogen/fibrin degradation products (FDP) in SARS-CoV-2 infected patients were analyzed among the different disease severity groups and results were compared to healthy controls in several studies (Table 1). Coagulation abnormalities were examined in a group of 22 COVID-19 patients with acute respiratory failure requiring ICU admission.[40] Patients were predominantly male and 67 ± 8 years old. Significantly higher plasma fibrinogen (517 ± 148 mg/dL, to convert to g/L multiply by 0.01) and D-dimer (5.3 ± 2.1 µg/mL) levels were observed compared to healthy controls (n=44).

Similar results were reported in another retrospective study of 94 patients with confirmed SARS-CoV-2 infection.[41] Levels of D-dimer (10.36 ± 25.31 µg/mL), fibrinogen (502 ± 153 mg/dL), and FDP (33.83 ± 82.28 µg/mL; to convert to mg/L multiply by 1) were significantly higher in all SARS-CoV-2 cases than in healthy controls (n=40). Moreover, D-dimer (19.1 ± 35.5 µg/mL

vs. 2.1 ± 2.9 µg/mL) and FDP (60 ± 10.9 µg/mL vs. 7.9 ± 11.4 µg/mL) values were **higher in** severe cases compared with mild ones.

Few selected retrospective studies compared coagulation function in patients with mild, severe, and critical disease. One large retrospective study (n=380) demonstrated that PT, D-dimer, and FDP were significantly higher in critically ill patients compared to those with mild and severe disease, and their levels correlated positively with disease severity.[30] Sepsis-induced coagulopathy and disseminated intravascular coagulation (DIC) scores were increased over time during disease progression.[30] Another study suggested that coagulation parameters have good **predictive value and ability to discriminate** between mild, severe, and critical disease states (AUC > 0.8).[42]

In summary, significant coagulation abnormalities were observed in patients with SARS-CoV-2 compared to healthy controls and correlated positively with disease severity. Monitoring coagulation parameters may prove helpful for the early identification of severe cases.

Biochemical Parameters And Inflammatory Biomarkers Including CRP, Ferritin, SAA, Procalcitonin, Albumin/Prealbumin, And Pro-Inflammatory Cytokines

One of the earliest host responses to viral or bacterial infection is the activation of acute-phase reactants including CRP, ferritin, SAA, albumin/prealbumin, procalcitonin, erythrocyte sedimentation rate (ESR), and pro-inflammatory cytokines, among others. CRP, a member of the short pentraxins group, is a 25-kDa protein synthesized in the liver and known to interact

with complement factor c1q in the activation of the complement cascade.[43] Cytokines are mainly

secreted from hematopoietic cells such as lymphocytes and macrophages and may play an

important role in the clinical course of disease and outcome. During the acute phase of illness, a

dysregulation of inflammatory response may occur with massive release of cytokines (cytokine

release syndrome or cytokine storm) causing single or multiple organ damage.

The most commonly measured cytokines in patients with SARS-CoV-2 infection were IL-2/2R,

IL-6, IL-10, TNF-α, and Interferon-γ (Table 2). Several studies reported that IL-6 was the most

sensitive cytokine and may be used as a biomarker for evaluating prognosis.[3,22,33,44] An elevated

level of IL-6 (>10 pg/mL) was proposed as the significant driving force for the cytokine storm

and as contributing to the multiple organ failure seen in advanced cases.[45] An extremely high

IL-6 level (up to 100 pg/mL) was closely correlated with the incidence of detectable serum viral

particles by RT-PCR.[44] Other inflammatory markers including Interferon-γ inducible protein

(IP-10) and human monocyte chemotactic protein (MCP-1/MCP-3) were also significantly

increased in severe cases.[5,45] A study examining cerebrospinal fluid (CSF) cytokine levels in a

case series of 3 patients with respiratory and neurologic complications have reported markedly

increased levels of IL-6, interleukin-8 (IL-8), and IL-10.[46]

A comparison study between survivors and non-survivors showed an upward trend of acute

phase proteins including CRP, ferritin, SAA, procalcitonin, and cytokine IL-6 in non-survivors

and stable or downward trend in survivors.[33] Within the survivor group, significant differences in

