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Evaluation Of the Effectiveness Of NLR, LMR, PLR, d-NLR, LeCR, LCR, NMR Bioparameters In the Course Of COVID-19

COVID-19 Seyrinde NLR, LMR, PLR, d-NLR, LeCR, LCR, NMR Biyoparametrelerinin Etkinliğinin Değerlendirilmesi

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Abstract

Objective: Severe inflammatory response of the immune system has a serious role in the progression of Coronavirus disease 2019 (COVID-19). The clinical benefits of early diagnosis of immune activation of COVID-19 have been emphasized repeatedly in the trials to this date. In this study, neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), derived neutrophil-to-lymphocyte ratio (d-NLR), lymphocyte-to-C reactive protein ratio (LCR), leukocyte-to-C reactive protein ratio (LeCR), neutrophil-to-monocyte ratio (NMR) biomarkers were evaluated for predicting clinical course of COVID-19.

Materials and Methods: In this retrospective cohort study, 383 laboratory-confirmed COVID-19 cases, who had been hospitalized in a tertiary care hospital between April and November 2020, were included. Patients, including 279 mild and 104 severe cases, were sequentially selected. Blood tests, conducted at the time of admission, were examined. Data was analyzed and ROC analysis was performed by using SPSS 22.0 program.

Results: 44.3% of the patients included in the study were female, 99.2% of the patients had viral pneumonia, 27.2% met clinical criteria for severe disease and median age was 58 years. Age, duration of hospitalization, white blood cell count, neutrophil count, ferritin, CRP, procalcitonin, D-dimer, troponin levels were higher and lymphocyte, monocyte counts were lower in the group with clinically severe disease. The diagnostic sensitivities of LCR, CRP, d-NLR, NLR, LeCR were found to be high (AUC> 0.8) for the prediction of clinical severity with cut-off values of 15, 74.65, 2.55, 4, 133 respectively.

Conclusion: High CRP, d-NLR, NLR and low LCR, LeCR are early predictors of the clinical severity, these patients should be under hospital follow-up for close monitoring and early intervention.

Keywords: COVID-19, d-NLR, LCR, LeCR, NLR

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Öz

Amaç: Bağışıklık sisteminin şiddetli inflamatuar yanıtı, 2019 Coronavirüs hastalığının (COVID-19) ilerlemesinde ciddi bir role sahiptir. COVID-19'un immün aktivasyonunun erken teşhisinin klinik faydaları, bugüne kadar yapılan çalışmalarda vurgulanmıştır. Bu çalışmada nötrofil-lenfosit oranı (NLR), lenfosit-monosit oranı (LMR), trombosit-lenfosit oranı (PLR), türetilmiş nötrofil-lenfosit oranı (d-NLR), lenfosit-to-lenfosit oranı -C reaktif protein oranı (LCR), lökosit-C reaktif protein oranı (LeCR), nötrofil-monosit oranı (NMR) biyoendeksleri, COVID-19'un klinik seyrini tahmin etmek için değerlendirildi.

Gereç ve Yöntemler: Bu retrospektif kohort çalışmasına, Nisan ve Kasım 2020 tarihleri arasında üçüncü basamak bir hastanede yatarak tedavi görmüş, laboratuvarca doğrulanmış 383 COVID-19 vakası dahil edildi. 279 hafif ve 104 ciddi vaka dahil olmak üzere hastalar sırayla seçildi. Başvuru sırasında yapılan kan testleri incelendi. Veriler SPSS 22.0 programı kullanılarak analiz edildi ve ROC analizi yapıldı.

Bulgular: Çalışmaya alınan hastaların %44,3'ü kadındı, hastaların %99,2'sinde viral pnömoni vardı, %27,2'si ciddi hastalık klinik kriterlerini taşıyordu ve medyan yaş 58 idi. Klinik olarak ciddi hastalığı olan grupta yaş, hastanede yatış süresi, lökosit sayısı, nötrofil sayısı, ferritin, CRP, prokalsitonin, D-dimer, troponin düzeyleri daha yüksek, lenfosit, monosit sayıları daha düşüktü. LCR, CRP, d-NLR, NLR, LeCR'nin sırasıyla 15, 74,65, 2,55, 4, 133'lük eşik değerleri ile klinik şiddeti öngörmek için tanısal duyarlılıkları yüksek (AUC> 0.8) bulundu.

Sonuç: Yüksek CRP, d-NLR, NLR ve düşük LCR, LeCR klinik ciddiyetin erken belirteçleridir, bu hastalar yakın izlem ve erken müdahale için hastanede takip edilmelidir.

