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Original Scientific Paper



# IS LYMPHOPENIA A PREDICTOR OF MORTALITY IN PATIENTS WITH COVID-19?

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SUMMARY – In this study, we evaluated the relation between the presence of lymphopenia and the need of intensive care unit (ICU) or mortality. A total of 1670 COVID-19 patients were divided according to the severity of lymphopenia developing at the time of COVID-19 infection. According to the symptoms and need of ICU, the infection was classified as mild or severe. The rates of severe infection, ICU admission, and mortality were evaluated between the groups. Among 1670 patients, 576 (34.4%) patients had severe disease and 1094 (65.6%) patients had a mild form of the disease; 213 (12.7%) patients with severe COVID-19 died. The severe form of COVID-19 was more common in patients with low lymphocyte levels (<500) than in those with normal lymphocytes count (64.7% vs. 5.2%; p<0.001). The odds ratio of lymphopenic patients was 2.4 (1.8-3.0; p=0.001). The risk of severe COVID-19 infection and mortality was 8.9 and 12.4 times higher in patients with low lymphocyte count compared to patients with normal lymphocyte count subsequently. ROC analysis showed that lymphocyte counts lower than 615 lym/mcL had 96.4% sensitivity for severe disease (AUC:0.89 (0.842-0.938); p<0.001). There was a significant negative correlation between lymphocyte count and mortality rate and severe COVID-19 disease (for severe COVID-19 r=-0.590; p<0.001and for mortality r=-0.511; p=0.001). In conclusion, we found a strong correlation between lymphopenia and COVID-19 outcomes. Lymphopenia in patients with COVID-19 was a prognostic factor in the course of the disease. Lymphopenia is an easy and inexpensive prognostic factor that can be used in the management of COVID-19 patients.

Key words: *COVID-19*, *Lymphopenia*, *Mortality*

## Introduction

Coronavirus disease (COVID-19) has currently caused death of more than 2 million people worldwide as it has still been spreading around the world[1]. Manifestation of COVID-19 mainly involves the respiratory system but can show different symptoms among patients, The symptoms such as fever, cough, tiredness, weakness, loss of taste and smell, headache, myalgia, nausea, vomiting, and diarrhea can be seen as presenting symptoms. The disease might progress to pneumonia and acute respiratory distress syndrome (ARDS)[2-3]. Once the disease has progressed to ARDS, admission to the intensive care unit (ICU) and the mortality risk are high[4].

In COVID-19 patients, the most common laboratory findings are lymphopenia, thrombocytopenia, impaired liver function tests, increased markers indicating inflammation such as C-reactive protein (CRP), interleukin (IL)-1, IL-6, tumor necrosis factor alpha (TNF-α) and deterioration in coagulation parameters. According to a study conducted on 393 patients, lym-

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phopenia and thrombocytopenia are detected subsequently in 90% and 27% of patients, respectively[5]. It is suggested that high levels of D-dimer, lactate dehydrogenase (LDH), troponin, ferritin, and creatinine may be associated with progression of the course of COVID-19 infection and mortality at the time of admission to the hospital[6].

Incubation time varies between 1 and 14 days, it is generally clinically asymptomatic and lymphocyte count is normal or slightly low during this time. After viremia, the virus begins to cause clinical symptoms *via* binding to angiotensin-converting enzyme-2 (ACE-2) receptors. In some patients, cytokine storms caused by increased inflammatory markers can develop following the symptoms within 7-14 days. Lymphopenia becomes more evident during this period. Although there are many unknown underlying causes, it is considered that lymphocytes also express ACE-2 receptor on the cell surface, and therefore lymphocytes are lysed by the virus[7]. Moreover, it is thought that high levels of interleukin (IL-6, IL-2, IL-7, G-CSF, interferon-γ inducible protein 10, MCP-1, MIP1-a) and TNF-α developed in cytokine storm might cause lymphocyte apoptosis[8].

Diseases such as autoimmune diseases and infections can cause lymphopenia. The presence and severity of lymphopenia might affect the course of these diseases[9]. In this study, we aimed to assess the relation between the severity of lymphopenia and the need of severe COVID-19 outcomes.

## Patients and Methods

In this study, we evaluated hospitalized patients with COVID-19 infection confirmed with a positive polymerase chain reaction (PCR) test between March and September 2020 in our hospital. The study received approval by the Ethics Committee of the Gazi Yaşargil Education and Training Hospital.

The following patients were excluded from the study: patients with hematologic disease; patients using drugs that cause bone marrow suppression; patients with lymphoproliferative diseases; patients with immunodeficient diseases; patients with hepatitis B, C, and HIV; patients using immunoglobulin and/or cytotoxic agents; patients with granulomatosis; patients with other viral infections and negative PCR results.

