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Neutrophil/Lymphocyte Ratio – A Marker of COVID-19 Pneumonia Severity

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Abstract

Aim: To determine the efficacy of neutrophil/lymphocyte ratio (NLR) as a marker of the severity of COVID-19 pneumonia in the South-Asian population. Methods: This was a prospective, cross-sectional, analytic study conducted at HDU/ICU of District Headquarter Hospital, Faisalabad, Pakistan, from May through July 2020. Sixty-three eligible patients, admitted to the HDU/ICU, were prospectively enrolled in the study. Their NLR, C-reactive protein, serum albumin, and serum fibrinogen were measured. Patients' demographic characteristics, comorbidities, clinical manifestations of COVID-19 infection, medication use, and history of lung malignancy were retrieved from their medical history. Patients were categorized into either a general group (with mild COVID-19) or a heavy group (with moderate to severe COVID-19). Results: There were significant differences between the two groups in diabetes prevalence, NLR, C-reactive protein, and serum albumin. NLR and C-reactive protein were positively correlated ($P < 0.001$, $P = 0.04$ respectively) whereas serum albumin was negatively correlated ($P = 0.009$) with severe COVID-19. NLR was found to be an independent risk factor for severe COVID-19 pneumonia in the heavy group (OR = 1.264, 95% CI: 1.046˜1.526, $P = 0.015$). The calculated AUC using ROC for NLR was 0.831, with an optimal limit of 4.795, sensitivity of 0.83 and specificity of 0.75, which is highly suggestive of NLR being a marker for early detection of deteriorating severe COVID-19 infection. Conclusion: NLR can be used as an early warning signal for deteriorating severe COVID-19 infection and can provide an objective basis for early identification and management of severe COVID-19 pneumonia.

What is already known about this subject?

- Patients with Severe COVID-19 infection have a high mortality rate.
- Neutrophil/lymphocyte ratio (NLR) may be used as an early warning signal of severe and deteriorating COVID-19 infection and can help the physicians to intervene and manage the patients ' clinical situation early.

What does this study contribute to the literature?

- Our study further adds to the evidence that NLR has been associated with severe and deteriorating COVID-19 infection in the South-Asian population also.
- It can be used as an early warning sign in this population and provide an objective basis for early identification and management of severe COVID-19 pneumonia.

1 INTRODUCTION

The new coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a highly communicable infectious disease. It has rapidly and widely spread worldwide,[1]

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and was declared a pandemic in March 2020 by World Health Organization.[2] As of 27th August 2020, the cumulative number of confirmed cases in Pakistan is 294,193 with 8,987 active cases and 6,267 deaths.[3]

Despite significant advancements in medicine and science, we are still in the age of infancy regarding COVID-19 infection and its management. COVID-19 infection can be categorized as asymptomatic, mild, moderate, or severe disease. Moderate and severe COVID-19 infections have been associated with long hospital stays, difficult clinical management and high mortality rate.[4] Clinically, if early warning signs of severe COVID-19 infection can be identified, timely intervention and treatment may help reduce the mortality, improve the cure rate, shorten the hospital stay and reduce the consumption of resources.

Different immunity parameters in the human body have been identified to combat the disease.[5,6] These tests are often expensive and time delay occurs in the results of these tests, jeopardizing the patient's treatment. Therefore, we ought to have a clinical indicator or marker that is inexpensive, has a rapid turnaround time, and is simultaneously specific and sensitive. One such marker is a complete blood count (CBC) profile, out of which neutrophils and lymphocytes are especially important. The neutrophils are increased in bacterial infection, and lymphocytes are reduced during viremia. Examining these two parameters can greatly help assess the COVID-19 infection.

A recent systematic review and metanalysis by Feng *et al.*regarding immune-inflammatory parameters in COVID-19 infection concluded that neutrophil/lymphocyte ratio (NLR) is associated with the progression of the infection and can be utilized by the physicians to identify high risk or deteriorating patients at an early stage.[7] Several other studies have reported their findings that NLR can be used as an early warning signal of severe COVID-19 infection,[8] and cohnsidered as an independent marker for poor clinical outcomes and mortality in COVID-19 infection.[9-13] However, the majority of these studies have been reported from China and some from European countries. To our knowledge, no study has been conducted in this regard in the South-Asian population. Our study aimed to prospectively analyze the clinical data of COVID-19 patients admitted to the high dependency unit/intensive care unit (HDU/ICU) at District Headquarter (DHQ), Faisalabad (Pakistan) in order to determine the risk factors and markers, with particular focus on NLR, associated with severity of COVID-19 infection in the South-Asian population.

2. METHODS

2.1 Study design

This was a prospective, cross-sectional, analytic study conducted at HDU/ICU DHQ Hospital, Faisalabad (Pakistan), from May through July 2020. This facility was designated as a referral center by the provincial health department to cater to approximately 13 million population from the surrounding districts. The patients from these districts were referred to this facility for the management of COVID-19 patients.