Anahtar Kelimeler: COVID-19, d-NLR, LCR, LeCR, NLR

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Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), demonstrates a wide spectrum of conditions from asymptomatic to moderate clinical symptoms to progressive organ failure, leading to sepsis, acute respiratory distress syndrome (ARDS), and even death (1). In patients with mild clinical course, symptoms include nausea, vomiting, diarrhea, loss of taste and smell sensations, sore throat, headache, weakness, muscle-joint pain, fever, cough and abdominal pain. In more severe cases, symptoms, such as shortness of breath or abnormalities in lung imaging are also involved (2). The immune system's strong inflammatory response plays a significant role in the progression of COVID-19 (3). Consequently, it is believed that the illness manifestation is connected with an increase in inflammatory markers and acute phase reactants such C-reactive protein (CRP), D-dimer, serum ferritin, and procalcitonin as well as other hematological abnormalities, particularly lymphopenia (2,4).

The clinical advantages of early COVID-19 identification and treatment have been emphasized repeatedly in the trials conducted to this date. According to several studies, clinical severity may be predicted with a high neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), derived neutrophil-to-lymphocyte ratio (d-NLR) and neutrophil-to-monocyte ratio (NMR) and low lymphocyte-to-monocyte ratio (LMR), lymphocyte-to-CRP ratio (LCR), and leukocyte-to-CRP ratio (LeCR) (1,3,5,6,7). Our objective was to assess the blood metrics and biomarkers, checked on the day of admission. In order to forecast severe clinical deterioration and hospitalizations, which last longer than five and ten days, we also evaluated the diagnostic sensitivity, specificity and cut-off values for these biomarkers.

Materials and Methods

Study Population and Design

In this retrospective cohort analysis, 500 laboratory confirmed COVID-19 cases, who had been hospitalized and followed-up in a tertiary care hospital between April and November 2020, were selected. 383 participants, who had eligible data for the research, were included in the study and 117 patients were excluded due to incomplete data. Figure 1 depicts a flowchart of patient inclusion, COVID-19 severity and prognosis.

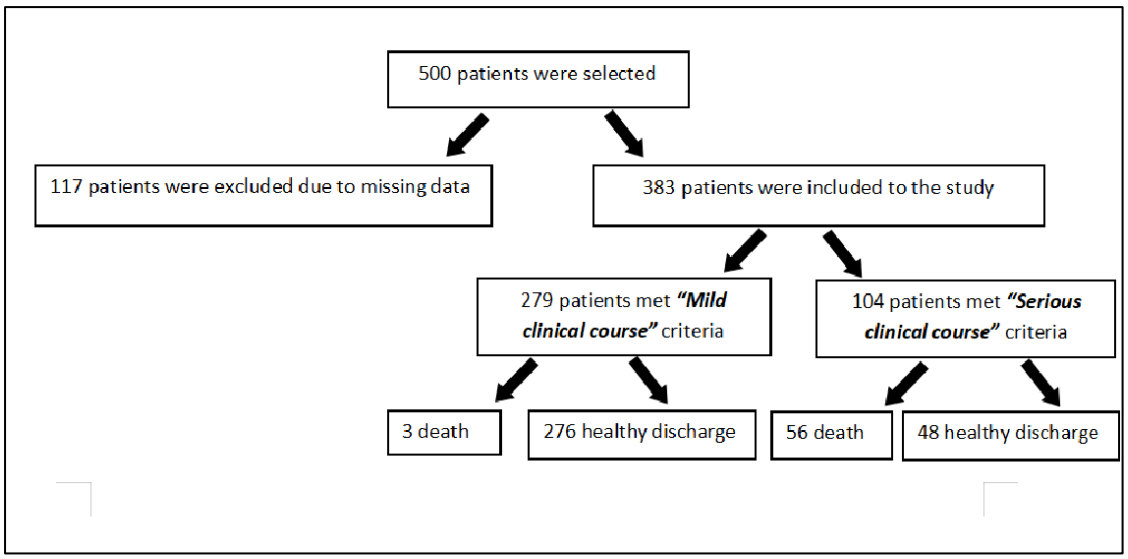

Figure 1. Flowchart of patient inclusion, COVID-19 disease severity and prognosis

According to the current diagnosis and treatment recommendations for COVID-19, cases were diagnosed and treated (8,9). The patients had to meet certain criteria for admission to hospital, including having

certain abnormal blood values (lymphocyte count < 800 / μl, CRP > 10 × Upper laboratory limit, ferritin > 500 ng / ml, D-dimer > 1000 ng / ml), an age of over 60 years, having a comorbid disease, such as hypertension, diabetes, coronary artery disease, heart failure, obesity, malignancy, chronic obstructive pulmonary disease, asthma or having an immunosuppressive condition or taking an immunosuppressant medication or having tachypnea, desaturation, oral intake disorder (10). Retrospective data screening from patient files was conducted for demographic information, laboratory results and vital signs. The study excluded patients under 18, pregnant women and patients with insufficient data. The patients were divided into two groups based on their clinical severity: severe and mild. Patients were sequentially chosen, including 279 mild and 104 severe cases. Symptoms of patients with mild clinical course included fever or respiratory symptoms, typical COVID-19 CT image abnormalities and positive results from real-time reverse transcriptase-polymerase chain reaction (RT-PCR) for SARS-CoV-2 RNA in nasopharyngeal swap samples. Clinically serious patients additionally met at least one of the following conditions: respiratory rate ≥ 30 times/min, shortness of breath, oxygen saturation (resting state) ≤ 93%, arterial blood oxygen pressure (PaO2) / inspired oxygen concentration (FiO2) ≤ 300 mmHg (1 mm Hg= 0.133 kPa)2. All biomarkers, except d-NLR, were calculated using related laboratory parameters, while d-NLR was calculated as follows: neutrophil count divided by the result of white blood cell count, minus neutrophil count.

