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Haematological Parameters in COVID-19 and their Association with Severity and Mortality

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Abstract

Background: Since the COVID-19 global pandemic emerged, the worldwide medical fraternity has been facing multiple challenges regarding its management. Patients with severe/critical illness have a poor prognosis. Hence, early detection and assessment of disease severity is vital to offer timely management. Recent studies indicate that altered haematological parameters may predict the disease severity and mortality. We aimed to investigate associations between haematological parameters and disease severity in patients with SARS CoV 2 infection.

Objectives: This study was undertaken to find out the optimal cut-off values of haematological parameters that may significantly relate to the clinical severity of COVID-19 and to evaluate their utility as parameters to predict mortality.

Methods: It was a hospital based prospective cohort study, conducted over a period of 4 months, from May, 2020 to August, 2020 at a level 3 designated COVID-19 facility in Uttar Pradesh. In our cohort, there were a total of 211 patients out of which 125 were non-ICU admissions and 86 were ICU admissions. Cases were classified as severe, moderate and mild based on their oxygen requirements and ICU care needs.

Results: The mean age of non-ICU patients was 47.1 ± 16.2 years, ICU survivors, were aged 57.4 ± 11.4, and ICU non-survivors were aged 57.3 ± 15.2. There was no mortality in the non-ICU group. Of the 86 ICU admissions, 69 were male, of which 27 were non-survivors and 17 were female, of whom 5 were non-survivors. Amongst the deceased patients, there was a significant leucocytosis ($P < 0.001$), neutrophilia ($P < 0.001$) and increased NLR ($P = 0.026$). The pooled analysis revealed that the NLR cut-off of > 3.85 was associated with severity and prediction for ICU admission, while NLR of > 5.2857 was associated with mortality.

Conclusion: In conclusion, advanced age, male sex, a high white blood cell count, neutrophilic leucocytosis or neutrophilia along with the elevated NLR were significantly associated with both the clinical severity and mortality.

Key words: SARS COV-2, COVID-19, complete blood count, absolute neutrophil count, neutrophil lymphocyte ratio, acute respiratory distress syndrome.

Introduction

On December 31, 2019, the World Health Organisation was notified of a cluster of pneumonia cases of unknown aetiology in Wuhan, China[1]. The aetiologic agent was identified as a novel beta-coronavirus, subsequently named SARS CoV-2 and the disease was designated COVID-19[2]. It has subsequently spread rapidly. The World Health Organisation (WHO) on March 11, 2020, had declared the novel coronavirus (COVID-19) outbreak a global pandemic[3].

Current information suggests that the incubation period ranges from 1 to 12.5 days (with median estimates of 5 to 6 days). Patients with SARS COV-2, develop a myriad of clinical symptoms like fever, dry cough, myalgia, dyspnoea, anorexia, rhinorrhoea, sore throat, anosmia, ageusia. Major complications include ARDS, arrhythmias, metabolic acidosis, coagulopathy, and septic shock. Most critically ill patients were older, around 60 years and had more underlying co-morbidities. Most patients require oxygen therapy and a minority of the patients need non-invasive and invasive ventilation[4-5].

There are no specific clinical symptoms which can accurately predict the severity and progression of COVID-19; consequently, we opted to rely on laboratory parameters to assess the severity of the disease. Complete blood count (CBC) is a simple, readily obtainable and affordable haematological investigation, that can provide comprehensive, yet reliable, information regarding the disease progression. SARS CoV-2 infection is characterised by the development and progression of inflammatory responses. Haematological parameters, such as white blood cells (WBCs) and their subpopulations like neutrophils and

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lymphocytes and also red cell distribution width, platelet count, mean platelet volume, platelet distribution width and derived markers such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and lymphocyte to monocyte ratio (LMR), are established biomarkers of inflammatory responses[6-9]. Several studies have been carried-out, comparing the various values and ratios obtained from CBC, in COVID-19 patients and COVID-19 negative individuals[10,11].

However, there is a lacuna regarding the haematological parameter comparison in COVID-19 patients and their association with disease severity. We aimed to study the differences in haematological parameters between ICU and Non-ICU cases of COVID-19.

Material and methods

The present work was a hospital-based prospective cohort study, conducted over a period of 4 months, from May, 2020 to August, 2020 at Sharda Hospital, a level 3 designated COVID-19 facility. Patients were triaged on admission according to Indian Central Medical Research (ICMR) guidelines and were categorised as mild, moderate, severe and critically ill[12]. Mild/moderate category of patients were admitted to wards and severe/critically ill cases were transferred to ICU. COVID -19 diagnosis was confirmed by SARS-COV-2 real time PCR (Qualitative) by obtaining nasopharyngeal or oropharyngeal swab. COVID-19 test was conducted as per kits approved by ICMR/CE-IVD/USFDA.

