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Differential white blood cell count in the COVID-19: A cross-sectional study of 148 patients

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ABSTRACT

Background: SARS-CoV-2 infection alters various blood parameters, which may indicate disease severity and thus help in better clinical management.

Aim: To study the association between various hematological parameters and disease severity of COVID-19. To analyze the effects of hypertension and diabetes on neutrophil-lymphocyte ratio and neutrophil-monocyte ratio in patients suffering from COVID-19.

Materials and methods: The study was a cross-sectional study involving 148 laboratory-confirmed cases of SARS-CoV-2 infection. The patients were divided into three groups on the basis of disease severity. Various hematological parameters were analyzed. The effects of hypertension and diabetes on NLR and NMR in COVID-19 patients were evaluated.

Results: Of the 148 patients, 78.4%, 8.1% and 13.5% cases were in the mild, moderate and severe groups, respectively. Mean age was 42.63 ± 16.04 years (IQR: 29, 54.75; Range: 7—74). 58.8% patients were male while the rest (42.2%) were female. Mean TLC (cells/mm^3), neutrophil (%), lymphocyte (%), monocyte (%), eosinophil (%), neutrophil-lymphocyte ratio (NLR) and neutrophil-monocyte ratio (NMR) among mild, moderate and severe COVID-19 was statistically significant ($p < 0.05$). Basophil (%) and lymphocyte-monocyte ratio (LMR) was statistically insignificant among the three groups. Lymphocyte (%), monocyte (%) and eosinophil (%) were negatively correlated to disease severity. Among diabetics, both NLR and NMR were statistically significant ($p < 0.05$). However, among hypertensive cases, only the NLR was statistically significant.

Conclusion: Older age, higher TLC, neutrophilia, lymphopenia, eosinopenia, high NLR and high NMR are associated with severe COVID-19. High NLR and high NMR are indicative of severe disease among diabetic patients. High NLR also indicates severe disease among hypertensive patients.

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1. Introduction

Ever since the emergence of COVID-19, the disease has affected 36,754,395 individuals worldwide and has resulted in deaths of 1,064,838 patients until the time of writing this report [1]. The agent responsible for this pandemic is a coronavirus, which was named SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2) by the W.H.O on 11 February 2020 [2].With a case fatality rate (CFR) of around 4% and no effective vaccine or treatment expected to come to the fore in near future, the problems posed by COVID-19 pandemic is unprecedented. The knowledge regarding the spectrum of effects that SARS-CoV-2 may have on its host and the modes of its transmission among humans is still evolving. The common clinical features of COVID-19 include fever, dry cough, dyspnea, fatigue, myalgia, headache, anosmia, ageusia and diarrhea [3,4]. Severe cases may develop life threatening complications like acute respiratory distress syndrome (ARDS), coagulopathy etc. Also, according to WHO, current evidences suggest that SARS-CoV-2 spreads among people via direct contact routes and by droplet, airborne and, fomite transmission [5].

COVID-19 may involve many organ systems in its host. Studies suggest that hematological profiles change during the course of SARS-CoV-2 illness. Neutrophils are involved in early anti-viral

defense. However, during severe pneumonia, neutrophils become cytotoxic through degranulation and lysis [6]. Studies have suggested that neutrophil recruitment may exacerbate COVID-19 immunopathology [7]. A study involving 90 hospitalized COVID-19 patients reported an association between lymphopenia and disease severity [8].Another study involving 93 laboratory-confirmed cases of COVID-19 identified neutrophil-lymphocyte ratio (NLR) as an independent marker of poor clinical outcome in COVID-19 [9,10].Age≥60 years, hypertension, diabetes mellitus, chronic lung/liver/kidney disease, cerebrovascular disease, obesity (BMI > 25 kg/m^2), cancer and smoking are a few risk factors that complicate the disease course in COVID-19 [4,11]. Dysregulated glucose metabolism in diabetes may lead to immune dysfunction and hypercoagulable state which may complicate SARS-CoV-2 infection. Hence, diabetes mellitus was found to be an independent risk factor for progression of COVID-19 [12]. SARS-CoV-2 enters its target cell via angiotensin converting enzyme-2 (ACE-2) receptors [13]. Treatment of hypertension with ACE inhibitors and ARBs (angiotensin receptor blockers) is postulated to increase ACE-2 receptor expression, thus facilitating SARS-CoV-2 infection [14].

India caters the health requirements of a large population with very limited resources. Therefore, the challenges posed by the COVID-19 pandemic to the Indian healthcare system are different from those of developed countries. Ministry of Health and Family Welfare, Government of India has published guidelines to combat the pandemic effectively. The guidelines stratify the COVID-19 disease severity on the basis of clinical parameters like, arterial oxygen saturation and respiratory rate (Table 1) [4].