Ethics Committee Approval and Informed Consent

The study protocol was initially approved by the Ministry of Health of the Republic of Turkey and then by Bakırçay University local ethics committee with the decision, numbered 91 and dated October 12, 2020. Informed consents were obtained from the patients according to the regulations of the committee. All procedures were carried out in compliance with the current recommendations, the Declaration of Helsinki, and the committee rules.

Laboratory Tests

On the day the admission of patients to the hospital, blood tests were conducted. Hematologic tests were made on a Beckman Coulter LH 780 (Beckman Coulter Ireland Inc., Mervue, Galway, Ireland). Blood biochemical parameters, consisting of blood urea nitrogen (BUN) (mg / dL), creatinine (mg / dL), alanine transaminase (ALT) (IU / L), aspartate transaminase (AST) (IU / L), total bilirubin (Tbil) (mg / dL), ferritin (ng / ml), CRP (mg / dL) were analyzed by COBAS C702 auto-analyzer (Roche Diagnostics, Belgium) and procalcitonin (ng / ml), troponin (ng / L) were analyzed by COBAS E601 auto-analyzer (Roche Diagnostics, Belgium). Automated coagulation analyzer Sysmex® CS-2500 System was used to measure D-dimer (SysmexCorporation, Kobe, Japan). Nasopharyngeal swab samples were delivered to the molecular virology lab of VNAT viral transport, where they were examined using the Biospedy (Bioeksen, Turkey) rRT-PCR kit that was supplied by the Turkish Ministry of Health.

Statistical Analysis

The Statistical Package for Social Sciences version 22 was used to analyze the data. The categorical variables were presented as frequency and percentage. Normal distribution was examined by the Kolmogorov-Smirnov test. Since the variables had an irregular distribution, continuous variables were reported as the median (IQR). The non-parametric Mann-Whitney U test was used for comparison of variables and for the comparison of nominal data Fisher's exact test was used. Spearman correlation test was used for the correlations. The area under the curve (AUC) was found by using Receiver Operating Curves (ROC) analysis. AUC is defined as a test with excellent diagnostic power between 1 - 0.9, good between 0.9 - 0.8, moderate between 0.8 - 0.7, weak between 0.7 - 0.6, unworthy between 0.6 - 0.5 and AUC≤ 0.5 was considered as the test has no diagnostic value (11). All the P values were 2-sided and P values less than 0.05 were considered statistically significant.

Results

Study Population

The median age of the COVID-19 patients, all of whom were Caucasian, was 58 years (21 - 99 / years) and 44.3% of the patients were female. 28.2% of the male patients and 25.9% of the female patients had a serious clinical course. Median age for mild cases was 54 years (IQR= 23) and the median number of hospitalization days was 5 (IQR= 3); in severe clinical cases, the median age was 68 years (IQR = 17), and the median number of hospitalization days was 15 (IQR= 11). The median length of stay for the entire group was 6 days (range: 1 to 61 days). Males were substantially more likely to have a hospitalization period of more than ten days (P= 0.03). The median age and length of hospital stay were significantly higher in the group with a severe clinical course. The Fisher's exact test revealed no statistically significant gender differences (P= 0.645) in both mild and severe clinical situations. Of the patients included in the study, 99.2% had viral pneumonia and 27.2% (n= 104) met serious clinical criteria. Out of the 383 hospitalized patients, a total of 59 (13.6%) people died as a result of COVID-19 and 324 patients recovered and were discharged. Three of the individuals, who died while being monitored in the hospital, did not have critical clinical conditions.

Evaluation Of Blood Parameters and Biomarkers

Age, duration of hospitalization, white blood cell (WBC) count, neutrophil count and BUN, creatinine, AST, Tbil, ferritin, CRP, procalcitonin, D-dimer, and troponin levels were significantly higher in the severe clinical group whereas lymphocyte and monocyte counts were significantly lower (P< 0.05). However, no significant difference was observed between the groups in terms of platelet (P= 0.703) and ALT (P= 0.54) levels. When the values of the biomarkers NLR, PLR, d-NLR, and NMR were compared between the two groups, the values of the severe clinical group were found to be significantly higher; the values of the biomarkers LMR, LCR, and LeCR were found to be significantly lower (P< 0.05). Median (IQR) values, comparison of the blood parameters and biomarkers are given in table 1. The ROC curves of the tests for predicting severe clinical course for COVID-19 patients are shown in figure 2.

