

ORIGINAL RESEARCH

Evaluation of routine hematological parameters in predicting disease severity in COVID-19 patients in northern India

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ABSTRACT

Background: Hematological parameters like total leukocyte count (TLC), neutrophil, lymphocyte, and absolute eosinophil counts (AEC), and neutrophil-to-lymphocyte ratio (NLR) are known to predict the severity of novel corona virus disease 2019 (COVID-19) patients. In the present study, we aimed to study the role of Hematological parameters in predicting disease severity in Covid 19 patients. **Methods:** A retrospective study was done over a period of 2 months. Patients, who were ≥ 18 years of age with COVID-19 confirmed on SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) and whose routine hematology counts were sent within 24 h of admission, were included in the study. **Results:** Total 282 patients included in the study, 62.8% were male and the average age was 47.6 ± 16.9 years. The most common complaints were fever (37.2%) and cough (15.2%). Comparisons made according to real-time PCR test results revealed that while no statistically significant difference was observed between test groups (negative-positive) regarding lymphocyte and platelet lymphocyte ratio values ($p > 0.05$), a statistically significant difference ($p < 0.05$) was found between the test groups regarding platelet, hemoglobin, leukocyte, neutrophil, NLR and SII values. **Conclusions:** In conclusion, the low values for NLR, SII, absolute neutrophil and lymphocyte counts, and PLTs may have diagnostic properties concerning COVID-19.

Key words: Haematological parameters, COVID-19 disease, neutrophil lymphocyte ratio, platelet lymphocyte ratio, systemic inflammatory index

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INTRODUCTION

Novel corona virus disease 2019 (COVID-19) resembles severe acute respiratory syndrome corona virus (SARS CoV). Most patients are asymptomatic and those with symptoms develop mild flu-like conditions to severe acute respiratory syndrome or death¹. Considering the infectivity and serious harm of COVID-19, it is of paramount importance to identify various circulating biomarkers, which can predict the severity of COVID-19². The CBC is widely available, inexpensive and can provide the clinicians with reliable information regarding the patients' condition, that in turn, will be helpful in making a reasonable allocation of medical resources³. Except for clinical symptoms and pulmonary computed tomography (CT) findings, most confirmed COVID-19 patients revealed laboratory fluctuations in different serological parameters, including renal and

liver function tests, coagulation parameters, and inflammatory, biochemical and hemocytometric parameters⁴⁻⁵. To show the prognosis and hyper inflammation state, a combination of laboratory tests has been evaluated. The combination of the various test includes platelet-to-lymphocyte (PLR) and neutrophils to lymphocyte ratio (NLR). COVID-19 leads to variation in the hematological parameters, including lymphocytes, white blood cells, platelets, neutrophils, etc.⁶⁻⁷. These variations are different from case to case and level of the disease severity. Lymphopenia has been previously reported in about 35%-85% of patients and was the most common blood count abnormality⁸. Complete blood counts (CBC) are easily performed and inexpensive. Included in the CBC are values such as white blood count, neutrophil, lymphocyte and platelet count (PLT), mean platelet volume and certain ratios of these

values. These can be used as inflammatory markers⁹. Because of the large number of COVID-19 patients flooding the healthcare system, these routine markers are especially important. Therefore, a simple CBC which includes TLC, neutrophil, lymphocyte and eosinophil counts, and NLR may be extremely useful in predicting the severity and triaging of these patients especially in developing countries with limited resources¹⁰.

AIM

The study was undertaken to assess the trend of routine hematology parameters and their ability to predict the severity and mortality in COVID-19.

MATERIAL AND METHODS

This retrospective study was carried out in the department of pathology, in a tertiary care hospital, northern India. This study also agrees with the principles of the Declaration of Helsinki of the World Medical Association.