CRP[3], ferritin[37], SAA[22,47–49], procalcitonin[47], and IL-6[37], were reported between mild, severe, and

critically ill patients. There was also a downward trend of prealbumin and albumin as the disease progressed from mild to severe or critical condition.[47] Increased ESR levels have also been reported.[50,51]

The inflammatory markers among mild, severe and critically-ill patient groups were analyzed to identify their correlation with disease progression.[37] Significant differences in serum CRP, ferritin, and procalcitonin were reported between the groups. Interleukin-2 receptor (IL-2R), IL-6, Interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α) levels were significantly lower in mild compared to severe and critically ill groups ($P < .001$). The study concluded that CRP > 3.07 mg/dL, ferritin > 2252 ng/mL (to convert to µg/L multiply by 1), IL-2R > 0.7935 U/L, and IL-6 < 100 pg/mL were associated with disease progression.

Several studies reported increased levels of SAA among patients with mild and severe disease.[22,47–49] Gradual increase of SAA was seen as the disease progressed from mild to severe.[49] A markedly increased SAA level (> 300 µg/mL; to convert to mg/L multiply by 1) was observed in severe and critically ill patients and positively correlated with disease severity.[22,47] Serial measurements of SAA may aid in monitoring the extent of pneumonia and the levels correlated well with the dynamic changes seen on serial computerized tomography (CT) scans.[49,52]

One study of 75 patients in Italy measured the level of Presepsin (PSP) in addition to routine laboratory tests.[53] PSP or soluble cluster of differentiation CD14-subtype (sCD14-ST) is a

regulatory factor that modulates immune responses by interacting with T and B cells and has been demonstrated to be a better marker for early diagnosis of sepsis compared to other sepsis markers in non-COVID19 patients.[54] PSP was significantly higher in ICU patients and fatal cases than non-ICU patients. High PSP level (>25 ng/dL) correlated with longer ICU stay, but the level correlated poorly with CRP, LDH, and procalcitonin levels measured on the same day. The study suggested that PSP may be used for risk stratification of SARS-CoV-2 patients and early identification of disease severity and longer hospitalization times.

In summary, commonly used inflammatory biomarkers such as CRP, ferritin, procalcitonin, SAA, and IL-6, and less commonly utilized, PSP, were significantly increased in patients suffering from COVID-19. The level of these biomarkers correlated with disease severity. Initial and serial measurement of these markers along with other parameters might aid in risk stratification and follow-up assessment of disease progression and improvement. At present, assays for SAA and PSP are not available in the United States but their implementation should be considered.

Other Biomarkers Including LDH, Creatine Kinase, AST, ALT, Blood Urea Nitrogen, And Creatinine

Significant elevations of LDH, creatine kinase (CK), liver enzymes (AST and ALT), total bilirubin, blood urea nitrogen (BUN) and creatinine were commonly reported in severe and critically ill patients (Table 2).

The liver biochemistry and its association with other biomarkers were analyzed in COVID-19 patients among different disease severities (n=60).[55] On admission, the majority of non-intubated patients (69%) demonstrated elevated AST (median=46 U/L) and ALT (median=30 U/L). The dynamics of AST levels correlated with the level of LDH, CK, and ferritin, but not with CRP. In critically ill and intubated patients, a marked AST predominant increase was observed (69 U/L and 364 U/L, respectively). No clear association was found between preexisting liver disease and clinical outcome of COVID-19 patients. Other study of the liver enzymes concluded that higher levels of bilirubin, AST, and gamma-glutamyl transferase (GGT) were predominantly observed in fatal cases.[56]

The clinical features and outcome of patients with high brain natriuretic peptide (BNP) levels (>100 pg/mL; to convert to ng/L multiply by 1×10^3) were analyzed and the findings compared with a group of patients with normal BNP levels.[57] The high BNP group demonstrated a higher CRP, AST, and c-Tn-I and were more likely to develop pneumonia requiring ICU admission.

