Clinical Research Development Center
Ghaem Hospital
*Corresponding author: Nasrin Milani.
Department of Internal Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail: nasrinmilani@gmail.com
Tel: 09155023323
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Introduction
In December 2019, numerous patients with clinical presentations such as respiratory symptoms were reported in Wuhan, Hubei, China (1). Clinical features of patients resembled previous viral respiratory infections like Middle-east respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) (2,3). Middle-East respiratory syndrome coronavirus (MERS-CoV) and Severe acute respiratory syndrome coronavirus (SARS-CoV) (4-6) lead to the death of many infected patients (7-10). Since the declaration of the outbreak by World Health Organization in January 2020 and recognizing that as a pandemic in March 2020, the COVID-19...
virus has caused severe acute respiratory syndrome (SARS-CoV-2) in millions of people across the world leading to the death of 1.9 million people as of January 2021. Coronavirus categorizes as the Coronaviridae family (11). Symptoms of most patients included fever, dyspnea, dry cough, and lung opacities in computed tomography (CT) images (12,13). Many hospitalized patients develop severe conditions and often death (13), and the broad majority showed abnormal laboratory results. Studies on SARS patients showed that the increase of cytokines resembled the inflammatory responses, such as IL6, IFNy, IL1B, MCP1, IP10, and IL12 (14).

In MERS-CoV infection, the increase of IL15, IL17, TNFα, and IFNγ cytokines has been observed (15). Previous studies reported that COVID-19 patients showed activated T- helper and a high amount of IP10, MCP1, IFNy, and IL1B (16). Individuals admitted to the Intensive Care Unit (ICU) revealed greater levels of TNFα, MIP1A, MCP1, IP10, and GCSF, compared to non-ICU patients, recommending that severe cases were accompanied by cytokine storm (1).

Studies proved a correlation between cytokine storm and the severity of infections (17,18). This review examines the immune responses in COVID-19 patients, reviews the role of pro-inflammatory factors in confirmed COVID-19 cases, and introduces possible components to predict the prognosis of disease with infection and cytokine storm. This review also defines cytokine storm and similar conditions like macrophage activation syndrome (MAS) and hemophagocytic lymphohistiocytosis (HLH), explaining the importance of cytokine storm in severe COVID-19 cases, and also examines similar conditions where cytokine storm plays an important role in mortality.

This article also points out the potential design to identify and diagnose severe cases beforehand, in order to anticipate and block the cytokine storm. By having laboratory data from severe COVID-19 patients, we will be able to diagnose the severe cases in early stages, and reduce the mortality rate and morbidity, and lower the chance of progressing to severe conditions.

**Literature review**

**Evidence Acquisition**

Databases such as Scopus, Google Scholar, MEDLINE, PubMed, and Science Direct were explored and keywords such as “COVID-19”, “coronavirus”, “cytokine storm”, “prognostic factor” were used. Inclusion criteria included English studies on humans, published from 1953 to 2020, and the associated references.

About 52 studies were explored in this search, and 29 studies were excluded because of non-related methods. Twenty-three studies were included in this study, such as studies performed in China, Italy, and Iran, with confirmed COVID-19 tests. Studies used different sample sizes, ranging from 5 to 1099 cases. The study design included prospective and retrospective methods. All the studies were conducted over 3 months of the COVID-19 pandemic (December 2019 to February 2020).

1. **COVID-19 Immune Response**

COVID-19 patients showed different clinical presentations, most patients remain asymptomatic and some developed severe conditions. Patients present with a wide range of symptoms from cough to hospitalization and ICU admission (17,19). However, studies showed that severe condition depends on criteria such as age and co-morbidity (20). The COVID-19 pathologic mechanism is not completely understood. However, studies showed a correlation between the increase in pro-inflammatory cytokines and lung damage, as seen in pulmonary inflammation in SARS (20) and MERS-CoV infection (21), and recently in COVID-19 (1).

Among severe COVID-19 infections, the number of patients showing laboratory abnormalities remains unclear and only the most constant abnormalities among non-severe patients are an increase of D-dimer, lactate dehydrogenase (LDH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) (22). Given the physiopathology of the COVID-19 and immune system hyperactivity, we started to investigate the background pathology of these circumstances, aiming to find the prognostic laboratory factors in COVID-19, as these circumstances and cytokine storm have been seen in HLH.

