Evaluation of inflammatory and hematological parameters in patients diagnosed with COVID-19

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Abstract
Introduction: This study aims to research the effects of hematological and inflammatory parameters on the prognosis of COVID-19 disease and hospitalization duration.
Methodology: One hundred and eighty-six patients with COVID-19 and a control group consisting of 187 healthy individuals were included in the study. Hematological variables and inflammatory parameters of the patients were recorded on the first and the fifth days of hospitalization. Results: White blood cell count, lymphocyte count, and platelet count were statistically lower, and mean platelet volume (MPV), neutrophil to lymphocyte ratio (NLR), and platelet to lymphocyte ratio (PLR) levels were higher in the patient group compared to the control group. It was observed that the neutrophil count and MPV level were lower, and the platelet count and ferritin level were statistically higher on the fifth day of follow-up compared to the admission day. In contrast, there was a significantly positive correlation between the duration of hospitalization and the fifth day D-dimer (r = 0.546, p < 0.001) and ferritin (r = 0.568, p < 0.001); in addition, there was a negative correlation between the duration of hospitalization and admission day lymphocyte count and the fifth-day lymphocyte count.
Conclusions: Increased levels of ferritin and D-dimer, and decreased count of lymphocytes are among the important factors affecting the duration of hospitalization for COVID-19 patients. Furthermore, we think that neutrophil count and MPV levels are low, and platelet count and ferritin levels are high during the disease. Therefore, these parameters can be used as prognostic indicators of the disease.

Key words: COVID-19; infection; markers; pandemic.

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Introduction
COVID-19 is a multisystemic infection caused by a coronavirus, a single-stranded RNA virus, and may affect neurological, gastrointestinal, hepatic, and pulmonary systems [1,2]. Many possible candidates have been tested for treating patients with COVID-19 disease which usually present flu-like symptoms [2].

COVID-19 was declared a pandemic by the World Health Organization (WHO). The virus is a member of the beta-coronavirus family and can affect all age groups, however infected persons may present different clinical pictures [1]. The clinical and laboratory indicators of COVID-19 leading to the progression towards severe and lethal forms need to be urgently identified. These predictors will enable risk classification, guide interventional efforts to target patients at high risk of developing severe disease and optimize the allocation of limited healthcare workers and technical resources. Moreover, defining the laboratory parameters that could predict the cases' clinical course will increase clinical situational awareness. Therefore, monitoring COVID-19 patients and effective early intervention are the most fundamental measures to improve survival.

Based on observations so far, the inflammatory response plays a critical role in the progression of COVID-19. Although the changes in acute phase reactants in COVID-19 cases that affect the disease's course remains unclear, it is recognized that these play a significant role in disease severity and mortality [1,2]. In some studies, hematological parameters are defined as potential indicators of disease severity [3,4].
However, to the best of our knowledge, few studies reported on hematological parameters, especially mean platelet volume (MPV), in adults infected with COVID-19.

Hematological and inflammatory parameters such as white blood cell count (WBC), lymphocyte count, neutrophil count, platelet count, neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), MPV, ferritin, D-dimer, fibrinogen, procalcitonin and C-reactive protein (CRP) in COVID-19 patients have been defined as prognostic indicators of the disease [5-8].

Our findings will provide additional information about the role of these indicators in determining the clinical course of patients diagnosed with COVID-19. This study evaluates whether hematological and inflammatory biomarkers have prognostic effects on the duration of hospitalization in patients who had been diagnosed with COVID-19 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) test and followed up by hospitalization.

Methodology
In this retrospective study conducted between 16 March and 24 July 2020, 186 patients, who were aged between 15-92 years, hospitalized in the pandemic hospital of Diyarbakir Dicle University, located in the southeast of Turkey, and diagnosed with COVID-19, were compared with a control group of 187 individuals of similar age and gender.