Gong et al. reported that on admission, higher levels of serum LDH, CRP, direct bilirubin, and BUN, and lower albumin were found to be associated with higher odds for severe disease and disease progression.[58] Lastly, COVID-19 patients mounted with a marked acute cortisol stress response and the level is significantly higher compare to non-COVID-19 individuals, 22.44 µg/dL vs. 18.81 µg/dL (to convert to nmol/L multiply by 27.588), respectively.[59]

Significant Biomarkers As Predictors Of Disease Severity In Mild, Severe, And Critically Ill Cases

Several studies compared the hematological, biochemical, inflammatory, and coagulation

parameters in mild, severe, and critical disease and reported comparable findings.[8,60] However, in

some of the studies statistical analysis was limited due to unavailability of serial test specimens

in many patients, resulting in smaller total sample size. The median age in mild, severe, and

critical groups ranged from 50 to 51.4 years[8,60], 63.9 to 64 years[30,60], and 66 years[7], respectively.

Chen et al. reported that CRP, ferritin, LDH, and ALT were significantly higher in severe cases

compared to mild cases.[60] As the disease progressed from mild to severe or critical, a downward

trend for lymphocytes, prealbumin, and albumin was observed , whereas the opposite was seen

for WBC, neutrophil count, CRP, and LDH.[8,47] Other significant findings, including increased

SAA, NLR, PT, D-dimer, FDP, and inflammatory cytokines IL-2R, TNF-α, and IL-10 have been

reported.[30,47,60]

Association of serum viral load, disease severity and outcome was analyzed in several studies.
[44,51,61,62] In critically ill patients, serum viral load was correlated with extremely high levels of

IL-6 (up to 100 pg/mL) and higher mortality.[44] Prolonged viral shedding (up to 57 days) as

demonstrated by serial RT-PCR assay and anti-SARS-CoV-2 IgM, and higher levels of ESR,

CRP, ferritin, and IL-4 were observed among patients in the poor recovery group.[51] The risk of

intubation and in-hospital mortality increased significantly with high viral load on admission

compared to low or mild viral load.[61] Other reported laboratory characteristics of critically ill

patients requiring ICU admission were progressive decrease in lymphocyte count with worsening

lymphopenia, decreased eosinophil count, thrombocytopenia, elevated CRP, elevated

procalcitonin, increased IL-6 and IL-10, increased liver enzymes, increased total bilirubin,

decreased renal function, hypercoagulable state higher and higher incidence of complications.[3,7,44] In contrary to previously described findings, another study found no significant association between viral load, admission to ICU, and outcome.[62] Higher viral load was associated with shorter duration of symptoms and shorter hospital stay.[62]

In summary, progressive worsening of laboratory parameters including decreased lymphocyte count, and increased NLR, CRP, ferritin, IL-6, IL-10, certain coagulation parameters and serum viral load were observed as the disease progress. However, lymphocytopenia and age appeared to be the most important determinant of disease severity.[32]

Significant Biomarkers As Predictors Of Case Mortality

Retrospective studies analyzing the non-survivor/fatal cases demonstrated a median patient age from 71 to 72.5 years.[22,23] An unfavorable prognosis was observed predominantly in male patients and those with underlying medical conditions including diabetes mellitus, hypertension, and cardiac disease.[22]

On admission, the majority of the patients demonstrated lymphopenia, neutrophilia, thrombocytopenia, prolonged PT, elevated D-dimer, high lactate level, lower oxygen saturation, high NLR (>5.0) and systemic immune-inflammation (SII) index (>500).[22,23] All patients had high initial CRP and IL-6 (>10 pg/mL) levels, and a large subset of patients had elevated LDH, D-dimer, procalcitonin and c-Tn-I levels. These groups were reportedly more likely to develop sepsis, rapid disease progression, and early death (within 3 days of admission).[23] Laboratory results obtained 24 hours prior to death showed elevated serum AST, ALT, CK-MB, myoglobin,

BUN and creatinine levels.[22] In this study, the leading cause of death was respiratory failure followed by sepsis and multiple organ failure.[22]

