Two Consecutive Visits to the Emergency Department: Potential Role of CBC Parameters in COVID-19 Patients Whose PCR Tests Change from Negative to Positive

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ABSTRACT

Introduction: The coronavirus disease-2019 (COVID-19) pandemic is an important health problem worldwide. In this study, we aimed to investigate the relationship between the clinical features and complete blood count (CBC) parameters of COVID-19 patients who were admitted to the emergency department (ED) and whose first polymerase chain reaction (PCR) test was negative and turned positive on the second admission.

Methods: The study was designed as a single-center, retrospective, cross-sectional, and observational study. Demographic characteristics, vital signs, complaints, comorbidities, duration of complaints, time between two admissions, and defined CBC parameters in both the first and second admissions were recorded. Positive parameters were recorded in patients whose first PCR test was negative and turned positive and who were admitted to the ED again within ten days. The relationship between the PCR tests and these parameters was investigated.

Results: A total of 123 patients were included in the study at the first admission, 89 of them were symptomatic. Of these, in the symptomatic group, body temperature was found higher and peripheral oxygen saturation percentage was found lower. Hypertension was the most common comorbidity, followed by diabetes mellitus and coronary artery disease, respectively. The most common symptom was fever, followed by cough and pain. The median time between the two PCR tests was six days. The leukocyte count, neutrophil count, lymphocyte count, platelet count, and hematocrit decreased on the second admission, whereas the platelet-lymphocyte ratio (PLR) increased.

Conclusion: CBC parameters defined in repeated evaluations with suspicion of COVID-19 may support the predictions of a positive test. Regardless of symptoms, the increase in PLR and decrease in leukocyte count, neutrophil count, lymphocyte count, platelet count, and hematocrit should raise suspicion that the COVID-19 test, which was initially negative, may turn positive in the process.

Keywords: COVID-19, emergency department, platelet-lymphocyte ratio, polymerase chain reaction

Introduction

Coronaviruses pose significant threats to humans and animals. Toward the end of 2019, a new coronavirus was pinpointed as the root cause of a pneumonia outbreak in Wuhan, a city in China. Rapid transmission led to an epidemic within China, followed by an increasing number of cases in various countries worldwide. In February 2020, the World Health Organization officially named the illness coronavirus disease-2019 (COVID-19), an abbreviation for "COVID-19" (1). Swiftly collecting and testing appropriate specimens from individuals who meet the suspected case criteria for COVID-19 is a top priority for the clinical management and control of outbreaks. Nevertheless, it is essential to understand that obtaining one or more negative results through polymerase chain reaction (PCR) tests does not definitively rule out the possibility of COVID-19 infection (2).

In the context of a highly sensitive test, negative results should not be solely relied upon to rule out infection, especially when the pretest likelihood of infection is high. It is crucial for clinicians to exercise caution with unexpected negative outcomes. Assessing test sensitivity in individuals who have had contact with confirmed cases and those who are asymptomatic is a pressing concern. Additionally, it is essential to develop methods to estimate the pretest probability of infection for both asymptomatic and symptomatic individuals, enabling the calculation of

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posttest probabilities following either positive or negative results. While PCR tests used for diagnosing COVID-19 are highly specific, there have been reports of false-negative results (3-6).

Recognizing COVID-19 early is of paramount importance because of its swift transmission and the potential strain it may place on healthcare systems, particularly in cases where patients initially test negative for COVID-19 using PCR tests. Complete blood count (CBC) is a widely available and cost-effective blood test commonly used in emergency departments, even in resource-constrained settings, and is routinely administered. In our study, we aimed to explore the connection between CBC parameters and clinical characteristics among patients who were readmitted to the emergency department with similar symptoms and subsequently tested positive for COVID-19 after initially testing negative in the preceding ten days.