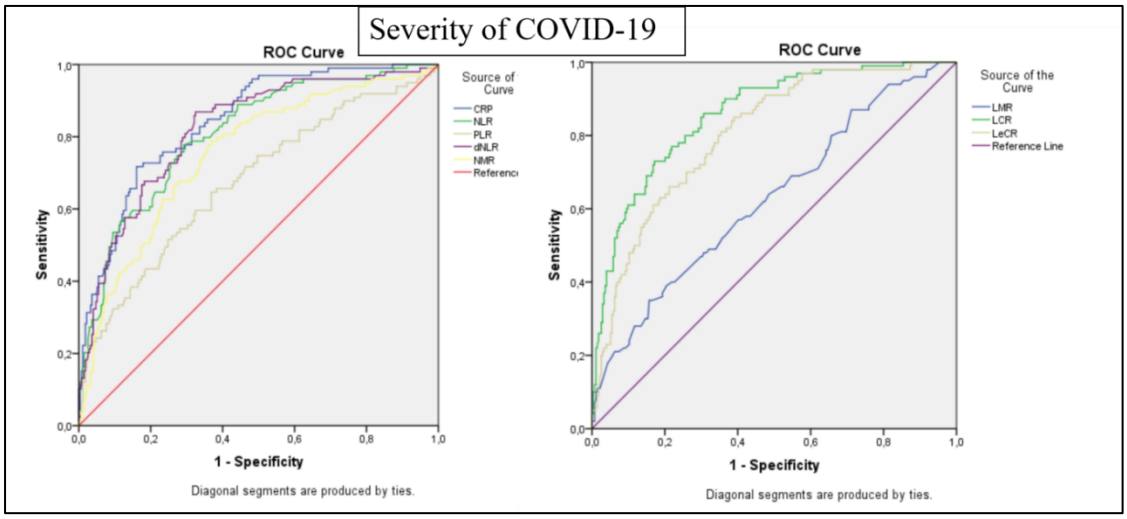

Figure 2. The ROC Curves of the Tests for Predicting Severe Clinical Course of COVID-19 Patients

Abbreviations: CRP, C reactive protein; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio

Table 1

The Comparison of Demographic and Clinical Features of Mild Clinical and Severe Clinical Course in COVID-19 Patients

Clinical Values	Entire Population (n=383) Median (IQR)*	Mild Clinical Group (n=279) Median (IQR)*	Severe Clinical Group (n=104) Median (IQR)*	P-value**
Demographic data				
Age (year)	58 (23)	54 (23)	68 (17)	**<0.001**
Duration of Hospitalization (days)	6 (8)	5 (3)	15 (11)	**<0.001**
Hematological parameters (normal values)				
WBC (4-10 × 10^9 L^{-1})	6 (3.1)	5.8 (2.7)	7 (5)	**<0.001**
Neutrophil (1-7 × 10^9 L^{-1})	4.2 (3.1)	3.7 (2.4)	5.7 (4.7)	**<0.001**
Lymphocyte (1-4.2 ×10^9 L^{-1})	1.1 (0.8)	1.2 (0.8)	0.8 (0.6)	**<0.001**
Monocyte (0.3-0.8 ×10^9 L^{-1})	0.5 (0.3)	0.5 (0.3)	0.4 (0.3)	**0.019**
Platelet (150-450 × 10^9 L^{-1})	206 (92)	208.5 (96)	205 (81)	0.703
Biochemical parameters (normal values)				
BUN (16.6-48.5 mg/dL)	31 (18)	29.4 (13)	40 (27)	**<0.001**
Creatinine (0.7-1.2 mg/dL)	0.91 (0.4)	0.87 (0.34)	1.07 (0.4)	**<0.001**
AST (0-40 IU/L)	29 (19)	26 (16)	37.5 (28)	**<0.001**
ALT (0-40 IU/L)	23 (21)	23 (20)	22 (21)	0.54
TBil (0-1.2 mg/dL)	0.42 (0.29)	0.39 (0.27)	0.49 (0.32)	**0.001**
Ferritin (30-400 ng/mL)	258 (433)	208.5 (264)	490 (749)	**<0.001**
CRP (0 - 5 mg/dL)	44.89 (84.2)	28.3 (56.1)	112.26(117.7)	**<0.001**
Procalcitonin (0-0.05 ng/ml)	0.099 (0.14)	0.07 (0.07)	0.195 (0.28)	**<0.001**
D-dimer (0-550 ug / L)	700 (705)	570 (700)	830 (950)	**<0.001**
Troponin (< 14 ng / L)	6.89 (9.2)	5.52 (6.1)	12.06 (13.5)	**<0.001**
Biomarkers				
NLR	3.62 (3.98)	3.03 (2.9)	7.22 (5.7)	**<0.001**
LMR	2.5 (1.7)	2.66 (1.75)	2.25 (1.6)	**<0.001**
PLR	184 (130)	168.6 (111.6)	234.54 (218.8)	**<0.001**
d-NLR	2.44 (2.36)	2.04 (1.58)	4.36 (3.7)	**<0.001**
LCR	25 (97.47)	39.52(189.7)	7.36 (11.6)	**<0.001**
LeCR	129.29(333.8)	192.3(634.1)	64.57 (63.3)	**<0.001**
NMR	9.33 (8.28)	7.66 (5.9)	14.08 (12.3)	**<0.001**

