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The diagnostic value of neutrophil to lymphocyte ratio in determining the severity of COVID-19

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Abstract

Background: Changes in hematological parameters play a role in the pathogenesis of coronavirus disease 2019 (COVID-19). We aimed to investigate the significance of neutrophil-lymphocyte ratio (NLR) and hematologic parameters in determining the severity of COVID-19.

Methods: This retrospective cross-sectional study was conducted on adult patients diagnosed with COVID-19 in two pandemic hospitals between 01, April, and 01, July 2020. Using the COVID-19 diagnostic criteria of the world health organization (WHO), the patients were divided into two groups as severe and non-severe. Demographic and clinical characteristics, white blood cell (WBC), neutrophil, lymphocyte and platelet counts, and NLR of all patients were examined at the first admission. Multivariate analyzes were performed to determine the independent predictive data and ROC analysis to test the diagnostic accuracy of the hematological parameters.

Results: Of the 381 patients included in the study, 42 (11%) had severe COVID-19 infection. While the mean NLR was 7.61±7.48 in patients with severe COVID-19, the mean NLR of non-severe patients was 2.97±2.37 (95% CI: 2.294 to 6.984, p<0.001). Long duration of hospital stay, elevated NLR ratio, female gender were predictive variables of severe COVID-19 cases (OR =0.833, 95% CI: 0.744 to 0.934, p=0.002; OR=0.195, 95% CI: 0.057 to 0.6731, p=0.010; OR=0.664, 95% CI: 0.501 to 0.881, p=0.005, respectively). In ROC analysis, NLR ratio had 2.625 optimum cut-off value, 60% specificity (95% CI: 54.7 to 65.4), 86% sensitivity (95% CI: 71.5 to 94.6), positive likelihood ratio (PLR) of 4.2 (95% CI: 2.0 to 8.9) and negative likelihood ratio (NLR) of 0.46 (95% CI: 0.4 to 0.6) for severe COVID-19 cases.

Conclusion: The results of this study revealed that there might be a relationship between elevated NLR and severity in COVID-19 cases.

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Introduction

Coronavirus disease 2019 (COVID-19) may lead to severe acute respiratory syndrome. COVID-19 first appeared in Wuhan, China, and spread from there, causing an epidemic across China and then a pandemic around the world [1-3]. A large number of infected patients were seen due to a lack of immunity to COVID-19, and complications that occur during this viral disease. It usually manifests itself with fever (>80%), cough (>60%), and myalgia or fatigue (>40%) in patients [3]. About 60% of male cases in the middle age are affected around

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the age of 50 [4]. Clinical manifestations can be asymptomatic, and vary from very mild to severe disease to sepsis and death. Looking at the available data, most of the COVID-19 diseases are mild, while 16% of cases were severe [5]. In clinically severe cases, infection-related complications were reported to activate systemic coagulation and inflammatory responses, which are vital for patients' defense but can cause DIC [6-10].

Neutrophilia is a parameter that indicates a response to systemic inflammation, while lymphopenia, in general, indicates that cellular immunity is weak. The ratio of these two parameters indicates the adequacy of the cellular immune response against this inflammatory state by the size of the systemic inflammation. Neutrophil lymphocyte ratio (NLR) is an indicator of ability to generate immune responses and subclinical inflammation. NLR is an economical, easy and repeatable measurement parameter. The reason NLR shows a poor prognosis is that neutrophils are dominant, which can suppress cytotoxic T cells. NLR increases in the presence of infection, especially sepsis, and also in the increased severity of these clinical conditions [9-11].

New studies on the characteristics and treatment of the virus and the disease are added everyday to the literature since the emergence of COVID-19 in China. However, despite the large number of scientific studies included in the literature from day to day, there is not yet sufficient and accurate information about COVID-19 and its treatment. Considering the pathogenesis of the disease, the clinical manifestation, and test results in patients, it is observed that hematological parameters, especially neutrophil and lymphocyte counts are affected in this infection. In the literature, few publications examined hematological parameters in relation to severity of COVID-19 [12,13]. Thus, this study aimed to investigate the neutrophil-to-lymphocyte ratio (NLR) and other hematological parameters for the diagnosis of severe COVID-19 patients.

Methods

Study settings and protocol: This hospital-based retrospective cross-sectional study was conducted by investigating the files of COVID-19 patients who were brought to Adıyaman Training and Research Hospital and Sakarya University Training and Research Hospital between April 2020 and July 2020. Both hospitals were among the hospitals designated as COVID-19 pandemic hospitals in Turkey. Before starting the study, approvals of the Ministry of Health and the local ethics committee were obtained and, the Declaration of Helsinki was followed.

