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Evaluation of WBC Parameters and Their Significance in COVID-19 Patients in Western Maharashtra, India

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ABSTRACT

Background and objectives: In patients with coronavirus disease 2019 (COVID-19), white blood cell (WBC) abnormalities have been found worldwide with significant inter-regional differences. In the present study, we evaluated WBC parameters concerning COVID-19 positivity and severity.

Methods: In this cross-sectional study, total WBC count, absolute count of each type of WBC, neutrophil to lymphocyte ratio (NLR), and lymphocyte to monocyte ratio (LMR) were compared between 150 COVID-19 patients and 150 non-COVID-19 patients presenting with COVID-19-like symptoms. Also, COVID-19 patients were divided into severe and non-severe cases.

Results: The severity of the disease had no significant association with age or gender ($p>0.05$). Total WBC count, absolute neutrophil count, absolute monocyte count, and NLR were significantly lower ($p<0.05$), while LMR was significantly higher in COVID-19 patients compared to non-COVID-19 patients ($p<0.05$). Total WBC count, absolute neutrophil count, and NLR were significantly higher ($p<0.05$), while absolute eosinophil count and absolute lymphocyte count were significantly lower ($p<0.05$) in severe COVID-19 patients compared to non-severe patients.

Conclusions: Age is not a predictive factor for the severity of COVID-19. Routine WBC parameters are useful in predicting the severity of the disease in COVID-19 patients and can be used as prognostic indicators. Routine WBC parameters can also be used for repeat RT-PCR testing in COVID-19 suspected patients.

Keywords: COVID-19 Testing, Injury Severity Score, India.

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS‑CoV‑2), a positive-sense single-stranded RNA virus, has been declared a pandemic on March 11, 2020, by the World Health Organization (1, 2). Amongst all states in India, Maharashtra has demonstrated a rapidly growing epidemic curve with the highest slope (3).

In COVID-19 patients, hematological abnormalities have been observed during the course of the disease, amongst which, white blood cell (WBC) abnormalities are markedly seen (4-6). Inter-regional differences have been noted in WBC parameters among COVID-19 patients worldwide (7-11). For example, a study by Mardani et al. from Iran showed a significantly higher neutrophil count in COVID-19 patients (9); on the contrary, many studies from China and a study from Taiwan showed a significantly lower neutrophil count (10). A study by Fan et al. in Singapore showed lymphopenia in 28% of cases (7) compared to 63% in a study by Huang et al. in Wuhan (8) and 42% in a study by Xu et al. outside Wuhan in China (11). An analysis done by Soraya et al. showed a slightly lowered lymphocyte count in COVID-19 patients as compared to non-COVID cases, although it did demonstrate a significantly lower lymphocyte count in severe cases as compared to non-severe cases (10).

Limited data is available on the WBC parameters of COVID-19 patients in the Western Maharashtra population of India (12, 13). In the present study, we evaluated WBC parameters in COVID-19 patients and those presenting with COVID-19-like symptoms but who turned out to be negative by reverse transcriptase polymerase chain reaction (RT-PCR) testing. We also compared WBC parameters between severe and non-severe COVID-19 patients.

MATERIALS AND METHODS

This study was undertaken in the Pathology Section of the Central Clinical Laboratory collected in the CCL for the diagnostic purpose were allowed to be used for research. The present study was a cross-sectional study consisting of 150 in-patients (patients admitted to the hospital with positive RT-PCR) and 150 patients presenting with COVID-19-like symptoms but who turned out to be negative after RT-PCR testing (non-COVID-19 patients). In both groups, a consecutive case series of patients presenting to our institute between June 2020 to September 2020 was selected.

The COVID-19-positive patients were segregated further into severe and non-severe (mild and moderate) categories according to the guidelines given in the ‘Updated Clinical Management Protocol for COVID-19’ by the Ministry of Health and Family Welfare of India (14) (Table1).

