INTERNATIONAL JOURNAL OF
MEDICAL BIOCHEMISTRY

DOI: 10.14744/ijmb.2021.03164
Int J Med Biochem 2021;4(3):143-9

Research Article

Predictive value of immature granulocyte count and other inflammatory parameters for disease severity in COVID-19 patients

Murat Alisik, Utku Gorkem Erdogan, Merve Ates, Mehmet Alp Sert, Ozgur Mehmet Yis, Guler Bugdayci

Department of Medical Biochemistry, Bolu Abant Izzet Baysal University, Bolu, Turkey

Abstract

Objectives: This study was designed to compare the immature granulocyte (IG) count, IG-to-lymphocyte ratio (IGLR), complete blood count (CBC) values, and inflammatory parameters of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP), ferritin level, and CRP-to-albumin ratio (CAR) measured at hospital admission in patients with coronavirus disease 2019 (COVID-19) and non-COVID-19 patients and to compare these parameters between subgroups according to disease severity. In addition, these parameters were evaluated for predictive value related to the severity of COVID-19.

Methods: The data of adult patients admitted with a suspected COVID-19 infection confirmed with real-time polymerase chain reaction testing of nasal and pharyngeal swab specimens were included in this retrospective study. Outpatient COVID-19-positive patients were enrolled in the mild group, hospitalized patients were classified in the moderate group, and patients admitted to the intensive care unit were categorized in the severe group.

Results: A total of 1213 COVID-19-positive patients and 1034 COVID-19-negative patients were included in the study. The IGLR, NLR, PLR, CRP, CAR, and ferritin levels were significantly higher, and the leukocyte, IG, neutrophil, lymphocyte, monocyte, basophil, and eosinophil levels were significantly lower in the COVID-19-positive group than the COVID-19-negative group ($p<0.05$ for all). The severe group had higher median IG, IGLR, neutrophil, NLR, PLR levels than the mild, moderate, and COVID-19-negative groups ($p<0.05$ for all). Receiver operating characteristic analysis revealed an area under the curve value for IGLR, CAR, CRP, IG, NLR, and ferritin of 0.868, 0.860, 0.834, 0.848, 0.845, 0.841, and 0.827, respectively, which differentiated severe COVID-19 patients from mild and moderate COVID-19 patients.

Conclusion: The results suggest that the IGLR may be useful to distinguish severe COVID-19 patients at the time of admission. Further exploration is warranted to fully determine the potential value of the IGLR in disease monitoring.

Keywords: C-reactive protein, complete blood count, COVID-19, immature granulocyte, immature granulocyte-to-lymphocyte ratio, inflammatory markers

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection, was declared a pandemic by the World Health Organization in March 2020 [1]. Since the first case, identified in late 2019, COVID-19 has affected over 145.2 million people around the world and caused more than 3.08 million deaths as of April 24, 2021 [2]. COVID-19 infection may be asymptomatic or have clinical manifestations that range from mild to severe, but understanding the signs upon presentation remains complex [3]. Greater disease severity is associated with poor outcomes and

Address for correspondence: Murat Alisik, MD. Department of Medical Biochemistry, Bolu Abant Izzet Baysal University, Bolu, Turkey
Phone: +90 537 323 19 50 **E-mail:** muratalisik@gmail.com **ORCID:** 0000-0003-0434-3206

Submitted Date: April 25, 2021 **Accepted Date:** June 14, 2021 **Available Online Date:** September 10, 2021

higher mortality [4, 5]. Therefore, the ability to determine factors that will identify severe disease at the time of diagnosis is critical.

Immature granulocytes (IGs) are white blood cells that have not fully developed during leukopoiesis before release from the bone marrow. They may include promyelocyte, myelocyte, and metamyelocyte cells. In healthy individuals, IGs are not typically released into blood circulation [6-8]. However, infection or inflammation can cause the release of IG to peripheral blood [6, 7]. Therefore, IGs can be used as inflammatory parameter or an indicator of the severity of infection [6, 7]. The development of new automated hematology analyzers has facilitated the easy identification of IGs, in contrast to a time-consuming manual platelet count peripheral blood smear [9].

Inflammation has an important role in COVID-19, as in other viral infections [10]. Several hematological and systemic inflammatory parameters have diagnostic and predictive values [10-12]. The severity of COVID-19 is associated with older age, male sex, obesity, and comorbidities, such as diabetes mellitus, cancer, cardiovascular disease, chronic kidney disease, chronic lung disease, and hypertension [11]. In addition, a number of hematological and inflammatory parameters have been proposed as predictors of disease severity, including the level of C-reactive protein (CRP) [13] and ferritin [14], the lymphocyte (LYM) [15], white blood cell (WBC) [15], basophil (BASO) [12], neutrophil (NEU) [12], and eosinophil (EOS) counts [16], and the neutrophil-to-lymphocyte ratio (NLR) [4]. Although it has been shown that IG levels were elevated in severe COVID-19 patients [17, 18], the ability of the IG and IG-to-lymphocyte ratio (IGLR) values to distinguish severe COVID-19 patients from others has not been well established. The objective of this study was to compare the IG count and IGLR, complete blood count (CBC) parameters, and other hematological and systemic inflammatory parameters, namely the NLR, platelet-to-lymphocyte ratio (PLR), CRP, ferritin, and the CRP-to-albumin ratio (CAR), measured at hospital admission in patients with COVID-19 and non-COVID-19 patients and to compare these parameters between subgroups of disease severity to evaluate the value for predicting the severity COVID-19.

