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Ferritin Lymphocyte Ratio and D-dimer C-Reactive Protein Ratio as Diagnostic Parameters in Pre-COVID-19 Healthy Individuals

İbrahim Koç • **Serdar Doğan**
Department of Pulmonary Medicine, Bursa City Hospital, Bursa, Turkey
Medical Biochemistry, Mustafa Kemal University, Faculty of Medicine, Hatay, Turkey

Introduction: The gold standard diagnostic tool for COVID-19 is real-time reverse-transcription polymerase chain reaction (PCR) which is not available in every healthcare institution. There is a need for faster, cheaper, and available tests. This study aimed to determine if eosinophil levels, neutrophil-lymphocyte ratio (NLR), ferritin C-reactive protein (CRP) ratio (FCR), and D-dimer to CRP ratio (DCR) can be used as biomarkers in the early diagnosis of pre-COVID-19 healthy individuals.
Materials and Methods: 289 previously healthy COVID-19 patients and 152 SARS-CoV-2 PCR negative healthy controls were included in the study. Two patient groups were compared by examining the parameters such as white blood count (WBC), neutrophil, monocyte, eosinophil, and red blood cell distribution width (RDW) obtained at the time of hospital admission before using any kind of medication.
Results: Eosinophil levels, FLR, DCR, and NLR remained significantly lower, whereas DCR, FLR, and CRP lymphocyte ratio (CLR) were found to be higher in the COVID-19 group.
Conclusion: High levels of RDW, FLR, and CLR whereas low levels of WBC, monocyte, NLR, FCR, and DCR may predict COVID-19 in patients with no comorbidities.
Keywords: Ferritin lymphocyte ratio, monocyte lymphocyte ratio, COVID-19

Introduction

The COVID-19 disease, which was detected in December 2019, spread all over the world in the following period, causing the death of millions of people. It caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has been affecting not only individual with chronic disease but also those without. Given the rapid spread and serious harm of SARS-CoV-2, it is urgent to continuously improve and enrich its clinical diagnosis and treatment modalities. Today, the gold standard diagnostic tool is testing naso-pharyngeal swab samples by real-time reverse-transcription polymerase chain reaction (PCR). Yet PCR is an expensive tool that is not available in every healthcare institution due to its cost

Corresponding Author: İbrahim Koç, MD; Department of Pulmonary Medicine, Bursa City Hospital, Bursa, Turkey
ORCID: 0000-0003-4926-7331
E-mail: ibrahimkoc1981@gmail.com
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and working technique. There is a need for faster cheaper and available tests that will help doctors during the diagnosis. Some biomarkers derived from the peripheral blood count, complete blood count parameters such as white blood cell count (WBC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) neutrophil-lymphocyte ratio (NLR), and platelet-lymphocyte ratio (PLR) were analyzed as predictive parameters for prognosis of systematic inflammatory diseases (1, 2). In the daily rutin, all the above-mentioned parameters are studied in most of the COVID-19 patients by routine complete blood count tests that might be overlooked by clinicians. This study aimed to determine whether monocyte levels, eosinophil level, RDW, NLR, PLR, Ferritin to C-reactive protein (CRP) ratio (FCR), and D-dimer to CRP ratio (DCR) can be used as biomarkers in the early diagnosis of COVID-19, to determine optimal cut-off values and to estimate the probability of COVID-19. To the best of our knowledge, this is the first study investigating above mentioned parameters in previously healthy COVID-19 patients.

Materials and Methods

Study Design

Files of patients admitted to a tertiary hospital in Turkey between April and June-2021 with complaints compatible with COVID-19 disease were investigated retrospectively. Laboratory-confirmed 289 previously healthy COVID-19 patients and 152 SARS-CoV-2 PCR(-) healthy controls which consisted of asymptomatic contacts and healthcare workers were included. The demographic and clinical data of all the patients are shown in Table-1. Two groups were compared by examining hemogram parameters (WBC, RDW, neutrophil, lymphocyte, monocyte, eosinophil, and platelets) and biochemical parameteres obtained at the time of hospital admission before using any kind of medication. CRP and D-dimer were examined using the particle-enhanced immunoturbidometric essay. Ferritin levels were assayed using the sandwich principle. Leukocytes were measured using fluorescent flow cytometry; erythrocytes and platelets were measured using the impedance method. NLR, ferritin lymphocyte ratio (FLR), monocyte lymphocyte ratio (MLR), and CRP lymphocyte ratio (CLR) values were calculated by dividing neutrophil, ferritin, monocyte, and CRP levels to lymphocyte count respectively. FCR, ferritin eosinophil ratio (FER), and ferritin neutrophil ratio (FNR) values were calculated by dividing ferritin levels into CRP, eosinophil, and neutrophil levels respectively. Patients with chronic diseases, and who used a medication, and pregnants were excluded.