Every patient with COVID-19 RT-PCR positivity was hospitalized since the start of the pandemic in Turkey on March 11, 2020, in line with the guidelines of the Ministry of Health. For this reason, patients selected for the study included asymptomatic individuals who were hospitalized with COVID-19 PCR positivity, regardless of pneumonia in lung imaging findings. The individuals in the control group included patients who did not have any systemic disease, who were admitted to the hospital with general health issues and wanted to have an enzyme-linked immunosorbent assay (ELISA) test scan (those who have high-risk sexual behaviors or because of post-vaccination antibody titer), endocrinological patients who were monitoring blood glucose, and outpatients who applied to the hospital and wanted to have routine tests such as measurement of the prostate. Medical information of the patients and control groups was obtained from the hospital records.

COVID-19 was diagnosed using the RT-PCR test (Roche Company, Basel, Switzerland) based on the presence of SARS-CoV-2 nucleic acid in a combined nasal/oral pharyngeal swab sample. Age, gender, duration of hospitalization, WBC, neutrophil count, lymphocyte count, platelet count, MPV, NLR and PLR, ferritin, D-dimer, fibrinogen, procalcitonin and C-reactive protein (CRP) values of patients on the first and fifth day of hospitalization after being diagnosed with COVID-19 were collected retrospectively and recorded on the follow-up form. The age, gender, and hematological parameters (WBC, neutrophil count, lymphocyte count, platelet count, MPV, NLR, and PLR) of the control group were recorded. NLR and PLR were calculated as the ratio of neutrophils to lymphocytes and the ratio of platelets to lymphocytes, respectively. All blood samples taken for hematological parameters were tested with the same analyser that was calibrated regularly.

Patients who had negative RT-PCR results and only COVID-19-compatible thorax tomography findings, patients who missed blood tests on the first and fifth days, those who were younger than 15 years, and those who were not hospitalized were excluded from the study. Persons with inflammation-related diseases such as acute bacterial infections, rheumatic, connective tissue, various bone and joint-related issues, fever-related with other etiologies and diseases such as cardiac and hematological, and patients whose medical records are missing were excluded from the control group.

The Dicle University Faculty of Medicine Ethics Committee approved this retrospective study protocol. All the authors have signed the Helsinki Declaration.

Statistical Analysis
Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) (version 19.0 for Windows; SPSS Inc., Chicago, IL, USA). Categorical data were presented as numbers (percentage) and tested using the Chi square test or Fisher’s exact test. Continuous variables with normal distribution were presented as mean ± SD (standard deviation) and tested with a paired t test. The Chi square test was used to compare categorical variables between the groups. Correlations between numerical variables were evaluated using Pearson’s or Spearman’s correlation analysis. We selected correlation strength by an absolute correlation |r| > 0.2. The p values based on two-sided tests were considered statistically significant at less than 0.05.

Results
One hundred and eighty-six adults diagnosed with COVID-19 infection and 187 healthy control subjects were retrospectively evaluated. The mean age of the
patient's group was 47.0 ± 18.4 years (15–92 years, min-max), and 54.3% (n = 101) of the patients were male. The mean age of the control group was 44.0 ± 12.7 years (18-68 years, min-max), and 55.6% (n = 104) were male. There were no significant differences in the mean age (p = 0.067) or gender distribution (p = 0.799) between the patient and the control subjects (p > 0.05). The mean hospitalization duration was 7.2 ± 4.1 days (1-24 days, min-max) in the patient group.

WBC (p = 0.014), lymphocyte count (p < 0.001) and platelet count (p = 0.001) were statistically lower in the patient group compared to the control group, while neutrophil count (p = 0.293) and MPV (p = 0.001), NLR (p < 0.001) and PLR (p < 0.001) levels were higher. No significant difference was found between the two groups in terms of neutrophil count (Table 1).

When comparing the first and fifth day laboratory parameters of the patients, it was found that neutrophil count (p = 0.035) and MPV (p = 0.001) levels were significantly lower, while platelet count (p < 0.001) and ferritin (p = 0.026) levels were significantly higher on the fifth day of the follow-up compared to the time of admission. There was no statistically significant difference between the first and fifth day in terms of WBC, lymphocyte count, RDW, NLR, PLR, D-dimer, fibrinogen, CRP, and procalcitonin (Table 2).