This study aimed to analyze the association between the COVID-19 disease severity and various hematological parameters. This study also tried to analyze the effects of comorbidities like hypertension and diabetes on various hematological parameters in COVID-19 patients.

2. Materials and methods

This study was a single-center, cross-sectional study which was done in Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. 148 real-time polymerase chain reaction confirmed SARS-CoV-2 infected patients who were admitted in the center for treatment were included in the study. The subjects were chosen from the population of patients by simple random sampling. Necessary approvals and consent from patients and the Institutional ethics committee, Rajendra Institute of Medical Sciences, Ranchi, Jharkhand, India were taken. Patients were then divided into 3 groups on the basis of the clinical severity.(Table 1) Group I included asymptomatic and mild cases, group II included moderate cases while group III included patients suffering from severe COVID-19. Diabetic and hypertensive patients included in the study were also identified and grouped accordingly.

2.1. Inclusion criteria

Real-time polymerase chain reaction confirmed SARS-CoV-2 infected patients were included in this study.

2.2. Exclusion criteria

Patients suffering from hematological malignancies and immunodeficient states (excluding diabetes mellitus) were excluded from the study.

Proper history and detailed clinical examination was conducted for each patient. Blood samples were withdrawn at the time of admission. Various laboratory parameters, such as differential count for neutrophil (normal = 55–70%), lymphocyte (normal = 20–40%), monocyte (normal = 2–8%), eosinophil (normal = 1–4%) and basophil (normal = 0.5–1%) were measured using standard methods [15]. Values were considered abnormal if they were either above the upper limit of normal (ULN) or below the lower limit of normal (LLN).

2.3. Statistical analysis

Categorical data were expressed in percentages. Chi-square analysis (X^2) was done to compare sex with the severity of the disease. One way analysis of variance (ANOVA) was used as the statistical test of significance to compare each hematological parameter with different severity groups. Independent t-tests were used to compare the significance of different laboratory values among diabetic/non-diabetic and hypertensive/non-hypertensive groups. The level of statistical significance was p < 0.05. All hypothesis tests were 2-tailed. SPSS software version 23.0 was used to perform the analysis.

3. Results

148 patients were included in this study. The mean age of the group was 42.63 ± 16.04 years (IQR: 29, 54.75; Range: 7–74). 87 patients (58.8%) were male and 61 patients (41.2%) were females. (Table 2).

Group I, group II and group III included 116 (78.4%), 12 (8.1%) and 20 (13.5%) patients, respectively. The mean age of patients in group I, group II and group III was 39.41 ± 15.73 years (IQR: 27, 51.75; Range: 7–74), 48.58 ± 9.86 years (IQR: 42, 55.75; Range: 27–62) and 57.70 years (IQR: 50.5, 65.5; Range: 38–74), respectively (p = 0.002). Among males, 63 (72.41%) patients suffered from mild, 9 (10.3%) patients suffered from moderate while 15 (17.2%) patients suffered from severe COVID-19. Among females, 53 patients (86.88%) were either asymptomatic or had mild symptoms. 3 (4.9%) patients presented with moderate illness while only 5 (8.2%) patients developed severe COVID-19 manifestations. (Table 2).

The statistical analysis of total leukocyte count (TLC) and differential leukocyte count (DLC) has been presented in Table 2. The mean TLC of the study population was 8631.82 ± 4284.27 cells/mm^3. Mean TLC in group I was 7225.78 ± 2657.82 cells/mm^3, in group II was 11778.50 ± 3666.66 cells/mm^3 while in group III, it was 14899.50 ± 5639.44 cells/mm^3 (p < 0.05). Differential counts (in %age) for neutrophil (mean = 64.92 ± 17.14), lymphocyte (mean = 24.96 ± 14.22), monocyte (mean = 6.86 ± 3.77) and eosinophil (mean = 2.32 ± 2.53) among the three groups suggested significant statistical differences with p < 0.05 for each test category. However, the basophil differential count (in %age)

Table 1
Clinical severity of COVID-19.

	MILD	MODERATE	SEVERE
Clinical Criteria			
Arterial oxygen saturation (SPO$_2$)	>94% in Room Air	90–94% in Room Air	<90% in Room Air
Respiratory rate (RR)	<24/min	24–30	>30

Table 2
Characteristics of differential counts in different severity groups of COVID-19.