Ethical Statement

The ethics committee approval was obtained from a Clinical Research Ethics Committee of a tertiary hospital (Ethics Committee Approval No:2021-20/4). All the statistical analyses were carried out using SPSS v25 statistical software. A Kolmogorov-Smirnov test was performed for the normality of the sample data, and the continuous variables were defined by the mean±standard deviation, median(interquartile range %25-75), while the categorical variables were expressed as frequency and percent. To compare the independent groups, the Student's t-test for parametric test assumptions and a Mann-Whitney U test for non-parametric assumptions were used. The ROC analysis was performed for optimal cut-off values to predict SARS-CoV-2 positivity. Youden Index values wax exploited to identify the optimal cut-off values. In addition, a p-value less than 0.05 was set as the statistical significance level. Logistic regression analysis was carried out to estimate the COVID-19 risk.

Results

The median age turned out to be 35 (19-70) in the control, whereas 38 (19-81) in the COVID-19 group (Table-1). The median age was higher in the COVID-19 group (p=0.04). Although the

gender distribution in the control group was 33.1% (50) women and 66.9% (101) men, it was 45.8% (131) women and 54.7% (158) men in the COVID-19 group. No difference was found between death rates. In the COVID-19 group, 34.9 % (101) of patients were on home follow up whereas 64.7% (187) were hospitalized. WBC, neutrophil, lymphocyte, monocyte, and eosinophil levels, NLR, FCR, and DCR remained significantly lower in the COVID-19 group.

On the other hand RDW, D-dimer, ferritin, CRP, CRP D-dimer ratio (CDR), FLR, MLR, FER, CNR, FNR, and CLR were higher in the COVID-19 group. Optimal cut-off values calculated by the ROC analysis and the ROC curves are presented in Figure 1 and 2. When COVID-19 compared to the healthy controls the areas under the curve (AUC) of WBC, basophil, eosinophil, neutrophil, DCR, CNR, FLR, CLR, FNR and FER were as 0.83, 0.81, 0.77, 0.78, 0.66, 0.79, 0.68, 0.74, 0.72 and

Table-1. Demographics and Laboratory Findings of SARS-CoV-2 PCR Positive and Negative Patients

Parameters	Heathy Controls *(n=152)*	COVID-19 *(n=289)*	P value
Age (years)	35(19-70)	38 (19-81)	0.04*
Number of Females	50 (33.1%)	131 (45.3%)	0.01*
Death rates	1 (0.7%)	3 (1%)	0.17
Home follow up	152 (100%)	101 (34.9%)	0.001*
SpO_2	96 (93-98)	94 (90-97)	0.09
WBC	9.07 (4.6-17.5)	5.3 (2.7-14)	0.001*
Neutrophil	5.2 (0.65-85)	2.9 (0.02-72)	0.001*
Lymphocyte	2.4 (0.6-14)	1.6 (0.2-4.9)	0.001*
Monocyte	0.69 (0.3-9.9)	0.59 (0.19-1.9)	0.008*
Eosinophil	0.12 (0-6.4)	0.03 (0-0.9)	0.001*
RDW	13.3 (11-43)	13.6 (10.8-47)	0.03*
D-dimer	0.2 (0.012-1.2)	0.22 (0.15-33)	0.001*
Ferritin	76 (8.5-445)	105 (33-1670)	0.001*
CRP	2 (0.3-98)	6.3 (0.3-237)	0.001*
NLR	2.05 (0.16-24)	1.8 (0.02-70)	0.04*
CDR	8.2 (0.32-449)	19 (0.28-490)	0.001*
FCR	32 (0.65-741)	16 (0.04-531)	0.01*
FLR	33 (0.27-262)	72 (0.12-1134)	0.001*
MLR	0.27 (0-4.5)	0.33 (0.09-2.5)	0.001*
FER	519 (21-4380)	2436 (4.7-16000)	0.001*
CNR	0.33 (1-14)	1.9 (0.06-475)	0.001*
DCR	0.12 (0.6-3.1)	0.05 (0.3-3.59)	0.001*
FNR	12 (0.04-332)	37 (0.11-4349)	0.001*
CLR	0.6 (0.03-59)	3.3 (0.21-217)	0.001*