Patients with no respiratory distress, only fever, headache, loss of taste and smell, diffuse body pain, respiratory rate with or without oxygen <20 RR/min, and oxygen saturation >92% were accepted as mild COVID-19 infection. Patients with severe respiratory symptoms (low PaO2 according to blood gas analysis) and/or circulatory problems requiring intensive care treatment with noninvasive or mechanical ventilation were accepted as severe COVID-19 infection[10]. We examined laboratory parameters, especially lymphocyte values in patients with mild and severe COVID-19 to evaluate any differences between the two groups.

Considering the guideline regarding the definition and degree of lymphopenia, patients were divided into 3 groups according to lymphocyte count within the first 72 hours from the time of admission to the hospital: group 1 <500 Lym/mcL; group 2 500-1500 Lym/mcL; and group 3 >1500 Lym/mcL. These groups were evaluated in terms of mortality rate, need of ICU, and rate of development of severe COVID-19.

### *Statistics*

The data recorded in the groups, either valid and suitable for normal distribution, were tested by the Shapiro-Wilk test, coefficient of variation, skewness, and kurtosis. Continuous variables were specified with mean and standard deviation, whereas categorical variables were specified with percentage and frequency. While the mean and standard deviation were specified for the normally distributed parameters, minimum and maximum values were specified for the non-normally distributed parameters. A two-way ANOVA test was performed in the groups of patients (variance homogeneity) divided according to lymphocyte count. Bonferroni correction was used to determine which groups had a significant difference. Welch ANOVA and Kruskal-Wallis tests were performed in the groups with non-homogeneous variances. ROC analyses were performed to determine the effect of lymphocyte count on mortality. Their results were reported as the area under the curve (AUC) and 95% confidence interval (CI). The level of statistical significance was set at $p<0.05$. Statistical analysis was performed using SPSS 26.0 for Windows (SPSS Inc., Chicago, IL, USA) package program.

## Results

In this study, 1670 patients were included, i.e., 1094 patients with mild COVID-19 infection and 576 patients with severe symptoms of COVID-19 infection. The mean age of patients with mild COVID-19 was 41.08±17.80 years, while the mean age of

patients with severe COVID-19 was 65.6±19.24 years (p<0.001). The duration of hospitalization in patients with severe infection was longer (15.9 *vs*. 7.1 days). The mean lymphocyte count was 1702±726 cell/μL in patients with mild COVID-19 and 628±424 cell/μL (p<0.001) in patients with severe COVID-19 (p<0.001). However, there were significant differences in inflammation parameters between the patients with mild and severe COVID-19. The mean procalcitonin level was 2.34±4.76 ng/mL in patients with mild COVID-19 and 4.09±9.38 ng/mL in patients with severe COVID-19 (p=0.002). Similarly, ferritin level was different between the patients with mild and severe COVID-19 (221.0±334.9 μg/L *vs*. 655.0±554.6 μg/L; p=0.001). Significant differences were detected in D-dimer levels between the patients with severe COVID-19 and mild COVID-19 ( 259.8±419.2 ng/mL *vs*. 1495.4±3296.1 ng/mL; p<0.001) (Table 1).

Although 64.7% of patients with lymphocyte count below 500 developed severe disease, this rate was only 5.2% in patients without lymphopenia (p=0.001). While 25.9% of patients with deep lymphopenia (group 1) died, this rate was 2.9% in patients with high lymphocyte levels. The odds ratio for mortality in patients with lymphopenia was 2.4 (1.8-3.0; p=0.001) compared to all patients. Patients with lymphopenia had a 12.4 times higher risk of severe COVID-19 infection and 8.9 times higher risk of mortality than the group without lymphopenia (Table 2).

*Table 1. Demographic data and laboratory parameters of patients with mild and severe COVID-19*

| Parameters | Mild COVID-19 (n=1094) | Severe COVID-19 (n=576) | p* |
|---|---|---|---|
| Age, mean± SD | 41.08±17.80 (17-74) | 65.6±19.24 (22-96) | **<0.001** |
| Gender:<br>Female<br>Male | <br>562 (51.4%)<br>532 (48.6%) | <br>265 (46%)<br>311 (54%) | |
| Days of hospitalization | 7.1 (3-20) | 15.9 (2-55) | **0.001** |
| WBC (cell/μL) | 7570±4512 | 9702±3871 | **0.021** |
| Lymphocytes (cell/μL) | 1702±726 | 628±424 | **<0.001** |
| Platelets ($10^3$ cell/μL) | 222.4±65.6 | 231.7±105.9 | 0.878 |
| CRP (mg/L) | 42.9±77.8 | 149.7±85.2 | **<0.001** |
| Procalcitonin (ng/mL) | 2.34±4.76 | 4.09±9.38 | **0.002** |
| Ferritin (μg/L) | 221.0±334.9 | 655.0±554.6 | **0.001** |
| D-dimer (ng/mL) | 259.8±419.2 | 1495.4±3296.1 | **<0.001** |
| Creatinine (mg/dL) | 0.9±0.6 | 1.2±1.0 | **0.006** |
| Albumin(g/L) | 4.1±0.5 | 3.0±0.4 | **0.001** |
| AST (IU/L) | 41.5±67.2 | 58.3±93.4 | **0.001** |
| ALT (IU/L) | 34.1±21.3 | 42.4±50.2 | **0.002** |
| LDH (IU/L) | 223.2±107.9 | 405.7±182.3 | **<0.001** |