Abbreviations: WBC, white blood cell; BUN, blood urea nitrogen; AST, aspartate transaminase; ALT, alanine transaminase; TBil, total bilirubin; CRP, C reactive protein; IQR, interquartile range; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio.

*The data with abnormal distribution were shown as median and interquartile range.

**P value < 0.05 was described statistically significant and it was written in bold.

The Comparisons of Diagnostic Performance of Tests In COVID-19

When the diagnostic sensitivity of blood parameters and biomarkers were examined, it was concluded that all of WBC count, lymphocyte count, monocyte count and CRP, ferritin, troponin, D-dimer, NLR, LMR, PLR, d-NLR, LCR, LeCR, NMR levels could significantly predict the severe clinical course (AUC> 0.5, P< 0.05). In addition, LCR (AUC= 0.860, P< 0.001, CI= 0.819-0.900) was the best diagnostic test for predicting severe clinical course. For the estimation of the severe clinical course, cut-off value for LCR score was found to be 15 (sen= 74.26%, spec= 79.56%, negative predictive value (NPV)= 87%). With cut-off values of 15, 74.65, 2.55, 4, and 133, respectively, the diagnostic sensitivity of the LCR, CRP, d-NLR, NLR, and LeCR tests was determined to be significantly high with an AUC value of greater than 0.8 in the ROC analysis. The diagnostic performances of the biomarkers are presented in table 2.

Table 2

Roc Analysis and Cut-off Values of the Tests for the Prediction of Severity of COVID-19

TESTS	AUC*	Confidence Interval	Cut-off	Sen (%)	Spec (%)	NPV (%)	*P-Value*†	Youden index
Clinical Severity								
WBC	0.650	0.600-0.698	>6.7	54.81	69.53	80.5	**<0.001**	0.24
LYM	***0.734***	0.687-0.778	≤0.9	65.05	73.02	84.94	**<0.001**	0.38
CRP	***0.845***	0.805-0.880	>74.65	72.55	83.64	89.02	**<0.001**	0.56
FERRITIN	***0.764***	0.709-0.812	>380	62.92	78.42	81.77	**<0.001**	0.41
TROPONIN	***0.766***	0.715-0.812	>10.92	65.62	80.75	83.90	**<0.001**	0.46
D-DIMER	0.655	0.600-0.708	>530	85	47.47	86.96	**<0.001**	0.32
NLR	***0.812***	0.769-0.850	>4	78.43	69.78	89.81	**<0.001**	0.48
LMR	0.626	0.576-0.675	≤1.67	35.29	83.81	77.93	**<0.001**	0.19
PLR	0.683	0.634-0.729	>194	65.69	62.72	83.33	**<0.001**	0.28
d-NLR	***0.819***	0.776-0.856	>2.55	8.41	67.63	93.07	**<0.001**	0.54
LCR	***0.860***	0.819-0.900	≤15	74.26	79.56	87.00	**<0.001**	0.54
LeCR	***0.807***	0.760-0.854	≤133	84.31	61.5	90.43	**<0.001**	0.45
NMR	***0.751***	0.705-0.794	>9.43	78.43	63.31	88.89	**<0.001**	0.42

Abbreviations: AUC, Area under curve; Sen, sensitivity; Spec, specificity; NPV, negative predictive value; WBC, white blood cell; LYM, lymphocyte; CRP, C reactive protein; NLR, neutrophil-to-lymphocyte ratio; LMR, lympocyte-to-monocyte ratio; PLR, platelet-to-lympocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio

* Those with AUC > 700 are written in bold and italic.

† P value < 0.05 was described statistically significant and it was written in bold.

In a different ROC analysis, it was shown that d-NLR had high diagnostic sensitivity with a cut-off value of 2.55 (AUC= 0.819, sen= 86.41%, spec= 67.63%) in the prediction of hospitalization duration of more than five days. The areas under the curve of other biomarkers for the prediction of >5 days of hospitalization ranged from 0.571 to 0.674. In terms of predicting duration of hospitalization longer than ten days, LCR had the best performance (AUC= 0.768, sen= 77.87, spec= 63.64, cut-off= 24.94). In addition, with an AUC> 0.7, CRP, NLR, d-NLR, LCR, and LeCR scores were among the moderately suitable tests for predicting hospitalization for more than 10 days. Figures 3-4 display the ROC curves for predicting the duration of hospitalizations of >5 days and >10 days for COVID-19 patients. Also, table 3 presents the diagnostic results of tests for the prediction of duration of hospitalization of greater than five and 10 days.