.	All	CLINICAL SEVERITY OF COVID-19			SIGNIFICANCE
		Mild	Moderate	Severe	p-value
Age (Mean ± S.D)	42.63 ± 16.04	39.41 ± 15.73	48.58 ± 9.86	57.70 ± 10.58	**0.002**
Sex (n, %)	87 (58.8%)	63 (72.41%)	9 (10.3%)	15 (17.2%)	0.109
Male	61 (41.22%)	53 (86.88%)	3 (4.9%)	5 (8.2%)	
Female					
TLC (Mean ± S.D)	8631.82 ± 4284.27	7225.78 ± 2657.82	11778.50 ± 3666.66	14899.50 ± 5639.44	**<0.05**
NEUTROPHILS (Mean ± S.D)	64.92 ± 17.14	59.35 ± 14.13	80.16 ± 13.24	88.04 ± 8.05	**<0.05**
LYMPHOCYTES (Mean ± S.D)	24.96 ± 14.22	29.19 ± 12.35	13.0 ± 10.90	7.58 ± 7.34	**<0.05**
MONOCYTE (Mean ± S.D)	6.86 ± 3.77	7.62 ± 3.61	5.11 ± 3.62	3.52 ± 2.52	**<0.05**
EOSINOPHIL (Mean ± S.D)	2.32 ± 2.53	2.72 ± 2.63	1.23 ± 2.03	0.68 ± 0.87	**<0.05**
BASOPHIL (Mean ± S.D)	0.25 ± 0.53	0.28 ± 0.59	0.17 ± 0.22	0.14 ± 0.21	0.166
NLR (Mean ± S.D)	7.79 ± 17.72	3.82 ± 10.28	15.18 ± 14.87	26.39 ± 34.15	**<0.05**
NMR (Mean ± S.D)	25.12 ± 88.54	11.57 ± 15.38	31.66 ± 39.69	99.76 ± 226.47	**<0.05**
LMR (Mean ± S.D)	5.00 ± 6.64	5.16 ± 6.36	3.16 ± 45.47	5.14 ± 8.82	0.565

Categorical data was expressed in frequency and percentage (%). Quantitative data was expressed in mean ± S.D. p values were calculated from Chi-square tests and One way ANOVA. Bold data indicated $p < 0.05$. TLC- Total leukocyte count, NLR- Neutrophil-Lymphocyte ratio, NMR- Neutrophil-Monocyte ratio, LMR- Lymphocyte-Monocyte ratio.

Table 3
Comparison of NLR and NMR among diabetic and non-diabetic.

	Diabetics	Non-diabetics	P-value
NLR(Mean ± SD)	15.21 ± 30.69	6.14 ± 12.86	**0.016**
NMR (Mean ± SD)	66.30 ± 199.94	15.93 ± 21.78	**0.007**

Data was expressed in mean ± SD. P-value was calculated using independent sample *t*-test.
NLR: Neutrophil-lymphocyte ratio, NMR: Neutrophil-monocyte ratio.

Table 4
Comparison of NLR and NMR among hypertensive and non-hypertensive

	Hypertensives	Non-hypertensives	p-value
NLR(Mean ± SD)	16.14 ± 14.91	6.34 ± 17.82	**0.016**
NMR (Mean ± SD)	40.98 ± 93.32	22.35 ± 87.77	**0.364**

Data was expressed in mean ± SD. P-value was calculated using independent sample *t*-test.
NLR: Neutrophil-lymphocyte ratio, NMR: Neutrophil-monocyte ratio.

(mean = 0.25 ± 0.53) between the three groups was found to be statistically insignificant (p = 0.166). (Table 2).

The mean NLR (neutrophil-lymphocyte ratio) of the study population was 7.79 ± 17.72. The mean NLR for patients in group I was 3.82 ± 10.28, for group II, it was 15.18 ± 14.8, while for group III, it was 26.39 ± 34.15 (p < 0.05). The mean NMR (neutrophil-monocyte ratio) of the study population was 25.12 ± 88.5, while for groups I, II and III, it was 11.57 ± 15.3, 31.66 ± 39.6 and 99.76 ± 226.4 respectively (p < 0.05). Mean LMR (lymphocyte-monocyte ratio) of the study population was 5.00 ± 6.64. For group I, group II and group III, mean LMR was 5.16 ± 6.36, 3.16 ± 45.47 and 5.14 ± 8.82 respectively (p = 0.565). (Table 2).

27 patients (18.2%) were diabetic and 22 patients (14.9%) were known hypertensive. Mean NLR among diabetics was 15.21 ± 30.69, while among non-diabetics, it was 6.14 ± 12.86 (p = 0.016). Statistical analysis of NMR among diabetic patients was also significant (p = 0.007) (Table 3). The NLR was also significantly higher among hypertensive group (mean = 16.14 ± 14.91). Among non-hypertensive population, the mean NLR was 6.34 ± 17.82 (p = 0.016). However, mean NMR for hypertensive patients was not found to be statistically significant (p = 0.364) (Table 4).