Inclusion criteria

- All patients aged > 18 years.
- Patients with a positive RT PCR for COVID 19/positive COVID-19 rapid antigen test.

Exclusion criteria

- Pregnancy or breastfeeding.
- Patients with documented haematological disorders like thalassaemia, sickle cell disease, haemolytic disorders, etc.

Study design

A total of 211 subjects were studied, out of which 86 were classified as severe cases of COVID-19 requiring ICU admission, while the rest were mild-to-moderate cases admitted to the ward.

An informed consent was taken from all subjects included in the study and prior approval of the institutional ethics committee of, Sharda Hospital and School of Medical Science and Research, (SMSR) Greater Noida, Uttar Pradesh was obtained. Thorough history was taken and examination was carried-out.

As per evaluation protocol, CBC was sent for all patients along with the other relevant investigations. A general blood picture was performed on the same blood sample that was used for CBC in order to corroborate the findings of the automated cell counter. CBC was done by using hydrodynamic focusing on Sysmex automated analyser XT1800i available in the central laboratory of the hospital.

Statistical methods

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and mathematical functions were then applied using the same. Continuous variables were summarised in the form of means and standard deviations and categorical variables were expressed as frequencies and percentages. Graphically the data was presented by bar and pie diagrams. ANOVA test was employed for comparing continuous variables. Chi-square test or Fisher's exact test, whichever appropriate, was applied for comparing categorical variables. A p value of less than 0.05 was considered statistically significant. All p values were two-tailed.

Results

A total of 211 patients were enrolled, of which 125 were mild/moderate cases, admitted to wards (Non-ICU admissions) and 86 were severe/critically ill cases, hence considered for ICU admissions. The mean age of non-ICU patients was 47.1 ± 16.2 years; ICU survivors were aged 57.4 ± 11.4, and ICU non-survivors were aged 57.3 ± 15.2. Patients with mild disease were significantly younger than those with severe disease with a mean age difference of 10.29 years ($p < 0.001$). However, age did not have statistical significance ($p < 0.966$) in the mortality prediction between ICU survivors and non-survivors. Of the 125 non-ICU admissions, 83 were male (66.4%) while 42 were female (33.3%) with no mortality noted. Of the 86 ICU admissions 69 were male of which 27 were non-survivors and 17 were female of whom 5 were non-survivors. There was a significant difference between both genders as to the requirement of ICU admission and mortality. We found that males had an overwhelming higher percentage of ICU admission and mortality than female patients. When the parameters were compared between the three groups it was found that there was a significant difference in all parameters except lymphocyte-monocyte ratio (LMR) and PDW. We did intergroup analysis to find the parameters that could provide a better prognostication of disease severity and mortality.

Table I: Characteristics of the three groups of patients.

Characteristics	Severity			p value
	Non ICU	ICU		
		Survivors	Non-survivors	
Age (years)	47.1 ± 16.2	57.4 ± 11.4	57.3 ± 15.2	< 0.001*
Sex	M = 83 (66.4%) F = 42 (33.6%)	M = 42 (77.7%) F = 12 (22.2%)	M=27 (84.4%) F=5 (15.6%)	
Haemoglobin (g/dl)	12.89 ± 1.61	12.07 ± 2.02	12.5 ± 2.6	0.03*
TLC	6466.4 ± 2051.4	9696.3 ± 4401.7	15560.9 ± 6036.9	< 0.001*
Neutrophils	4093.2 ± 1792.4	7777 ± 3972.7	13372.5 ± 5982.8	< 0.001*
Lymphocytes	1814.1 ± 766.3	1360.6 ± 1418.1	1443.9 ± 781.2	0.009*
Monocytes	449.5 ± 184.1	462.2 ± 314.7	620.1 ± 326.4	0.002*
Platelets	220104 ± 108211	266037 ± 125074.8	207187.5 ± 105968.2	0.02*
RDW	14.59 ± 1.62	14.95 ± 1.82	16.86 ± 2.71	< 0.001*
PDW	16.05 ± 3.27	14.89 ± 3.55	15.60 ± 2.52	0.09
MPV (fl)	11.95 ± 1.35	11.41 ± 1.25	11.29 ± 1.08	0.005*
NLR	2.859 ± 2.35	7.939 ± 5.31	12.161 ± 9.51	< 0.001*
LMR	4.532 ± 2.84	4.091 ± 4.58	3.169 ± 2.85	0.125
PLR	142.79 ± 93.61	266.12 ± 165.53	180.85 ± 147.59	< 0.001*

In the intergroup comparison between non-ICU and the ICU survivor group of patients, we found that there was a significant difference between the two groups when compared for age, haemoglobin, TLC, neutrophils, lymphocytes, platelets, PDW, MPV, NLR and PLR.