Table 3

Roc Analysis and Cut-off Values of Tests for the Prediction of Duration of Hospitalization

TESTS	AUC*	Confidence Interval	Cut-off	Sen (%)	Spec (%)	*P- value* †	Youden index
Duration of Hospitalization (> 5 days)							
LYM	0.607	0.556- 0.657	≤1.6	84.23	33.06	**<0.001**	0.17
CRP	0.644	0.594 - 0.693	>56.41	50.78	73.95	**<0.001**	0.25
FERRITIN	0.664	0.605 - 0.719	>231	64.77	67.44	**<0.001**	0.32
TROPONIN	0.674	0.618 - 0.726	>4.73	76.81	52.94	**<0.001**	0.3
D-DIMER	0.621	0.565 - 0.674	>470	76.04	49.00	**<0.001**	0.25
NLR	0.629	0.578 - 0.677	>3.47	60.62	64.46	**<0.001**	0.25
LMR	0.571	0.520 - 0.622	≤1.75	28.19	83.47	**0.023**	0.12
PLR	0.591	0.540 - 0.641	>172	60.77	56.20	**0.003**	0.17
d-NLR	***0.819***	0.776 - 0.856	>2.55	86.41	67.63	**<0.001**	0.54
LCR	0.658	0.698 - 0.718	≤24.94	58.59	68.91	**<0.001**	0.27
LeCR	0.644	0.584 - 0.703	≤288.12	75.58	47.06	**<0.001**	0.23
NMR	0.573	0.522- 0.624	>7.5	66.02	51.04	**0.019**	0.17
Duration of Hospitalization (>10 days)							
LYM	0.691	0.642 - 0.737	≤1.1	69.11	57.75	**<0.001**	0.27
CRP	***0.749***	0.702 - 0.792	>63.65	65.85	74.02	**<0.001**	0.40
FERRITIN	0.685	0.627 - 0.739	>336	58.59	72.78	**<0.001**	0.31
TROPONIN	0.690	0.635 - 0.741	>11	51.89	77.83	**<0.001**	0.30
D-DIMER	0.578	0.521 - 0.633	>530	73.87	43.20	**0.010**	0.17
NLR	***0.715***	0.667 - 0.760	>4.75	61.48	73.26	**<0.001**	0.35
LMR	0.574	0.523 - 0.624	≤2.11	45.08	68.99	**0.020**	0.14
PLR	0.643	0.592 - 0.691	>264.44	39.34	84.56	**<0.001**	0.24
d-NLR	***0.709***	0.661 - 0.754	>3.4	55.28	79.84	**<0.001**	0.35
LCR	***0.768***	0.718 - 0.818	≤24.94	77.87	63.64	**<0.001**	0.42
LeCR	***0.733***	0.681- 0.785	≤115.32	70.73	66.14	**<0.001**	0.37
NMR	0.674	0.624- 0.721	>10.8	59.02	70.93	**<0.001**	0.30

Abbreviations: AUC, Area under curve; Sen, sensitivity; Spec, specificity; NPV, negative predictive value; LYM, lymphocyte; CRP, C reactive protein; NLR, neutrophil-to-lymphocyte ratio; LMR, lympocyte-to-monocyte ratio; PLR, platelet-to-lympocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio

*Those with AUC > 0.700 are written in bold and italic.

† P value < 0.05 was described statistically significant and it was written in bold.