*Student's t-test or Mann Whitney U-test; SD = standard deviation; ALT = alanine transaminase; AST = aspartate transaminase; CRP = C-reactive protein; IU = international unit; WBC = white blood cell

*Table 2. Mortality rate and rate of severe COVID-19 infection among three groups according lymphocyte count*

| | Group 1 (lymphocyte count <500) (n=547) | Group 2 (lymphocyte count 501-1500) (n=572) | Group 3 (lymphocyte count >1500) (n=551) | p value |
|---|---|---|---|---|
| Severe COVID-19 infection | 354 (64.7%) | 193 (33.7%) | 29 (5.2%) | **<0.001** |
| Exitus | 142 (25.9%) | 55 (9.6%) | 16 (2.9%) | **<0.001** |
| Odds ratio for exitus (95%) | 2.4 (1.8-3.0) | 0.7 (0.5-0.9) | 0.4 (0.2-0.5) | |

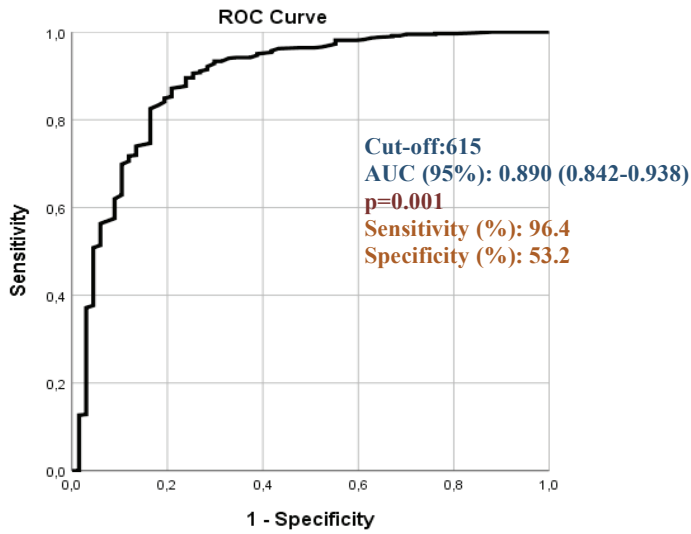


*Fig. 1. ROC curve for severe COVID-19 infection.*

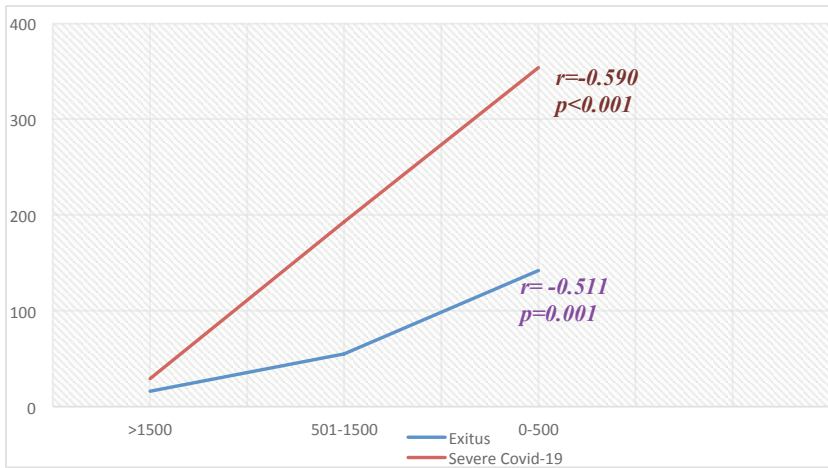


*Fig. 2. Correlation of lymphocyte count and severe infection and mortality in patients with COVID-19 infection.*

According to ROC analysis, sensitivity for severe infection was 96.4% when lymphocyte count falls below 615 (AUC: 0.89 (0.842-0.938); p=0.001) (Fig. 1).

In addition, a moderate negative correlation was found between lymphocyte count and severe COVID-19 infection and mortality (severe disease: r=-0.590; p<0.001; mortality: r=-0.511; p=0.001) (Fig. 2).

