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Association of hematological parameters with disease severity in Covid-19 patients in a tertiary care teaching hospital in Rajasthan

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ABSTRACT

Introduction

Severe acute corona virus 2 (SARS-CoV-2) has rapidly evolved from an epidemic outbreak into a pandemic affecting the whole world. Hematological parameters may help in aiding diagnosis and evaluating prognosis of the patients. Early diagnosis and recognition of cases with severe disease requiring admission in intensive care unit (ICU) may help in triaging the patients.

Methods

Complete blood counts (CBC) and peripheral smear examination were studied in 510 patients in ICU and non-ICU settings. The findings were compared to know the association between hematological parameters and disease severity.

Results

Patients with age above 60 years were seen to be more severely affected. Males were affected twice more commonly than females. High Absolute neutrophil counts (ANC), low Absolute lymphocyte count (ALC), high neutrophil lymphocyte ratio (NLR), high platelet lymphocyte ratio (PLR) were significantly associated with ICU admission.

Conclusion

A routine investigation like CBC, if studied in a proper clinical context may help in evaluating the prognosis in patients of Covid-19 as CBC parameters in patients requiring ICU admission differ significantly from those with patients not requiring ICU admission.

Keywords: SARS-CoV-2, hematological parameters, NLR, PLR

INTRODUCTION

Coronaviruses belong to the virus family Coronaviridae and are enveloped, positive-sense RNA viruses. The coronavirus genome is approximately 31 Kb, making these viruses the largest known RNA viruses.[1,2]

SARS-CoV-2 causing corona virus disease 2019 (COVID-19) has rapidly evolved from an epidemic outbreak into a pandemic infecting more than 100 million individuals all over the world (Source: www.worldometers.info/coronavirus/)and more than 10 million individuals in India (Source: www.covid19india.org). United States, India and Brazil are the worst infected countries. Among North Indian states, Delhi and Uttar Pradesh have been

affected severely followed closely by Rajasthan. Owing to the population size and little adherence to safety protocols, the numbers of affected individuals are rapidly rising and they might soon outnumber the PCR tests that must be performed for correct diagnosis. Although it is well documented that COVID-19 is primarily manifested as a respiratory tract infection, emerging data indicate that it should be regarded as a systemic disease involving cardiovascular, respiratory, gastrointestinal, neurological, hematopoietic and immune systems. ARDS, heart failure, renal failure, shock and multiorgan failure results in the death of patients.[3]

Blood tests especially CBC and coagulation parameters are essential, particularly in a resource limited country like India, as they may give clue for the diagnosis as well as help in detection and monitoring of severity of disease and hematological complications.

AIMS AND OBJECTIVES

1. To study the CBC parameters and smear findings in the COVID-19 patients at the time of admission.
2. To calculate the NLR and PLR in these patients and correlate them with ICU requirement.
3. To study the demography of the COVID-19 patients in the institution.

Methods

CBC and peripheral smear examination were studied in 510 patients over the period of three months i.e. May – July, 2020 who had come positive for SARS-CoV-2 infection as diagnosed by reverse transcriptase-polymerase chain reaction (RT-PCR). Patients were divided in two groups: ICU and Non ICU depending on their clinical condition of Covid-19 severity.

CBC samples were run in six part analyzers (Sysmex XN-1000 and Beckman Coulter DXH900). Smears were prepared, stained with Leishman stain and examined for red cell morphology, differential leucocyte count (with special reference to lymphocyte morphology) and platelet count.

Analysis of data

Data were compiled in an excel sheet and were analyzed using SPSS V 22. Descriptive statistics including frequency and percentages were used to summarize demographic characteristics of study population and categorical variables. Mean and standard deviation was calculated for continuous variables. To compare the continuous variables from different groups, t-test was applied. The frequencies of categorical variables were compared using Chi square test. A p-value of <0.05 was considered statistically significant and <0.001 as highly significant.