In this study, 282 patients who have admitted to the emergency department in our hospital during the study period (between June 2020 to December 2020) and who have been considered as possibly having COVID-19 were included. The recommended criteria established by the Scientific Committee of the Ministry of Health were used for the selection of possible COVID-19 patients¹⁴. Patients from whom a throat swab was obtained and those who were thereafter hospitalized with an initial diagnosis of COVID-19 were studied retrospectively. Only patients above the age of 18 years were included in the study. The presenting complaints, epidemiological features and blood test results of patients were obtained from the patient files. Only the results of the initial RT-PCR and CBC tests were used and no further tests were performed on these patients. The decision to obtain a CBC from incoming patients was made by the ER's attending physician, and later, the blood sample was taken by the ER nurse. The blood sample was studied in the ER laboratory by the laboratory technician using the Horiba Medical Pentra DF Nexus analyzer. The CBC results obtained from this analysis were studied and approved by a biochemistry specialist.

The analysis of the data was done using the IBM SPSS 25.0 and Med Calc 15.8 statistical package programs. A Chi-square test was used to analyze the categorical variables of the patients, which were expressed as a number and percentage. For parametric continued variables, the independent samples t-test was used for analysis and they were presented as a mean and standard deviation. The 95% CI was calculated whenever appropriate, and a two-tailed $p < 0.05$ was considered statistically significant.

RESULTS

A total of 282 patients included in the study, 62.8% were male and the average age was 47.6 ± 16.9 years. RT-PCR analyses of 163 patients (57.8%) were Covid 19 positive. The most common complaints during admission were fever (37.2%), cough (15.2%), sore throat (5.3%), shortness of breath (3.9%) and myalgia (3.2%) [Table 1].

Comparisons made according to the PCR test results of patients included in the study, revealed that no difference of statistical significance regarding age and test results (negative or positive) was found ($p > 0.05$). It was determined that age of the patients was not a risk factor for COVID-19. Furthermore, a significant difference was observed regarding gender and test results ($p < 0.05$). A positive test result among males was more commonly observed than in females. Also, those with a negative test result were more commonly symptomatic than those with a positive test result (Table 1).

Comparisons made according to the RT-PCR test results revealed that while no statistically significant difference was observed between test result groups (negative or positive) regarding lymphocyte and platelet lymphocyte ratio values ($p > 0.05$), a statistically significant difference ($p < 0.05$) was found between the test result groups regarding platelet, hemoglobin, leukocyte, neutrophil, NLR and SII values. In patients with negative test results, it was found that platelet, leukocyte, neutrophil, NLR and SII values were higher, whereas hemoglobin was found to be higher in patients with positive test results. Also, hemoglobin was found to be higher in male patients who tested positive for COVID-19 (Table 2).

Table 1: Comparison of patients features according to PCR test results

Patient features		Positive	Negative	Total	P-Value
Gender	Female	50 (30.7%)	55 (46.2%)	105 (37.2%)	0.008
	Male	113 (69.3%)	64 (53.8%)	177 (62.8%)	
Age (years)	General	46.2±15.5 43 (34–58)	49.6±18.5 49 (33–64)	47.6±16.9 46 (18–91)	0.097
	Female	53.1±15.3 56 (41–61)	54.1±19.8 57 (36–68)	53.6±17.7 56 (18–91)	0.780
	Male	43.1±14.7 40 (31–55)	45.7±16.5 45 (31–57)	44.1±15.4 41 (19–83)	0.286
Symptoms	Fever	105 (37.2%)	70 (58.8%)	35 (21.5%)	
	Cough	43 (15.2%)	15 (12.6%)	28 (17.2%)	
	Sore throat	15 (5.3%)	3 (2.5%)	12 (7.4%)	

	Dyspnea	11 (3.9%)	7 (5.9%)	4 (2.5%)	
	Myalgia	9 (3.2%)	6 (5.0)	3 (1.8%)	
	Headache	2 (0.7%)	2 (1.7%)	–	