Materials and Methods

This retrospective study was approved by the Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (no: 2020/304) and the Republic of Turkey Ministry of Health. Adult patients admitted with suspected COVID-19 infection to BAIBU Izzet Baysal Education and Research Hospital between July 1 and November 1, 2020 were enrolled. All of the patients had at least one of the symptoms of fever, muscle/joint pain, cough, or sore throat. The laboratory test results of patient samples obtained at the time of presentation to the special area of the emergency department reserved for the COVID-19 pandemic in the emergency department were used for the study analysis. The COVID-19-positive group comprised patients who were confirmed to be COVID-19-positive with real-time polymerase chain reaction (RT-PCR) testing of nasal and pharyngeal swab specimens. Patients who presented with the suspicion of COVID-19 infection but whose RT-PCR test was negative were included in the COVID-19-negative group. The COVID-19-positive patients were divided into 3 groups: mild (outpatient), moderate (hospitalized), and severe (intensive care unit [ICU] hospitalization).

Age, gender, CBC (Sysmex XN-1000; Sysmex, Kobe, Japan), CRP, albumin (Architect c8000; Abbott Diagnostics, Lake Forest, IL, USA), and ferritin (Architect i2000, Abbott Diagnostics, Lake Forest, IL, USA) data were collected from the laboratory information system, in addition to routine hematological parameters of hemoglobin (HGB) level, hematocrit (HCT), red blood cell (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) level, mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet (PLT) count, platelet distribution width (PDW), mean platelet volume (MPV), plateletcrit (PCT) value, and WBC, NEU, LYM, monocyte (MONO), BASO, EOS, and IG counts from the CBC analysis. The NLR, PLR, IGLR, and CAR values were calculated using the relevant data.

The analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). The normality of distribution of the parameters was examined using the Kolmogorov-Smirnov test. The descriptive statistics were presented as median (1^{st}-3^{rd} quartile values) for non-parametric distributions and the number of cases (percentage) was used for categorical variables. Pearson's chi-squared test was used to compare categorical variables. Non-parametrically distributed continuous variables were compared with the Mann-Whitney U test or the Kruskal-Wallis test. Post-hoc analysis of the Kruskal-Wallis test findings was conducted with a Dunn-Bonferroni pairwise comparison test. The diagnostic value of significant parameters to differentiate severe COVID-19 patients was assessed using receiver operating characteristic (ROC) and area under the ROC curve (AUC) analysis with 95% confidence intervals (95% CI). Youden's index was used to determine an optimum cut-off value of associated parameters and corresponding sensitivity (95% CI), specificity (95% CI), positive likelihood ratio (+LR), and negative likelihood ratio (-LR) values to distinguish severe COVID-19 patients. $P<0.05$ was used as the significance level.

Results

A total of 1213 COVID-19-positive patients and 1034 COVID-19-negative patients were included in the study. Demographic data, CBC results, and inflammatory parameters of the 2 initial groups are provided in Table 1. No statistically significant difference was observed between the median age of the COVID-19-positive group (52 years [IQR: 37-69.5]) and the COVID-19-negative group (51 years [IQR: 35-68]) ($p<0.063$). No statistically significant difference was observed in terms of gender between the groups ($p=0.121$). The IGLR, NLR, PLR, CRP, CAR, and ferritin levels were significantly higher, and the WBC, IG, NEU, LYM, MONO, BASO, and EOS levels were significantly lower in the group that was positive for COVID than those of the COVID-19-negative group ($p<0.05$ for all).

Table 1. Comparison of demographic data, complete blood count, and inflammatory parameters between patients with and without COVID-19 infection