SpO_2: Median Oxygen Saturation, WBC: White Blood Count, CRP: C-Reactive Protein, NLR: Neutrophil-Lymphocyte Ratio, CDR: CRP D-dimer ratio, FCR: Ferritin CRP Ratio, FLR: Ferritin-Lymphocyte Ratio, MLR: Monocyte Lymphocyte Ratio, FER: Ferritin Eosinophil Ratio, CNR: CRP Neutrophil Ratio, DCR: D-Dimer CRP Ratio, FNR: Ferritin-Neutrophil Ratio, CLR: CRP Lymphocyte Ratio.
*$p<0.05$: statistically significant

0.74, respectively (p=0.001) (Table-2). A multivariable model was developed to predict the risk of COVID-19, and odds ratios for significant results are depicted in Table-3.

Table-2. ROC Analysis of SARS-CoV-2 PCR (-) and (+)

Variable	AUC	Cut-off	Sens.	Spec.	P
WBC	0,83	7.19	76	75	0,001*
BASO	0,81	0.025	74	75	0,001*
EOS	0,77	0.7	70	72	0,001*
NEU	0,78	4.16	71	72	0,001*
DCR	0,66	0.08	64	63	0,001*
CNR	0,79	0.78	73	72	0,001*
FLR	0,68	47.8	63	63	0,001*
CLR	0,74	1.56	71	70	0,001*
FNR	0,72	21.25	65	63	0,001*
FER	0,74	983	66	65	0,001*

AUC: Area under ROC Curve, WBC; White Blood Cell Count, Baso; Basophil, Eos; Eosinophil, Neu; Neutrophil, DCR; D-dimer C-reactive Protein (CRP) Ratio, CNR; CRP Neutrophil Ratio, FLR; Ferritin Lymphocyte Ratio, CLR; CRP Lymphocyte Ratio, FNR; Ferritin Neutrophil Ratio, FER; Ferritin Eosinophil Ratio.

* p<0.05 means statistically significant.

Patients with low WBC and FCR had an adjusted multivariable odds ratio of 0.54 (p=0.001), and 0.99 (p=0.05) respectively. Patients with high RDW, Ferritin, CDR, FCR, MLR, CNR and CLR had an increased risk of SARS-CoV-2 positivity (respectively; 1.059: p=0.005, 1: p=0.025, 0.95: p=0.002, 17: p=0.039; 4.89: p=0.001, and 0.8: p=0.001).

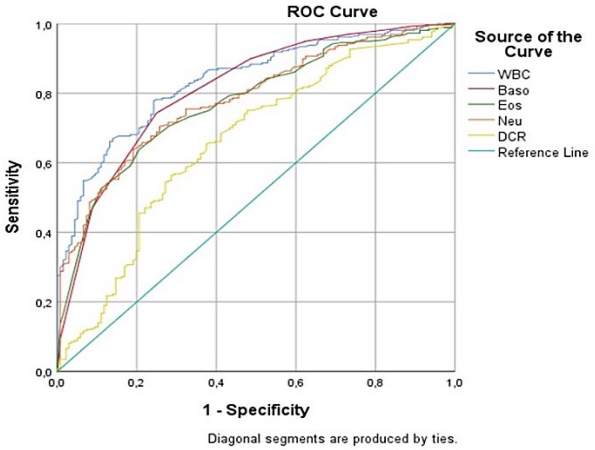


Figure 1. ROC Curves Comparing The Prediction of SARS-CoV-2, Variables For White Blood Cell Count (WBC), Basophils (Baso), Eosinophils (Eos), Neutrophils (Neu) and D-dimer C-reactive protein Ratio (DCR).