RESULTS

The results of the present study have been presented under various heads below:

Demography

The patients were of wide age range i.e. from 1 year to 84 years (Mean ± SD - 44.5±16.45 years). 43 out of 510 patients required ICU admission (Figure – 1).

Only 5.5 percent of the patients were below 20 years of age and 2 of them required ICU admission. Maximum numbers of patients were in the age range of 41 to 60 years accounting for 40.4 percent of the total patients. However, maximum ICU requirement was noted in patients above 60 years of age (Table – 1). Males outnumbered females in our study population as Male: Female ratio came out to be approximately 2:1 and the ratio was same for the patients in ICU and non-ICU settings.

Hematological Findings

The results of the CBC parameters have been presented in Table-2, 3 and 4.

Hemoglobin levels

Two thirds of the non-ICU patients in the study had hemoglobin levels ≥ 12 g/dl (Mean – 10.4 g/dl).

However, 45 percent of ICU patients had anemia (Mean – 9.8 g/dl). The difference might be owing to the older age patients requiring more ICU admission compared to young population. The data of other RBC parameters were in line with the above findings. (Table – 2)

Platelet count

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76.4 percent of the Non-ICU patients had normal platelet count (>150 x 10^3/µl) compared to 62.8 percent of the ICU patients. (Table – 2)

Total leucocyte count (TLC)

79.6 percent of the patients in non-ICU settings had normal TLC and rest of these patients had almost equal incidence of leucopenia and leucocytosis. While in the ICU patients, incidence of leucopenia was similar to non-ICU patients but leucocytosis was observed in 41.9 percent of ICU patients with the mean TLC of 15.61x10^3 in these patients. (Table – 2, Figure – 2)

Absolute neutrophil count

ANC were significantly different in ICU and Non-ICU patients. While ANC above 7x10^3/µl was recorded in 65.1 percent of ICU admissions, it was recorded in only 20.4 percent of Non ICU patients. In the present study ANC <2x10^3/µl was not found in any of the ICU patients. (Table – 3, Figure – 3)

Absolute lymphocyte count

ALC was normal (1-3x10^3/µl) in 70.8 percent of Non-ICU patients and only 34.9 percent of ICU patients. ALC less than 0.5x10^3/µl correlated even better with disease severity as 30.2 percent of the ICU patients compared to 5.2 percent of Non ICU patients had ALC counts below this level.

Monocytopenia and eosinopenia were also found to be associated more with ICU admissions (Table – 3, Figure – 3).

Neutrophil lymphocyte ratio

As the patients requiring ICU admissions had shown higher association with neutrophilia and lymphopenia, likewise NLR was significantly associated with disease severity in the similar fashion. The cutoff of 4.7 has been described by Xia X *et al* [4] as well as Alexander NI [5] and a significant correlation was also found at 4.7 in the present study. 79.1 percent of the ICU patients had NLR >4.7 compared to around 30.8 percent of the Non-ICU patients. (Table – 4, Figure – 4)

Platelet lymphocyte ratio

Alexander NI [5] described 216 as upper limit of reference value for PLR. PLR of more than 216 was positively associated with ICU admitted Covid patients as 58.2 percent of ICU patients (compared to 23.9 percent of Non-ICU patients) had PLR>216. (Table – 4, Figure – 4)

On **peripheral smear examination**, schistocytes and microspherocytes were noted in 13 cases. Covid disease is a thrombotic disease which commonly shows deranged d-dimer levels. However, fragmented RBCs or spherocytes were only seen in 2.5 percent of the total cases. Leucoerythroblastic picture was seen in 3 cases, while, nRBCs alone were seen in 5 other cases. Absolute neutrophilia was observed in 123 cases but mild left shift was noted only in 9 cases.

Reactive lymphocytes "covicytes" were also noted in 14.7 percent of cases, though, they didn't correlate with ICU admission. No definite morphological difference between covicytes and other virocytes was observed.