	COVID-19 negative (n=1034)	COVID-19 positive (n=1213)	p
Age; years	51 (35-68)	52 (37-69.5)	0.063
Gender; Female n (%)	527 (51%)	658 (54.2%)	0.121
HGB; g/dL	14 (12.7-15.3)	13.5 (12.2-14.7)	<0.001
HCT; %	42.8 (39.5-46.1)	41.4 (38-44.7)	<0.001
RBC; 10^{12}/L	4.9 (4.52-5.29)	4.78 (4.39-5.16)	<0.001
MCV; fL	87.5 (84.5-90.2)	87.2 (84.2-90.1)	0.133
MCH; pg	28.8 (27.5-29.9)	28.6 (27.4-29.7)	0.005
MCHC; g/dL	32.8 (32-33.5)	32.7 (31.9-33.3)	0.016
RDW; %	12.7 (12.2-13.6)	13 (12.3-13.9)	<0.001
PLT; 10^9/L	263.5 (221-310)	218 (175-264)	<0.001
PDW; fL	12.1 (11-13.6)	12.4 (11.1-13.9)	0.004
MPV; fL	10.5 (9.9-11.1)	10.7 (10.1-11.4)	<0.001
PCT; %	0.27 (0.23-0.32)	0.23 (0.19-0.28)	<0.001
WBC; 10^9/L	8.59 (6.93-10.42)	5.98 (4.75-7.67)	<0.001
IG; 10^9/L	0.03 (0.02-0.04)	0.02 (0.01-0.04)	<0.001
NEU; 10^9/L	5.15 (3.98-6.91)	3.75 (2.74-5.24)	<0.001
LYM; 10^9/L	2.14 (1.55-2.77)	1.31 (0.94-1.82)	<0.001
MONO; 10^9/L	0.71 (0.56-0.91)	0.57 (0.42-0.78)	<0.001
BASO; 10^9/L	0.04 (0.02-0.05)	0.02 (0.01-0.03)	<0.001
EOS; 10^9/L	0.12 (0.05-0.21)	0.03 (0.01-0.08)	<0.001
IGLR	0.013 (0.008-0.025)	0.017 (0.009-0.039)	<0.001
NLR	2.3 (1.6-3.86)	2.73 (1.72-4.73)	<0.001
PLR	126.1 (92.9-172.2)	160.9 (114.7-225.2)	<0.001
CRP; mg/L	3.4 (0.1-15.5)	12.9 (2.2-54.3)	<0.001
CAR	0.08 (0-0.36)	0.29 (0.05-1.44)	<0.001
Ferritin; µg/L	50.1 (21.1-102.2)	112.7 (40.3-269.8)	<0.001
Albumin; g/L	45.1 (42.5-47.3)	42.5 (38.1-45.5)	<0.001

Data shown as median (1^{st}-3^{rd} quartile). BASO: Basophil; CAR: CRP-to-albumin ratio; CRP: C-reactive protein; EOS: Eosinophil; HCT: Hematocrit; HGB: Hemoglobin; IG: Immature granulocyte; IGLR: Immature granulocyte-to-lymphocyte ratio; LYM: Lymphocyte; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MONO: Monocyte; MPV: Mean platelet volume; NEU: Neutrophil; NLR: Neutrophil-to-lymphocyte ratio; PCT: Plateletcrit; PDW: Platelet distribution width; PLT: Platelet; PLR: Platelet-to-lymphocyte ratio; RBC: Red blood cell; RDW: Red cell distribution width; WBC: White blood count

The demographic data, CBC results, and inflammatory parameters of the patients who tested negative for COVID-19 and the COVID-19 patients according to classification as mild, moderate, or severe are shown in Table 2. The mean age was significantly higher in the severe and moderate groups compared with the mild and negative groups ($p<0.05$ for all). The distribution of male gender was significantly higher in the severe group ($p<0.05$). The severe group had higher mean IG, IGLR, NEU, NLR, and PLR levels than the mild, moderate, and COVID-19-negative groups ($p<0.05$ for all). The mild, moderate, and severe groups had significantly lower LYM levels than the COVID-19-negative group ($p<0.05$ for all).

ROC analysis revealed that the IGLR, CAR, CRP, IG, NLR, and ferritin had greater AUC values (0.868, 0.860, 0.848, 0.845, 0.841 and 0.827, respectively) in the leukocyte count parameters of the CBC as well as hematological and systemic inflammatory parameters, which distinguished severe COVID-19 patients from mild and moderate COVID-19 cases (Table 3).

Discussion

One of the burdens caused by the COVID-19 pandemic is the increased hospital workload. This added workload has been associated with lower quality of care and higher patient mortality [11]. It is therefore of great importance to identify severe cases that have a higher mortality risk in order to direct resources appropriately. Several laboratory parameters, such as the CRP, NLR, NEU, ferritin, and PLR, have been proposed as a means to classify severe patients [19, 20]. The results of this study indicate that IG values, which can be easily measured with new hematology analyzers, and the calculated IGLR were significantly higher in severe COVID-19 patients. This differentiation was also seen in other hematological inflammatory

Table 2. Comparison of demographic, complete blood count and inflammatory parameters between groups