Methods

The study was designed as a single-center, retrospective, cross-sectional, and observational study. The current study protocol was reviewed and approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 2581, date: 13.11.2020). The population of the study consisted of patients who were admitted to the ED between 01.04.2020 and 30.05.2020, were evaluated as a pre-diagnosis of COVID-19, and requested PCR test. We included ED patients whose first PCR test was negative and became positive within the next ten days. We excluded patients who were younger than 18 years of age, whose clinical data were missing, and whose CBC parameters and PCR tests could not be studied for technical reasons.

The patient's demographic characteristics, vital parameters, comorbidities, and symptoms on admission were recorded. We recorded leukocyte count, neutrophil count, lymphocyte count, platelet count, hematocrit, immature granulocyte count, and neutrophil percentage from the CBC parameters studied both in the first and second admissions. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) values were calculated. The relationship between defined CBC parameters and PCR tests that became positive and negative at the first admission was investigated.

Statistical Analysis

Statistical analyses were performed using the SPSS version 22.0 program. The normality of continuous variables was evaluated using the Kolmogorov-Smirnov test. Continuous variables are expressed as mean ± standard deviation, and categorical variables are expressed as numbers and percentages. Categorical variables were compared using Fisher's exact test. The Wilcoxon test was used for dependent variables and the Mann-Whitney U test was used for independent variables when examining the changes in non-normally distributed (non-parametric) variables. In the correlation analysis, the p-value and correlation coefficient (r) were given together using the Spearman correlation test. All analyses were performed using a 95% confidence interval. Statistical significance was accepted as p<0.05.

Results

A total of 12,140 patients were retrospectively analyzed for appropriate the International Classification of Diseases-10 diagnoses using the Patient Information Management System between 01.04.2021 and 30.05.2021. Among these patients, one thousand nine hundred and fifty-six patients (16.1%) were excluded because their first PCR tests were positive. Of the remaining 10,184 patients, 3,268 were re-admitted to the ED at least once within 10 days. A total of 2,297 (70.3%) patients did not have a contact history in terms of COVID-19. We identified 971 patients whose first PCR test was negative, who had a second PCR test within ten days, and whose contact status was known. Among these, only 123 patients underwent CBC tests in both admissions (Figure 1). According to the symptom status, the "asymptomatic group" included 34 (27.6%) patients who declared possible contact but had no symptoms on the first admission. "Symptomatic group" included 89 (72.4%) patients who had symptoms possible due to COVID-19 on the first admission and who had a positive second PCR test while the symptoms were still in progress by re-admission. Of the patients, 76 patients (61.8%) were male and 47 patients (38.2%) were female. The median age was 41 years (range: 18-93), and the number of patients ≥65 years was 17 (13.8%). Body heat was higher and peripheral oxygen saturation percentage was lower in the symptomatic group. Hypertension was the most common comorbid disease, followed by diabetes mellitus and coronary artery disease, respectively. The most common symptom was fever, followed by pain and cough. The median times between the two tests were six days (4-7) and six days (5-7), respectively (Table 1). When the difference between the two groups in terms of CBC parameters was investigated, it was observed that on the first admission, the immature granulocyte count and PLR were higher in the symptomatic group than in the
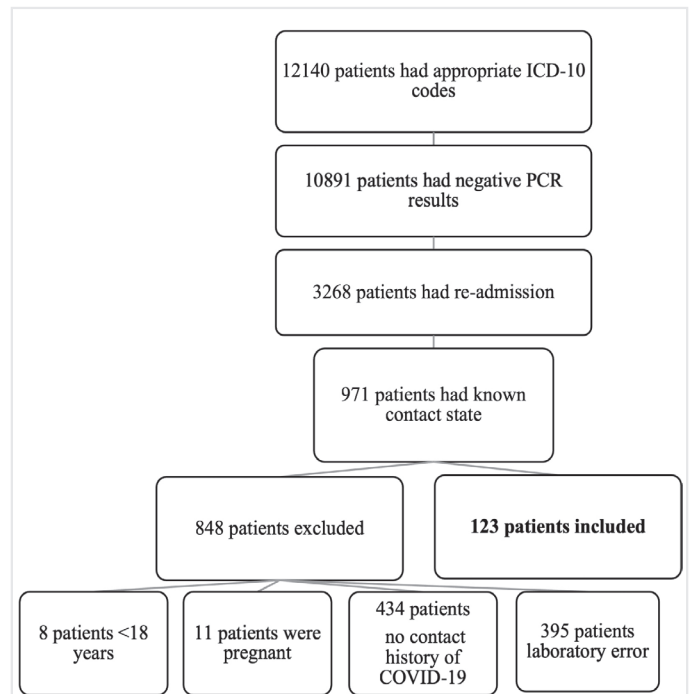

