ORIGINAL ARTICLE

# Severity of Coronavirus Disease 19: A Profile of Inflammatory Markers in Iraqi Patients

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## ABSTRACT

**Introduction:** Accumulating evidence indicates that inflammatory responses play a major role in the development and/or severity of coronavirus disease 2019 (COVID-19). Therefore, a retrospective, cross-sectional study was performed to provide an inflammatory profile in COVID-19. **Methods:** The study included 139 patients infected with COVID-19, who were admitted to inpatient wards and intensive care units in Baghdad Teaching Hospital. There were 105 patients suffering from non-severe illness and 34 patients had severe disease. This study simultaneously evaluated six peripheral blood markers of inflammation to determine their predictive value in COVID-19 severity. These were C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, D-dimer, lactate dehydrogenase (LDH) and neutrophil-to-lymphocyte ratio (NLR). **Results:** The medians of age, CRP, ESR, ferritin, D-dimer and NLR were significantly elevated in severe cases of COVID-19 compared to non-severe cases. The LDH also tended to have increased levels in severe cases but the difference was not significant compared to non-severe cases. Logistic regression analysis demonstrated that D-dimer was the most significant risk factor, followed by NLR, ferritin and CRP. Receiver operating characteristic (ROC) curve analysis identified that the best cut-off values of CRP, ESR, ferritin, D-dimer, LDH and NLR for predicting severity in COVID-19 patients were 22.7 mg/L, 59.5 mm/h, 719.4 ng/mL, 367.5 ng/mL, 468.5 U/L and 12.9, respectively. **Conclusion:** Age and the inflammatory markers CRP, ESR, ferritin, D-dimer, and NLR showed higher medians in severe cases of COVID-19 compared to non-severe cases. In this context, D-dimer and NLR are suggested to be important predictive markers of severe disease.

**Keywords:** Inflammation, Ferritin, D-dimer, Lactate dehydrogenase, Neutrophil-to-lymphocyte ratio

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## INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a highly contagious disease caused by the severe acute respiratory syndrome (SARS-CoV-2) virus that was identified in late 2019. The World Health Organization (WHO) declared on January 30, 2020 that the COVID-19 outbreak constituted a public health emergency of international concern (1). Since then, COVID-19 has become a pandemic, and more than 200 countries have been affected by the disease, leading to increased morbidity and mortality rates (2). To date (March 11, 2021), 118,946,985 cases have been reported worldwide with 2,637,080 deaths (2.1%) (3). The most common direct causes of death are septic shock and multi-system failure, often due to purulent respiratory infections (4). However, a wide range of clinical symptoms are observed in affected patients, and although a proportion of patients is asymptomatic, others may have mild symptoms (fever, muscle pain, cough, shortness of breath and fatigue) or develop severe clinical manifestations (acute respiratory distress syndrome, pulmonary edema, or weakness syndrome) (5).

There has been accumulating evidence indicating that inflammatory responses play a major role in the development and / or severity of COVID-19 (6). It has been found that SARS-CoV-2 specifically reduces type I and III interferons (IFNs) responses and, on the contrary, leads to the production of various pro-inflammatory and inflammatory cytokines, such as CXCL1, CXCL5, CXCL10 and interleukin-6 (IL-6), which are associated with severe inflammatory reactions and widespread lung damage (7). Besides, several other inflammatory markers are suspected to contribute to the severity of COVID-19. Among these are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), serum ferritin, D-dimer, lactate dehydrogenase (LDH) and neutrophil-to-lymphocyte ratio (NLR) (8). These markers have been described to be elevated in patients with severe COVID-19 compared to patients with mild disease; however, data across studies are not completely consistent (9). Therefore, this study simultaneously evaluated six peripheral blood markers

of inflammation (CRP, ESR, ferritin, D-dimer, LDH and NLR) in non-severe and severe cases of COVID-9 to determine their predictor value of severity in the disease.

## MATERIALS AND METHODS

### COVID-19 patients

During the period from September 1 to December 15, 2020, a single-center cross-sectional study was conducted retrospectively on a cohort of 139 COVID-19 patients (mean age ± standard deviation [SD] = 51.6 ± 13.5 years; 67.6% males and 32.4% females), who were admitted to inpatient wards and intensive care units (ICUs) at Baghdad Teaching Hospital (Baghdad, Iraq). Of these, 105 patients were moderately ill (mean age ± SD = 49.6 ± 13.9 years; 66.7% males and 33.3% females), while 34 patients were in severe condition (mean age ± SD = 57.6 ± 10.3 years; 70.6% males and 29.4% females). Patients were randomly selected, and only those who gave written consent were included.