	COVID-19 negative (n=1034)	Mild (n=867)	Moderate (n=281)	Severe (n=65)	p
Age; years	51 (35-68)	45 (33-60)[a]	69 (59-78)[ab]	76 (66-83.5)[ab]	<0.001
Gender; Female n(%)	527 (51%)	500 (57.7%)	134 (47.7%)	24 (36.9%)	<0.001
HGB; g/dL	14 (12.7-15.3)	13.8 (12.6-14.9)[a]	12.8 (11.3-14.1)[ab]	12.6 (10.9-13.7)[ab]	<0.001
HCT; %	42.8 (39.5-46.1)	42.2 (39-45.2)[a]	39.4 (35.1-43.1)[ab]	39.1 (33.6-42.3)[ab]	<0.001
RBC; 10^{12}/L	4.9 (4.52-5.29)	4.84 (4.49-5.21)	4.6 (4.01-5.05)[ab]	4.48 (3.83-4.89)[ab]	<0.001
MCV; fL	87.5 (84.5-90.2)	87.2 (84.4-89.7)	87.2 (83.4-90.9)	87.6 (83.5-92.1)	0.333
MCH; pg	28.8 (27.5-29.9)	28.6 (27.5-29.7)	28.5 (27.1-29.9)	28.3 (26.7-29.5)	0.023
MCHC; g/dL	32.8 (32-33.5)	32.7 (32-33.4)	32.6 (31.6-33.3)[ab]	32.3 (31.5-33.1)[ab]	<0.001
RDW; %	12.7 (12.2-13.6)	12.8 (12.2-13.5)	13.4 (12.6-14.45)[ab]	14.2 (13.35-15.35)[abc]	<0.001
PLT; 10^9/L	263.5 (221-310)	220 (179-262)[a]	203 (158-266)[a]	232 (174-292)[a]	<0.001
PDW; fL	10.5 (9.9-11.1)	10.7 (10.1-11.3)[a]	10.8 (10.2-11.5)[a]	11 (10.4-11.6)[ab]	<0.001
MPV; fL	12.1 (11-13.6)	12.3 (11.1-13.8)	12.4 (11-14.2)	12.8 (11.35-15.05)	0.011
PCT; %	0.27 (0.23-0.32)	0.23 (0.19-0.28)[a]	0.22 (0.17-0.29)[a]	0.26 (0.18-0.33)	<0.001
WBC; 10^9/L	8.59 (6.93-10.42)	5.74 (4.64-7.26)[a]	6.48 (4.85-8.63)[ab]	8.63 (6.21-12.89)[bc]	<0.001
IG; 10^9/L	0.03 (0.02-0.04)	0.02 (0.01-0.03)[a]	0.04 (0.02-0.06)[ab]	0.09 (0.04-0.21)[abc]	<0.001
NEU; 10^9/L	5.15 (3.98-6.91)	3.47 (2.51-4.61)[a]	4.73 (3.33-6.61)[ab]	7.13 (4.83-11.42)[abc]	<0.001
LYM; 10^9/L	2.14 (1.55-2.77)	1.42 (1.07-1.95)[a]	1.07 (0.78-1.45)[ab]	0.91 (0.63-1.34)[ab]	<0.001
MONO; 10^9/L	0.71 (0.56-0.91)	0.61 (0.46-0.81)[a]	0.48 (0.33-0.7)[ab]	0.46 (0.26-0.66)[ab]	<0.001
BASO; 10^9/L	0.04 (0.02-0.05)	0.02 (0.01-0.03)[a]	0.01 (0.01-0.03)[ab]	0.02 (0.01-0.03)[ab]	<0.001
EOS; 10^9/L	0.12 (0.05-0.21)	0.04 (0.01-0.1)[a]	0.01 (0-0.03)[ab]	0 (0-0.02)[ab]	<0.001
IGLR	0.013 (0.008-0.025)	0.013 (0.008-0.026)	0.035 (0.019-0.068)[ab]	0.107 (0.042-0.225)[abc]	<0.001
NLR	2.3 (1.6-3.86)	2.28 (1.5-3.6)	4.43 (2.89-7.03)[ab]	7.78 (4.34-16.53)[abc]	<0.001
PLR	126.1 (92.9-172.2)	150.2 (109.8-204.7)[a]	192.8 (130.4-271.6)[ab]	265.9 (177.7-438.7)[abc]	<0.001
CRP; mg/L	3.4 (0.1-15.5)	6.65 (1-21.23)[a]	78.2 (32.2-131.4)[ab]	128.4 (71.18-189.38)[ab]	<0.001
CAR	0.08 (0-0.36)	0.15 (0.02-0.49)[a]	2.2 (0.85-3.6)[ab]	4.03 (2.37-6.38)[ab]	<0.001
Ferritin; µg/L	50.1 (21.1-102.2)	83.1 (30.8-166)[a]	269.5 (131.5-627)[ab]	606.9 (265.8-1413.2)[ab]	<0.001
Albumin; g/L	45.1 (42.5-47.3)	43.8 (41.2-46.2)[a]	37.4 (34.9-40.5)[ab]	34.1 (29.7-36.7)[ab]	<0.001

Data shown as median (1st-3rd quartile). [a]: Significantly differs from COVID-19 negative group; [b]: Significantly differs from mild group; [c]: Significantly differs from moderate group. BASO: Basophil; CAR: CRP-to-albumin ratio; CRP: C-reactive protein; EOS: Eosinophil; HCT: Hematocrit; HGB: Hemoglobin; IG: Immature granulocyte; IGLR: Immature granulocyte-to-lymphocyte ratio; LYM: Lymphocyte; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume; MONO: Monocyte; MPV: Mean platelet volume; NEU: Neutrophil; NLR: Neutrophil-to-lymphocyte ratio; PCT: Plateletcrit; PDW: Platelet distribution width; PLT: Platelet; PLR: Platelet-to-lymphocyte ratio; RBC: Red blood cell; RDW: Red cell distribution width; WBC: White blood count

parameters, such as the NEU, NLR, and PLR, as well as systemic inflammatory parameters of CRP, CAR, and ferritin level.