Figure 1. Study flowchart
ICD: International Classification of Diseases-10, PCR: Polymerase chain reaction, COVID-19: Coronavirus disease-2019

asymptomatic group, but on the second admission, only the immature granulocyte count was higher in the symptomatic group than in the asymptomatic group, which was statistically significant.

When we examined the CBC parameters of 123 patients regardless of symptoms, leukocyte count, neutrophil count, lymphocyte count, platelet count, and hematocrit decreased, whereas PLR increased at the second admission (Table 2). The immature granulocyte count, neutrophil percentage, and NLR were not statistically significant. Decreases in leukocyte and immature granulocyte counts were not statistically correlated with the asymptomatic group. The statistically correlated parameters in both groups are shown in Table 3. While we found decreases in neutrophil count, lymphocyte count, platelet count, hematocrit, and NLR in both groups, we found increases in neutrophil percentage and PLR. In the symptomatic group, the decrease in leukocytes and neutrophils had a higher correlation coefficient, whereas in the asymptomatic group, the decrease in platelets and hematocrit had a higher correlation coefficient. The obvious correlation was the reduction in the neutrophil count in the symptomatic group (R: 0.768).

Discussion

COVID-19 is a highly complex disease and a serious health threat worldwide. Overall, COVID-19 resulted in serious economic costs. Thus,

Table 1. Characteristics of the patients

Characteristics		Symptomatic	Asymptomatic	p
Demographics				
Age	Median (IQR)	43 (31-57)	40,5 (33-51)	0.565*
Elderly age (>65 years)	n (%)	13 (14.6)	4 (11.8)	0.467#
Male	n (%)	56 (62.9)	20 (60.6)	0.684#
Smoker	n (%)	34 (38.2)	12 (35.3)	0.467#
Vital signs				
Systolic blood pressure (mmHg)	Median (IQR)	130 (124-135)	135 (125-140)	0.103*
Diastolic blood pressure (mmHg)	Median (IQR)	75 (70-80)	75 (70-80)	0.569*
Pulse (/min)	Median (IQR)	90 (85-100)	87.5 (81-97)	0.251*
Body temperature (°C)	Median (IQR)	37 (36.4-37.5)	36.9 (36.7-37)	**0.023***
SpO_2 (%)	Median (IQR)	96 (94-97)	98 (96-98)	**<0.001***
Comorbidities				
Hypertension	n (%)	20 (22.5)	6 (17.7)	0.375#
Diabetes mellitus	n (%)	11 (12.4)	5 (14.7)	0.468#
Coroner artery disease	n (%)	10 (11.2)	4 (11.8)	0.578#
Asthma/COPD	n (%)	10 (11.2)	1 (2.4)	0.136#
Malignancy	n (%)	6 (6.7)	1 (2.4)	0.375#
Immunosuppression	n (%)	7 (7.9)	1 (2.4)	0.296#
Time				
Contact time (day)	Median (IQR)	3 (2-4)	2.5 (2-4)	0.787*
Time between two PCR tests (day)	Median (IQR)	6 (4-7)	6 (5-7)	0.783*

*Mann-Whitney U test, #Fisher's exact test, COPD: Chronic obstructive pulmonary disease, PCR: Polymerase chain reaction