DISCUSSION

SARS-CoV2 has disrupted the normal life of humans all over the world. The virus has not just changed the way of living but has significantly affected the economy of the society. So, the foremost priority of the health care agencies is to control the disease and bring back life the way it was in pre Covid era.

The demographic characteristics of the study showed that males were almost twice as affected by Covid-19 as females and the results are in conformity with already reported by Huang C *et al* [6] and Guan WL *et al* [7] as they also noted male predominance. However, Zhang JJ *et al* [8] reported equal incidence in males and females. Median age of the patients in the present study is approximately similar to the median age reported by Huang C *et al* [6] and Guan WL *et al* [7] which is 49 and 47 years, respectively.

Absolute lymphocyte count alone can also be studied as independent biomarker as a significant association of ICU admissions with low ALC has been observed. Lymphopenia, in Covid patients, occurs because of various factors. Terpos E *et al*. [9] have described in the study that firstly, SARS-CoV2 directly infects lymphocytes due to presence of ACE2 receptors on their surface and secondly, there is increased apoptosis of lymphocytes due to markedly increased levels of cytokines. Lastly, these cytokines may also be associated with atrophy of lymphoid organs.

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Neutrophils are the cells which lead the innate immunity during infections. Chemo-attractant molecules for neutrophils resulting in their recruitment can be seen in viral infections as described by Bordon J *et al*.[10] Leucopenia and relative lymphocytosis is routinely encountered in viral infections. Corona virus is different in this regard guiding us towards the diagnosis of Covid infection as it has neutrophilia along with lymphopenia.

Eosinopenia has earlier been reported to be commonly associated with Covid disease and has significant association with severe cases and monocytopenia is noted during the cytokine storm of COVID-19. [11, 12, 13].

Qin C *et al* [14] had reported changes in NLR which are in conformity to the present findings. The ANC was higher and ALC was lower in the patients with severe disease. Higher NLR has been associated with severe disease including systemic manifestation, thus it has been considered as a new biomarker for systemic inflammation. Channappanavar R *et al* [15] revealed in their study that increased neutrophil production is due to stimulation of inflammatory response and it also enhances the apoptosis of lymphocytes.

Liu Y *et al* [16] reported that in-hospital mortality due to Covid had increased with the increase in NLR. There was 10 percent increase in risk of mortality with each unit increase in NLR.

Various initial studies have taken different NLR cut offs to determine its correlation with disease severity. Liu J *et al* [17] had taken 3.13, while Yang AP *et al* [18] had taken a cut off of 3.3 which is associated with lower survival. Ciccullo *et al* [19] conducted a study in Italy and their results were in agreement with other studies including the present study that NLR is a useful marker for prognosis, and they had taken 4.0 as the cut off. Yan X *et al* [20], correlated NLR >11.75 with all cause in-hospital mortality. Therefore, risk of mortality seems to increase with the increase in the value of NLR and hence such patients should be considered for intensive care.

Gustavo *et al* [21] also concluded that NLR being a low cost marker is a useful indicator of poor prognosis at the initial moment of hospitalization.

PLR is related to the cytokine storm as described by Rong Qu *et al* [22]. These workers concluded that patients with PLR>126.7 require early intervention to prevent further deterioration of the disease.

Leucoerythroblastic reaction is rarely seen in viral infections (e.g. parvovirus). Mitra A *et al* [23] reported a single case of Covid with leucoerythroblastic reaction. Similarly, only 3 cases with leucoerythroblastic reaction were observed in this study. However, none of these cases showed presence of blasts in the peripheral smear.