Alnor et al. [17] reported that COVID-19 patients had lower IG levels than those who tested negative, and that severe COVID-19 patients had a higher IG value than non-severe patients. It has also been noted that COVID-19 patients who needed to be admitted to an intensive care unit (ICU) had a higher IG level than those who did not require intensive care [18]. Linssen et al. [21] compared critical and non-critical COVID-19 patients and reported that the severe patient group had higher IG and IGLR values. Similarly, we observed that the median IG value was higher in the more patient groups with more significant disease. The IGLR value was also significantly higher in the severe disease group than in the other groups. IGs are progenitor cells and immature granulopoiesis has been reported to mirror activation and left shift in the bone marrow [22]. NEU left shift is known to be associated with bacterial infection [23]. Therefore, the increase in the IG value observed in the severe and moderate patient groups was thought to be related to COVID-19 rather than a bacterial superinfection.

Several parameters have been proposed to distinguish severe COVID-19 patients. Wang et al. [20] evaluated the diagnostic efficacy of several hematological parameters as a method of differentiating moderate from severe COVID-19 cases and reported AUC values for WBC, NEU, NLR, and PLR of 0.652, 0.726, 0.890, and 0.842, respectively. Yang et al. [10] reported NLR, CRP, and PLR AUC values of 0.841, 0.714, and 0.784. In another study that set out to examine a means to discern severe cases, the NLR, WBC, NEU, LYM, CRP, and ferritin AUC was 0.831, 0.693, 0.765, 0.737, 0.816, and 0.752, respectively [24]. Other researchers observed an AUC for NLR and LYM of 0.98 and 0.092, respectively [25]. We found that the IG and IGLR values could reliably discriminate severe COVID-19 cases. The IGLR had a higher AUC value (0.868) than the IG, CRP, ferritin, CAR, NLR, PLR, NEU, LYM,

Table 3. The receiver operating characteristic curve analysis of the hematological and systemic inflammatory markers for severe COVID-19 patients

	AUC (95% CI)	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	+LR	-LR	p
IG; 10^9/L	0.845 (0.823 to 0.865)	>0.03	89.2 (79.1-95.6)	70.1 (67.4-72.8)	2.99	0.15	<0.001
IGLR	0.868 (0.848 to 0.887)	≥0.041	78.5 (66.5-87.7)	79.7 (77.3-82.0)	3.87	0.27	<0.001
WBC; 10^9/L	0.758 (0.733 to 0.782)	>8.14	60.0 (47.1-72.0)	81.5 (79.1-83.7)	3.23	0.49	<0.001
NEU; 10^9/L	0.824 (0.801 to 0.845)	>4.58	83.1 (71.7-91.2)	68.1 (65.3-70.8)	2.61	0.25	<0.001
LYM; 10^9/L	0.718 (0.692 to 0.743)	≤1.16	72.3 (59.8-82.7)	62.6 (59.8-65.4)	1.93	0.44	<0.001
MONO; 10^9/L	0.630 (0.602 to 0.657)	≤0.47	56.9 (44.0-69.2)	66.9 (64.1-69.6)	1.72	0.64	0.002
BASO; 10^9/L	0.534 (0.506 to 0.563)	>0.06	12.3 (5.5-22.8)	96.9 (95.7-97.8)	3.92	0.91	0.362
EOS; 10^9/L	0.702 (0.676 to 0.728)	≤0.02	80.0 (68.2-88.9)	52.2 (49.2-55.1)	1.67	0.38	<0.001
NLR	0.841 (0.819 to 0.861)	≥3.88	83.1 (71.7-91.2)	69.9 (67.2-72.6)	2.76	0.24	<0.001
PLR	0.725 (0.699 to 0.750)	>210.84	66.2 (53.4-77.4)	73.0 (70.3-75.5)	2.45	0.46	<0.001
CRP; mg/L	0.848 (0.825 to 0.868)	>49.5	82.8 (70.6-91.4)	76.8 (74.2-79.3)	3.57	0.22	<0.001
CAR	0.860 (0.838 to 0.880)	≥1.21	86.2 (74.6-93.9)	75.9 (73.2-78.5)	3.58	0.18	<0.001
Ferritin; µg/L	0.827 (0.803 to 0.849)	>237.67	82.1 (69.6-91.1)	75.4 (72.7-78.0)	3.34	0.24	<0.001

WBC: white blood count, NEU: neutrophil, LYM: lymphocyte, MONO: monocyte, BASO: basophil, EOS: eosinophil, IG: immature granulocyte, CRP: C-reactive protein, NLR: neutrophil to lymphocyte ratio, PLR: platelet to lymphocyte ratio, CAR: CRP to albumin ratio, IGLR: immature granulocyte to lymphocyte ratio; AUC: Area under curve; BASO: Basophil; CAR: CRP-to-albumin ratio; CI: Confidence interval; CRP: C-reactive protein; EOS: Eosinophil; IG: Immature granulocyte; IGLR: Immature granulocyte-to-lymphocyte ratio; LR: Likelihood ratio; LYM: Lymphocyte; MONO: Monocyte; NEU: Neutrophil; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; WBC: White blood count

and WBC measures (Table 3). As seen in the literature, hematological parameters (such as NEU, NLR, and PLR) and systemic inflammatory parameters (such as CRP and ferritin) can separate severe patients from mild and moderate. However, our results indicated that an IGLR cut-off of 0.041 and an AUC of 0.868 produced a sensitivity of 78.46% and a specificity 79.7% for segregating severe patients from non-severe patients, which suggests that the IGLR is the most effective of the studied markers as a method to distinguish the severity of disease.