Participants: Patients over the age of 18 years admitted to the hospital with a definitive diagnosis of COVID-19 were included in the study. Patients under the age of 18 years, pregnant women, patients with missing data in hospital records, patients with the hematological disease were excluded from the study. Patients definitively diagnosed with COVID-19 based on typical CT image of COVID-19 viral pneumonia and/or with a positive result of RT-PCR for SARS-CoV-2 RNA were divided into two groups as severe and non-severe patients. Based on the COVID-19 Infection Diagnosis and Treatment Guideline [13], the World Health Organization (WHO) defines severe patients as the patients with clinical signs of pneumonia (fever, cough, dyspnea, rapid breathing) with at least one of the following criteria (respiratory rate ≥ 30 times/min, severe respiratory distress, oxygen saturation (room air) ≤ 93%). White blood cell (WBC), neutrophil, lymphocyte and platelet counts, and NLR of the patients were examined at the first admission to the COVID-19 pandemic service of the emergency clinic of hospitals. An experienced researcher confirmed the severity of the patients.

Data collection and laboratory investigations: Data regarding age, gender, present diseases and comorbidities, length of stay (day) in hospital, and laboratory investigation of each patient were obtained from hospital records. Two researchers independently examined the accuracy of the patient data and COVID-19 diagnosis. The venous blood samples used for laboratory analysis were collected in hemogram tubes containing ethylenediaminetetraacetic acid (EDTA). WBC, neutrophil, lymphocyte, and platelet count were studied in CELLDYN 3700 device (Abbott, USA) within one hour of collection of blood samples. The

reference values for total WBC, neutrophil, lymphocyte and platelet were 4.6 to 10.2 x 10^9/L, 2.0 to 6.9 x 10^9/L, 0.60 to 3.40 x 10^9/L and 140 to 424 x 10^9/L respectively. Throat and nasal swab samples for SARS-CoV-2 diagnosis were analyzed with the qRT-PCR kit as per the WHO guidelines (BioGerm, Shanghai, China).

Statistical analysis: Data analysis was performed using the Statistical Package for Social Sciences for Windows software, version 17 (SPSS Inc., Chicago, IL, United States) and Medcalc version 12.7.0.0. Data were expressed as mean ± SD for continuous variables and frequencies and proportions for categorical variables. Student's t-test was used to analyze mean differences between groups. Categorical data were analyzed using Pearson's chi-square test. Determining the best predictors that affect severity was evaluated by multiple logistic regression analysis. Any variable having a significant univariate test along with all other variables of known clinical importance were selected as candidates for the multivariate analysis. Odds ratios and 95% confidence intervals (CI) for each independent variable were calculated.

For the cut-off points of each clinical variable, severe and non-severe patient were evaluated by receiver operating characteristic (ROC) analyses, a calculating area under the curve (AUC) as giving the maximum sum of sensitivity and specificity for the relevant test. Sensitivity, specificity, and positive and negative likelihood values were also calculated at the best cut-off point for each clinical variable and presented with 95% CI. A p-value of <0.05 was considered statistically significant.

Results

Medical records of 462 COVID-19 patients were examined. However, 81 patients whose data could not be reached were excluded from the study. Finally, a total of 381 COVID-19 patients who met the research criteria were included in the study. Of these patients, 42 (11.02%) were severe COVID-19 patients, and 339 were non-severe COVID-19

Table-1: *Demographic and clinical characteristics of COVID-19 patients*

Variables	Severe case n=42	Non-severe case n=339	95% CI	p value
Age (Mean ± SD, in years)	67.33 ± 16.45	48.81 ± 18.47	13.052-23.998	<0.001
Gender (n, %)				0.043
Female	25 (59.5)	146 (43.1)		
Male	17 (40.5)	193(56.9)		
Length of hospital stay (day)	10.96 ± 5.68	5.79 ± 3.48	2.890-7.458	<0.001
Co-morbidity, **(n, %)**				
Hypertension	12(30.8)	53(16.2)	0.029-0.026	0.024
CAD	13(33.3)	55(16.8)	0.017-0.015	0.012
COPD	5(12.8)	29(8.8)	0.558-0.287	0.418
Hematological values (mean ± SD)				
Neutrophil, cells x10^9/L	7.01 ± 3.94	4.31 ± 2.38	1.445-3.954	<0.001
Lymphocyte, cells x10^9/L	1.64 ± 1.61	1.78 ± 0.84	-0.741-0.282	0.372
NLR	7.61 ± 7.48	2.97 ± 2.37	2.294-6.984	<0.001
WBC, cells x10^9/L	7.73 ± 3.19	6.88 ± 2.74	-0.051-1.749	0.065
Platelet, cells x10^9/L	206.88 ± 214.57	90.94 ± 70.01	-31.033-15.649	0.517

Note: COPD: chronic obstructive pulmonary disease, NLR: neutrophil-lymphocyte ratio, CAD: coronary artery disease, WBC: white blood cell, SD: standard deviation, CI: confidence interval.

