Original Article

Association of haematological biomarkers with severity of COVID-19 pneumonia

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Abstract

Background: Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China in December 2019. It is caused by SARS-CoV-2, a beta coronavirus. In this study, we assessed the association of biomarkers such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte monocyte ratio (LMR) with the severity of COVID-19 in patients. **Methods:** This retrospective observational study was carried out at a tertiary care hospital of the sub-Himalayan region of Uttarakhand over a period of six months from May to October 2020. A total of 350 patients with confirmed RT-PCR COVID-19 infection were included in the study. Detailed clinical, demographic and biochemical data of each patient was obtained from the hospital record section after permission from the Institute Ethical Committee. NLR, PLR and LMR ratios were calculated and compared with the outcomes in each patient. The patients were subdivided into two sub-groups: those with saturation less than 94% and those with saturation more than 94%. The patients were categorised as mild (with SpO_2 of > 94%) and moderate-severe (with SpO_2 of ≤94%) based on oxygen saturation. **Results:** A total of 350 patients with Covid-19 pneumonia were enrolled in the study. The mean age of the patients with oxygen saturation of ≤94% was 54.91 ± 13.29 years, which was comparable to the other group. Absolute neutrophil count (ANC) and NLR were significantly higher in patients with a saturation of < 94%. However, LMR and PLR were significantly lower in the group with saturation of <94%. Thus, a significant association was found between haematological inflammatory ratios and the severity of COVID-19 infection. **Conclusion:** NLR, LMR and PLR ratios can be utilised as point of care markers to assess severity in patients with COVID-19 pneumonia.

Keywords: Association of NLR, LMR, PLR, severity of COVID-19

Introduction

COVID-19 infection has emerged as a global pandemic. It was detected in Wuhan, China in December 2019. Since then, it has rapidly spread to almost all the countries of the world, taking a heavy toll on human life.[1,2] As per the worldometer, on 2nd March, 2021, there were approximately 11.12 million cases with 157385 reported deaths due to COVID-19 infection in India. Kerala and Maharashtra, which are the two worst-hit Indian states, continue to report a high daily positivity rate.[3]

The common signs and symptoms of the disease include fever, breathlessness, cough, loose stools, headache and fever. The presentation of the patients spans from mild to severe form of the disease and a more catastrophic form as acute respiratory distress syndrome (ARDS). Besides respiratory system involvement, neurological, cardiovascular, renal and hepatic complications of SARS-CoV-2 infection have also been reported.

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The gold standard diagnostic modality of COVID-19 infection is real-time–polymerase chain reaction (RT-PCR). However, it is time-consuming. Thus, there is an urgent need to analyse serum biomarkers of inflammation, which can have both diagnostic and prognostic implications. Haematological biomarkers such as total leucocyte count (TLC), neutrophil-lymphocyte ratio (NLR) and lymphocyte-monocyte ratio (LMR) have been individually studied as prognostic markers and to predict severity in patients with COVID-19 infection.[4,5]

As in patients with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), there is dysregulated host response leading to cytokine storm in patients with COVID-19 infection. Robust inflammatory response coupled with weak adaptive immunity contributes to multi-organ involvement in COVID-19 infection. In addition, NLR has been assessed in patients with COPD, pancreatitis and cardiovascular disease.[6,7]

Several case series have observed a significant relationship between NLR and mortality in patients with COVID-19 infection.[8] Few studies have also highlighted platelet-lymphocyte ratio (PLR) and LMR as inflammatory markers.[9] In this study, we assessed the association of all the three markers—NLR, LMR and PLR—with the severity of COVID-19 patients.

Materials and Methods

The retrospective study was carried out at the Emergency Medicine Department of a tertiary care hospital of Uttarakhand over a period of six months. A total of 350 RT-PCR-confirmed cases of COVID-19 infection were enrolled in the study. Detailed demographic (age, sex), clinical, haematological and biochemical parameters, co-morbidity history (diabetes, hypertension, asthma, COPD and ischaemic heart disease), CT findings and CT score were noted for each patient from the hospital record section. The study was conducted after obtaining approval from the Institutional Ethical Committee dated 12-12-2020.

All the patients were subdivided into two groups, one with oxygen saturation of >94% (mild group) and the other with oxygen saturation of <94% (moderate-severe group). NLR, plasma-lymphocyte ratio (PLR) and LMR were calculated in all the patients and were compared in both groups. The clinico-demographic, biochemical, haematological and outcomes of all the patients were compared for both groups. Clinical and haematological parameters of alive and expired patients were also compared.

The data was collected after taking permission from the institute's ethical committee and research cell.