Table 2. CBC parameters of patients at the first and second admission

CBC parameters	First admission Median (IQR)	Second admission Median (IQR)	p*	R#	p**
Leukocyte	7.12 (5.73-8.55)	6.31 (5.07-6.93)	**<0.001**	**0.747**	**<0.001**
Neutrophil	4.47 (3.37-5.36)	3.54 (2.86-4.32)	**<0.001**	**0.768**	**<0.001**
Lymphocyte	1.80 (1.26-2.37)	1.38 (1.09-1.80)	**<0.001**	0.605	**<0.001**
Platelet	234 (191-282.5)	214 (186-265.5)	**<0.001**	0.665	**<0.001**
Hematocrit	41.9 (36.2-44.9)	40.1 (35.1-43.4)	**<0.001**	0.708	**<0.001**
Immature granulocyte	0.02 (0.01-0.03)	0.02 (0.01-0.03)	**<0.001**	0.694	0.270
Neutrophil percentage	62.2 (53.7-69.4)	63.1 (56.2-68.7)	**<0.001**	0.485	0.218
Neutrophil-lymphocyte ratio	2.43 (1.57-3.61)	2.38 (1.74-3.18)	**<0.001**	0.616	0.950
Platelet-lymphocyte ratio	126.9 (103.5-179.4)	155.1 (116.3-203.7)	**<0.001**	0.512	**<0.001**

*Spearman correlation, #Linear regression coefficient, **Wilcoxon test, CBC: Complete blood count, IQR: Interquartile range

Table 3. Comparison of CBC parameters in the first and second admissions of patients by distinguishing between symptomatic and asymptomatic groups

CBC parameters	Group	First admission Median (IQR)	Second admission Median (IQR)	p*	R#	p**
Leukocyte	A	6.87 (5.54-8.51)	6.07 (5-6.88)	**<0.001**	**0.797**	**<0.001**
	B	7.88 (6.13-8.75)	6.70 (5.09-7.32)	0.078	0.306	**<0.001**
Neutrophil	A	4.17 (3.11-5.60)	3.54 (2.81-4.28)	**<0.001**	**0.801**	**<0.001**
	B	4.61 (3.87-5.47)	3.54 (3.12-4.53)	**0.046**	0.345	**<0.001**
Lymphocyte	A	1.71 (1.25-2.20)	1.38 (1.08-1.69)	**<0.001**	**0.668**	**<0.001**
	B	2.04 (1.49-2.55)	1.38 (1.11-1.92)	**0.011**	0.430	**<0.001**
Platelet	A	233 (181-281)	214 (187-273)	**<0.001**	0.650	**0.007**
	B	242.5 (193-284)	214 (185-244)	**<0.001**	**0.740**	**0.001**
Hematocrit	A	41.4 (35.5-45)	40.1 (34.7-43.4)	**<0.001**	0.687	**0.002**
	B	42.2 (38.1-44.8)	40.1 (36.6-44.1)	**<0.001**	**0.786**	**0.004**
Immature granulocyte	A	0.02 (0.01-0.03)	0.02 (0.01-0.03)	**<0.001**	0.606	0.681
	B	0.02 (0.02-0.03)	0.02 (0.01-0.02)	0.189	0.231	0.152
Neutrophil percentage	A	61.8 (53.2-68.7)	63.1 (56-68.6)	**<0.001**	0.448	0.400
	B	62.6 (54.2-71.2)	63.1 (57.1-69.4)	**<0.001**	0.614	0.288
Neutrophil-lymphocyte ratio	A	2.43 (1.59-3.69)	2.38 (1.73-3.19)	**<0.001**	0.617	0.711
	B	2.41 (1.55-3.45)	2.44 (1.88-3.16)	**<0.001**	0.583	0.578
Platelet-lymphocyte ratio	A	129.7 (103.4-196.7)	155.1 (119.1-205.9)	**<0.001**	0.530	**0.002**
	B	121.9 (106.1-148.4)	147.2 (112.4-176.3)	**0.012**	0.426	**0.011**