In the intergroup comparison between the ICU survivor and non-survivor group of patients we found that there was a significant difference between the two groups when compared for TLC, neutrophils, monocytes, platelets, RDW, NLR and PLR.

In the intergroup comparison between non-survivors and the non-ICU group of patients we found that there was a significant difference between the two groups when compared for age, TLC, neutrophils, lymphocytes, monocytes, RDW, MPV, NLR and LMR.

Table II: Intergroup comparison between the Non-ICU group and ICU survivor group.

Characteristics	Mean difference	p value
Age (years)	10.29	< 0.001*
Haemoglobin (g/dl)	- 0.82	0.01*
Total leucocyte count	3229.89	< 0.001*
Neutrophils	3683.76	< 0.001*
Lymphocytes	- 453.56	0.031*
Monocytes	12.64	0.785
Platelets	45933.04	0.022*
RDW	0.35	0.221
PDW	- 1.16	0.043*
MPV (fl)	- 0.54	0.011*
NLR	5.07	< 0.001*
LMR	- 0.44	0.517
PLR	123.32	< 0.001*

Table III: Intergroup comparison between the ICU survivor group and Non-survivor group.

Characteristics	Mean difference	p value
Age (years)	- 0.13	0.966
Haemoglobin (g/dl)	0.43	0.427
Total leucocyte count	5864.64	< 0.001*
Neutrophils	5595.58	< 0.001*
Lymphocytes	83.36	0.727
Monocytes	157.85	0.032*
Platelets	- 58849.53	0.023*
RDW	1.91	0.001*
PDW	0.71	0.282
MPV (fl)	- 0.11	0.651
NLR	4.22	0.026*
LMR	- 0.92	0.256
PLR	- 85.26	0.016*

Table IV: Intergroup comparison between the Non-Survivors group and Non-ICU group.

Characteristics	Mean difference	p value
Age (years)	-10.16	0.001*
Haemoglobin (g/dl)	0.39	0.424
Total leucocyte count	-9094.53	<0.001*

Neutrophils	-9279.34	<0.001*
Lymphocytes	370.19	0.02*
Monocytes	-170.49	0.007*
Platelets	12916.5	0.543
RDW	-2.27	<0.001*
PDW	0.45	0.403
MPV (fl)	0.66	0.005*
NLR	-9.30	<0.001*
LMR	1.362	0.019*
PLR	-38.06	0.173

The pooled analysis revealed that the NLR at admission was significantly elevated for ICU survivors, when compared to ward patients with the NLR cut-off of > 3.85 associated with severity and prediction for ICU admission with a sensitivity of 77.8% and a specificity of 84.0% (area under the curve (AUC): 0.852, 95% confidence interval (CI) 0.791 to 0.900 (p < 0.001). It had a diagnostic accuracy of predicting severity of 82.12%.

The pooled analysis was similarly elevated for non-survivors, when compared to survivors of ICU (p < 0.001). The NLR of > 5.2857 was associated with mortality, with a sensitivity of 78.1% and a specificity of 77.7% (area under the curve (AUC): 0.811, 95% confidence interval (CI) 0.752 to 0.862, (P < 0.001). The diagnostic accuracy of predicting mortality was 77.25%.

Table V: Receiver operating characteristic curve of NLR for predicting ICU and mortality.

NLR	ICU	Mortality
Area under the ROC curve (AUC)	0.852	0.811
Standard error	0.0334	0.0451
95% confidence interval	0.791 to 0.900	0.752 to 0.862
p value	< 0.0001	< 0.0001
Cut-off	> 3.85	> 5.2857
Sensitivity (95% CI)	77.78% (64.4 - 88.0%)	78.12% (60.0 - 90.7%)
Specificity (95% CI)	84% (76.4 - 89.9%)	77.65% (70.8 - 83.5%)
PPV (95% CI)	67.7% (54.7 - 79.1%)	38.5% (26.7 - 51.4%)
NPV (95% CI)	89.7% (82.8 - 94.6%)	95.2% (90.4 - 98.1%)
Diagnostic accuracy	82.12%	77.25%