Statistical analysis

Statistical analysis was performed by the SPSS program for Windows, version 17.0 (SPSS, Chicago, Illinois). Continuous variables are presented as mean ± SD, and categorical variables are presented as absolute numbers and percentages. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the unpaired *t*-test, whereas the Mann–Whitney U test was used for variables not normally distributed. Categorical variables were analysed using either the Chi-square test or Fisher's exact test. Kaplan–Meier survival analysis was used to estimate the mortality of study patients as admission was based on SpO_2 (>94% or ≤94%). For all statistical tests, a *P* value of <0.05 was taken to indicate a statistically significant difference.

Results

As shown in Table 1, a retrospective analysis of 350 patients with COVID-19 pneumonia was done in this study. They were subdivided into two groups: one with oxygen saturation of ≤94% (80, 22.8%) and the other with oxygen saturation of >94% (270, 77.1). Of these, 263 (75.1%) were males and 87 (24.8%) were females. There was no statistically significant difference in gender and age in both groups. The mean age of the patients with oxygen saturation of ≤94% was 54.91 ± 13.2 years, which was almost similar to the mean age in the other group. Mean diastolic blood pressure (72.6 ± 11.58 mm of Hg) was significantly lower in the group of patients with SPO_2 of ≤94%. Similarly, Glasgow Coma Scale (GCS) was significantly lower in the former group. Mean heart rate (100.31 ± 16.5 beats/min) and respiratory rate (25.4 ± 2.48 per min) were higher in the group with SPO_2 of ≤94%. Among the co-morbidities, hypertension (99, 28.2%) and diabetes mellitus (125, 35.7%) were the most common. Hypertension was significantly more common in the group with oxygen saturation of ≤94%. The

Table 1: Demographic, clinical and hospital stay variables of patients with COVID-19 pneumonia with oxygen saturation of more than and less than 94%

Variables	SpO_2 <94% (*n*=80)	SpO_2 >94% (*n*=270)	*P*
Age (1 year)	54:91±13.29	55.75±15.54	0.663
Sex			
F	16 (20%)	71 (26.3%)	0.252
M	64 (80%)	199 (73.1%)	
Systolic Blood Pressure (SBP)	124.61±19.02	126.99±19.53	0.337
Diastolic Blood Pressure (DBP)	72.6±11.58	76.05±13.76	0.042
Mean Arterial Pressure (MAP)	89.9±12.3	94.5±29.4	0.177
Heart Rate (HR)	100.31±16.5	92.7±14.1	<0.001
Respiratory rate (RR)	25.4±2.48	19.2±2.3	<0.001
GCS	13.46±2.9	14.3±2.2	0.005
Haemoglobin (HB)	16.91±19.3	12.15±3.08	0.001
Hypertension	33 (41.2)	66 (24.4)	0.003
Ischaemic Heart Disease (IHD)	18 (22.4)	36 (13.3)	0.06
Diabetes mellitus	29 (36.2)	96 (35.5)	0.6
Asthma	3 (3.7)	8 (2.9%)	0.8
COPD	6 (7.5)	11 (4.0%)	0.7
Referred	4 (5%)	3 (1.1%)	0.027
Mortality	36 (45%)	115 (42.6%)	0.027
Discharged	32 (40%)	139 (51.5%	0.027
Length of Stay	13.15±9.24	11.21±7.11	0.142

other co-morbidities were ischaemic heart disease (54, 15.4%), asthma (11, 3.1%) and COPD (17, 4.8%). Mortality (36, 45%) was higher in the group with SPO_2 of ≤94%. Length of stay was not significantly longer in the group with SPO_2 of ≤94%. In the mild group, significantly more patients were discharged as compared to the severe group. Among the haematological parameters, TLC (12.71 ± 17.8/cumm) were significantly higher in the group with SPO_2 of ≤94%. NLR was significantly higher in the group with SpO_2 of ≤94% [Table 2]. Lymphocyte-monocyte ratio (LMR) (1.48 ± 0.9) and platelet-lymphocyte ratio (PLR) (406.13 ± 251.1) were significantly lower in the group with SPO_2 of ≤94%. No significant association was observed with the other biochemical parameters [Table 3]. Table 4 shows the association of various parameters with mortality in patients with COVID-19 pneumonia. Age (65.23 ± 13.7 years), respiratory rate (33.7 ± 2.3 per min), absolute neutrophil count (ANC) (14618.2) and NLR (14.29) were significantly higher in the group who succumbed to death [Figure 1].