A: Symptomatic group. B: Asymptomatic group. *Spearman correlation, #Linear regression coefficient, **Wilcoxon test, CBC: Complete blood count, IQR: Interquartile range

the rapid detection of COVID-19 is crucial to avoid contamination and adverse outcomes and reduce morbidity by properly initiating treatment before irreversible damage occurs. Recent studies reported that the sensitivity and specificity of PCR tests were 80% and 98-99%, respectively (7). Even though a test is highly sensitive, the results cannot rule out infection if the clinical probability is high. Therefore, clinicians should pay attention to unexpected negative results. Early diagnosis of COVID-19 is still difficult today because it is characterized by many clinical manifestations. Thus, it is important to identify a reliable biomarker for screening high-risk patients and candidates for COVID-19. Among all the investigated COVID-19 biomarkers, CBC parameters could represent valuable tools. Indeed, CBC has several advantages. First, it is the most commonly ordered test in all clinical settings, including EDs; second, clinicians order it almost routinely. Especially in terms of limited resources, it is more important to identify early symptomatic or asymptomatic patients whose PCR test is negative at first admission and whose PCR test will probably turn positive in the process. Studies have reported that lymphopenia is more common at first admission. Ferrari et al. (8) reported in a study that COVID-19 patients with positive PCR tests had a lower lymphocyte count on the first admission. We found similar results in terms of lymphocyte count, but the platelet count on admission was lower, unlike in this study. A study by Guan et al. (9) reported thrombocytopenia in approximately one-third of patients. This may be because of the inclusion of asymptomatic patients with a clinically milder infection in our study. Inflammatory biomarkers play an important role in the diagnosis and prognosis prediction of various diseases, including COVID-19. Many publications report that NLR and PLR are associated with diagnosis, clinical course, and outcome because they're reflecting the increased inflammatory process (10-13). In this study, we did not find any relationship with NLR. We think that this may be because symptomatic patients were included in the study at a very early stage and asymptomatic patients. Similarly, Bedel et al. (14) reported that plasma inflammatory biomarkers such as NLR and PLR are time-sensitive and dynamic parameters accompanying the course of the disease, and the time elapsed since the onset of symptoms may affect NLR and PLR. We found that the PLR was higher on the second admission, which was statistically significant. Many studies in the literature have reported that PLR can be used in diagnosing and predicting the prognosis in patients with COVID-19. We found that the PLR was higher on the second admission as statistically significant (15).

Study Limitations

There are some limitations in this study. Our study was a single-center, retrospective study, and the results cannot be generalized to the population. The sample size is small, and studies with more patients are needed. Finally, the negative results of the PCR tests on the first admission may be false negative. We could not confirm this because we did not perform a second test within 24-48 h.

Conclusion

In conclusion, repeated CBC tests of suspected COVID-19 patients may predict a positive PCR result on the second admission. Regardless

of symptoms, the increase in PLR and a decrease in leukocyte count, neutrophil count, lymphocyte count, platelet count, and hematocrit should raise suspicion of a COVID-19 test, which was initially negative and may turn positive afterwards.

Ethics Committee Approval: The current study protocol was reviewed and approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Local Ethics Committee (approval number: 2581, date: 13.11.2020).

Informed Consent: Retrospective study.

Authorship Contributions: Surgical and Medical Practices - B.E., A.B.E., Özl.D.; Concept - B.E., Ö.D., A.V., A.B.E., Özl.D.; Design - B.E., Ö.D., A.V., A.B.E., Özl.D.; Data Collection or Processing - B.E., A.B.E.; Analysis or Interpretation - B.E., Ö.D., A.V., A.B.E., Özl.D.; Literature Search - B.E., Ö.D., A.V., A.B.E., Özl.D.; Writing - B.E., Ö.D., A.V., A.B.E., Özl.D.

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