Alnor et al. [17] found that both the WBC count and individual WBC parameters were lower in COVID-19 positive patients compared with negative patients. Another study also indicated that COVID-19-positive patients had lower WBC levels, but in that research, the WBC parameters were expressed as percentages and the NEU percentage was higher while the percentage of LYM was lower in COVID-19-positive patients [26]. It was also stated in another study that COVID-19-positive patients had lower WBC and NEU levels [27]. These results were consistent with research in which COVID-19 patients were grouped according to their need for ICU care, and significantly higher WBC, NEU, and IG counts as well as low LYM values were seen in those who needed ICU hospitalization [18]. Wang et al. [20] reported that high WBC and NEU values and low LYM and EOS values were recorded in severe COVID-19 patients. Anurag et al. [28] also reported that WBC and WBC parameters were lower in the COVID-19 positive group compared with a negative group. We also found that the WBC and WBC parameters were lower in the mild COVID-19 group in comparison with the COVID-19 negative group. In addition, we observed a left shift, supported by the increase in IG, with an increasing trend seen in WBC and NEU levels and a decreasing trend in LYM, MONO, EOS, and BASO values in the severe patient group. These results support the idea that COVID-19 infection has an effect of decreasing the WBC count and its parameters. The virus is thought to invade lymphocytes via angiotensin-converting enzyme 2 receptors, resulting in apoptosis or the suppression of lymphocyte production in the bone marrow due to a cytokine storm [15, 29]. Decreased BASO and EOS values in severe patient groups may be associated with consumption while clearing the virus. An elevated NEU count in severe and moderate groups may be related to inflammation induced by the virus, which is also supported by higher levels of CRP and ferritin in these COVID-19 patients.

CRP and ferritin are acute phase reactants and are used as biomarkers of inflammation and infection [14, 30]. The CAR has previously been used as a marker of sepsis and activity of autoimmune disease [31]. In addition, NLR and PLR values are also used as inflammatory markers [5]. It has been demonstrated that the CRP and ferritin values were high in COVID-19 patients, and that they were particularly high in severe cases requiring ICU care [18]. The NLR, PLR, and CRP values have been shown to be higher in COVID-19 positive patients [32]. In addition, the NLR and PLR values have been shown to be higher in patients with severe COVID-19 disease [10, 20, 33]. The findings of a meta-analysis indicated that COVID-19 patients had higher CRP values, and demonstrated a correlation between disease severity and CRP level [15]. Similarly, the NLR and PLR have been shown to be markers of disease severity [29]. In the present study, the NLR, PLR, CRP, CAR, and ferritin values were higher in COVID-19-positive patients. All of these parameters, with the exception of NLR, were found to be higher in the mild COVID-19 group when compared with the COVID-19-negative group. In the moderate group, all of the parameters were significant-

ly higher than those of both the mild group and the negative group. Similarly, in the severe COVID-19 group, all of the parameters were significantly higher than those of the mild and negative groups. When the severe COVID-19 group was compared with the mild COVID-19 group, although a higher value was detected in all parameter values, a statistically significance was only found in the NLR and PLR parameters. This suggests that COVID-19 disease causes an increase in inflammatory parameters and that this increment grows in relation to the severity of the disease. The current study findings also indicate that both hematological and systemic inflammation markers were higher in the presence of COVID-19 disease, and that this increase was greater in the severe disease group. In addition, the CAR had a higher AUC value (0.860) with a cutoff point of ≥1.21 in ROC analysis than CRP (AUC: 0.848, cutoff: 49.5). Thus, we think that this ratio may be more beneficial than using CRP alone as a tool for distinguishing severe COVID-19 cases.

A lower RBC count and associated parameters in patients with more severe disease activity may be associated with the resulting hypoxia and inflammation related to COVID-19 [21]. Studies have shown that HGB and RBC values were lower in severe cases [18, 20, 21]. The results of a meta-analysis also found that the HGB level was lower in severe disease than mild infection [34]. In contrast, some studies have shown no significant difference in HGB level according to the severity of COVID-19 [35, 36]. In the current study, the erythrocyte count and its associated parameters were significantly lower in COVID-19 patients. The RBC, HCT, HGB, and MCHC values were lower in the moderate and severe groups than the mild and negative COVID-19 groups. The HGB values in moderate and severe patients were found to be lower than those of the mild and negative patients. An insignificant decrease was observed between the severe and moderate groups. These different results may be related to hypoxia and inflammation processes according to the time of evaluation of patients after the onset of disease.

Ok et al. [5] reported that COVID-19-positive patients had lower PLT values. We also found that the PLT level was lower in the COVID-19-positive group than in the negative group. While some studies have indicated no difference in the PLT value when comparing severe and non-severe patients [20, 21, 36], it has also been demonstrated that patients with severe disease had a lower PLT level [17]. In the current study, no significant difference was found in the PLT level of the severe and non-severe COVID-19 groups. These differences between studies may be related to the proportion of patients enrolled with various comorbidities that may affect the PLT level.