CONCLUSION

The hematological findings in population of state of Rajasthan were similar to the results as described in literature from different countries. Baseline CBC findings revealed neutrophilia, lymphopenia along with eosinopenia and monocytopenia. Thrombocytopenia was seen in a few cases and most of those cases had mild decrease in platelet count. A significant variation was recorded in the values of NLR and PLR in ICU and non-ICU cases, reflecting that these parameters can be helpful in predicting the severity of the disease. Although Covid disease is known to cause a prothrombotic state, peripheral smear is not very helpful to predict the thrombo-embolic events in such patients as schistocytes or spherocytes, which are commonly seen in conditions like microangiopathic hemolytic anemia, are rarely seen in covid-19 patients. Hence, coagulation profile must be performed regardless of the peripheral smear findings.

CBC - a routinely performed test can really be helpful in Covid-19 patients, if studied carefully in proper clinical context. CBC can guide us through diagnosis and prognosis in patients with Covid-19 infection.

Compliance with Ethical Standards:

The present study was conducted after taking ethical approval from ethical committee and there is no conflict of interest amongst the authors.

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TABLES AND FIGURES

Table 1 - Age Distribution of Covid-19 patients

Age group (in years)	Frequency	%
<20	28	5.5
21-40	185	36.3
41-60	206	40.4
61-80	90	17.6
>80	1	0.2
Total	510	100

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Figure – 1 – Distribution of study subjects according to place of admission

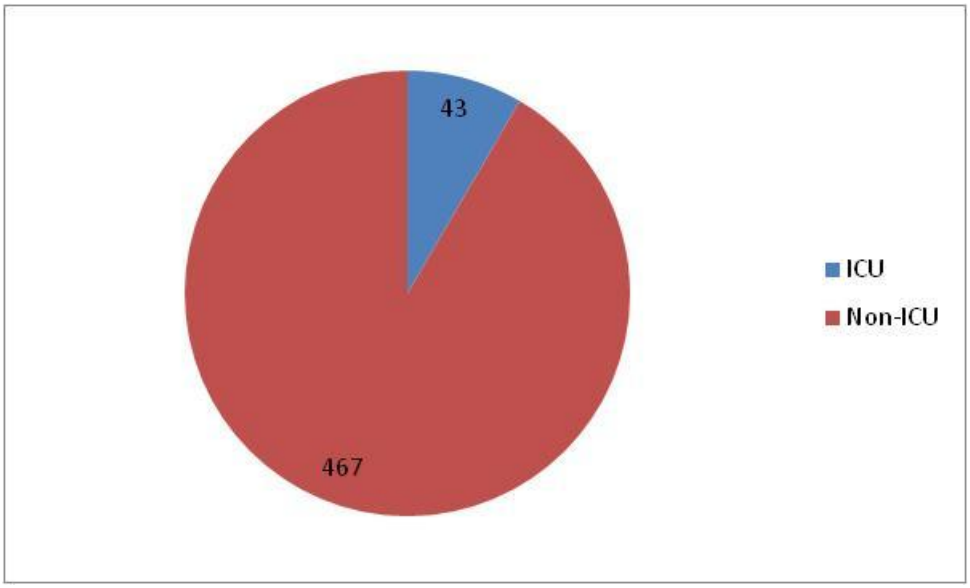

Table 2 – Association of Hb, TLC and platelet count values between ICU and Non-ICU patients

Variables	**ICU patients (N = 43)**	**Non ICU patients (N=467)**	**p value**
Haemoglobin			
Mean	11.69	12.05	**0.002**
Standard Deviation	2.08	1.99	
TLC ($x10^3$/ µl)			
Mean	10.88	7.45	**<0.001**
Standard Deviation	5.34	4.62	
Platelet count ($x10^3$/ µl)			
Mean	221.78	216.44	0.749
Standard Deviation	103.03	87.56	

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Figure -2 – Distribution of ICU and Non-ICU patients on the basis of Total leucocyte count