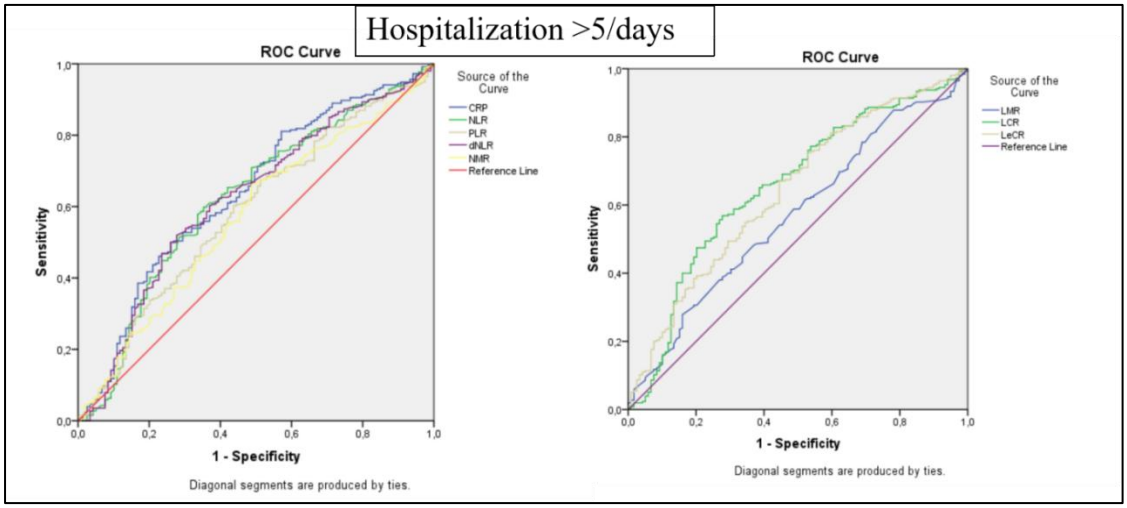

Figure 3. The ROC Curves of the Tests for Predicting Hospitalization Duration of >5 days for COVID-19 Patients

Abbreviations: CRP, C reactive protein; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio.

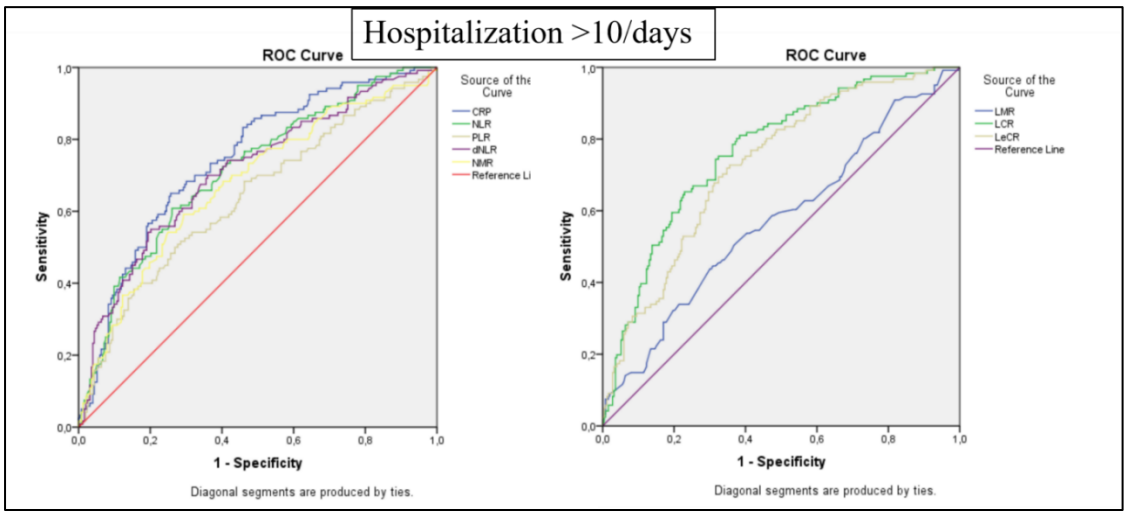

Figure 4. The ROC Curves of the Tests for Predicting Hospitalization Duration of >10 days for COVID-19 Patient

Abbreviations: CRP, C reactive protein; NLR, neutrophil-to-lymphocyte ratio; LMR, lymphocyte-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; d-NLR, derived neutrophil-to-lymphocyte ratio (neutrophil count divided by the result of white blood cell count minus neutrophil count); LCR, lymphocyte-to-C reactive protein ratio; LeCR, leukocyte-to-C reactive protein ratio; NMR, neutrophil-to-monocyte ratio

Discussion

COVID-19 is a systemic infectious disease and it can affect multiple organs. In our study, the results showed significantly elevated WBC and neutrophil counts, D-dimer, and troponin levels, while decreased lymphocyte and monocyte counts in the severe clinical course group. A significant increase was detected in AST, Tbil, BUN, and creatinine levels due to impaired liver and kidney functions in the severe clinical group. Inflammatory/infection markers CRP, ferritin, procalcitonin were higher in the severe clinical course group. In the course of COVID-19, microthrombin aggregation and hemostasis abnormalities occur as a

result of senility, immobility, hypoxia, damage to pulmonary vasculoendothelial tissues, disseminated intravascular coagulation and sepsis-induced coagulopathy. D-dimer reflects intravascular coagulation and fibrinolysis activation and is a marker of hypercoagulability (12). In our study, in line with the literature, an increase of D-dimer, as a coagulation function test, was positively correlated with COVID-19 severity (13,14).

In China, the total case fatality rate has been reported as 2.3 percent, whereas in Italy, 16 percent of all hospitalized patients had been admitted to the intensive care unit with an estimated overall case fatality rate of 7.2 percent (15). In our study, almost all of the patients had pneumonia and poor prognostic factors were used as the hospitalization criteria according to the guideline (8,10). Therefore, the percentages of the patients with severe clinical manifestation (27.2%) and fatality rate (13.6%) were found to be high in our study.