Peripheral venous blood (2.5 ml) was collected from all subjects in EDTA tubes. The samples were processed in the CCL using an automated hematology analyzer (Mindray BC 6000, China). Total WBC count, absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, absolute eosinophil count, and absolute basophil count were determined. Neutrophil to lymphocyte ratio (NLR) and lymphocyte to monocyte ratio (LMR) was also calculated. In COVID-19 patients, morphological details of WBCs were also observed in Leishman-stained peripheral blood smears.

Results were presented as mean value ± standard deviation. The two-sample t-test was used to evaluate differences in mean values between the two groups. A *p*-value of ≤0.05 was considered statistically significant. All data were analyzed using IBM SPSS software (version 28.0).

Table 1- Updated clinical management protocol for COVID-19

Variable	Mild	Moderate	Severe
SpO_2	> 94% in Room Air	90-94% in Room Air	< 90% in Room Air
Respiratory rate (breaths per minute)	< 24	24-30	>30

RESULTS

The mean age and gender did not differ significantly between the two groups. As shown in table 2, WBC count, absolute neutrophil count, and absolute monocyte count were significantly lower in COVID-19 patients compared to non-COVID-19 patients. In addition, COVID-19 patients had significantly lower NLR and significantly higher LMR compared to non-COVID-19 patients.

Age and gender had no significant association with the severity of the disease. Total WBC count, absolute neutrophil count, and NLR were significantly higher in severe cases compared to non-severe cases. On the other hand, absolute eosinophil count, absolute lymphocyte count, and LMR were significantly lower in severe cases compared to non-severe cases (Table 3).

Table 2- Comparison of WBC parameters between COVID-19 and non-COVID-19 patients

Variable	Total Mean (SD)	COVID patients	Non-COVID patients	*p*-value
Demographics				
Mean age (years)	50.05 (15.65)	49.55 (15.32)	50.55 (16.00)	0.58
Gender (% males)		60%	65.3%	0.34
WBC parameters				
Total WBC count (per µL)	8835.80 (6310.48)	7237.94 (3773.45)	10433.67 (7780.24)	<0.001*
Absolute neutrophil count (x10^3 per µL)	6.86 (6.12)	5.38 (3.62)	8.34 (7.59)	<0.001*
Absolute lymphocyte count (x10^3 per µL)	1.38 (0.9)	1.35 (0.7)	1.41 (1.06)	0.56
Absolute monocyte count (x10^3 per µL)	0.51 (0.34)	0.44 (0.33)	0.57 (0.33)	0.001*
Absolute neutrophil count to absolute lymphocyte count ratio	7.62 (9.85)	5.38 (5.34)	9.87 (12.50)	<0.001*
Absolute lymphocyte count to absolute monocyte count ratio	3.33(2.30)	3.64 (1.95)	3.03 (2.57)	0.02*
Absolute eosinophil count (x10^3 per µL)	0.07(0.15)	0.05 (0.09)	0.09(.019)	0.06
Absolute basophil count (x10^3 per µL)	0.02(0.02)	0.01(0.01)	0.02(0.03)	0.12

***Statistically significant difference between COVID-19 and non-COVID-19 patients**
SD: standard deviation.

Table 3- Comparison of WBC parameters between severe and non-severe COVID-19 patients

Variable	Total Mean (SD)	Non-severe (n=86) Mean (SD)	Severe (n=64) Mean (SD)	*p*-value
Demographics				
Age (years)	49.55 (15.32)	47.79 (15.96)	51.91 (14.21)	0.10
Gender (% males)		64%	54.7%	0.25
WBC parameters				
Total WBC count (per µL)	7237.94 (3773.45)	6169.3 (2303.88)	8673.9 (4781.32)	< 0.001*
Absolute neutrophil count (x10^3 per µL)	5.38 (3.62)	4.13 (1.95)	7.05 (4.57)	<0.001*
Absolute lymphocyte count (x10^3 per µL)	1.35 (0.7)	1.52 (0.7)	1.14 (0.66)	0.001*
Absolute monocyte count (x10^3 per µL)	0.44 (0.33)	0.44 (0.22)	0.45 (0.45)	0.75
Absolute neutrophil count to absolute lymphocyte count ratio	5.38 (5.34)	3.4 (2.85)	8.04 (6.62)	<0.001*
Absolute lymphocyte count to absolute monocyte count ratio	3.64 (1.95)	3.96 (1.83)	3.21 (2.03)	0.02*
Absolute eosinophil count (x10^3 per µL)	0.5 (0.9)	0.07 (0.1)	0.02 (0.5)	0.001*
Absolute basophil count (x10^3 per µL)	0.01 (0.01)	0.02 (0.01)	0.01 (0.01)	0.07