### Ethical clearance

Written consent was obtained from patients after the approval of the Ethics Committee at the Iraqi Ministry of Health and Environment (Approval No. 2619 on August 26, 2020).

### Sampling method

Two types of samples were collected from patients. The first included a nasopharyngeal swab, which was used to diagnose COVID-19. The second included 5 mL of blood, which was distributed into two tubes; 3 mL in ethylenediamin tetra-acetic acid (EDTA) tube and 2 mL in plain tube. EDTA blood was used to determine ESR and NLR. The plain tube blood was centrifuged in temperature-controlled centrifuge (3000 rpm for 5 minutes at 4 C) to collect serum, which was used to assess CRP, ferritin, D-dimer and LDH.

### Diagnosis and assessment of severity

The viral RNA was isolated from nasopharyngeal swabs using ExtractNow™ Virus RNA Swab Kit (Minerva Biolabs GmbH). The RealLine SARS-CoV-2 kit was used to detect the RNA of SARS-CoV-2 by reverse real-time polymerase chain reaction (rRT-PCR). In both cases the manufacturer's instructions were followed. To confirm diagnosis of COVID-19, chest computed tomography (CT) scan was also applied. Included patients are those hospitalized and with a positive rRT-PCR result and the CT scan indicated COVID-19 infection. The patients were classified as moderate (non-severe) and severe on the basis of their clinical condition during hospitalization. Criteria established by the world health organization (WHO) Interim Guidance were followed to define severe disease (10). If patients had one of the following conditions, they were classified as severe cases: severe respiratory distress, respiratory rate ≥ 30 breaths/minute and pulse oxygen saturation (SpO2) ≤ 93% on resting state. Patients with non-severe disease (moderate illness) were those with clinical or radiographic evidence of lower respiratory tract disease and the oxygen saturation ≥ 94%, and did not meet the criteria of severe disease (11).

### Inflammatory markers

Serum levels of CRP, ferritin, D-dimer and LDH were determined using commercial kits produced by Abbott Laboratories (Multigent CRP Vario, AxSYM Ferritin, AxSYM D-dimer and Lactate dehydrogenase kits, respectively), and the manufacturer's instructions were followed. The detection ranges of kits were 0.2-320 mg/L, 0-1000 ng/mL, 0-9000 ng/mL, and 5-4500 U/L), respectively. The Westergren method was used to measure ESR, while NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count.

### Statistical analysis

Data of the six inflammatory markers were tested for normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). The test revealed that these data showed a skewed distribution. Accordingly, they were given as median and interquartile range (IQR: 25%-27%). Significant differences were assessed using Mann–Whitney U test. Logistic regression analysis was applied (severe versus non-severe) to estimate odds ratio (OR) and 95% confidence interval (CI). Two-tailed Fisher exact probability was used to assess the significance of OR. The predictive significance of markers in severity was determined via receiver operating characteristic (ROC) curve analysis, and the results were expressed as area under curve (AUC), cut-off value, sensitivity and specificity. Bonferroni principle was applied to correct probability (p), and only corrected $p \leq 0.05$ was taken statistically significant. Bivariate Spearman rank correlation test was used to analyze the correlation between variables. The statistical package IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was used to perform these analyses. G*Power software (version 3.1.9.7) was used to calculate power of sample size.

## RESULTS

The power of sample size was calculated using G*Power software. At a one-tailed α error probability of 0.05 and an effect size of 0.3, a sample size of 139 had an actual power of 0.95 (1-β error probability).

The medians (IQR: 25-75%) of age (55 [52-58] vs. 50 [40-58] year; $p = 0.021$), CRP (30.7 [19.7-75.1] vs. 17.2 [11.9-31.2] mg/L; $p = 0.006$), ESR (68 [53-87] vs. 51 [30-70] mm/h; $p = 0.012$), ferritin (810 [721-915] vs. 454.0 [309-827] ng/mL; $p = 0.024$), D-dimer (720 [450-1103] vs. 215 [153-281) ng/mL; $p < 0.001$) and NLR (15.8 [13.8-18.5] vs. 8.0 [5.5-10.7]; $p < 0.001$) were significantly elevated in severe cases of COVID-19 compared to non-severe cases. The LDH median also tended to have increased levels in severe cases but the

difference was not significant compared to non-severe cases (497 [432-590] vs. 423 [328-517] U/L; p = 0.378) (Fig. 1).