The multivariable model had an area under the receiver operating characteristic curve estimate of 0.9 (95%CI: 0.85, 0.95).

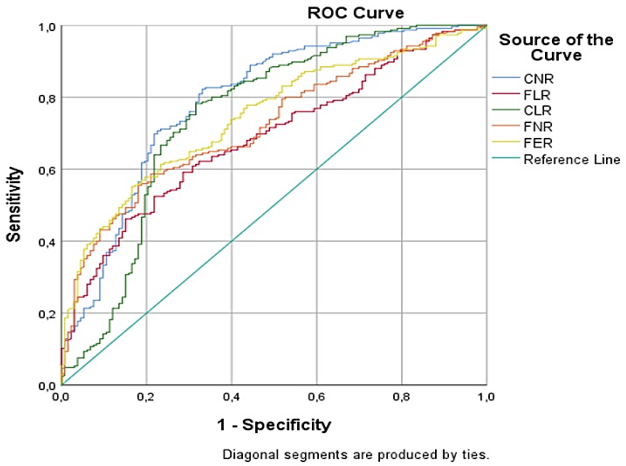


Figure 2. ROC Curve comparing the Prediction of SARS-CoV-2. Variant variables for C-reactive Protein (CRP), Neutrophil Ratio (CNR), Ferritin Lymphocyte Ratio (FLR), CRP Lymphocyte Ratio (CLR), Ferritin Neutrophil Ratio (FNR), Ferritin Eosinophil Ratio (FER).

Table-3. Laboratory parameters to predict COVID-19

Variable	P	OR
WBC	0,001*	0,545 (0.4-0.73)
RDW	0,005*	1,059 (1.01-1.1)
Ferritin	0,02*	1,009 (1.01-1.01)
CDR	0,002*	0,954 (0.92-0.98)
FCR	0,05*	0,993(0.98-1)
MLR	0,03*	17,519(1.14-266)
CNR	0,001*	4,895(1.99-12)
CLR	0,001*	0,818(0.7-0.9)

Data in parentheses are 95% confidence intervals. OR: Odds Ratio, WBC; White Blood Cell Count, RDW: Red Blood Cell Distribution Width, CDR: C-Reactive Protein to D-dimer Ratio, FCR: Ferritin CRP Ratio, MLR: Monocyte Lymphocyte Ratio, CNR: CRP Neutrophil Ratio, CLR: CRP Lymphocyte Ratio.

Discussion

In suspicious patients, PCR is still the gold standard diagnostic method in the diagnosis of COVID-19. Yet PCR is not available in every healthcare institution. Therefore, there is a need for simpler and more practical methods to detect COVID-19 cases. The data on neutrophil levels in COVID-19 are incomplete and have not been widely studied. The available data suggest neutrophilia is an expression of the cytokine storm and hyper-inflammatory state which

have an important pathogenetic role in COVID-19 and related infections. Neutrophilia was reported in patients treated in the intensive care unit (3). Some studies demonstrated low neutrophil levels in pediatric COVID-19 and adults as well (4, 5). In our study, neutrophils remained lower in the COVID-19 (p=0.001). The discrepancy between studies might be due to heterogeneity of COVID-19 disease or patient characteristics. Since neutrophil levels may be affected by many secondary diseases, in the present study to minimize secondary effects patients with chronic diseases and those using any medication were excluded. Lymphocytes are cells that play an important role in the maintenance of immune system function.