## Discussion

In this study, we concluded that there was a high risk of mortality and severe infection in patients with lower lymphocyte count compared to patients without lymphopenia. In addition, there was a significant correlation between the severity of lymphopenia and severe COVID-19 infection and mortality.

Patients with lymphopenia had a 1.4 times higher risk of severe COVID-19 infection and an 8-9 times higher risk of mortality than the group without lymphopenia. The odds ratio for mortality was less than one in patients without lymphopenia. This situation showed that the mortality rate of patients without lymphopenia during infections was also low.

Our study was important by showing lymphopenia to be a good indicator of the severity of COVID-19 infection. According to the WHO, while clinical findings and imaging (x-ray, computed tomography) findings are included in the classification of the severity of COVID-19 infection, there is no laboratory classification to determine the course of the disease[11,12]. Lymphocyte levels might be a helpful and additional parameter to determine the course of the disease and administration of a more aggressive treatment earlier.

According to the study by Henry *et al*. on 4969 patients with COVID-19 infection, a low lymphocyte count and high neutrophil level at the time of admission were found to be associated with the severity of the disease[13]. In the study by Huang and Pranata, the mean lymphocyte count of patients with severe COVID-19 infection was found to be lower[14].

In the study by Tan *et al*., patients were classified according to lymphocyte count and the authors demonstrated that the severity of lymphopenia could be a safe marker in determining the severity of the disease[15]. Similarly, the study by Zhao *et al*. on 2282 patients with COVID-19 infection demonstrated that lymphopenia could increase the probability of severe infection up to 3 times[16].

An important limitation of our study was that we did not consider other comorbid diseases while classifying patient groups and therefore the groups were not established in a heterogeneous way. Although it was a shortcoming of our study to ignore the lymphopenia-causing effect of the drugs used in the treatment of COVID-19 infection, we observed that all patients in the group were treated with similar antivirals and antibiotics. In addition, the inability to determine the subtypes of lymphocytes in patients with lymphopenia was an obstacle to detecting which lymphocyte series decreased more.

İt can be concluded that lymphopenia in patients with COVID-19 infection at the time of hospital admission is associated with severe infection and mortality. Lymphopenia developing with COVID-19 infection appears to be an independent marker to determine the prognosis of the COVID-19 infection. This can give clinicians the upper hand to determine the algorithm for treatment. In the upcoming period, strategies to prevent the development of lymphopenia or treatment studies aimed at increasing the number of lymphocytes in the treatment of COVID-19 patients will increase our knowledge and shed light on this issue.

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Sažetak

## JE LI LIMFOPENIJA PREDIKTOR SMRTNOSTI U BOLESNIKA S COVID-19?

*J. Kılıc, B. Ebik, F. Bacaksız, N. Ekin* i *B. Sami Kalın*

U ovoj studiji procijenili smo odnos između prisutnosti limfopenije i potrebe za jedinicom intenzivnog liječenja (JIL) ili smrtnosti. Ukupno je 1670 bolesnika oboljelih od COVID-19 podijeljeno prema težini limfopenije koja se razvila u vrijeme bolesti COVID-19. Prema simptomima i potrebi za intenzivnim liječenjem infekcija je klasificirana kao blaga ili teška infekcija. Među skupinama procijenjene su stope teških infekcija, prijma u JIL i stope smrtnosti. Od 1670 bolesnika 576 (34,4%) ih je imalo tešku bolest, a 1094 (65,6%) bolesnika je imalo blaži oblik bolesti. Umrlo je 213 (12,7%) bolesnika s teškim oblikom COVID-19. Teški COVID-19 bio je češći u bolesnika s niskim razinama limfocita (<500) od onih s normalnim brojem limfocita (64,7% naspram 5,2%, p<0,001). Omjer vjerojatnosti limfopeničnih bolesnika bio je 2,4 (1,8-3,0, p=0,001). Rizik od teške infekcije COVID-19 i smrtnosti bio je 8,9 i 12,4 puta veći u bolesnika s niskim brojem limfocita u usporedbi s bolesnicima s normalnim brojem limfocita kasnije. ROC analiza je pokazala da broj limfocita manji od 615 lym/mcL ima 96,4% osjetljivost za tešku bolest (AUC: 0,89 (0,842-0,938, p<0,001). Postojala je značajna negativna korelacija između broja limfocita i stope smrtnosti i teške bolesti COVID-19 (za teški oblik COVID-19, r=-0,590; p<0,001 i za smrtnost r=-0,511; p=0,001). U zaključku; otkrili smo snažnu korelaciju između limfopenije i ishoda bolesti COVID-19. Limfopenični bolesnici s COVID-19 bili su prognostički čimbenik u tijeku bolesti. Limfopenija je jednostavan, jeftin prognostički čimbenik koji se može koristiti u liječenju bolesti COVID-19.

Ključne riječi*: COVID-19; Limfopenija; Smrtnost*