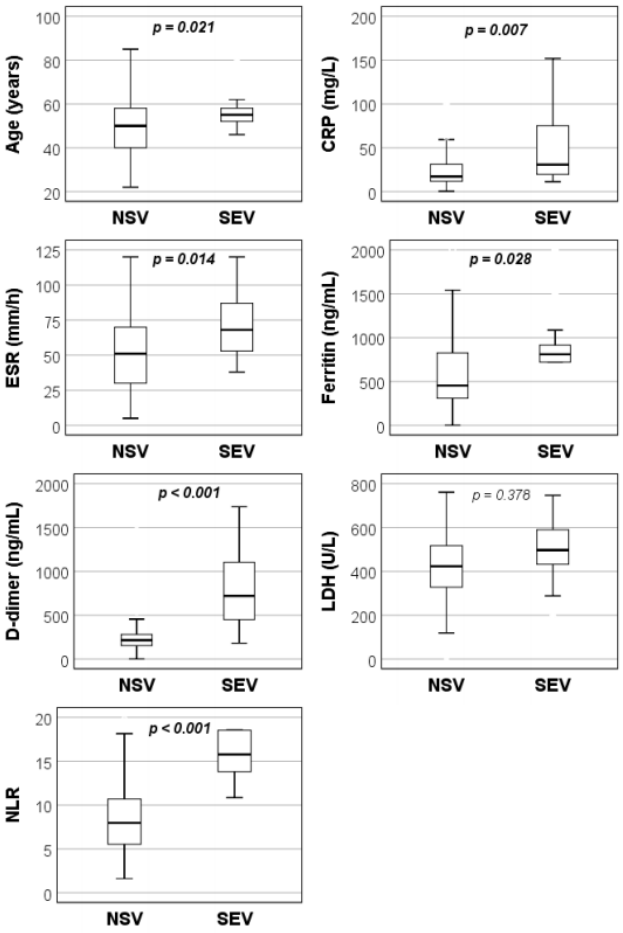


**Figure 1: Box-plot presentations of age, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, D-dimer, lactate dehydrogenase (LDH) and neutrophil-to-lymphocyte ratio (NLR) in non-severe (NSV) and severe (SEV) cases of COVID-19.** Boxes represent the interquartile range (IQR) between the first and third quartiles and the line inside the box represents the median. Whiskers indicate the lowest and highest values from the first and third quartiles. p: Bonferroni-corrected Mann–Whitney U test probability (significant p-value is indicated in bold).

To assess the most significant marker associated with risk of severity, OR was estimated. It was found that D-dimer was the most significant risk factor (OR = 13.78; 95% CI = 4.58-41.51; p < 0.001), followed by NLR (OR = 9.82; 95% CI = 3.56-27.07; p < 0.001), ferritin (OR = 4.69; 95% CI = 1.96-11.20; p = 0.002), age (OR = 3.46; 95% CI = 1.52-7.88; p = 0.021) and CRP (OR = 3.20; 95% CI = 1.41-7.28; p = 0.036) (Table I).

ROC curve analysis identified that the best cut-off values of CRP, ESR, ferritin, D-dimer, LDH and NLR for predicting severity in COVID-19 patients were 22.7 mg/L (sensitivity = 70.6%; specificity = 67.6; AUC = 0.694), 59.5 mm/h (sensitivity = 58.8%; specificity = 56.2%; AUC = 0.679), 719.4 ng/mL (sensitivity = 76.5%; specificity = 72.4%; AUC = 0.666), 367.5 ng/mL (sensitivity = 82.4; specificity = 81.0%; AUC = 0.866), 468.5 U/L (sensitivity = 58.8%; specificity = 59.0%; AUC = 0.610) and 12.9 (sensitivity = 82.4%; specificity = 81.9%; AUC = 0.839), respectively (Fig. 2 and Table II).

The stratification of non-severe and severe cases of COVID-19 by gender did not reveal significant differences between males and females with respect to the distribution of age, CRP, ESR, ferritin, D-dimer, LDH and NLR medians. However, in severe cases, females were significantly characterized by increased median of ESR compared to males (100.0 [IQR: 87.0-110.0) vs. 61.0 [IQR: 52.0-75.0] mm/h; p = 0.02) (Table III).