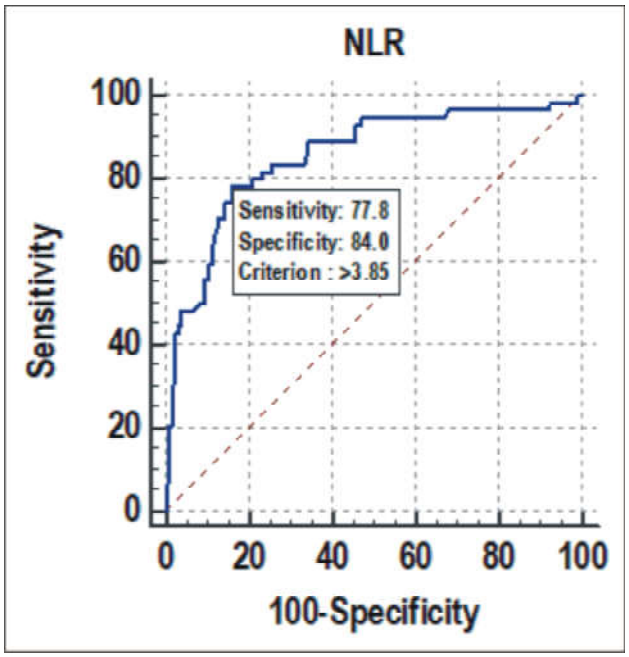

Fig. 1: *Receiver operating characteristic curve of NLR for predicting ICU admission.*

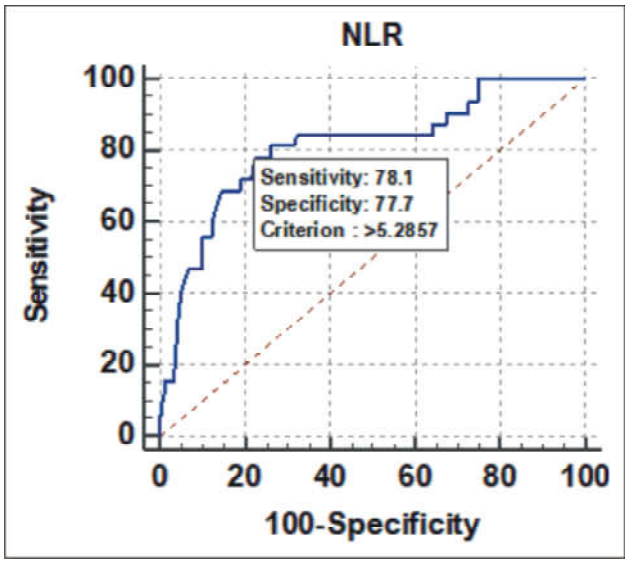

Fig. 1: *Receiver operating characteristic curve of NLR for predicting mortality.*

Discussion

Complete blood count (CBC) is simple, readily obtainable and affordable haematological investigation, that can provide comprehensive yet reliable information regarding disease progression. Haematological parameters, such as white blood cells (WBCs) and their subpopulations like neutrophils and lymphocytes and red cell distribution width, platelet count, mean platelet volume, platelet distribution

width and derived markers such as neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and lymphocyte to monocyte ratio (LMR), are established biomarkers of inflammatory responses[6-9]. There is a pressing need to rely on easily obtainable and cost-effective indicators to simplify the diagnostic process and evaluate the disease severity[13].

In our study, we enrolled 211 patients (Table I), of which 125 were mild/moderate cases, admitted to wards (Non-ICU admissions) and 86 were severe/critically ill cases who were admitted to ICU. Of the 86 patients admitted to ICU 32 expired and were grouped as non-survivors. Younger population, age < 50 years were less prone to develop severe disease. Clinical improvement was predicted by younger age with no associated co-morbidities, female gender and a low NLR. Transfer to ICU was instead forecasted by leucocytosis, lymphopenia, neutrophilia and advanced age along with an increased NLR. Wang *et al*[4], have shown similar association for the factors that predict severity of COVID-19. In their findings, compared with patients who did not receive ICU care (n = 102), patients who required ICU care (n = 36) were significantly older (median age, 66 years [IQR, 57 - 78] vs 51 years [IQR, 37 - 62]; P < .001) and were more likely to have underlying comorbidities. They also found that most patients had marked lymphopenia, and non-survivors developed more severe lymphopenia over time. White blood cell counts and neutrophil counts were higher in non-survivors than those in survivors. In another study, Tao *et al*[13], found that age, leucocytosis, neutrophilia, and lymphopenia were the predictors of worse clinical outcomes in patients. These results corroborate the findings of our study. In our intergroup comparison (3 groups of Non-ICU admissions, ICU survivors and ICU non-survivors), there was no statistically significant relationship noted between the other haematological variables such as haemoglobin, platelet parameters like PLT count, PDW, PLR and also Lymphocyte to monocyte ratio. Hence, these variables alone may not be considered for predicting severity or mortality outcomes in COVID-19 patients (Table II, III, IV).