2.2 Sample

We screened all the hospitalized COVID-19 patients who were admitted to HDU/ICU of DHQ Hospital, for eligibility, based on the inclusion and exclusion criteria listed below.

2.3 Inclusion criteria

Following patients were considered for enrolment in the study:

1. Adult patients age $>$ 18 years.
2. All the patients who meet the diagnostic criteria of the National Institute of Health (NIH) Pakistan for COVID-19 infection,[14] which are as follows:

1. Clinical manifestations that include fever and pulmonary symptoms (cough, shortness of breath, chest pain, and tightness).
2. Radiological findings of consolidation, ground-glass opacities (GGOs) either on chest X-ray or high-resolution computed tomography (HRCT).

3. Real-time fluorescent reverse transcription-polymerase chain reaction (RT-PCR) of respiratory samples (nasal/oropharyngeal swab or tracheal secretions) positive for SARS-CoV-2.

After being enrolled in the study, the eligible patients were then segregated into the following two groups, based on diagnostic criteria and classification criteria of the NIH Pakistan:[14]

1. The 'general group' that includes asymptomatic and mild cases.
2. The 'heavy group' that includes moderate and severe cases.

The following criteria were used to define the two groups.

2.4 General group diagnostic criteria

Patients presented with fever, respiratory symptoms, and radiological findings consistent with COVID-19 infection.

1. Respiratory rate < 30/min at rest.
2. Oxygen saturation (SpO_2) $> 93\%$ on room air.
3. Arterial oxygen partial pressure (PaO_2)/oxygen uptake concentration (FiO_2) > 300.

2.5 Heavy group diagnostic criteria

Patients presented with general group diagnostic criteria and any one of the following:

1. Respiratory rate [?] 30/min at rest.
2. Oxygen saturation (SpO2) [?] 93% on room air.
3. Arterial oxygen partial pressure (PaO_2)/oxygen uptake concentration (FiO_2) [PaO_2/FiO_2] (PF ratio) [?] 300.

2.6 Severe disease criteria

Patients from any group presented with any one of the following were categorized to have a severe disease:

1. Oxygen requirement of more than 10 liters for 90% saturation.
2. 50% of lung involvement on either chest X-ray or HRCT.
3. Multi-organ dysfunction
4. CRP > 10 mg/L, D-dimer > 1000 mg/mL, serum ferritin > 1000 ng/mL.
5. Secondary infection (diagnosed by blood culture and sensitivity test or raised procalcitonin).
6. Shock or use of inotropes.
7. [PaO2/FiO2] (PF ratio) < 118 mmHg.

2.7 Exclusion criteria

Following patients were excluded from the study:

1. Patients with negative detection of novel coronavirus nucleic acid.
2. Patients in whom monitoring of blood oxygen saturation (SpO_2 or PaO_2) was not feasible due to any reason.
3. Patients with suspected bacterial pneumonia (confirmed by sputum bacterial culture).
4. Patients with interstitial pneumonia (previously diagnosed based on radiological findings).
5. Patients with heart failure associated with pulmonary edema (non-COVID-19 heart disease).
6. Patients with allergic pneumonia (acute or chronic eosinophilic pneumonia).
7. Patients with active lung tumors.
8. Patients on immunosuppressive drugs (including long-term steroids).
9. Patients with incomplete chest computed tomography (CT) examination due to any reason.
10. Patient refusal to be enrolled in the study.

2.8 Sample size

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A total of 106 cases were screened for eligibility based on the inclusion and exclusion criteria. Sixty-three patients were found to be eligible and enrolled in the study.

2.9 Data collection and procedure

After taking informed consent from the enrolled patients, their blood sample was drawn according to standard operating procedures within 24 hours of admission and sent to the laboratory for routine laboratory tests that included complete blood count (CBC), liver function tests (LFTs), renal functional tests (RFTs) and serum electrolytes. CBC was analyzed using the hospital laboratory system, 'Sysmex analyzer'. The inflammatory markers such as neutrophil/lymphocyte ratio (NLR), C-reactive protein (CRP), serum albumin (ALB), and serum fibrinogen (FIB) were also ordered. The patients were then sent for a chest X-ray. HRCT chest scan was conducted on the advice of the respiratory physicians.

NLR ratio was calculated using the simple formula:

If a patient belonged to the heavy group, additional investigations (D-dimer level and serum ferritin) were also ordered. Where indicated, their sputum or blood culture, or serum procalcitonin was also ordered.

Simultaneously, patient demographic characteristics (gender and age), comorbidities (hypertension (HTN), diabetes mellitus (DM), coronary heart disease (CHD), lung diseases such as asthma or chronic obstructive pulmonary disease (COPD)), clinical manifestations of COVID-19 (fever, chest pain, cough and shortness of breath), medication history (including the use of long-term steroid), and history of lung malignancy were recorded on a data collection form.