Correlation analyses of age and the six inflammatory markers were performed (CRP, ESR, ferritin, D-dimer, LDH and NLR) using the bivariate Spearman rank correlation test. The results are given in Table IV. Most of the markers were positively correlated, but significant correlations at p ≤ 0.001 were only nine. They were age with ESR (correlation coefficient = 0.316), ferritin (correlation coefficient = 0.277) and LDH (correlation coefficient = 0.291), CRP with ferritin (correlation coefficient = 0.267), D-dimer (correlation coefficient = 0.385) and NLR (correlation coefficient = 0.351), ESR with ferritin (correlation coefficient = 0.324), ferritin

**Table I: Age and inflammatory markers stratified according to median in non-severe and severe cases of COVID-19**

| Variable | Non-severe cases; N = 105 | | | | Severe cases; N = 34 | | | | OR | 95% CI | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | > Median | | ≤ Median | | > Median | | ≤ Median | | | | |
| | N | % | N | % | N | % | N | % | | | |
| Age | 43 | 41.0 | 62 | 59.0 | 24 | 61.5 | 10 | 9.5 | 3.46 | 1.52-7.88 | **0.021** |
| CRP | 45 | 42.9 | 60 | 57.1 | 24 | 70.6 | 10 | 29.4 | 3.20 | 1.41-7.28 | **0.036** |
| ESR | 46 | 43.8 | 59 | 56.2 | 20 | 58.8 | 14 | 41.2 | 1.83 | 0.84-3.98 | 0.167 |
| Ferritin | 43 | 41.0 | 62 | 59.0 | 26 | 76.5 | 8 | 23.5 | 4.69 | 1.96-11.20 | **0.002** |
| D-dimer | 37 | 35.2 | 68 | 64.8 | 30 | 88.2 | 4 | 11.8 | 13.78 | 4.58-41.51 | **< 0.001** |
| LDH | 48 | 45.7 | 57 | 54.3 | 20 | 58.8 | 14 | 41.2 | 1.70 | 0.78-3.68 | 0.237 |
| NLR | 39 | 37.1 | 66 | 62.9 | 29 | 85.3 | 5 | 14.7 | 9.82 | 3.56-27.07 | **< 0.001** |

OR: Odds ratio (severe *vs.* non-severe); CI: Confidence interval; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; *p*: Corrected Two-tailed Fisher exact probability (Bonferroni correction). Significant *p*-value is indicated in bold.

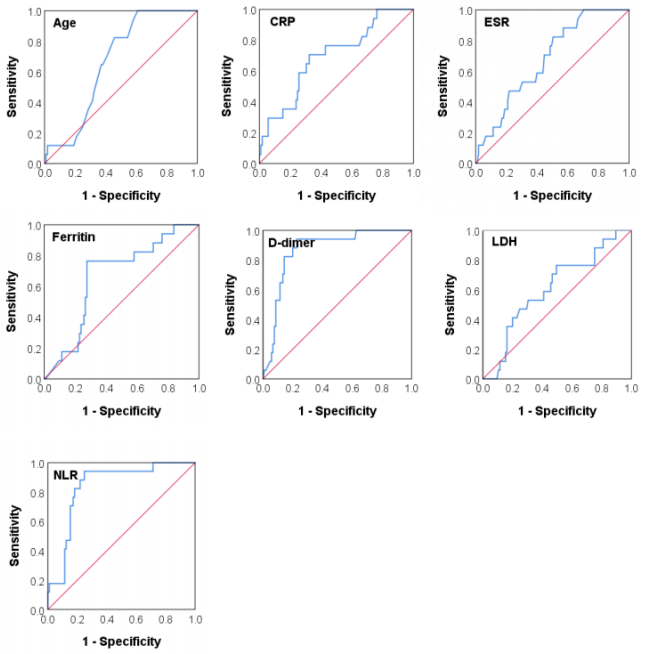


**Figure 2: Receiver operating characteristic curve for age, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), ferritin, D-dimer, lactate dehydrogenase (LDH) and neutrophil to lymphocyte ratio (NLR) as parameters for predicting severity in COVID-19 patients** (data of the figure is given in Table II).

with LDH (correlation coefficient = 0.385) and D-dimer with NLR (correlation coefficient = 0.420). There was no significant correlation between ESR and NOR (p = 0.132), or D-dimer and LDH (p = 0.082) (Table IV).