Furthermore, a study by McRae et al. analyzed the COVID-19 Severity Score and clinical decision support tools to predict mortality.[63] It combined measurements of multiple biomarkers: CRP, N-terminus pro-B type natriuretic peptide (NT-pro-BNP), myoglobin, D-dimer, procalcitonin, CK, and c-Tn-I, and risk factors in a statistical learning algorithm to predict mortality. The analysis found that COVID-19 Severity Scores were significantly higher for the group that died versus the group that was discharged with median (interquartile range) scores of 59 (40–83) and 9 (6–17), respectively. These encouraging results have the potential to empower healthcare sustenance to save lives by prioritizing critical care for patients at risk of adverse outcomes.

Summary Of Selected Systematic Reviews With Or Without Meta-Analysis

Several systematic reviews with or without meta-analysis of hematologic, biochemical, and inflammatory marker abnormalities associated with disease severity were also reviewed and the results are summarized in Table 3. Lymphopenia[24,64–69], increased neutrophil count[67,69], leukocytosis[24], and thrombocytopenia[64,66,69] were the most common significant hematological findings. One study demonstrated normal WBC count accompanied by lymphopenia.[70] Prolonged PT[41,71] or elevated D-dimer/ FDP[67–69,71] represented derangements in the coagulation profile. Elevated inflammatory markers – CRP[24,65,66,68,69,72,73], ferritin[64,72,73], procalcitonin[24,66], SAA[69,72], and IL-6[64,67,69,72] and other biomarkers – LDH[24,66–69], CK[24] and CK-MB[67],

AST/ALT[24,41,66,67], bilirubin[24,74], BUN[67,69], and creatinine[67,69] - were also among significant findings. Patients with severe and fatal disease had significantly higher WBC and lower lymphocyte and platelet counts compared to non-severe disease or survivors.[64] Thrombocytopenia and elevated levels of procalcitonin and c-Tn-I were associated with severe disease.[66] In severe and fatal disease, biomarkers of inflammation, cardiac and muscle injury, renal and liver function, and coagulation parameters were significantly elevated.[64] Additionally, IL-6, IL-10, and serum ferritin were strong discriminators for severe disease.[64]

DISCUSSION AND CONCLUSIONS

A great proportion of COVID-19 studies concluded risk factors contributing to severe disease and adverse outcomes included advanced age (> 60 years) and male gender with comorbidities such as hypertension, diabetes mellitus, and cardiovascular disease. The most common initial clinical symptoms were fever, cough, dyspnea, and fatigue.[4–8] However, it is unclear whether the symptoms will become more insidious as the pandemic progresses and gradually evolve into a virus similar to influenza or remain latent in humans for a long period of time, as suggested by Chen et al.[48]

Wide ranges of laboratory abnormalities were reported with different disease severity but marked changes were more commonly seen in samples from severe and critically ill patients.[24] Hematological parameters including lymphopenia, leukocytosis with increased neutrophil count, increased NLR, and thrombocytopenia were the most common findings observed and positively correlated with disease severity.[22,30,47] A strikingly decreased lymphocyte count was associated with severe disease and higher complication rate. The decreases in both CD4 and CD8 T-

lymphocytes are best explained by the roles these T-lymphocyte subsets play in eliminating virus-infected cells and this is consistent with low lymphocyte counts being associated with poor case outcomes.[32,64]