2. **Cytokine storm and HLH**

Cytokine storm is defined as an uncontrolled immune system and a hyper-response reaction (23). Cytokine storm first was mentioned in 1993 on graft-versus-host disease (24). Reviews focused on potential cellular and molecular mechanisms, leading to the cytokine storm in viral diseases (25, 26), such as cytomegalovirus (27), Epstein-Barr virus-associated HLH (28) infections, influenza virus (29), and SARS-CoV (23, 30). Infection with SARS-CoV, influenza virus infections and COVID-19 induces cytokine storm and severe condition named acute lung injury (ALI) (31-33). Cytokine storm components contain interferons (IFNs) (34, 35) interleukins (ILs) (36), IgM antibody, CD4 T cells (37), and tumor necrosis factor (TNF) (38).
HLH characterizes severe clinical syndrome and inflammation as a result of an unrestrained immune response (39). HLH classifies as primary (familial) and secondary (acquired) (40). Acquired HLH forms in adults because of infection, autoimmune diseases, and malignancy (41). This excessive inflammatory response and hypersecretion of cytokines result in a “cytokine storm” (42). The prognosis of HLH is poor and in untreated circumstances, the median survival of less than 2 years is estimated (43).

3. HLH in COVID-19 and similar conditions

As discussed earlier, the mechanism of mortality of severe cases believed to be immune response hyper-reactivity, and also secondary HLH in COVID-19 patients. Wu et al. confirmed the idea of hemophagocytic lymphohistiocytosis state (shHLH), developed in severe COVID-19 patients, and also stated that elevated serum ferritin, high levels of LDH, high CRP, and elevated coagulation function (prothrombin time (PT) and D-dimer) are associated with progression to acute respiratory distress syndrome (ARDS) (44).

Many studies also suggest secondary HLH in autoimmune diseases (45-48). A study demonstrated that auto-inflammatory diseases like adult-onset Still’s, rheumatoid arthritis, and systemic juvenile idiopathic arthritis (SJIA) develop secondary HLH and macrophage activation syndrome (MAS). The majority of laboratory findings reported hyper-ferritinemia, high CRP, and decrease in platelet, and also high levels of triglyceride (47,49-57).

4. Laboratory criteria for hyper-inflammation in COVID-19

COVID-19 outbreak in December 2019 infected many patients every day all across the world and became pandemic in 2020 (51). Most of the critical patients are elderly with comorbidities (51, 58), and severe COVID-19 patients developed cytokine storm (59), secondary HLH, and hyper-cytokinemia (59); however, in adults, secondary HLH mostly triggered due to viral infection (48).

Studies showed that in COVID-19 patients, cytokine storms happened in response to infection (48). Additionally, multiple studies stated that laboratory findings in COVID-19 patients include elevation of liver enzymes, CRP, ferritin, D-dimers, coagulation parameters like prothrombin time (PT), partial thromboplastin time (PTT), and LDH (9, 58, 60). Thus, by early detection of these laboratory parameters, severe cases could be detected in early stages, and the progression to poor outcome and occurrence of the cytokine storm could be prevented. For instance, one study related to the hyper-inflammation state suggests the early detection of serum ferritin as a biomarker (61-64). Several studies approved laboratory parameters in COVID-19 cases. One study performed in New York on 408 COVID-19 positive, African-American patients demonstrated that elevated serum ferritin, CRP, and D-dimer as independent predictors for mortality (65).