Neutrophil and lymphocyte counts are two biomarkers that can be easily studied in routine laboratory tests and lymphopenia indicates immune depletion, while neutrophilia indicates immunological activation (4). In COVID-19, the virus targets and damages lymphocyte producing tissues, while also interfering with immune system regulation. By secreting large quantities of cytokines, chemokines and different proteolytic enzymes at an advanced stage following activation by pathogens, immune system of the host causes a hyperinflammatory response, which leads to ARDS and septic shock (16,17). In our study, patients, who were more likely to advance to the critical phase, had increased neutrophil counts but decreased lymphocyte counts, resulting in disproportionately high NLR levels. Furthermore, elevation of neutrophil count causes a rapid increase in NMR, which, in turn, suggests that the disease may progress. The NLR and NMR scores could be regarded as a predictive criterion for the advancement of COVID-19 patients to a critical clinical condition within the 4-week term and according to the study done by Zhang H., et al., the cut-off value of NLR could be accepted as 5.92 (16). Consistently, in this present study, the NPV of NLR with 4-threshold was 89.81% whereas the NPV of NMR with 9.43-thresholds was 88.89%.

The lymphocyte-to-monocyte ratio, which typically ranges from 3 to 9, has been used to predict outcomes in chronic infections such tuberculosis, autoimmune diseases and cardiac conditions. Increased death rates and a worsening prognosis are indicated by a decrease in lymphocytes and an increase in monocytes (7). In our investigation, the median LMR index value for all COVID patients was found as 2.5, and as 2.25 in cases with a severe clinical course.

Normal PLR score is between 50 to 150 and a rise in PLR is seen in conditions such as malignancies, chronic autoimmune disorders, and viral infections, such as flu (7-13). In the study conducted by Wang H., et al., conducted on 61 patients, PLR > 200.8 and NLR > 4.4 have been identified as risk factors for the severe course of COVID-19 (1). In a meta-analysis of 20 recent studies, PLR and NLR biomarkers have been found to be affordable, practical and valuable predictive assays (18). In our study, it was discovered that while PLR levels were elevated in patients with severe clinical outcomes, its ability to predict such outcomes was limited (AUC = 0.683).

C-reactive protein is a component of typical inflammatory response and contributes to the activation of the complement system, which balances the immune system through phagocytosis (1). Today, it is frequently employed to determine the severity of an infection. LCR has been being used as a prognostic marker in a number of carcinomas and is a reliable predictor of the systemic inflammatory response (5). The course of COVID-19 is correlated with CRP and in patients with severe clinical course, LCR and LeCR indices decrease due to the elevated CRP. In a retrospective observational study by Asghar MS, et al., it was determined, in line with our study that LCR could predict severe clinical progression (7). The clinical course is aggravated by co-infections or secondary bacterial infections, which also contribute to the elevations in blood leukocyte count, neutrophil count and CRP and procalcitonin levels.

Overall, based on available results, NLR, LMR, PLR, d-NLR, LCR, and NMR have a good capability to differentiate patients, who will be critically ill in the later stages of the disease. For the prediction of disease

course in COVID-19, we recommend the use of, in particular, CRP, NLR, d-NLR, LCR, and LeCR biomarkers. These biomarkers are also well-validated for prediction of serious illness as well as hospitalization durations longer than ten days. While the best bioindicator for predicting severe clinical course was LCR with a cut-off value of 15 and AUC of 0.860, it was followed by CRP, d-NLR, NLR, LeCR, NMR, respectively. In particular, negative predictive values of d-NLR and LeCR biomarkers were over 90% (93.07%, 90.43%, respectively). In this investigation, it was observed that LMR's diagnostic value in predicting clinical severity was only slightly strong (AUC= 0.626). Since blood parameters can be affected by cortisol use, immunosuppressive conditions, cytotoxic chemotherapy and bone marrow metastasis of malignancies, the use of these biomarkers should be evaluated on an individual basis, also considering comorbidities and additional treatments of the patients (5).

Due to the retrospective nature of our study, it has some limitations, the most notable of which was the difficulty in accessing all patients' data. In addition, since the study was regional and single-center, it was challenging to generalize the findings due to exclusion of patients of different racial and national. Additionally, since Sars-CoV-2 sequence analyses were not provided, the analyses concerning COVID-19 subvariants could not be done. Also, one of our shortcomings was the presence of unanswered questions and contradictory data in COVID-19 diagnosis and treatment techniques.

Conclusions

Complete blood count and CRP are two common blood biochemical markers, which are inexpensive and accessible in most settings. LCR, CRP, d-NLR, NLR, and LeCR are early predictors of a severe clinical course and these biomarkers, derived with the use of blood parameters and NLR, d-NLR, LCR and LeCR are effective tests to predict long-term hospitalization. Patients with high blood CRP, d-NLR, NLR and low LCR and LeCR levels should be under hospital follow-up for close monitoring and early intervention, since a deterioration of the condition of these patients may be anticipated at any time.

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Committee of Izmir Bakırçay University (date: 12.10.2020 and Decision No: 91, Research No: 73).

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