There was a moderately positive correlation between the duration of hospitalization and admission day neutrophil count (r = 0.293, p < 0.001), NLR (r = 0.273, p = 0.001), D-dimer (r = 0.295, p < 0.001), CRP (r = 0.418, p < 0.001), procalcitonin (r = 0.284, p < 0.001), fibrinogen (r = 0.420, p < 0.001) and fifth day NLR (r = 0.413, p < 0.001), fifth day PLR (r = 0.345, p < 0.001), fifth day CRP (r = 0.480, p < 0.001), fifth day procalcitonin (r = 0.387, p = 0.004), and fifth day fibrinogen (r = 0.301, p < 0.001) levels. There was a highly positive correlation between the fifth day D-dimer (r = 0.546, p < 0.001) and the fifth day ferritin level (r = 0.568, p < 0.001). Moderately negative correlation was found between hospitalization duration and lymphocyte count on admission day (r = 0.256, p = 0.006) and the fifth day lymphocyte count (r = 0.325, p < 0.001).

**Discussion**

COVID-19 can affect many systems, and knowing the follow-up and prognostic parameters will lead to more insight into the disease. This study shows that low neutrophil count and MPV level, high platelet count,

### Table 1. Laboratory findings of patient and control groups at admission.

<table>
<thead>
<tr>
<th>Hematological Variables</th>
<th>Study Group Mean ± SD (n = 186)</th>
<th>Control Group Mean ± SD (n = 187)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (K/µL)</td>
<td>7.3 ± 2.9</td>
<td>8.0 ± 2.4</td>
<td>0.014 *</td>
</tr>
<tr>
<td>Neutrophil count (10^3/µL)</td>
<td>4.7 ± 2.5</td>
<td>4.7 ± 1.7</td>
<td>0.954</td>
</tr>
<tr>
<td>Lymphocyte count (10^3/µL)</td>
<td>1.7 ± 0.9</td>
<td>2.6 ± 0.9</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>Platelet count (10^3/µL)</td>
<td>245 ± 81</td>
<td>273 ± 75</td>
<td>0.001 *</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>8.7 ± 1.6</td>
<td>7.8 ± 1.2</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>NLR</td>
<td>3.3 ± 3.1</td>
<td>1.9 ± 1.0</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>PLR</td>
<td>167.3 ± 101.6</td>
<td>116.8 ± 50.2</td>
<td>&lt;0.001 *</td>
</tr>
</tbody>
</table>

WBC: white blood cell; MPV: mean platelet volume; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; SD: standard deviation. *: Statistically significant.

### Table 2. Comparison of laboratory findings on the 1st and 5th day in the patient group.

<table>
<thead>
<tr>
<th>Hematological Variables</th>
<th>1st day Mean ± SD</th>
<th>5th day Mean ± SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (K/µL)</td>
<td>7.3 ± 2.9</td>
<td>7.2 ± 2.9</td>
<td>0.623</td>
</tr>
<tr>
<td>Neutrophil count (10^3/µL)</td>
<td>4.7 ± 2.5</td>
<td>4.3 ± 2.5</td>
<td>0.035 *</td>
</tr>
<tr>
<td>Lymphocyte count (10^3/µL)</td>
<td>1.7 ± 0.9</td>
<td>1.9 ± 0.8</td>
<td>0.057</td>
</tr>
<tr>
<td>Platelet count (10^3/µL)</td>
<td>245 ± 81</td>
<td>270 ± 89</td>
<td>&lt;0.001 *</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>8.7 ± 1.6</td>
<td>8.4 ± 1.6</td>
<td>0.001 *</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>10.8 ± 1.2</td>
<td>10.7 ± 1.4</td>
<td>0.284</td>
</tr>
<tr>
<td>NLR</td>
<td>3.3 ± 3.1</td>
<td>3.1 ± 4.4</td>
<td>0.617</td>
</tr>
<tr>
<td>PLR</td>
<td>167.3 ± 101.6</td>
<td>173.2 ± 148.0</td>
<td>0.571</td>
</tr>
<tr>
<td>D-dimer (mg/L)</td>
<td>1.6 ± 2.9</td>
<td>3.8 ± 15.6</td>
<td>0.189</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>282 ± 373</td>
<td>419 ± 854</td>
<td>0.026 *</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>336 ± 147</td>
<td>356 ± 147</td>
<td>0.068</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>3.3 ± 5.2</td>
<td>2.9 ± 5.1</td>
<td>0.146</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.350</td>
</tr>
</tbody>
</table>

WBC: white blood cell; MPV: mean platelet volume; RDW: red cell distribution width; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; SD: standard deviation. *: Statistically significant.
and ferritin levels might be important parameters to monitor in the course and follow-up of COVID-19 infection. In addition, we found that the laboratory levels of infection have important correlations with the duration of hospitalization.