Discussion

The COVID-19 pandemic, first detected in December 2019, has spread exponentially all over the world. Although the mortality of COVID-19 infection is only 2.5%, it poses a humongous challenge to identify and initiate timely management in these patients. The common clinical characteristics of the infection are fever, cough and breathlessness.[1]

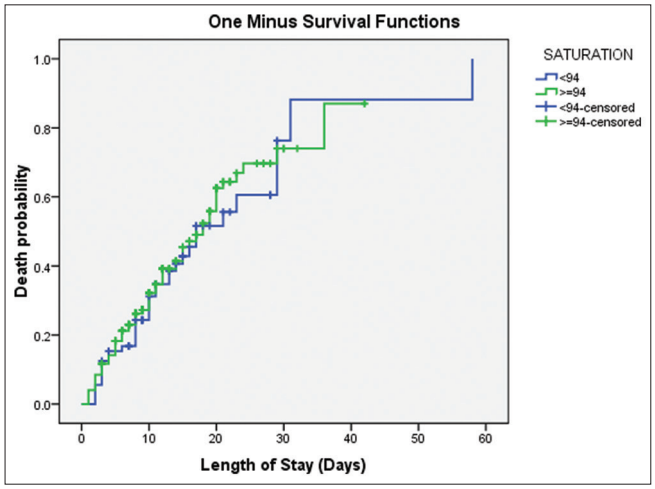

Figure 1: Kaplan–Meier curves for mortality in COVID-19 patients grouped into two groups based on SpO_2 (>94% or ≤94%) at admission. (SpO_2: Peripheral venous blood oxygen saturation value)

The gold standard diagnostic modality of COVID-19 infection is RT-PCR test.[10] In our study, the majority of the patients were more than 50 years of age. However, there was no statistically significant difference in age in the two subgroups. As per the previous studies, age is found to be significantly associated with mortality in patients with COVID-19 pneumonia.

The geriatric population has been found to be vulnerable to severe form of the disease due to co-existent co-morbidities as well as weak immune system.[11] In our study, patients with COVID-19 pneumonia who succumbed to death belonged to a higher age group as compared to patients who were discharged in stable condition. In this study, diastolic blood pressure was significantly lower in the group with SpO_2 of ≤94%. Both heart rate and respiratory rate were significantly higher in the moderate-severe group. Both the parameters have been identified by previous studies as prognostic and mortality markers in COVID-19 pneumonia patients.[12] Hypertension, followed by diabetes mellitus, was found to be the most common co-morbidities in patients with severe COVID-19 infection. However, hypertension was significantly more common in patients with SPO_2 of ≤94%. Similar results were observed by studies conducted by Mertz *et al.*[13]

Chronic conditions such as cardiovascular diseases, hypertension, diabetes mellitus and chronic obstructive pulmonary diseases can have been found to have a significant impact not only on mortality but also on prognostic parameters in viral infections such as SARS-COV-2, MERS and SARS.[14]

Lately, researchers have highlighted the significant role of multiple ratios such as NLR, LMR and PLR in many chronic inflammatory conditions. These ratios can be used as diagnostic and prognostic predictors of severity in patients with recently emerged COVID-19 infection. The NLR, PLR and LMR ratios can be utilised as low-cost diagnostic and prognostic biomarkers in COVID-19 patients.[15]

Table 2: Haematological variables of patients with covid-19 pneumonia with oxygen saturation greater than and less than 94%

Variables	SpO_2 ≤94%	SpO_2 >94%	*P*
HB	16.91±19.39	12.15±3.08	0.001
RBC Count	4.27±1.01	4.06±1.06	0.126
TLC	12.71±4.78	9.90±5.00	<0.001
Absolute neutrophil count (ANC) (/mm^3)	11817.66±4797.57	8107.37±3981.2	<0.001
Absolute lymphocyte count (ALC) (/mm^3)	675.8±519.8	917.5±727.6	0.029
Absolute monocyte count (AMC) (/mm^3)	659.4±500.3	4.96±373.5	0.044
Neutrophil-lymphocyte ratio (NLR)	25.7±17.8	15.4±14.7	<0.01
Lymphocyte-monocyte ratio (LMR)	1.48±0.9	3.9±14.7	0.001
Platelet-lymphocyte ratio (PLR)	406.13±251.1	433.28±695.6	0.006
PT INR	1.25±0.72	1.05±0.24	0.005

In the present study, the ANC and NLR ratio were significantly higher in the group with oxygen saturation of ≤94% and in the patients who expired. Thus, the NLR ratio should be integrated with the prognostic nomograph in patients with COVID-19 pneumonia. Shang *et al.* studied the role of NLR, CRP and platelets as predictors of disease serenity and emphasised NLR as the determinant of COVID-19 pneumonia severity.[16]

A Chinese study has highlighted that NLR cut-off value of >3.3 in COVID-19 pneumonia is associated with poor prognosis and lower survival rate. NLR has also been studied as a marker of endothelial dysfunction and is significantly associated with cardiovascular mortality.[17] The endothelial dysfunction leads to viral alveolar damage in patients with COVID-19 infection. SARS-CoV-2 utilises angiotensin-converting enzyme-2 (ACE-2) receptor to enter the cells. This ACE-2 is expressed in multiple organs including endothelial cells.[18]

The patients with multiple co-morbidities such as hypertension and diabetes have pre-existing endothelial dysfunction. Thus, these patients are more vulnerable to the severe form of the disease. Endothelial damage triggers the inflammatory cascade stimulating activation of complement and increasing endothelial permeability, resulting in cytokine storm.