## DISCUSSION

The results of this study indicated that age and the inflammatory markers examined were related to the severity of COVID-19. The median age for both severe and non-severe cases was 50 and over, but significantly higher for severe cases. Patients over the age of 55 were 3.46 times more likely to have severe disease. Accordingly, it is suggested that increased age may be a risk factor for the severe consequences of COVID-19. A widespread increase in the severity of COVID-19 has been observed with increasing age of patients, and an increase in mortality has also been described with advancing age (12–14). However, it has been debated that there is a significant drawback in assessments of the association between age and risk of severity in patients with COVID-19 because they rely on data that recognize age as an independent risk factor. Other risk factors, such as cardiovascular diseases, diabetes and conditions that lead to impaired immunity, also increase with age, and must be considered to reveal the effect of age on severity

**Table III: Age and inflammatory markers stratified according to gender in non-severe and severe cases of COVID-19**

| Variable | Gender | Median (IQR: 25%-75%) | | *p* |
|---|---|---|---|---|
| | | Non-severe cases N = 105 | Severe cases N = 34 | |
| Age; year | Male | 51.0 (42.0-58.0) | 55.5 (54.0-58.0) | 0.06 |
| | Female | 46.0 (33.0-60.0) | 52.0 (52.0-60.0) | 0.085 |
| | *p* | 0.265 | 0.564 | |
| CRP; mg/L | Male | 17.2 (11.8-37.8) | 29.9 (21.3-54.0) | **0.016** |
| | Female | 17.9 (12.8-22.5) | 47.6 (12.1-75.7) | 0.099 |
| | *p* | 0.747 | 0.897 | |
| ESR; mm/h | Male | 56.5 (33.0-78.0) | 61.0 (52.0-75.0) | 0.235 |
| | Female | 46.0 (29.0-66.0) | 100.0 (87.0-110.0) | **0.002** |
| | *p* | 0.187 | **0.02** | |
| Ferritin; ng/mL | Male | 455.5 (373.1-827.1) | 810.0 (730.1-875.4) | **0.032** |
| | Female | 434.5 (222.0-826.5) | 790.0 (385.0-1509.7) | 0.262 |
| | *p* | 0.399 | 0.838 | |
| D-dimer; ng/mL | Male | 210.0 (151.0-346.0) | 675.0 (447.0-1028.0) | < **0.001** |
| | Female | 217.0 (163.0-251.0) | 875.0 (632.0-1103.0) | **0.008** |
| | *p* | 0.815 | 0.467 | |
| LDH; U/L | Male | 455.5 (368.0-520.0) | 508.5 (437.5-576.0) | 0.131 |
| | Female | 398.0 (312.0-484.0) | 470.0 (323.0-610.0) | 0.298 |
| | *p* | 0.22 | 0.669 | |
| NLR | Male | 7.8 (5.5-10.3) | 16.9 (14.8-18.5) | < **0.001** |
| | Female | 8.5 (5.2-11.9) | 13.1 (11.8-41.6) | **0.004** |
| | *p* | 0.729 | 0.467 | |

IQR: Interquartile range; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; *p*: Bonferroni-corrected Mann–Whitney *U* test probability (significant p-value is indicated in bold).

**Table II: Receiver operating characteristic curve analysis characteristics of C-reactive protein, erythrocyte sedimentation rate, ferritin, D-dimer, lactate dehydrogenase and neutrophil to lymphocyte ratio in severe cases of COVID-19**

| Variable | AUC | SE | Asymptotic *p* | Asymptotic 95% CI | | Cut-off value | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| Age | 0.669 | 0.045 | **0.003** | 0.580 | 0.757 | 54.5 | 64.7 | 62.9 |
| CRP | 0.694 | 0.051 | **0.001** | 0.593 | 0.794 | 22.7 mg/L | 70.6 | 67.6 |
| ESR | 0.679 | 0.048 | **0.002** | 0.585 | 0.772 | 59.5 mm/h | 58.8 | 56.2 |
| Ferritin | 0.666 | 0.051 | **0.004** | 0.566 | 0.765 | 719.4 ng/mL | 76.5 | 72.4 |
| D-dimer | 0.866 | 0.033 | **< 0.001** | 0.801 | 0.932 | 367.5 ng/mL | 82.4 | 81.0 |
| LDH | 0.610 | 0.054 | 0.054 | 0.504 | 0.716 | 468.5 U/L | 58.8 | 59.0 |
| NLR | 0.839 | 0.037 | **< 0.001** | 0.767 | 0.912 | 12.9 | 82.4 | 81.9 |

AUC: Area under curve; SE: Standard error; *p*: Probability (significant p-value is indicated in bold); CI: Confidence interval; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; h: hour.