An upward trend of CRP, ferritin, SAA, procalcitonin, and the most prominent cytokine, IL-6, and a downward trend of albumin and/or prealbumin were frequently observed during progression from mild to severe/critical condition, and in non-survivors. Serial measurements of these markers can be utilized to predict disease course, severity, and mortality.[22,37,47] It has been postulated that SARS-CoV-2 may target alveolar macrophages via the angiotensin converting enzyme 2 (ACE2) receptor, leading to increase in cytokines secretion including IL-6 and TNF-a, which subsequently induce the elevation of various APPs such as CRP, SAA, and complement factor which are significantly upregulated in the severely ill group. Changes in coagulation parameters including prolonged PT, elevated D-dimer, and elevated fibrinogen or FDP were common findings in severe disease and non-survivors.[4,30] Prolonged prothrombin time and higher serum D-dimer levels were postulated to demonstrate a hypercoagulable state rather than consumptive coagulopathy. It was proposed that hyperfibrinogenemia leads to fibrin polymerization, thrombus formation, and eventually complications or adverse outcome.[40] Other biomarkers such as LDH, CK, BNP, AST, and ALT have been associated in several studies with severe and critically ill disease and their levels likely indicate adverse outcome.[24,55,56] AST-dominant elevations were common in COVID-19 patients and appeared to reflect true hepatic injury.[55] Additionally, AST level correlated with markers for muscle injury including LDH and CK.[55]

To date, molecular testing identifying viral particles on nasopharyngeal specimens by RT-PCR remains the gold standard in the diagnosis of SARS-CoV-2 infection. Concurrent antibody testing can aid in increasing detection sensitivity.[75] Other specimen sources, such as self-collected saliva, which is less painful and does not require trained personnel for collection, should be considered as preferable alternatives for SARS-CoV-2 screening of healthcare workers and asymptomatic cases.[11,76] The sensitivity of SARS-CoV-2 detection from saliva samples was demonstrated to be comparable to that of nasopharyngeal swabs in early hospitalization.[76] The results were more consistent during extended hospitalization and recovery, most likely due to less temporal SARS-CoV-2 variability.[76] Routine blood tests may also be utilized as early predictors of the molecular testing result. They can serve as an alternative for identifying SARS-CoV-2 infection in countries with heavy outbreaks and shortage of RT-PCR reagents or specialized labs, as they have been shown to have detection rates comparable with molecular tests.[25] Moreover, early recognition of severe disease or disease that is likely to progress is absolutely essential for timely triage of patients. Measurement of soluble PSP or sCD14-ST in peripheral blood may be utilized for early diagnosis of sepsis and for risk stratification.[53] The use proteomic approaches and non-traditional samples like CSF may identify additional biomarkers that may be helpful in the pathophysiology and prognosis of COVID-19 disease.[20,46]

Interpretation of the results of several studies presented in this review is limited due to predominantly retrospective study designs, small sample sizes, multiple sampling biases (i.e. the majority were single center studies with cohorts from east Asian ethnic groups), lack of uniformity of disease severity definition based on variable RT-PCR methods, lack of exact timeline of laboratory sample collection, as well as lack of serial sample measurements. This

information is important as a defined timeline of collection and serial sampling performance may aid in clinical decision-making during the acute phase of disease. In addition, a great proportion of studies were cut short and failed to report final outcomes due to the need for prompt data publishing during the current pandemic. Overall, the reader should be cautioned when viewing *medRxiv* papers presented prior to peer review. It is recommended that updated peer-reviewed published versions of these original studies are evaluated when/if available.

The dynamic changes in biomarker levels may assist in predicting disease course, prognosis and outcome. Indicators of systemic inflammation such as NLR and SII index or coagulopathy screening using DIC scoring system could be appropriately utilized to predict disease severity, possible complications, and outcome. Finally, COVID-19 Severity Score and clinical decision support tools can additionally be utilized using combined measurements of multiple biomarkers to predict mortality. In summary, WBC, lymphocyte, and platelet counts, CRP, ferritin, and IL-6 may be potential prognosticators of progression to critical illness. Therefore, prospective studies with larger cohorts, clearly defined disease severity, and serial measurements with defined sampling collection timelines are imperative to further confirming the correlation and significance of the current findings.