Table 2: Comparisons of hematological parameters according to PCR test results

Haematological parameters	Positive (n = 163)	Negative (n = 119)	P-Value
Platelets	210.0 ± 72.3 198.0 (168.0-241.0)	233.0 ± 72.3 226.0 (185.0-275.0)	0.009
Hemoglobin	14.7 ± 1.7 14.8 (13.3-15.9)	13.9 ± 1.8 14.0 (12.5-15.4)	0.000
Leukocytes	6.0 ± 3.9 5.8 (4.5-7.0)	9.2 ± 3.7 8.4 (6.9-10.6)	0.000
Neutrophils	3.5 ± 2.0 3.1 (2.4-4.1)	6.2 ± 3.5 5.5 (4.1-7.6)	0.000
Lymphocytes	2.8 ± 5.5 1.8 (1.4-2.3)	2.2 ± 2.5 1.9 (1.4-2.4)	0.223
NLR	2.0 ± 1.5 1.7 (1.2-2.4)	3.8 ± 3.2 2.8 (1.9-5.0)	0.000
PLR	119.8 ± 58.5 113.3 (82.2-147.5)	133.5 ± 59.3 121.3 (91.2-164.1)	0.081
SII	427.5 ± 347.0 345.9 (218.5-501.2)	864.4 ± 718.0 659.8 (409.3-979.1)	0.000

NLR: Neutrophil lymphocyte ratio; PLR: Platelet lymphocyte ratio; SII: Systemic inflammatory index.

Table 3: Recommended cut-off values for significant markers in the prediction of Covid-19 disease

Markers	AUC	Cut off	Sensitivity	Specificity	95% CI	P-value
Platelets	0.618	≤211	62.6	63.0	0.558-0.675	0.001
Hemoglobin	0.612	>13.6	72.4	48.7	0.553-0.670	0.001
Leukocytes	0.828	≤7.2	82.2	71.4	0.779-0.871	0.000
Neutrophils	0.826	≤3.9	73.0	80.7	0.776-0.868	0.000
NLR	0.739	≤1.8	59.5	76.5	0.684-0.790	0.000
SII	0.760	≤479.1	74.9	68.9	0.706-0.808	0.000

Table 4: Comparison of results according to cut-off points

Hematological parameters	Cut off	Positive (n = 163)	Negative (n = 119)	P-value
Platelets	>211	61 (44.9%)	75 (55.1%)	0.000
	≤211	102 (69.9%)	44 (30.1%)	
Hemoglobin	≤13.6	45 (43.7%)	58 (56.3%)	0.000
	>13.6	118 (65.9%)	61 (34.1%)	
Leukocytes	>7.2	29 (25.4%)	85 (74.6%)	0.000
	≤7.2	134 (79.8%)	34 (20.2%)	
Neutrophils	>3.9	44 (31.4%)	96 (68.6%)	0.000
	≤3.9	119 (83.8%)	23 (16.2%)	
NLR	>1.8	72 (43.4%)	94 (56.6%)	0.000
	≤1.8	91 (78.4%)	25 (21.6%)	
SII	>479.1	41 (33.3%)	82 (66.7%)	0.000
	≤479.1	122 (76.7%)	37 (23.3%)	

DISCUSSION

Considering the high infectivity and mortality rates of COVID-19, early diagnosis of the disease is essential. The definitive diagnosis of this disease is made by proving a viral presence in real-time PCR analyses. Due to factors such as the high number of samples, limited number of staff trained in performing the aforementioned tests and insufficient lab capacities, the time it takes to receive results can be prolonged.

Therefore, every parameter allowing for early diagnosis is vital.

In our study, 57.8% (163/282) patients were confirmed positive for COVID-19 by RT-PCR. Out of all the patients diagnosed with COVID-19, 69.3% were males and the average age was 46.2 ± 15.5 years. The average age was found to be lower in males compared with females. In a study conducted by Guan *et al.*, the median age was 47 and 52.1% of the patients were male ¹¹. Another study by Li *et al.*

revealed that 56% of all patients were male and the median age was 59¹². Furthermore, another study conducted by Xu *et al.* showed a median age of 41 and 56% of the patients were male¹³. Thus, it can be said that COVID-19 is seen more frequently in males and in middle-aged patients.

Fever, cough and a sore throat were the most common complaints observed followed less often by myalgia, malaise and fatigue. The results of this study were found to be noticeably compatible with that of previous research. The research conducted by Yang *et al.* also revealed fever and cough to be the most common complaints¹⁴. In a study by Guan *et al.*, fever and cough and less frequently nausea, vomiting and diarrhea, were observed¹¹. The study by Huang *et al.* showed that fever (40/41 patients [98%]), cough (31/41 patients [76%]) and myalgia or fatigue (18/41 patients [44%]) were the most commonly seen symptoms at onset of the disease⁷.