This study has several limitations. First, we were unable to compare results obtained using different products or hemogram devices. Second, patient comorbidities, which could affect the test results, were not evaluated in this study, which limits the ability to generalize the results. Finally, due to the cross-sectional design, it is difficult to conclude prognostic value for mortality risk, despite the changes in parameters in severe COVID-19 patients.

Conclusion

In conclusion, the results of this study demonstrated significant CBC and systemic inflammatory parameter changes associated with COVID-19 and the changes varied according to disease severity. Erythrocyte and associated parameters decreased with greater disease severity. With greater severity of disease, the IG, WBC and NEU tended to increase, following an initial decrease. Our results suggest that the IGLR may enable differentiation of severe COVID-19 cases at the time of presentation; however, additional work in this area is warranted to further examine the potential value of the IGLR in the prognosis and monitoring of COVID-19.

Conflict of Interest: The authors have no disclosure of potential conflicts of interest or competing interest.

Ethics Committee Approval: This study was approved by the Bolu Abant Izzet Baysal University Clinical Research Ethics Committee (Approval Number 2020/304) and the Republic of Turkey Ministry of Health.

Financial Disclosure: We did not receive any funding from any source for this work.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept – M.A., U.G.E., O.M.Y.; Design – M.A., U.G.E., M.Ates, M.A.S., O.M.Y., G.B.; Supervision – M.A., O.M.Y., G.B.; Funding – M.A., O.M.Y., G.B.; Materials – M.A., U.G.E., M.A.S., M.Ates; Data collection &/or processing – M.A., U.G.E., M.Ates, M.A.S., O.M.Y.; Analysis and/or interpretation – M.A., O.M.Y., U.G.E.; Literature search – M.A., U.G.E., M.Ates, M.A.S., O.M.Y., G.B.; Writing – M.A., O.M.Y.; Critical review – M.A., U.G.E., M.Ates, M.A.S., O.M.Y., G.B.

References

1. Kashte S, Gulbake A, El-Amin Iii SF, Gupta A. COVID-19 vaccines: rapid development, implications, challenges and future prospects. Hum Cell 2021;34(3):711–33. [CrossRef]
2. World Health Organization. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/. Accessed on April 24, 2021.
3. Chilamakuri R, Agarwal S. COVID-19: Characteristics and therapeutics. Cells 2021;10(2):206. [CrossRef]
4. Zeng ZY, Feng SD, Chen GP, Wu JN. Predictive value of the neutrophil to lymphocyte ratio for disease deterioration and serious adverse outcomes in patients with COVID-19: a prospective cohort study. BMC Infect Dis 2021;21(1):80. [CrossRef]
5. Ok F, Erdogan O, Durmus E, Carkci S, Canik A. Predictive values of blood urea nitrogen/creatinine ratio and other routine blood parameters on disease severity and survival of COVID-19 patients. J Med Virol 2021;93(2):786–93. [CrossRef]
6. Zeng L, Wang S, Lin M, Chen Y, Deng Q, Zhong H, et al. Evaluation of time to positivity for blood culture combined with immature granulocytes, neutrophil-to-lymphocyte ratio, and CRP in identifying bloodstream coagulase-negative