One study on COVID-19 cases in Jinyintan Hospitals located in Wuhan, China approved that patients who died showed high inflammatory biomarkers in the serum such as IL-6, procalcitonin, ferritin, and CRP. In addition to these findings, the study expressed that the virus also activates inflammatory mediators that can cause vascular endothelium damage and lead to thrombosis (66). Siddiqi et al. determined that severe cases present hyper-inflammatory conditions, in which inflammatory markers seemed to be elevated (67); markers such as D-dimer, ferritin, CRP, and TNF elevated undoubtedly in severe cases (44). One study also stated the presence of ESR in addition to D-dimer and ferritin elevation in COVID-19 patients, which was performed in non-severe COVID-19 patients, and reported an increase of D-dimer, LDH, ESR, and CRP (22). Studies also showed leukopenia, lymphopenia, and thrombocytopenia in addition to CRP, serum LDH and D-dimer in COVID-19 patients. A single-center, non-interventional cohort study that enrolled 49 confirmed COVID-19 patients in the center of Beijing, divided patients into two groups of severe and non-severe and showed that age, comorbidity, serum ferritin, lymphocyte counts, serum LDH and D-dimer differ significantly between the two groups, and severe cases display more laboratory abnormalities like leukopenia, lymphopenia, thrombocytopenia, and elevated CRP levels. However, in this study, serum ferritin remained undefined (68). Studies magnify the role of D-dimer and the effect of D-dimer as an inflammatory component in severe cases. Huang et al. evaluated 41 hospitalized patients with confirmed COVID-19 and stated that D-dimer elevated five times higher in severe cases, compared with non-severe cases (1). In addition to serum ferritin and D-dimer, studies showed that an increase of cardiac troponin I and IL-6 were also seen in COVID-19 patients. A retrospective study on 191 confirmed COVID-19 cases, located in Wuhan Pulmonary Hospital showed an increase in D-dimer and cardiac troponin I, and also significantly high IL-6, LDH, and serum ferritin levels were observed among dead cases (69). Studies also showed end-organ damage in COVID-19 patients and elevation of laboratory parameters. One study reviewed COVID-19 severe cases and suggested elevation of alanine amino-
transferrin (ALT) and aspartate aminotransferase (AST), in addition to serum D-dimer levels in severe COVID-19 patients with end-organ damage (70). Studies also determined the appearance of embolism in COVID-19 hospitalized patients. One study analyzed the correlation between embolism and elevated D-dimer in COVID-19 patients. The study enrolled 25 patients for testing D-dimer and pulmonary embolism by CT pulmonary angiography (CTPA), and demonstrated an increase of D-dimer level in all 25 patients, and acute pulmonary embolism (APE) was detected in 10 patients, according to CTPA images and D-dimer levels; fifteen patients displayed APE negative and D-dimer levels with a median value (71). One study published in December 2020, developed criteria for COVID-19-associated hyperinflammatory syndrome, they included fever, hyperferritinaemia, neutrophil to lymphocyte ratio as hematologic dysfunction, LDH or aspartate aminotransferase for hepatic injury, D-dimer for coagulopathy and CRP, interleukin-6, or triglycerides for cytokinemia, and evaluated the patients based on the mentioned criteria and suggested that the proposed criteria are associated with death and progression to a mechanical ventilator (72).

A study on 183 cases demonstrated that patients infected with COVID-19 showed changes in coagulation parameters, including D-dimer, PT, PTT, fibrinogen, and anti-thrombin. Out of 183 COVID-19 patients, 84 cases remained in the hospital and 21 cases died. In this analysis, 15 patients out of 21 dead showed overt disseminated intravascular coagulation (DIC), in 4 days (median time) after admission, ranging from 1 to 12 days. All patients also showed decreased fibrinogen and increased D-dimer and PT, with no evidence of sepsis (73). Also, additional study by Ranucci et al. (74) reported coagulation states in confirmed COVID-19 patients and evaluated the status of coagulation in COVID-19 cases with ARDS. They found that platelet count and international normalized ratio (INR) are non-correlated, but a significant correlation was found between abnormal D-dimers, fibrinogen, and activated partial thromboplastin time (aPTT). At the end claimed that 100% of patients showed a relation with high fibrinogen level. In a study, the blood coagulation tests of 94 confirmed COVID-19 patients located in Wuhan and 40 control cases were examined in the same period. The study showed a higher level of D-dimer and fibrin/fibrinogen in SARS-CoV-2 cases, compared with those in healthy control and also declared higher D-dimer, in comparison with patients with milder symptoms (75). Another study showed fibrinogen changes in COVID-19 patients. The 22 cases of COVID-19 patients admitted to ICU were tested for coagulation abnormalities. The study showed significantly high D-dimer and fibrinogen levels in plasma in infected patients, compared with healthy controls. The result marked a hypercoagulable condition in COVID-19 patients. The study stated that COVID-19 patients that develop acute respiratory failure showed an increase of coagulation state, and resulted in hyper-coagulation. Fibrin formation and polymerization tend to be correlated with a poor outcome (76).