patients. Mean age of the severe and non-severe patients was 67.33±16.46 and 48.81±18.48 years respectively (95% CI: 13.05 to 23.99, p<0.001). In the comparison of severe and non-severe patients, female gender (59.5% vs. 43.1%, p=0.043), hypertension (30.8% vs. 16.2%; 95% CI: 0.029 to 0.026, p=0.024), presence of coronary artery disease (33.3 vs. 16.8; 95% CI: 0.017 to 0.015,

Table-2: *The results of multiple logistic regression analysis*

Variables	Wald	OR	95% CI	p-value
Gender	6.688	0.195	0.057-0.673	0.010
Length of stay (day)	9.890	0.833	0.744-0.934	0.002
Hypertension	0.221	0.674	0.130-3.492	0.638
CAD	1.035	2.116	0.499-8.968	0.309
COPD	0.391	0.430	0.030-6.064	0.532
Neutrophil	0.118	0.917	0.560-1.501	0.731
NLR	8.068	0.664	0.501-0.881	0.005
WBC	0.030	1.028	0.748-1.414	0.863
Platelet	1.093	1.005	0.995-1.015	0.296

Note: CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, OR: odds ratio, CI: confidence interval.

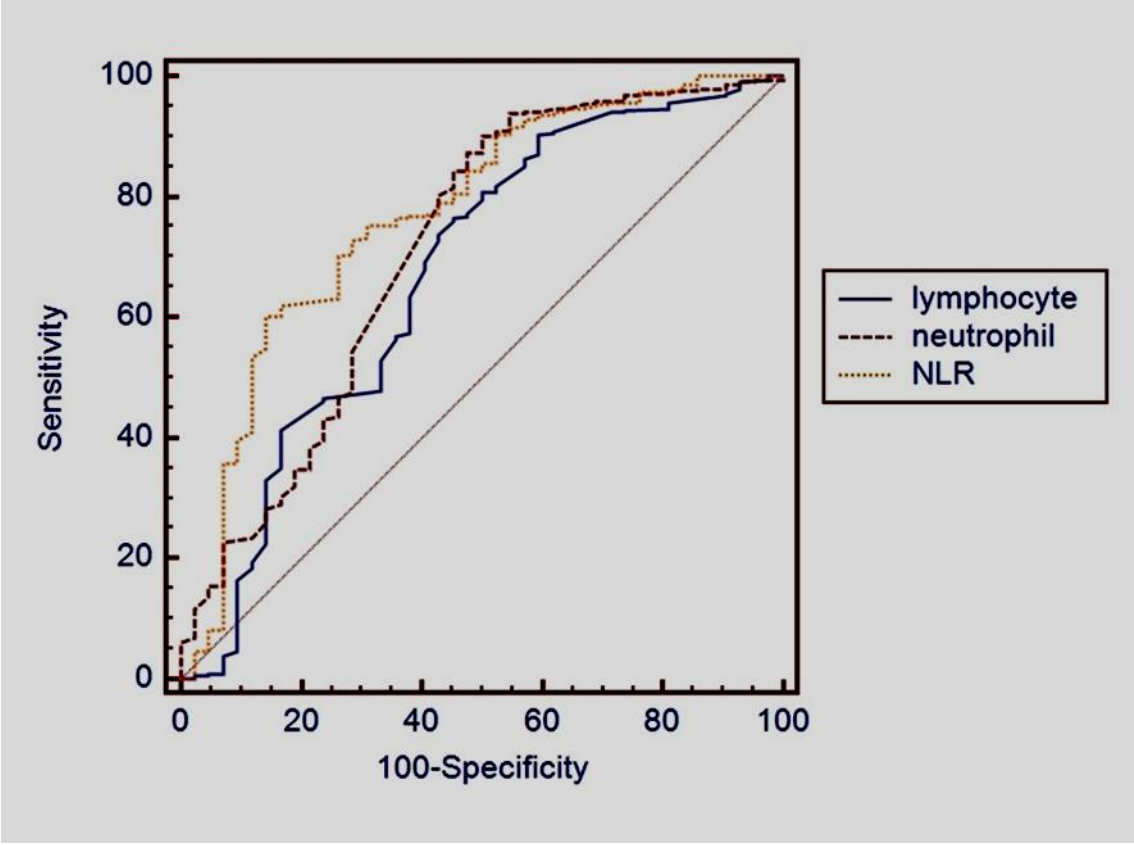

Figure-1: *Lymphocyte, neutrophil and neutrophil-lymphocyte ratio (NLR) ROC curve*

p=0.012), mean number of days hospitalized (10.96±5.68 vs. 5.79±3.49; 95% CI: 2.89 to 7.458, p<0.001) and mean NLR (7.61±7.48 vs. 2.97±2.37 95% CI: 2.294 to 6.984, p<0.001) were found to be higher in severe patients. The demographic and clinical characteristics of the patients are shown in Table-1.