**Table IV: Bivariate Spearman rank correlation analysis of age, C-reactive protein, erythrocyte sedimentation rate, ferritin, D-dimer, lactate dehydrogenase and neutrophil to lymphocyte ratio in COVID-19 patients.**

| Variable | Spearman bivariate correlation | Age | CRP | ESR | Ferritin | D-dimer | LDH | NLR |
|---|---|---|---|---|---|---|---|---|
| Age | Correlation coefficient | 1.000 | 0.233 | 0.316 | 0.277 | 0.228 | 0.291 | 0.200 |
| | Two-tailed *p*-value | | 0.006 | **< 0.001** | **0.001** | 0.007 | **0.001** | 0.018 |
| CRP | Correlation coefficient | | 1.000 | 0.224 | 0.267 | 0.385 | 0.189 | 0.351 |
| | Two-tailed *p*-value | | | 0.008 | **0.001** | **< 0.001** | 0.026 | **< 0.001** |
| ESR | Correlation coefficient | | | 1.000 | 0.324 | 0.172 | 0.198 | 0.128 |
| | Two-tailed *p*-value | | | | **< 0.001** | 0.043 | 0.020 | 0.132 |
| Ferritin | Correlation coefficient | | | | 1.000 | 0.194 | 0.385 | 0.234 |
| | Two-tailed *p*-value | | | | | 0.022 | **< 0.001** | 0.006 |
| D-dimer | Correlation coefficient | | | | | 1.000 | 0.148 | 0.420 |
| | Two-tailed *p*-value | | | | | | 0.082 | **< 0.001** |
| LDH | Correlation coefficient | | | | | | 1.000 | 0.167 |
| | Two-tailed *p*-value | | | | | | | 0.049 |
| NLR | Correlation coefficient | | | | | | | 1.000 |

CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; NLR: Neutrophil-to-lymphocyte ratio; *p*: Probability (*p*-value ≤ 0.001 is indicated in bold).

of COVID-19. Accordingly, a meta-analysis study has been performed and found that the crude effect of age on severity of COVID-19 substantially decreased when the risk factors associated with aging were controlled (15).

Among the inflammatory markers, D-dimer and NLR were of particular interest. The D-dimer increased the risk of severity by 13.78 folds, and it occupied a very good AUC, which was 0.866. A similar relationship was observed between D-dimer and disease severity in male and female patients. Thus, D-dimer could be proposed as a biomarker of severity in COVID-19 patients. Most severe COVID-19 cases have been characterized by up-regulated serum levels of D-dimer, and elevated levels of this inflammatory marker could be utilized as a sensitive biomarker marker of the disease severity (16,17). D-dimers are the fibrin degradation products of the fibrin system after thrombus formation, and in clinical practice, the marker is used in diagnosis of intravascular thrombosis and exclusion of deep vein thrombosis and pulmonary embolism (18). D-dimer levels have been correlated with various inflammatory conditions particularly those related to activation of pro-inflammatory cytokines, while anti-inflammatory cytokines such as IL-10 did not show this correlation. Accordingly, it has been proposed that D-dimer can be used as an indicator of an imbalance between pro-inflammatory and anti-inflammatory cytokines (19). Although this imbalance is still a speculation in COVID-19, the pathology of disease has been marked by pathological levels of cytokines (20). Therefore, D-dimer may contribute to the pathology and severity of COVID-19 through its effects on cytokines, because one of the critical characteristics of COVID-19 severity is the cytokine storm, which outcome in severe endothelial damage that can lead to acute respiratory distress, hyper-coagulation, and disseminated intravascular coagulation (21).