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Table 1. Summary of Studies with Reported Changes in Hematology and Coagulation Parameters/Biomarkers

Parameter/Biomarker Changes	Citations	Study Categories/Experimental Design
Hematology		
WBC - variable, mostly ↑	3,5,7,23,25,26,30,34,36-38,47,50,77–80	Small cohort size n<40 (refs 3,7,39,57,60)
↓ Lymphocyte count and/or %	3,5,6,8,22,23,25,26,30,32–38,47–51,53,58,60,77–81	Multicenter study (refs 8,33,58,59)
↓CD3+/CD4+/CD8+ T-lymphocyte count and/or %	22,23,32,33,45,51,60,77,78	Compare non-severe and severe disease only (refs 5,6,8,34–36,58,60,79)
Neutrophil count and/or %: mostly ↑, rarely ↓	5,7,22,23,25,26,30,33-35,36–38,47,58,59,73,77–79,80	Compare mild or moderate, severe, and/or critical cases (refs 3,30,32,33,37,38,42,45,47,49,80)
↑NLR	22,30,34,35,38,51,58,73,79	Assessment of fatal cases (refs 22,23,42,59,77,79)
Monocyte count and/or %: variable	25,36,39	Control or non-COVID-19 group present (refs 25,26,38,42,45,50,59,78)
↓ Eosinophil count and/or %	3,6,25,30,33,37,38	Lack of clear clinical severity definitions or comparisons (refs 22,25,26,30,39,48,50,51,53,57,59,77,78)

Table 1. (cont.) Summary of Studies with Reported Hematology and Coagulation Parameters/Biomarker Changes

↓ Platelet count	3,8,22,30,33,42,57,77–79	Serial sampling (>2 samples) performed: (refs 22,30,33,34,38,45,49)
		Comorbidities/risk factor data unclear or unavailable (refs 25,26,37–39,42,48,49,53,58,78,80)

Coagulation

↑ PT	23,30,42,47	Small cohort size n< 40 (refs 3,40)
↑ D-dimer	3,5,6,8,22,23,30,32–34,40–42,47,51,59,79	Multicenter study (refs 8,33,59)
↑ Fibrinogen and/or FDP	3,8,30,34,40–42,50,51,82	Compare non-severe and severe disease only (refs 5,6,8,34,79)
		Compare mild or moderate, severe, and/or critical (ICU) cases (refs 3,30,32,33,41,42,47)
		Assessment of fatal cases (refs 22,23,42,59,79)
		Control or non-COVID-19 group present (refs 40–42,50,59,79)
		Lack of clear clinical severity definitions or comparisons (refs 22,40,50–52)
		Clarified final outcome for all subjects (discharged or deceased) (ref 33)

Table 1. (cont.) Summary of Studies with Reported Hematology and Coagulation Parameters/Biomarker Changes

	Serial sampling (> 2 samples) performed (refs 22,33,34)
	Comorbidities/risk factor data unclear or unavailable (refs 40–42)

Abbreviations: ↑, increased; ↓, decreased; FDP, fibrin degradation products; ICU, intensive care unit, NLR, neutrophil-lymphocyte ratio; PT, prothrombin time; WBC, white blood cell count

Table 2. Summary of Studies with Reported Changes in Inflammatory and Biochemical Parameters/Biomarkers

Parameter/Biomarker Changes	Citations	Study Categories/Experimental Design
Inflammatory		
↑ CRP	3,5,8,22,23,25,26,32,34,36,37,44,47,49,50,51, 53,55–60,63,73,33,77–82	Small cohort size n<40 (refs 3,7,57,60,83)
↑ Ferritin	30,33,37,60,73	Multicenter study (refs 8,33,58,59)
↑ SAA	6,33,47–49,58,82,83	Compare non-severe and severe disease only (refs 5–8,34,36,56,58,60,79)
↑ Procalcitonin	5,6,8,22,23,33,36,37,47,53,58,63,79	Compare mild or moderate, severe, and/or critical (ICU) cases (refs 3,32,33,37,44,45,47,49,80)
↓ Albumin and/or prealbumin	3,8,22,23,26,47,51,56,58,73,82	Assessment of fatal cases (refs 22,23,45,56,59,63,77,79)
↑ ESR	50,51	Control or non-COVID-19 group present (refs 25,26,45,59,78,82)
↑ PSP or sCD14-ST	53	Lack of clear clinical severity definitions or comparisons (refs 22,25,26,48,53,55,57,59,77,78,83)
↑ Il-2 and/or IL2R	5,37,51,60	