5. Other pro-inflammatory factors

Recently studies on COVID-19 patients, especially critical patients showed elevated IL6 and more importantly claimed that inhibition of Janus kinase (JAK) resulted in the cellular entry of the virus, and inflammation in COVID-19 JAK-STAT transforms exogenous signals in the immune system and inhibits and blocks cytokine release in inflammatory diseases such as rheumatoid arthritis (77-79). A study on COVID-19, reviewing 89 related studies, demonstrated the role of IL6 and JAK, and also stated the role of JAK inhibitors (80). Studies also insisted on drugs, which act on JAK signaling and block the inflammation response.

Recent studies on COVID-19 cytokine storm showed elevation of G-CSF, IL-17, IL-7, IL-10, IL-9, IL-8, IL-2, IL-1β, interferon-gamma (IFNγ), granulocyte colony-stimulating factor (GM-CSF), TNFα, interferon-inducible protein (IP10), and monocyte chemotactic protein 1 (MCP1) in patients, especially ICU-admitted patients (1). On the other hand, an increase of IL-1β, TNFα, and T-helper 17 (TH17) was shown to correlate with vascular permeability and leakage (81). Studies showed that drugs are able to block Jak2 and Jak1 by inhibiting IL-6, IFN-β signaling (82); therefore, these mechanisms are responsible for cytokine storm (Table 1).

Conclusion

As discussed earlier, the major cause of COVID-19 in the critical patients was cytokine storm; therefore prognostic factors in cytokine storm also lead us to predict the prognosis in COVID-19 patients. Michel et al. investigated that individual response to inflammation is different and it can be measured by inflammation markers such as CRP; thus it can be utilized for prediction of prognosis of inflammation (83). Increased CRP level, elevated LDH, serum ferritin and creatine kinase (CK), higher D-dimer and FDP, IL-6, cardiac troponin I levels, and longer PT, are potential markers for the prediction of infection progress. Particularly, D-dimer elevated five-time higher in severe cases, compared with non-severe cases. Besides that, the lower lymphocyte count was re-
lated to the progress of the disease. Severe cases showed lymphopenia, neutrophilia, and thrombocytopenia. Significant changes were found such as prolonged aPTT, and also a decrease of fibrinogen levels could be seen in severe cases. A higher level of fibrin/fibrinogen in all SARS-CoV-2 cases and patients with milder symptoms can appear. CRP, ESR, and LDH levels were positively correlated with the

Table 1. Search the number of studies included for some prognostic factor predicting severity of COVID-19