Staphylococci infection in pediatric patients. J Clin Lab Anal 2020;34(11):e23473. [CrossRef]
7. Bedel C, Korkut M, Selvi F. New markers in predicting the severity of acute pancreatitis in the emergency department: immature granulocyte count and percentage. J Postgrad Med 2021;67(1):7–11. [CrossRef]
8. Incir S, Calti HK, Palaoglu KE. The role of immature granulocytes and inflammatory hemogram indices in the inflammation. Int J Med Biochem 2020;3(3):125–30. [CrossRef]
9. Ünal Y, Barlas AM. Role of increased immature granulocyte percentage in the early prediction of acute necrotizing pancreatitis. Ulus Travma Acil Cerrahi Derg 2019;25(2):177–82.
10. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol 2020;84:106504. [CrossRef]
11. Gallo Marin B, Aghagoli G, Lavine K, Yang L, Siff EJ, Chiang SS, et al. Predictors of COVID-19 severity: A literature review. Rev Med Virol 2021;31(1):1–10. [CrossRef]
12. Chen R, Sang L, Jiang M, Yang Z, Jia N, Fu W, et al. Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. J Allergy Clin Immunol 2020;146(1):89–100. [CrossRef]
13. Luo X, Zhou W, Yan X, Guo T, Wang B, Xia H, et al. Prognostic value of C-reactive protein in patients with coronavirus 2019. Clin Infect Dis 2020;71(16):2174–9. [CrossRef]
14. Ahmed S, Ansar Ahmed Z, Siddiqui I, Haroon Rashid N, Mansoor M, Jafri L. Evaluation of serum ferritin for prediction of severity and mortality in COVID-19- A cross sectional study. Ann Med Surg (Lond) 2021;63:102163. [CrossRef]
15. Huang Y, Zhang Y, Ma L. Meta-analysis of laboratory results in patients with severe coronavirus disease 2019. Exp Ther Med 2021;21(5):449. [CrossRef]
16. Xie G, Ding F, Han L, Yin D, Lu H, Zhang M. The role of peripheral blood eosinophil counts in COVID-19 patients. Allergy 2021;76(2):471–82. [CrossRef]
17. Alnor A, Sandberg MB, Toftanes BE, Vinholt PJ. Platelet parameters and leukocyte morphology is altered in COVID-19 patients compared to non-COVID-19 patients with similar symptomatology. Scand J Clin Lab Invest 2021;81(3):213–7. [CrossRef]
18. Pozdnyakova O, Connell NT, Battinelli EM, Connors JM, Fell G, Kim AS. Clinical significance of CBC and WBC Morphology in the diagnosis and clinical course of COVID-19 infection. Am J Clin Pathol 2021;155(3):364–75. [CrossRef]
19. Tahtasakal CA, Oncul A, Sevgi DY, Celik E, Ocal M, Turkkan HM, et al. Could we predict the prognosis of the COVID-19 disease? J Med Virol 2021;93(4):2420–30. [CrossRef]
20. Wang C, Deng R, Gou L, Fu Z, Zhang X, Shao F, et al. Preliminary study to identify severe from moderate cases of COVID-19 using combined hematology parameters. Ann Transl Med 2020;8(9):593. [CrossRef]
21. Linssen J, Ermens A, Berrevoets M, Seghezzi M, Previtali G, van der Sar-van der Brugge S, et al. A novel haemocytometric COVID-19 prognostic score developed and validated in an observational multicentre European hospital-based study. Elife 2020;9:e63195. [CrossRef]
22. Ihlow J, Michaelis E, Greuel S, Heynol V, Lehmann A, Radbruch H, et al. B cell depletion and signs of sepsis-acquired immunodeficiency in bone marrow and spleen of COVID-19 deceased. Int J Infect Dis 2021;103:628–35. [CrossRef]
23. Mare TA, Treacher DF, Shankar-Hari M, Beale R, Lewis SM, Chambers DJ, et al. The diagnostic and prognostic significance of monitoring blood levels of immature neutrophils in patients with systemic inflammation. Crit Care 2015;19(1):57.
24. Sayah W, Berkane I, Guermache I, Sabri M, Lakhal FZ, Yasmine Rahali S, et al. Interleukin-6, procalcitonin and neutrophil-to-lymphocyte ratio: Potential immune-inflammatory parameters to identify severe and fatal forms of COVID-19. Cytokine 2021;141:155428. [CrossRef]
25. Hammad R, Eldosoky M, Fouad SH, Elgendy A, Tawfeik AM, Alboraie M, et al. Circulating cell-free DNA, peripheral lymphocyte subsets alterations and neutrophil lymphocyte ratio in assessment of COVID-19 severity. Innate Immun 2021;27(3):240–50. [CrossRef]
26. Mardani R, Ahmadi Vasmehjani A, Zali F, Gholami A, Mousavi Nasab SD, Kaghazian H, et al. Laboratory Parameters in Detection of COVID-19 Patients with Positive RT-PCR; a Diagnostic Accuracy Study. Arch Acad Emerg Med 2020;8(1):e43.
27. Usul E, San I, Bekgoz B, Sahin A. Role of hematological parameters in COVID-19 patients in the emergency room. Biomark Med 2020;14(13):1207–15. [CrossRef]
28. Anurag A, Jha PK, Kumar A. Differential white blood cell count in the COVID-19: A cross-sectional study of 148 patients. Diabetes Metab Syndr 2020;14(6):2099–102. [CrossRef]
29. Rahman A, Niloofa R, Jayarajah U, De Mel S, Abeysuriya V, Seneviratne SL. Hematological abnormalities in COVID-19: a narrative review. Am J Trop Med Hyg 2021;104(4):1188–201.
30. Malik SUF, Chowdhury PA, Hakim A, Islam MS, Alam MJ, Azad AK. Blood biochemical parameters for assessment of COVID-19 in diabetic and non-diabetic subjects: a cross-sectional study. Int J Environ Health Res 2021:1–14. [CrossRef]
31. Tsai CM, Yu HR, Tang KS, Huang YH, Kuo HC. C-reactive protein to albumin ratio for predicting coronary artery lesions and intravenous immunoglobulin resistance in kawasaki disease. Front Pediatr 2020;8:607631. [CrossRef]
32. Seyit M, Avci E, Nar R, Senol H, Yilmaz A, Ozen M, et al. Neutrophil to lymphocyte ratio, lymphocyte to monocyte ratio and platelet to lymphocyte ratio to predict the severity of COVID-19. Am J Emerg Med 2021;40:110–4. [CrossRef]
33. Chan AS, Rout A. Use of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in COVID-19. J Clin Med Res 2020;12(7):448–53. [CrossRef]
34. Lippi G, Mattiuzzi C. Hemoglobin value may be decreased in patients with severe coronavirus disease 2019. Hematol Transfus Cell Ther 2020;42(2):116–7. [CrossRef]
35. Wan S, Xiang Y, Fang W, Zheng Y, Li B, Hu Y, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J Med Virol 2020;92(7):797–806. [CrossRef]
36. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. Clin Infect Dis 2020;71(15):769–77. [CrossRef]