Various studies on COVID-19 have reported that the duration of hospitalization may be prolonged due to comorbidity conditions (obesity, diabetes, hypertension, and coronary heart disease), lymphopenia, high D-dimer and ferritin levels [9-11]. However, studies on clinical and laboratory parameters that can determine the prognosis of patients are limited. As far as we know, there are few comprehensive studies on prognostic factors affecting the duration of hospitalization in COVID-19 patients.

In our study, the mean hospitalization period of the patients was 7.2 ± 4.1 days. A positive correlation was found between the duration of hospitalization and the first and fifth day NLR, CRP, procalcitonin, D-dimer, and fibrinogen, the fifth day PLR, and ferritin levels. The low course of lymphocyte count both at admission and follow-up was the most important factor in prolonging the duration of hospitalization. Therefore, parameters are factors affecting the duration of hospitalization in the follow-up of infection for COVID-19 patients in accordance with the literature.

Meta-analysis on COVID-19 patients concluded that the male gender was predominant at a rate of 60% and 63%, with an average age of 46.4 years [10,11]. Our patients’ mean age and gender were consistent with the literature.

While investigating the clinical characteristics of COVID-19 patients, 64.5% of the patients were found to have lymphopenia and 29.4% had leukopenia [10]. A study by Fu et al. found 45.3% lymphopenia, 21.3% leukopenia, and 12% thrombocytopenia [7]. In a study examining the laboratory parameters of 3377 patients, there was a slight increase in the WBC count in patients with severe disease, and there was a clinically significant increase in this parameter in patients who died [5]. Therefore, in patients with severe disease, a significant increase in WBC may indicate clinical worsening and an increased risk of poor outcomes. Lymphopenia is a common feature in these patients. A decrease in percentages of monocytes, eosinophils, and basophil cell percentages, CD4/CD8 T cells and natural killer cells was observed [1,5,12].

Studies have suggested that survival with COVID-19 may depend on the ability to regenerate lymphocytes damaged by the virus. Therefore, the lymphocyte count, especially CD4, can be used as a clinical predictor of severity and prognosis [12]. In our study, while the WBC and lymphocyte count were statistically lower in the patient group at the time of admission than in the control group, no significant difference was found between the two groups in terms of neutrophil count. It was observed that the neutrophil count was lower, and there was no significant difference in the WBC and lymphocyte count on the fifth-day of follow-up of the patients compared to the time of admission. This indicates that COVID-19 patients have lower WBC and lymphocyte count than the normal population; however, there was no significant change in the first five days of infection, and low levels of neutrophil should be closely monitored during the follow-up.

Increased neutrophil count and the associated increase in NLR levels are other findings observed in COVID-19 patients. It has been reported that NLR, which can be easily calculated from a routine blood test by dividing the absolute neutrophil count with the absolute number of lymphocytes, can indicate the general inflammatory status of a patient [6]. In a study, it was found that the neutrophil rate was 51.6%, and the increased NLR level was associated with the severity of the disease, poor prognosis, and duration of hospitalization [8]. In our study, while the NLR level was higher in the patient group than the control group at the time of admission, there was no significant difference in the patients’ first and fifth-day NLR levels. Therefore, we believe that the NLR level may be important in the diagnosis of the COVID-19 infection; however, it has no prognostic significance in the follow-up of the disease.