Table 3: Biochemical variables of patients with covid-19 pneumonia with oxygen saturation more and less than 94%

Variables	SpO_2 <94%	SpO_2 >94%	*P*
SGPT (U/L)	73.03±71.3	85.08±21.9	0.989
SGPT (U/L)	78.82±54.09	84.4±124.7	0.683
CREAT (mg/dl)	2.25±288	1.9±1.9	0.669
BUN (mg/dl)	75.2±56	64.4±48.5	0.121
K+ (Potassium)	4.87±0.99	4.86±0.78	0.878

There is an increase in NLR in the severe form of the disease. The values of both baseline NLR and peak values of NLR can be compared to assess the severity of the disease.[19] Hence, NLR can be used as a cost-effective and easily measurable biomarker of COVID-19 disease severity. In this study, the LMR was significantly lower in patients with severe COVID-19 (SPO_2 of ≤94%). This is in accordance with the previous studies. Neutrophils account for more than 60% of the leucocyte count. They release oxygen-free radicals and trigger DNA damage and release of virus from the cells, resulting in stimulation of humoral immune response. Neutrophils stimulate the production of various cytokines such as vascular endothelial growth factor, granulocyte-colony stimulating factor (G-CSF), granulocyte-monocyte colony stimulating factor (GM-CSF), IL-1 and TNF-alfa. This pro-inflammatory cascade leads to enhanced expression of CD8+ T-lymphocytes and increased NLR ratio.[20,21]

NLR ratio has been assessed as a useful marker in various oncological diseases, autoimmune disorders and bacterial pneumonias.[9,22,23] However, the ratio has been rarely reported in patients with viral pneumonia. Elevated NLR and leukopenia have been reported as independent risk factors in patients with COVID-19 pneumonia. Some studies have also reported eosinophilia and leucopenia as prognostic variables in patients with COVID-19 pneumonia.[24-26] Clearly, these biomarkers can aid in ruling out other causes of respiratory infections and undifferentiated fevers.

Summary

1. Higher levels of ANC and neutrophil-lymphocyte ratio are associated with increased severity of COVID-19 infection.
2. Lower levels of LMR and PLR are associated with increased disease severity in patients with COVID-19 pneumonia.

Table 4: Difference between clinico-demographic and biochemical profile patients with COVID-19 pneumonia alive and died

Variables Demographic	Alive (*n*=199) (50.8%)	Dead (*n*=151) (43.1%)	*P*
Age	52.3±15.56	65.23±13.73	<0.001
Sex			
F	41 (23.03)	42 (27.8)	0.320
M	137 (76.9)	109 (72.1)	
SBP	125.15±12.9	126.64±20.97	0.487
DBP	75.66±12.9	74.8±13.96	0.602
MAP	94.4±34.5	92.13±14.7	0.45
HR	93.8±14.9	96.04±14.9	0.185
R.R	23.13±2.39	23.7±2.37	0.028
Oxygen saturation	94.8±4.47	44.2±5.04	<0.001
GCS	14.4±1.95	13.6±29	0.011
HB	13.13±8.2	13.00±11.9	0.908
RBC	4.17±1.00	3.96±1.09	0.079
TLC	10.32±5	10.88±5.29	0.378
ANC/MM^3	8480.6 (5124.6-11825.12)	14618.2 (8179.6-15671.3)	0.002
NLR	10.91 (5.418-20.8)	14.29 (5.514-24.81)	0.014
LMR	1.6 (1.163-2.8)	1.6/(1.155-2.88)	0.947
PLR	228.8/(129.20-401.838)	35.7 (125.53-409.65)	0.855

3. Haematological biomarkers can be used as severity risk indicators in the quick assessment of patients with COVID-19 infection.

Conclusion

NLR, LMR and PLR ratios can be used as cheap and readily available biomarkers to assess severity in patients with COVID-19 infection. The focus of this study is early detection of COVID-19 infection and stratifying it as severe and non-severe using these ratios.

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Conflicts of interest

There are no conflicts of interest.

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