Neutrophil proliferation and lymphocyte apoptosis are physiological responses of the innate immune system to systemic inflammation. In several previous studies[7-10], NLR has been linked to conditions such as pancreatitis, appendicitis, lung cancer and pneumonias. Elevated NLR is also associated with increased mortality in patients with severe acute respiratory syndrome[11-12].

In our study, the ROC curve of NLR for predicting ICU admission and mortality (Table V) showed AUC of 0.852 and 0.811 to predict the severity and mortality, respectively. Confidence interval (95%) is 0.791 to 0.900 for severity prediction and 0.752 to 0.862 for mortality prediction, which confirms the strength of association between elevated NLR and disease severity. Fig. 1 shows NLR cut of value for predicting severity was > 3.85 (95% confidence interval (0.791 to 0.90), p value < 0.001) with a sensitivity of 77.78% and specificity of 84%. Fig. 2 shows NLR cut-off value for predicting mortality was > 5.29 (95% confidence interval (0.752 to 0.862), p value < 0.0001) with a sensitivity of 78.12% and specificity of 77.65%.

Yang *et al*[14], comparing the NLR, LMR, PLR and CRP amongst severe and non-severe patients of COVID-19, found that WBC count, NLR, LMR, PLR, and CRP of severe patients were significantly higher than those of non-severe patients. These results are similar to ours. In their study, Yang *et al*, also found that the optimal threshold at 3.3 for NLR showed a superior prognostic ablity and had the highest sensitivity and specificity (63.6% and 88%) and the largest AUC (0.841). The AUC is similar to the one found in the present study. However, our optimal cut-off value was slightly higher at 3.85 with a sensitivity and specificity of 77.78% and 84%, respectively.

In a systematic review and meta-analysis[15] of 19 studies, it was found that the sensitivity, specificity, AUC and cut-off value of NLR for predicting mortality and disease severity varied greatly among the studies. The cut-off value for severity ranged from 3.0 - 13.4, with an average of 5.24. The cut-off value for mortality ranged from 3 - 11.8, with an average of 7.14. In terms of predicting disease severity, the cut-off value in six studies was higher than 4.5 and was termed the "high cut-off value" subgroup. Seven others used a lower cut-off value, which were included in the "low cut-off value" subgroup. The AUC was 0.86 (95% CI 0.83 -0.89) and 0.82 (95% CI 0.78 - 0.85), respectively. Similarly, ten studies reporting the predictive value of NLR on mortality were divided into "high cut-off value" (cut-off e" 6.5) and "low cut-off value" (< 6.5) subgroups, and the AUC was 0.92 (95% CI 0.89 - 0.94) and 0.84 (95% CI 0.80 - 0.87), respectively. These findings were consistent with our findings.

The variability in the optimal cut-off for NLR can be explained by geography and race as most of the studies included in this meta-analysis were carried-out in China. Also, the timing of acquiring the sample from the day of disease onset may play an important role in the variability of NLR. As, in severe or non-survivor patients with COVID-19, the lymphocyte count decreases progressively, while the neutrophils count gradually increases. This may be due to excessive inflammation and immune suppression caused by SARS-CoV-2 infection. On the one hand, neutrophils are generally regarded as pro-inflammatory cells with a range of antimicrobial activities, which can be triggered by virus-related inflammatory factors, such as interleukin-6 and interleukin-8. On the other hand, systenic inflammation

triggered by SARS-CoV-2 significantly depresses cellular immunity, leading to a decrease in CD3 + T cells, CD4+ T cells and CD8+ T cells[16].

Strengths of study

NLR was statistically significantly different ($P < 0.001$) in predicting disease severity and mortality. NLR is an easily attainable early bio-marker of inflammation and also cost-effective when compared to other inflammatory markers.

Limitations of the study

Haematological parameters were not measured dynamically, hence it is unclear whether these parameters exhibit cumulative changes when the patients condition deteriorates. Also, inclusion of the other inflammatory markers would complement the initial test results in elucidating the underlying inflammatory mechanism, which contributes to the disease severity.

Conclusion and summary

In conclusion, advanced age (> 58 years), male gender, haematological variables like leucocytosis, neutrophilia, lymphopenia and NLR are useful in prognosticating the disease severity. NLR was notably associated with increased severity and mortality. We conclude that NLR can be considered as an initial screening test to assess the severity and mortality in patients of COVID-19.

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