Table 2. (cont.) Summary of Studies with Reported Changes in Inflammatory and Biochemical Parameters/Biomarkers

↑ IL-6	3,5,7,22,32,33,37,44,45,51	Clarified final outcome for all subjects (deceased or discharged)
↑ IL-8	32,37	(refs 33,53,63)
↑ IL-10	5,7,30,37,51,60	Serial sampling (>2 samples) performed (refs 22,33,34,45,49,55,56,63)
↑ TNF-α	5,37,60	
		Comorbidities/risk factor data unclear or unavailable
↓ IFN-γ	60	(refs 25,26,37,48,49,53,56,58,63,78,80)
↑ IP-10 and MCP	5,45	

Other Biomarkers

↑ LDH	5,22,25,26,30,32,47,50,51,58,60,78,80,81	Small cohort size (<40) (refs 3,57,60)
↑ CK and/or CK-MB	6,23,36,58,63,80	Multicenter study (refs 8,58,59)
↑c-Tn-I	22,23,36,44,51,57,63	Compare non-severe and severe disease only (refs 5,6,8,36,56,58,60)
↑ BNP	23,57	
		Compare mild, severe, and/or critical cases (refs 3,32,44,47,58,80)
↑ Myoglobin	22,23,36,57,63	
↑ Bilirubin (total and/or direct)	5,22,58,81	Assessment of fatal cases (refs 22,23,56,59,63)
↑ BUN	3,22,26,47,58	
		Control or non-COVID-19 group present (refs 25,26,50,59,78)
↑ Creatinine	3,22,59,73	

Table 2. (cont.) Summary of Studies with Reported Changes in Inflammatory and Biochemical Parameters/Biomarkers		
↑ Cortisol	59	Lack of clear clinical severity definitions/comparisons (refs 22,25,26,50,51,55,57,59,78)
		Clarified final outcome for all subjects (deceased or discharged) (refs 63)
		Serial sampling (> 2 samples) performed (refs 22,55,56,63)
		Comorbidities/risk factor data unclear or unavailable (refs 25,26,56,58,63,78,80)

Abbreviations: ↑, increased; ↓, decreased; BUN, blood urea nitrogen; BNP, brain natriuretic peptide; CK, creatine kinase; CK-MB, creatine-kinase-MB; CRP, C-reactive protein; C-Tn-I, cardiac troponin I; IFN-γ, interferon-gamma; IL-2, interleukin-2; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IP-10, interferon-γ inducible protein 10; ICU, intensive care unit; LDH, lactate dehydrogenase; MCP, human monocyte chemotactic protein; ESR, erythrocyte sedimentation rate; PSP, presepsin; SAA, serum amyloid A; sCD14-ST, soluble cluster of differentiation CD14-subtype; TNF-α, tumor necrosis factor-alpha

Table 3. Summary of 10 COVID-19 Systematic Reviews with or without Meta-Analysis of Reported Findings

Number of Studies (Sample size)	Major Findings	Comment
72 (n=3470) Reference 65 Publ 3/12/20	↓ Lymphocytes in 62.8% ↑ CRP in 64.8% Fever and abnormal CT findings in 83.0% and 88.2%, respectively ICU admission and CFR were 11.5% and 3.7%, respectively	Systematic review; early study with most data report from Wuhan, China No severity analogy
20 (n=4062) reference 24 Publ 4/1/20	Severe cases: Male>Female, older age, ↑BMI, and comorbidities ↓ Lymphocytes ↑ WBC, CRP, LDH, procalcitonin, CK, total bilirubin, AST, and ALT	One of the earliest systematic reviews with meta-analysis of severity risk factors in China
21 (n=3377) reference 64 Publ 4/10/20	Severe and fatal cases (*P*≤.05): ↓ Lymphocytes, eosinophil count, platelets, and albumin ↑ WBC (including neutrophils), CPR, ESR, IL-2R, IL-6, D-dimer, LDH, ferritin, procalcitonin, CK, CK-MB, AST/ALT, and creatinine	Systematic review with meta-analysis of 18 cohorts compared severe vs. non-severe, while 3 compared survivors vs. non-survivors All studies were from Asia/China