In the results of this study, which are also consistent with previous research, low thrombocyte, leukocyte and neutrophil counts were revealed in COVID-19 positive patients. Likewise, thrombocytopenia and leukopenia were noted in Guan *et al.*'s study¹¹. The thrombocyte count was also found to be low in the study by Assiriet *al.*¹⁵ and leukopenia was noted in another study conducted by Xu *et al.*¹³. In general, while the leukocyte count was lower than 10,000 in viral pneumonias, leukocytosis was seen in bacterial pneumonias with a leukocyte count of more than 50,000¹⁶. Additionally, Xu *et al.* revealed in their study that thrombocyte counts are significantly low in pneumonia patients and that this decrease is directly proportional to the patients' clinical status¹⁷. In a study by Fan *et al.* mild thrombocytopenia and leukopenia was observed in some patients at first admission who were COVID-19 positive¹⁸.

Also, hemoglobin levels in COVID-19 positive patients were found to be significantly higher than in COVID-19-negative patients. While no significant difference was observed among females regarding hemoglobin, higher hemoglobin levels were seen in COVID-19 positive male patients. It is possible that these results are also affected by other reasons, such as the presence of comorbidities or anemia, and habits such as cigarette smoking. The patient files used for this study did not include a detailed patient history, and thus, their effect on hemoglobin levels were not accounted for. Also, the normal hemoglobin level in the female population is lower than that of males¹⁹. Since around 70% of the positive patient group is comprised of males in this study, this is likely to also have an effect on the results.

An apparent relationship, although not certainly proven, exists between a bacterial infection and neutrophilia, and a viral infection and lymphocytosis. Accordingly, NLR of peripheral blood has been used to distinguish between these types of infections^{20,21}. In a retrospective study concerning hospitalized patients with a fever of an unknown origin, it has been

shown that NLR is higher in those with fever due to bacterial infections than those with fever due to a viral etiology²². In the study by Zhang *et al.*, NLRs were used as an early diagnostic marker for aiv-H7N9 patients²³. Ai-Ping Yang *et al.* found an AUC of 0.743, with a cut-off of 3.3, specificity of 0.636, and a sensitivity of 0.88 for NLR in determining the prognosis for seriously ill COVID-19 patients¹⁴. Moreover, in the study conducted by Sun *et al.*, an AUC of 0.88 for NLR was found for serious COVID-19 positive patients at their first visit to the ER²⁴. Similarly, this study found a significantly lower NLR at first visit to the ER for patients with positive test results. Low NLR levels can therefore possibly be used as a diagnostic marker for COVID-19.

A recently proposed prognostic score is the SII, which relies on thrombocytes, neutrophils and lymphocytes. As an index defining the instability in the inflammatory response, the SII has been proposed as a prognostic indicator in the follow-up of sepsis patients²⁵. In addition, SII has been found to be useful in predicting the prognosis of small cell lung cancer and hepatocellular carcinoma^{26,27}. In this study, SII was found to be significantly low for COVID-19-positive patients, meaning that it can also be used while diagnosing COVID-19. The cut-off value for SII was noted as being ≤ 479.1 and the AUC = 0.760 with a sensitivity of 74.9%, and a specificity of 68.9%.

In conclusion, the results demonstrate that low values for NLR, SII, absolute neutrophil and lymphocyte counts, and PLTs may have diagnostic properties concerning COVID-19. The widest area under the ROC curve was of absolute leukocyte and neutrophil counts, followed by the AUC's of SII and NLR.

CONCLUSIONS

The definitive diagnosis of COVID-19 was made by RT-PCR analysis, but this was a time-consuming and less accessible test. With this test, the time it takes to diagnose and treat patients can be delayed. In our study, low values of leukocytes, neutrophils, platelets and high values of haemoglobin found with a CBC test which is easily available in ERs were found to be valuable in terms of the initial diagnosis of COVID-19. In addition, low values of NLR and SII were also indicative of COVID-19.

CONFLICTS OF INTEREST

None.

SOURCE OF FUNDING

None.

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