4. Discussion

The mean age of patients included in this study was around 40 years. In this study, older age was associated with severe illness. Many similar studies have also found poorer outcome of COVID-19 among elderly population [16].Studies have found an independent association of male sex with poorer outcome in COVID-19 patients [17].However, in this study the clinical severity of the illness was not associated with the sex of the patient.

Increased total leukocyte count and differential neutrophil count was more commonly seen in patients having severe COVID-19. Yuan et al. reported similar findings in severe COVID-19 cases. [18]In this study, lymphopenia was strongly associated with severe illness. A study by Tan et al. involving 90 hospitalized patients also

arrived at the same conclusion [8].In our study, differential monocyte count and differential eosinophil count was negatively correlated with the severity of the illness. Basophil counts were unrelated to the severity of COVID-19. Also, neutrophil-lymphocyte ratio (NLR) and neutrophil-monocyte ratio (NMR) were found to be positively correlated with COVID-19 disease severity. A meta-analysis of 6 studies reported a higher NLR to be a poor prognostic marker for patients with SARS-CoV-2 infections.[19] A similar study by Yang et at also suggested that increased NLR predicts poor prognosis in COVID-19 [9].

Among diabetics, mean NLR and NMR was higher when compared to their non-diabetic counterpart. In this study, an association was also found between hypertension and high NLR in COVID-19 patients. However, no association between high NMR and hypertension was seen in this study. Despite our best efforts, this study had some limitations, such as-

5. Small sample size of both groups meant an increased chance of large sampling error

2. It is a single-center, non-externally validated, cross-sectional study. Bias associated with the study design could not be removed.
3. Definite association of NMR and NLR with hypertension and diabetes couldn't be ascertained as a few patients suffered from both hypertension and diabetes simultaneously.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] World Health Organization. WHO coronavirus disease (COVID-19) dashboard. 9 Oct. 2020, http://covid19.who.int. [Accessed 11 October 2020]. Accessed.

[2] *World Health Organization*. Naming the coronavirus disease (COVID-19) and the virus that causes it. Accessed, www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it. [Accessed 11 October 2020].

[3] Wan Suxin, Xiang Yi, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. J Med Virol 2020 April 1. https://doi.org/10.1002/jmv.25783.

[4] Clinical management protocol:COVID-19." *Ministry of Health and Family Welfare Government of India*. www.mohfw.gov.in/pdf/UpdatedClinicalManagementProtocolforCOVID19dated03072020.pdf. Accessed 11 Oct. 2020.

[5] *World Health Organization*. Transmission of SARS-CoV-2: implications for infection prevention precautions. Accessed, www.who.int/publications/i/item/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations. [Accessed 11 October 2020].

[6] Haick AK, Rzepka JP, Brandon E, Balemba OB, Miura TA. Neutrophils are needed for an effective immune response against pulmonary rat coronavirus infection, but also contribute to pathology. J Gen Virol 2014;95:578–90. https://doi.org/10.1099/vir.0.061986-0.

[7] Didangelos A. COVID-19 hyperinflammation: what about neutrophils? mSphere 2020;5(3). https://doi.org/10.1128/mSphere.00367-20.

[8] Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Sig Transduct Target Ther 2020;5:33. https://doi.org/10.1038/s41392-020-0148-4.

[9] Yang AP, Liu JP, Tao WQ. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharm 2020:84. https://doi.org/10.1016/j.intimp.2020.106504.

[10] Liu Y, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. J Infect 2020;81:e6–12.

[11] Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. BMJ 2020;368:m1198. https://doi.org/10.1136/bmj.m1198.

[12] Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes/metabolism research and reviews 2020:e3319.

[13] Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J Virol 2020. https://doi.org/10.1128/JVI.00127-20. published online Jan 29.

[14] Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 2020;8:e21. https://doi.org/10.1016/S2213-2600(20)30116-8.

[15] Pagana KD, Pagana TJ, Pagana TN. Mosby's diagnostic & laboratory test reference. fourteenth ed. St. Louis, Mo: Elsevier; 2019.

[16] Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: a comparison with young and middle-aged patients. J Infect 2020;80(6):e14–8.

[17] Palaiodimos L, Kokkinidis DG, Li W, et al. Severe obesity, increasing age and male sex are independently associated with worse in-hospital outcomes, and higher in-hospital mortality, in a cohort of patients with COVID-19 in the Bronx, New York. Metabolism 2020; 108:154262.

[18] Yuan J, Zou R, Zeng L, Kou S, Lan J, Li X, Liang Y, Ding X, Tan G, Tang S. The correlation between viral clearance and biochemical outcomes of 94 COVID-19 infected discharged patients. Inflamm Res 2020:1–8.

[19] Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. J Med Virol 2020.