Table 3 (cont). Summary of 10 COVID-19 Systematic Reviews with or without Meta-Analysis of Reported Findings

16 (n=3962) reference 72 Publ 4/17/20	Severe cases ($P \leq .05$): ↑ ESR, CRP, IL-6, ferritin, procalcitonin, and SAA Survivors had ↓ IL-6 vs. non-survivors	Systematic review with meta-analysis of inflammatory biomarkers associated with severity Most studies were from China
60 (n=59254) reference 66 Publ 4/22/20	↓ Lymphocytes (0.93 x 10^3/μl) and ↑ CRP (33.72 mg/dL) Severity/mortality correlated with ↑ age (>60) and male gender Fever found in 82-95%	A scoping review with meta-analysis with data from 1/1/20 to 2/24/20; 56 of 60 studies had confirmatory RT-PCR and limited laboratory data Not all studies had clinical data, laboratory findings, or severity analysis
11 (n=2437) reference 68 Publ 4/24/20	Severe cases ($P<.001$): ↓ Lymphocytes ↑ D-dimer, CRP, LDH	Systematic review with meta-analysis of biomarkers associated with severity
45 (n>5000) reference 67 Publ 4/29/20	Severe cases: ↓ Lymphocytes (mainly CD4) and albumin ↑ WBC (including neutrophils), NLR, ESR, IL-6, CRP, D-dimer, LDH, ferritin, CK-MB, BNP, BUN, creatinine, AST/ALT	Systematic review with meta-analysis of 45 studies from 6 countries (87% China) Comprehensive assessment of different laboratory biomarkers and association with severity

Table 3 (cont). Summary of 10 COVID-19 Systematic Reviews with or without Meta-Analysis of Reported Findings

18 (n=2862) reference 74 Publ 5/1/20	Fatal cases: ↑PT/INR, AST/ALT (mean 30.9U/L and 45.3U/L, respectively) and total bilirubin ↓ Albumin Advanced age correlated with fatal outcome	Systematic review with meta-analysis of liver function (survivors vs. non-survivors) All studies from China All patients were hospitalized and/or critically ill (pooled fatal cases 31.6%)
12 (n=772) reference 70 Publ 5/2/20	↓ Lymphocytes (mean 1.01 x 10^3/µl) ↑ CRP and NLR	Systematic review with meta-analysis of inflammatory biomarkers All studies from China; no severity analogy
34 (n>7000) reference 69 Publ 5/13/20	Severe cases: ↓ Lymphocytes and platelets ↑ Neutrophil count, NLR, IL-6, CRP, D-dimer, LDH, C-Tn-I, SAA, BUN, and creatinine Increased CRP strongly correlated with adverse outcome	Systematic review of biomarker correlation with severity and outcome
11 (n>2000) reference 71	↑ D-Dimer/FDP correlated with severity and prognosis Fibrinogen also significantly altered	Focused review of coagulation function alterations and severity

Table 3 (cont). Summary of 10 COVID-19 Systematic Reviews with or without Meta-Analysis of Reported Findings		
Publ 6/20	DIC may play role in advanced cases	All studies from China
	Anticoagulant therapy associated with better prognosis	

Abbreviations: ↑, increased; ↓, decreased; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; CFR, case fatality rate; CK, creatine kinase; CK-MB, creatine-kinase-MB; CRP, C-reactive protein; CT, computed tomography; ; C-Tn-I, cardiac troponin I; ESR, erythrocyte sedimentation rate; ICU, intensive care unit; IL-2R, interleukin-2 receptor; IL-6, interleukin-6; LDH, lactate dehydrogenase; NLR, neutrophil-lymphocyte ratio; PT/INR, prothrombin time/international normalized ratio; SAA, serum amyloid A; DIC, disseminated intravascular coagulation; WBC, white blood cell count; RT-PCR, reverse transcription polymerase chain reaction; FDP, fibrin degradation products.