In multiple logistic regression analysis, predictive variables that differed between severe and non-severe cases were identified as gender, the number of days of hospitalization, and NLR (Table-2).

ROC analysis of NLR and WBC and platelet count was performed to evaluate the use of optimal limit values in laboratory results to distinguish non-severe COVID-19 infection. The area below the ROC curve was found to be statistically significant for the NLR in determining severe COVID-19 patients (AUC: 0.770, 95% CI: 0.725 to 0.812, p<0.001). In distinguishing the two groups from each other, the NLR had 2.625 optimum cut-off value, 60% specificity (95% CI: 54.7 to 65.4), 86% sensitivity (95% CI: 71.5 to 94.6), positive likelihood ratio (PLR) of 4.2 (95% CI: 2.0 to 8.9) and negative likelihood ratio (NLR) of 0.46 (95% CI: 0.4 to 0.6). AUC (Area under the curve) values were not statistically significant in distinguishing the two groups in the ROC analysis performed to determine diagnostic values of lymphocytes, neutrophils, and platelets (p>0.05) (Figure-1).

Discussion

COVID-19 infection affects the respiratory tract, causing a wide range of clinical manifestations ranging from mild viral pneumonia to severe respiratory failure and death [10,12]. Alteration of many hematological and biochemical parameters related to inflammation, coagulation and tissue damage were found to be associated with the course of COVID-19 infection and mortality [14].

NLR correlates with the prognosis of systemic inflammatory diseases. Therefore, NLR levels were also investigated especially in diseases other than COVID-19 [15-19]. Besides, indices such as NLR were found to be significant in prognostic monitoring of diseases such as ulcerative colitis, obstructive sleep apnea, Sjogren's syndrome and systemic lupus erythematosus, where the inflammatory activity is dominant [19-21]. Studies in patients with squamous cell carcinoma of the esophagus and diseases accompanied by inflammation showed a significant association of the condition with NLR value [22,23]. A study conducted in patients with rheumatoid arthritis found NLR values as significantly higher in the patient group compared to the healthy control group [24]. Studies conducted in patients with cardiovascular disease have found that increase in mortality was correlated with the elevated NLR values [25,26].

In COVID-19 cases, a study conducted by Yang et al. found that among the hematological and inflammation biomarkers, increased NLR was associated with poor prognosis, duration of hospital stay, and clinical course [9]. A study that analyzed 548 COVID-19 cases reported an increase in neutrophil count and NLR in critically ill and terminal cases [13]. Similarly, increased NLR has been shown to be associated with increased severity and mortality in COVID-19 cases [27]. In our study of COVID-19 patients, when we compared the NLR values of severe patients with non-severe patients, we found a statistically significant high NLR values in severe cases. Published studies have reported different efficacy and power of the diagnostic value of NLR in determining the severity of COVID-19. While its diagnostic efficiency was high in some studies, it was low in others. A study examining NLR to predict all-cause mortality in COVID-19 patients found that NLR had 84% specificity and 100% sensitivity [28]. A study involving 1579 patients reported the sensitivity and specificity as 0.78 (95% CI: 0.70 to 0.84) and 0.78 (95% CI: 0.73 to 0.83) respectively for the predictive value of NLR on disease severity [29]. In our study, NLR had 60% specificity (95% CI: 54.7 to 65.4) and 86% sensitivity (95% CI: 71.5 to 94.6) in distinguishing severe COVID-19 cases.

Platelets are blood cells involved in coagulation. Recent studies have reported occurrence of thrombus and occlusion in small vessels in the lungs of severe cases of COVID-19 [3,30]. In our study of COVID-19 patients, we found that though there was an increase in platelet count in severe COVID-19 patients than that of non-severe cases, the increase was not significant statistically. Also,

higher leukocyte count was reported in 11.4% of severe COVID-19 cases than patients with mild to moderate severe disease [12]. In our study of COVID-19 patients, we also observed increased neutrophil and decreased lymphocyte counts in severe patients.

Conclusion

Severe COVID-19 infection occurred more commonly in the elderly, women, and patients with hypertension and coronary artery disease. Female gender, long duration of hospital stay, and increased NLR were predictive factors for a severe course of COVID-19 infection. The mean NLR was found to be higher in severe COVID-19 patients than non-severe cases. The NLR had 60% specificity and 86% sensitivity at the 2.625 optimum cut-off value in distinguishing severe COVID-19. However, the present study had some limitations. It was a retrospective study and had small number of severe COVID-19 cases.

Conflict of interest

All the authors declared that there is no conflict of interest.

Financial disclosure

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