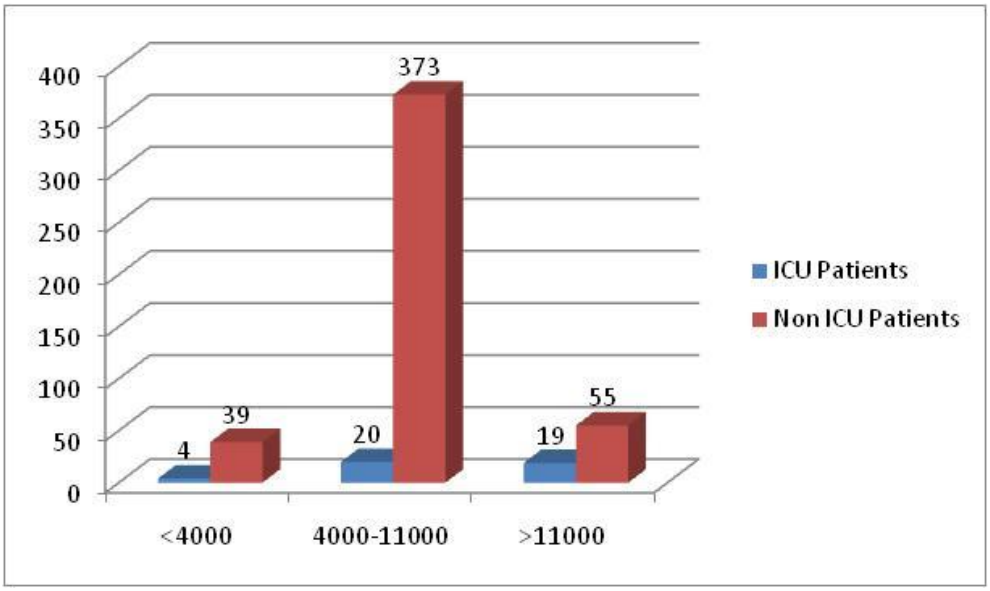

Table 3 - Association of differential counts between ICU and Non-ICU patients

Variables	**ICU patients (N = 43)**	**Non ICU patients (N=467)**	**p value**
ANC ($x10^3$/µl)			
Mean	0.51	0.42	**<0.001**
Standard Deviation	0.39	0.24	
ALC ($x10^3$/µl)			
Mean	0.99	1.53	**<0.001**
Standard Deviation	0.75	0.78	
AMC ($x10^3$/µl)			
Mean	0.51	0.42	0.212
Standard Deviation	0.39	0.24	
AEC ($x10^3$/µl)			0.677
Mean	0.05	0.06	
Standard Deviation	0.12	0.11	

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ANC – absolute neutrophil count;

ALC - absolute lymphocyte count;

AMC - absolute monocyte count;

AEC - absolute eosinophil count.

Figure -3 – Distribution of ICU and Non-ICU patients on the basis of differential count