***Statistically significant difference between COVID-19 and non-COVID-19 patients**
SD: standard deviation.

We also studied the morphological features of WBCs in COVID-19 patients. Morphological changes were noted in neutrophils and lymphocytes. The frequency of neutrophil morphological features is shown in table 4.

Table 4- Neutrophil morphological features in COVID-19 patients

Neutrophil morphology	Cases (N=150)
Hypolobated nuclei	36.6%
Ringed nuclei	4.6%
Left shift	41.3%
Toxic granules	15.3%
Cytoplasmic vacuoles	1.3%

Table 5 shows the morphological features of lymphocytes in COVID-19 patients.

Table 5- Lymphocyte morphological features n COVID-19 patients

Lymphocyte morphology	Cases
Large granular lymphocytes	12.6%
Plasmacytoid lymphocytes	15.3%
Monocytoid lymphocytes	8.6%
Lymphocytes with nucleoli	8.6%
lymphocytes with indented nuclei	30.6%

DISCUSSION

Changes in the WBC parameters were noted in COVID-19 patients. These changes vary in different parts of the world. In this study, we evaluated the WBC parameters in COVID-19 patients in Western Maharashtra, India.

In the present study, we did not find any association between COVID-19 severity and age or gender. Inconsistent with this finding, some previous studies reported a significant association between the age of the patients and the severity of COVID-19 (6, 15).

In the present study, the total WBC count was significantly lower in COVID-19 patients than in non-COVID-19 patients, which is in line with the study by Mardani et al. (9). Leucopenia in COVID-19 patients was also reported by two previous studies (16, 17). Low total WBC counts were found to be related to the direct invasion of hematopoietic cells or bone marrow stromal cells by the virus, thereby leading to hematopoietic inhibition (17).

In our study, the absolute neutrophil count was significantly lower in COVID-19 patients when compared to non-COVID-19 patients. Although this finding was reported by several studies (10), Mardani et al. found significantly higher neutrophil counts in COVID-19 patients (9).

It has been established that a reduction in circulating leukocytes and neutrophils commonly arises following the viral invasion, as a consequence of either bone marrow suppression or peripheral destruction during the initial stages of the infection (10).

We found no significant difference in the absolute lymphocyte count between the two groups. This is in contrast to the study by Mardani et al. where lymphocyte count in COVID-19 patients was significantly lower than that in non-COVID-19 patients (9).

In our study, the absolute monocyte count in COVID-19 patients was significantly lower than that in non-COVID-19 patients. Wang et al. also found similar findings when they compared absolute monocyte counts between COVID-19 patients and influenza patients (18). On the other hand, the absolute monocyte count was significantly higher in COVID-19 patients than in influenza patients in a study by Curtolo et al. (19).

In the present study, NLR was significantly higher in non-COVID-19 patients compared to COVID-19 patients, which may be attributed to other severe acute inflammatory conditions or sepsis in the non-COVID-19 group. On the other hand, a significantly higher LMR was found in COVID-19 patients compared to non-COVID-19 patients. However, this parameter was not commonly studied to differentiate between these two groups. Additional studies on larger populations are required to substantiate this finding. We did not find any significant difference in the absolute eosinophil count and absolute basophil count between the two groups.