Following the viral infection, alteration in lymphocytes might be observed depending on the virus types. Wang et al. reported decreased lymphocyte levels in patients with COVID-19 (6). Even though low lymphocyte levels have been demonstrated previously, this study showed that lymphocytes decrease in previously healthy COVID-19. Monocyte-macrophage activation is known to play an important role in the immunopathology of patients with COVID-19 (7). Previous studies reported morphological and inflammation-related phenotypic changes in peripheral blood monocytes that correlate with the patient's outcome during SARS-CoV-2 infection (8). In a study Kos, et al. reported a reduced rate of activated monocytes mainly observed in patients with severe COVID-19 (9). In the present study COVID-19 group had lower monocyte levels than those of healthy. Eosinophils are circulating leukocytes that have proinflammatory effects in some diseases. Eosinophils also have been shown to have various other functions like immunoregulation and antiviral activity. Yet their role in COVID-19 is not well known. Previously, it has been reported that COVID-19 had a decrease in circulating eosinophil counts, which was more frequent than other types of pneumonia (5). Zhang et al. reported eosinopenia in patients with acute respiratory deterioration during infection with SARS-CoV-2 (10). Zhang et al. included 140 COVID-19 with comorbidities that may affect eosinophil levels yet similar to their study. In our study, low levels of circulating eosinophils were found in previously healthy COVID-19. The results indicate that SARS-CoV-2 affects neutrophils, lymphocytes, eosinophils, a treatment strategy that increases the above-mentioned cell levels or targets reasons for the decline may help in dealing with COVID-19. High levels of RDW were reported to be a prognostic predictor for patients with severe COVID-19 (6). In a study, Henry et al. found an increase in RDW with advancing COVID-19 severity (11). In the present study high levels of RDW were associated with an increase of COVID-19 (OR:1) (P=0.005).

In recent years, some ratios have been in use by researchers in the diagnosis and prognosis of inflammatory conditions. Some of these are neutrophil / lymphocyte, platelet / lymphocyte, and monocyte/lymphocyte ratio. CRP, ferritin, D-dimer, and lymphocyte levels are important markers that are predictors of prognosis in COVID-19 (12). In this study, it was hypothesized that the ratios of the above-mentioned markers could help in the diagnosis of COVID-19.

In a study, Seyit et al. Reported high NLR in patients who tested positive for SARS-CoV-2 compared to the control (12). In a study from Wuhan/China Yang et al. reported elevated NLR significantly associated with illness severity (13). In the present study, NLR remained lower in the COVID-19. The discrepancy between studies might be due to the heterogeneity of COVID-19 or patient characteristics since we included only previously healthy patients. Yang et al. investigated 93 COVID-19 patients and found out that lymphocyte to monocyte ratio (LMR) levels of severe patients were significantly higher than those of non-severe patients. (13). In the present study, MLR was investigated and was found to be higher in the COVID-19.

COVID-19 has been found not only to cause lung damage but also to damage other organs which may alter iron hemostasis. High levels of ferritin have been associated with poor prognosis in patients with COVID-19 (6). Early diagnosis and timely treatment of severe COVID-9 are crucial. In the present study ferritin levels, FLR, FER, FNR were found to be higher whereas FCR was lower in the COVID-19. High levels of ferritin and low levels of FCR were predictive for COVID-19 (OR:1,p=0.025; OR:0.99, P=0.05 respectively).

The CRP is an important inflammatory marker in which high levels have been associated with severe COVID-19 (14). This study aimed to see if CRP, CDR, and CNR can help the diagnosis of COVID-19. CRP, CDR, and CNR were higher in the COVID-19 group. High levels of CDR were predictive for COVID-19 (OR:0.954, p=0.002).

D-dimer is a product of the degradation of fibrin and is usually elevated in thrombotic events. Previously in the early phase of COVID-19 infection high D-dimer levels have been reported (15). In the present study, D-dimer levels were higher whereas DCR was lower in the COVID-19 group. In the same group, MLR was higher which was highly predictive for COVID-19 (OR:17, p=0.03).

Conclusion

In conclusion, high levels of RDW, D-dimer, ferritin, CRP, CDR, FLR, MLR, FER, CNR, FNR, and CLR whereas low levels of WBC, neutrophil, lymphocyte, monocyte, eosinophil, NLR, FCR, and DCR may predict COVID-19 in patients with no comorbidities.

Conflict of Interest

All authors declare no conflict of interest.

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Contact Details

İbrahim Koç, MD
Department of Pulmonary Medicine,
Bursa City Hospital, Bursa, Turkey
ORCID: 0000-0003-4926-7331
E-mail: ibrahimkoc1981@gmail.com

Serdar Doğan, MD *(Corresponding)**
Medical Biochemistry, Mustafa Kemal University,
Faculty of Medicine, Hatay, Turkey
ORCID: 0000-0001-6854-2197
E-mail: drserdardogan@hotmail.com

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