<table>
<thead>
<tr>
<th>References</th>
<th>Study design</th>
<th>Study location</th>
<th>Factors studied</th>
<th>Primary outcome</th>
<th>Importance of prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai, T., et al. (66)</td>
<td>N=127 patients with confirmed COVID-19</td>
<td>Wuhan, China</td>
<td>CRP, Ferritin, Procalcitonin, IL6</td>
<td>Died patients showed high inflammatory biomarkers</td>
<td>High</td>
</tr>
<tr>
<td>Wu, C., et al. (44)</td>
<td>A retrospective cohort study, N=201 patients</td>
<td>Wuhan, China</td>
<td>CRP, Ferritin, D-dimer, TNF-α</td>
<td>Severe patients present hyperinflammatory</td>
<td>High</td>
</tr>
<tr>
<td>Ji, D., et al. (68)</td>
<td>A single-center, non-interventional cohort study N=49 patients (sever and non-sever)</td>
<td>Beijing, China</td>
<td>Age, co-morbidity disease, Ferritin, Lymphocyte counts, Serum LDH, D-dimer, CD4 and CD8 counts</td>
<td>Significantly difference between the two groups with p&lt;0.005</td>
<td>High</td>
</tr>
<tr>
<td>Huang, C., et al. (1)</td>
<td>Prospective data N=41 patients</td>
<td>Wuhan, China</td>
<td>D-dimer</td>
<td>D-dimer elevated five times higher in severe cases comparing with non-severe cases</td>
<td>High</td>
</tr>
<tr>
<td>Zhou, F., et al. (69)</td>
<td>N=191 patients</td>
<td>Wuhan, China</td>
<td>D-dimer, Cardiac troponin I, Serum ferritin, LDH, IL-6</td>
<td>Increase in D-dimer and cardiac troponin I, serum ferritin, lactate dehydrogenase, and IL-6</td>
<td>High</td>
</tr>
<tr>
<td>Zhang, B. (70)</td>
<td>N=82 death cases</td>
<td>Wuhan, China</td>
<td>D-dimer, Prothrombin, Platelet, Lymphocyte, LDH, CRP</td>
<td>Severe patients showed lymphopenia neutrophilia, thrombocytopenia and increased CRP level, LDH, and D-dimer</td>
<td>Medium</td>
</tr>
<tr>
<td>Chen, J., et al. (71)</td>
<td>N=25 hospitalized patient</td>
<td>Lausanne, Switzerland</td>
<td>D-dimer</td>
<td>An increase of D-dimer level in all 25 patients and in 10 patients acute pulmonary embolism (APE)</td>
<td>Medium</td>
</tr>
<tr>
<td>Tung, N., et al. (73)</td>
<td>N=183 patients</td>
<td>Wuhan, China</td>
<td>D-dimer, PT/PTT, Fibrinogen, Antithrombin</td>
<td>Elevated D-dimer and FDP are common in deaths</td>
<td>High</td>
</tr>
<tr>
<td>Study</td>
<td>Patients</td>
<td>Location</td>
<td>Measures</td>
<td>Results</td>
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<tr>
<td>Han, H., et al. (75)</td>
<td>N=94 patients and N=40 healthy controls</td>
<td>Wuhan, China</td>
<td>D-dimer, Fibrin/fibrinogen</td>
<td>A higher level of D-dimer and fibrin/fibrinogen in all SARS-CoV-2 cases and patients with milder symptoms.</td>
<td></td>
</tr>
<tr>
<td>Xiong, Y., et al. (83)</td>
<td>Number of 35 patients diagnosed with CT scan evaluated</td>
<td>Shanghai, China</td>
<td>ESR, CRP, LDH</td>
<td>CRP, ESR, and LDH level positively correlate with the severity of lung abnormalities quantified on initial CT.</td>
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<tr>
<td>Chen, N., et al. (58)</td>
<td>N=99 patients evaluated</td>
<td>Wuhan, China</td>
<td>LDH, Ferritin, Albumin, D-dimer, Prothrombin, AST, ALT, Serum creatinine and BUN</td>
<td>Among all elements that have been evaluated, ferritin CRP d-dimer elevation showed a significant correlation with severity</td>
<td></td>
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<tr>
<td>Mehta, P., et al. (9)</td>
<td>Retrospective, single-center</td>
<td>Wuhan, China</td>
<td>Prothrombin, LDH</td>
<td>Severe patients in ICU were older and had comorbidities and also showed elevated LDH and prothrombin</td>
<td></td>
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<tr>
<td>Chen, L., et al. (60)</td>
<td>N=29 patients (15 mild cases, 9 severe cases, and 5 critical)</td>
<td>Shanghai, China</td>
<td>CRP, LDH, Albumin, Alt, AST, Bilirubin, Creatinine</td>
<td>(LDH) increased (20/29), albumin decreased (15/29), other items showed no significant changes.</td>
<td></td>
</tr>
<tr>
<td>Webb, B. J., et al. (72)</td>
<td>N=299 patients evaluated</td>
<td>Salt Lake City, UT, USA</td>
<td>Fever, Ferritin, Neutrophil to lymphocyte ratio, Lactate dehydrogenase, D-dimer, C-reactive protein, interleukin-6, or triglycerides</td>
<td>The proposed criteria are associated with death and progression to a mechanical ventilator</td>
<td></td>
</tr>
<tr>
<td>Gayam, V., et al. (65)</td>
<td>N=408 patients</td>
<td>New York, USA</td>
<td>Ferritin, C-reactive protein, and D-dimer</td>
<td>Elevated serum ferritin, C-reactive protein, and D-dimer as independent predictors for mortality.</td>
<td></td>
</tr>
</tbody>
</table>

**Conflict of interest**

The authors declare no conflicts of interest.

**References**


25. Rouhbakhsh Zahmatkesh MR et al.