In the present study, severe COVID-19 patients had a significantly higher WBC count compared to their non-severe counterparts,

which is consistent with the findings of some previous studies (6, 8, 20). However, Xu et al. (11) and Guan et al. (21) found lower WBC counts in severe COVID-19 cases. Higher WBC counts may be due to severe acute inflammatory conditions. However, it is also known that some cases of sepsis may present with low WBC counts.

Absolute neutrophil count was significantly higher in severe cases of COVID-19 compared to non-severe cases, which was in line with the results reported by Yang et al. (6).

In the present study, absolute lymphocyte count in severe cases of COVID-19 was significantly lower than that in non-severe cases, which is in line with previous studies (6-8). However, some other studies reported comparable lymphocyte counts for severe and non-severe COVID-19 cases (10). Systemic inflammation significantly depresses cellular immunity, which in turn significantly decreases CD4+ T lymphocytes and increases CD8+ T lymphocytes. This may contribute to the low absolute lymphocyte count or no significant change in absolute lymphocyte count in some cases (6).

In our study, NLR was significantly higher in severe cases compared to non-severe cases, which is in line with the findings of Yang et al. (5) and Liu et al. (6).

Conversely, LMR was significantly lower in severe cases compared to non-severe cases, which is in line with findings of Yang et al. (6). However, Erdogan et al. found no significant difference between severe and non-severe COVID-19 patients in terms of LMR (22). Finally, we found a significantly lower eosinophil count in severe cases of COVID-19 compared to non-severe cases.

In the present study, the most frequently observed neutrophil morphology in COVID-19 patients was left shift (41.3%), followed by hypolobated nuclei (36.6%). A study by Nath et al. showed the presence of polymorphs having dyspoietic features like hypolobation, ring-shaped polymorph nucleus, cytoplasmic vacuoles, coarse granules mimicking toxic granules, some hypogranular polymorphs, apoptotic cells reminiscent of polymorphs with nuclear fragmentation, atypical lymphoid cells, large granular lymphocytes and monocytoid and plasmacytoid lymphoid cells (23). Podznyakova et al. found morphologic changes such as toxic granulation, cytoplasmic vacuolization, Howell-Jolly body-like inclusions, and Döhle bodies in neutrophils; large coalescing cytoplasmic vacuoles in monocytes; and cytoplasmic vacuoles in lymphocytes. They also found large granular lymphocytes and atypical lymphocytes including plasmacytoid forms (24). Neutrophils with clumped chromatin and smudged neutrophils were found in a study by Kaur et al. (25). Hypogranular neutrophils were found in a study by Alnor et al. (26). Acquired neutrophilic nuclear projections, acquired Pelger-Huet anomaly, and apoptotic cells were reported by Kannan et al. (27). Dyspoietic neutrophils with C-shaped or fetal shaped nuclei, ring forms, nuclear projections, and lymphocytes with blebbing were found reported by Agarwal et al. (28). Neutrophils with crowded dark granulations with the peripheral light blue agranular area, cytoplasmic hypogranularity, and abnormalities of nuclear shape were reported by Zini et al. (29). Finally, granulocytes with marked hypogranular cytoplasm and hyposegmented nucleus were reported by Ahnach et al. (30).

Limitations

This was a single-center study, and as a result, the findings might not be generalizable to the larger COVID-19 population. In addition, COVID-19 patients with sepsis may present with leucopenia and/or neutropenia; therefore, sepsis should be considered as a potential confounding factor in future studies. It should be also noted that the immune status of patients differs age-wise.

CONCLUSION

Routine WBC parameters including total WBC count, absolute neutrophil count, absolute lymphocyte count, absolute eosinophil count, NLR, and LMR are useful in predicting the severity of disease in COVID-19 patients and can be used as prognostic indicators. Some WBC parameters can also be used for repeat RT-PCR testing in COVID-19 suspected patients.

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DECLARATIONS

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Ethics approvals and consent to participate

This study was approved by the Ethics Committee of Maharashtra Institute of Medical Education and Research (MIMER) Medical College (Registration number: IEC/MIMER/2020/729).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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