The study revealed that NLR was another marker of disease severity in COVID-19. The marker was associated with an OR of 9.82, and ROC curve analysis showed an AUC of 0.839. At a cut-off value of 12.9, the sensitivity and specificity of NLR were 82.4 and 81.9%, respectively. Neutrophils and lymphocytes are the most effective cells in innate and adaptive immune responses, respectively, and their role is well recognized in controlling inflammatory responses during evolution of infectious diseases (22). A high neutrophil count predicts persistent inflammation and a low lymphocyte count is an indication of a poor prognosis; thus it is generally accepted that a combination of these two measures (i.e. NLR) predicts an inflammatory condition (23). Therefore, elevated NLR indicates an abnormality in the inflammatory response and can be considered a useful predictor of severity in infectious diseases (24). In COVID-19, it has been reported that NLR was significantly associated with disease severity, and the estimated AUC was 0.841, which was close to the AUC in the current study (25). These findings were confirmed by a meta-analysis study of 13 studies involving 1579 patients. The analysis indicated that NLR has good predictive value regarding disease severity and mortality in COVID-19 patients (26). It has also been concluded that NLR is an easy-to-measure, accessible, cost-effective and reliable biomarker in the continuous monitoring of patients with COVID-19 infection (27).

This study also indicated that CRP, ESR, and ferritin could be considered inflammatory markers of disease severity in COVID-19 patients. However, the estimated AUCs were less than 0.7, and thus the predictive significance of disease severity may be weak. CRP is an acute-phase protein synthesized by hepatocytes in response to IL-6 and its significance as a marker of systemic inflammation has been well defined. Further, elevated serum levels of CRP are associated with severe illness in infectious diseases (28). In patients with COVID-19,

there has been accumulating evidence indicating a strong association between elevated serum levels of CRP and venous thromboembolism, lung lesions and critical illness, and thus this marker may reflect disease severity (29,30). In this study, although CRP levels were significantly increased in patients with severe disease, the ROC curve analysis limited the significance of CRP and a poor AUC was recorded. However, the bivariate Spearman rank correlation analysis revealed that CRP was strongly correlated with other inflammatory markers, which were ESR, ferritin, D-dimer and NLR (correlation coefficient = 0.224, 0.267, 0.385 and 0.351, respectively). With regard to ESR, which is a marker of inflammation, it has also been indicated that patients with severe COVID-19 showed marked elevations in ESR compared to non-severe cases, and this may reflect the association between ESR, hyper-inflammation and COVID-19 severity (31). Besides, ferritin also showed up-regulated levels in serum of severe COVID-19 cases. High ferritin levels have been well demonstrated in COVID-19 patients, and the potential role of this inflammatory marker in amplifying the inflammatory response after disease progression has been recognized (32). Most importantly, ferritin has been linked to cytokine storm syndrome, which is perhaps one of the hallmarks of the severity of COVID-19 disease. It has been reported that several inflammatory cytokines, such as IL-6, can induce the synthesis of ferritin. Further, complex feedback mechanisms between ferritin and cytokines in controlling pro-inflammatory and anti-inflammatory reactions have been described (33). Ferritin in this study was also significantly correlated with LDH. However, LDH showed a tendency to higher levels in severe cases of COVID-19 but the difference was not significant compared to cases with non-severe disease. In a previous pooled analysis of nine published studies, inconsistent results were reported and LDH levels were significantly increased in severe cases of COVID-19 compared to non-severe cases with a 6-fold increase in odds (34).

Collectively, these data indicate that the six markers of inflammation, particularly D-dimer and NLR, showed significant predictive values of disease severity in patients with COVID-19. Their concurrent evaluation may represent an important approach in early identification of severely affected patients, which in turn could improve initial curative management and reduce morbidity and mortality from COVID-19.

The study had a few limitations. First, although the sample size achieved statistical power, it is still recommended to screen a larger number of patients. Second, the profile of pro-inflammatory and anti-inflammatory cytokines was not investigated and this may also limit the understanding of inflammatory markers in severity of COVID-19. Third, comorbidities in the current sample of COVID-19 patients; for instance, cardiovascular disease and diabetes, were not evaluated.

## CONCLUSION

Age and the inflammatory markers CRP, ESR, ferritin, D-dimer, and NLR showed higher medians in severe cases of COVID-19 compared to non-severe cases. In this context, D-dimer and NLR are suggested to be important predictive markers of severe disease. Therefore, it is recommended that these markers be used in the rapid identification of severe disease in COVID-19 patients to facilitate early initiation of effective therapy. Besides, the dynamics of inflammatory factors in COVID-19 patients can be a useful indicator of disease transition from mild to severe infection.

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