Thrombocytes are immune cells that play an important role in the human body. The incidence of thrombocytopenia in critically ill patients admitted to the intensive care unit varies between 15-60% [5]. A meta-analysis that investigated the relationship between thrombocytopenia and COVID-19 severity concluded that low platelet count increases the risk of severe disease and mortality by three times in COVID-19 patients and prolongs the length of hospitalization [3]. According to another study, platelet peaks and increased PLR levels observed in the follow-up of symptomatic and asymptomatic patients were associated with the severity of the disease and hospitalization duration [8]. The PLR level is calculated by dividing the absolute platelet count by the absolute lymphocyte count. The high level of PLR of the patients refers to the degree of cytokine storm that can provide a new indicator in the follow-up of patients with COVID-19 [8,13].

MPV is a laboratory marker associated with platelet function and activity. High MPV is hemostatically more
reactive and produces higher amounts of the prothrombotic factor, thromboxane. This increased thromboxane causes thrombotic sensitivity and therefore results in thrombotic complications [14]. According to a study by Elsayed and Mohamed, it was reported that increasing MPV and MPV/thrombocyte count ratio is an important risk factor in the development of thromboembolic events [15]. In the study by Pan et al., the increased MPV level was a distinctive feature in COVID-19 patients [16]. In our study, the number of platelets at the time of admission was significantly lower, while PLR and MPV levels were higher in the patient group than in the control group. It was found that the MPV level was lower, and the platelet count was higher on the fifth-day of patient follow-up compared to the time of admission. We think that the increase in the platelet count in the later days of the disease is related to the treatment and recovery process. However, due to lack of sufficient evidence about this situation, we believe that studies with a larger patient series should be carried out. We think that the high MPV level in the early stage of the disease and its low course in the follow-up may be related to the prognosis. Therefore, the MPV level can be a useful prognostic factor in the follow-up of COVID-19 infection.

Ferritin is used as an inflammation marker, and it significantly increased during the course of the disease in patients with severe COVID-19. Therefore, serum ferritin levels should be used to monitor prognosis in COVID-19 patients during hospitalization. Increase in the CRP level along with ferritin indicates the development of systemic inflammatory response syndrome. The exaggerated elevation that may lead to a cytokine storm can cause tissue damage progressing to acute lung injury and multi-organ failure [17,18]. In a study examining the relationship between the severity of the COVID-19 disease and procalcitonin, a significant difference was observed only in the procalcitonin level between severe and non-severe forms of the disease. However, in another study, an approximately five-fold higher risk of severe COVID-19 infection was observed in patients with increased procalcitonin [4,18]. D-dimer occurs due to the destruction of the fibrin clot formed by cross-links of plasmin with the activation of the coagulation system [5]. A study by Zhang et al. concluded that a high D-dimer level at the time of admission was an important factor affecting mortality [19]. D-dimer is significantly elevated in patients with both severe and fatal COVID-19 [5]. In our study, significantly high ferritin levels were observed on the 5th day compared to the time of admission. However, no significant difference was observed in CRP, procalcitonin, D-dimer, and fibrinogen levels in prognostic terms. With these results, we think that, instead of inflammatory markers such as CRP, procalcitonin and thrombosis indicators such as D-dimer, the ferritin level should be closely monitored to predict the infection's severity.

Our study had limitations including the fact that it was retrospective and performed at a single center. In addition, it covered a short duration and did not investigate other inflammatory indicators such as lymphocyte subtypes and cytokines.

Conclusions
The SARS-CoV-2 virus is spreading rapidly all over the world. The prognosis of the disease may vary depending on the nature of a person’s immune system. In this study, hematological and inflammatory characteristics of peripheral blood were analysed. It was shown that increased ferritin and D-dimer levels and decreased lymphocyte count are important factors affecting the duration of hospitalization for COVID-19 infection. Moreover, we identified low neutrophil count and MPV level and high platelet count and ferritin levels during the course of the disease. These parameters can be used as prognostic indicators of the disease.

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Authors’ Contributions
FA, CM, and SD: study conception and design, manuscript preparation, approval of the final version; NO: COVID-19 RT-PCR, critical revision of manuscript; RD, EA, MK, and RK: manuscript drafting, data interpretation, data acquisition; MKÇ, AKK, and RT: manuscript review and revision. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final version of the manuscript.

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