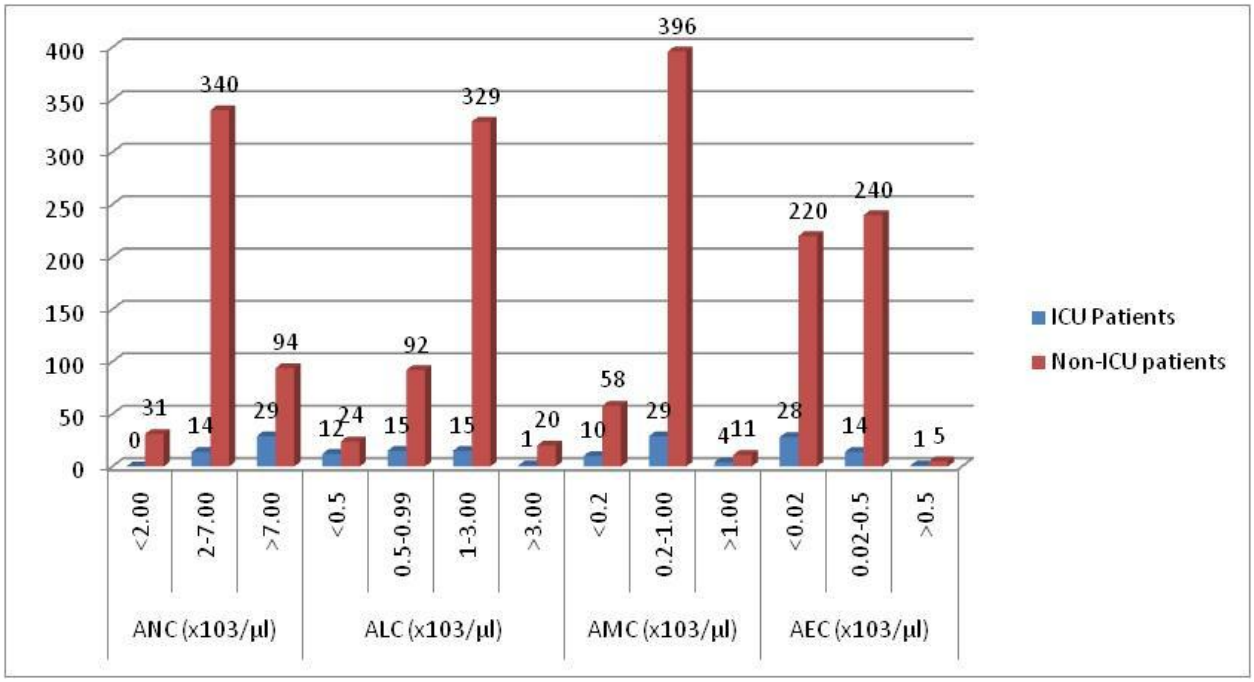

Table 4 - Association of NLR and PLR values between ICU and Non-ICU patients

Variables	**ICU patients (N = 43)**	**Non ICU patients (N=465)**	**p value**
NLR			
Mean	14.71	5.23	**<0.001**
Standard Deviation	12.02	8.67	
PLR			
Mean	360.26	185.12	**0.004**
Standard Deviation	378.73	172.00	

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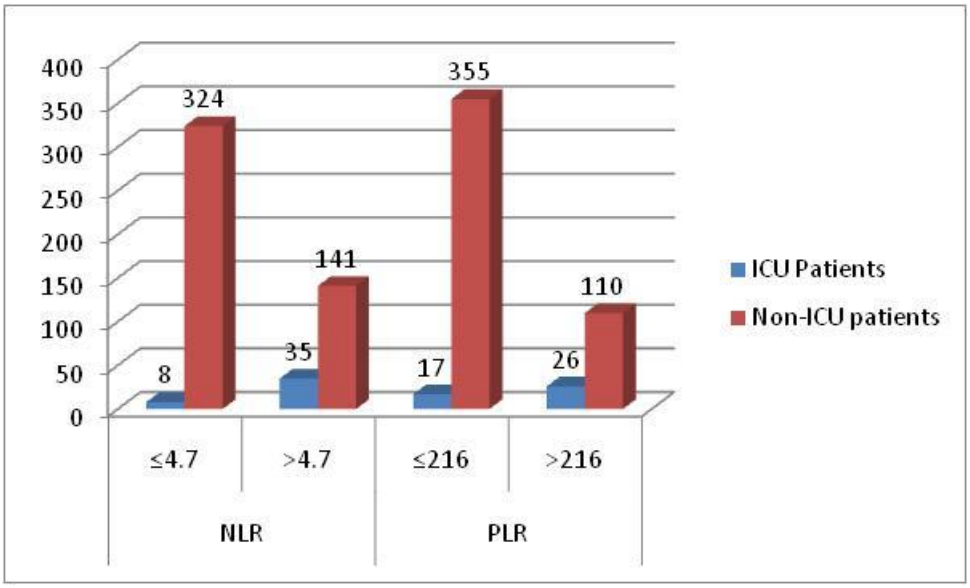
Figure - 4 – Distribution of ICU and Non-ICU patients on the basis of NLR and PLR



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