2.10 Ethical approval

The study was reviewed and approved by the DHQ hospital research ethics committee.

2.11 Data analysis

The data was analyzed using Statistical Package for Social Sciences (SPSS) version 25. For comparison of the measurements between the two groups, independent samples t -test was used for the data that met normal distribution, and the Mann-Whitney U test was used for the data that did not meet normal distribution. Chi-square test was used for comparison of categorical data between the groups; Pearson correlation test was used for linear correlation analysis; Spearman correlation test was used for rank correlation analysis; Z test was used for the area under Receiver Operating Characteristic (ROC) curve (AUC-ROC) comparison; multiple logistic regression was used for regression analysis. P values of less than 0.05 were considered statistically significant unless otherwise stated.

3. RESULTS

3.1 Demographic characteristics

A total of 63 patients met the inclusion and exclusion criteria and consented to be enrolled in the study, out of whom 32 patients were in the general group and 31 patients in the heavy group. The average age of the patients in the general group was 62.25±15.07 years with 15 male patients, whereas the average age of the patients in the heavy group was 64.55±14.88 years with 18 male patients. The differences in age and gender between the two groups were not found to be statistically significant ($P > 0.05$).

3.2 Comorbidities, clinical manifestations and laboratory parameters

As presented in Table 1, the difference in diabetes prevalence between the two groups was statistically significant ($P < 0.05$). However, the difference in HTN, CHD, lung malignancy, and other lung diseases was not statistically significant ($P > 0.05$). As shown in Table 2, no statistically significant difference in cough, fever, chest pain, tightness, and breathlessness was found between the two groups ($P > 0.05$). Moreover, no statistically significant difference in the FIB index was seen between the two groups ($P > 0.05$). However, significantly increased NLR and CRP, and significantly decreased ALB were observed in the heavy group ($P < 0.05$) as compared to the general group (Table 3).

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3.3 Correlation analysis and regression analysis

Correlation analysis showed that diabetes, NLR, CRP and ALB had a significant correlation with severe COVID-19 pneumonia in the heavy group ($P < 0.05$) (Table 4). Diabetes, NLR and CRP were positively correlated, whereas ALB was negatively correlated with severe COVID-19 pneumonia in the heavy group. Further, multi-factor logistic regression analysis of diabetes, NLR, CRP, ALB and severe COVID-19 pneumonia found that NLR was an independent risk factor for severe COVID-19 pneumonia in the heavy group (OR = 1.264, 95% CI: 1.046˜1.526, P = 0.015) (Table 5).

3.4 NLR prediction of heavy group COVID-19 pneumonia and critical value judgment

Using the ROC curve to calculate the area under the NLR curve, the results showed that: AUC was 0.831 (95% CI 0.730˜0.932), the Youden index was the largest 0.589, corresponding to the best cut-off value of 4.795, the sensitivity was 0.839, and the specificity was 0.750 (Figure 1).

4. DISCUSSION

The results of our study show that the two groups of patients had a significant difference in diabetes prevalence, NLR, CRP, and ALB. There was a positive correlation between diabetes, NLR, CRP and severe COVID-19 infection. However, ALB had a negative correlation with severe COVID-19 infection. Moreover, NLR was found to be an independent risk factor for severe COVID-19 infection.

Neutrophils are one of the human body's vital immune cells. When pathogenic microorganisms invade the body, immune cells tend to rapidly chemotactically gather to the infection site and play the role of host defense and immune regulation.[15] When the body's neutrophils are significantly reduced, the body's immunity is compromised, and thus the risk of infection is significantly increased.[16] Lymphocytes are the main effector cells of the human immune response. The number of lymphocytes in the body is closely related to the body's immunity and defense system against pathogenic microorganisms and is negatively correlated with the degree of inflammation.[9] NLR encompasses two types of leukocyte subtypes, reflecting the balance of the body's neutrophil and lymphocyte count levels and the degree of systemic inflammation. More accurately, it reflects the balance between the severity of the inflammation and the body's immunity status,[17] and is thus considered an important marker of systemic inflammatory response. Based on this, we speculated that heavy COVID-19 infection can have significant systemic inflammation and that NLR may have a role in predicting the infection's severity.

The outbreak of COVID-19 is caused by the SARS-CoV-2 virus infection. The disease spectrum varies from no symptoms to severe disease and death. Clinical observation has found that some patients with mild disease progress to severe disease within a short period of time with a high risk of mortality. The specific pathological mechanism is unknown. Some believe that the sudden aggravation of the condition is due to the rapid emergence of ARDS, and subsequent multiple-organ dysfunction in the later period which may be related to the "cytokine release storm".[18] The concept of an inflammatory factor storm was first proposed in 1993.[19] In 2003, the SARS-CoV virus infection caused ARDS and multiple-organ failure, resulting in an exceedingly high rate of mortality – the underlying pathology of inflammatory factor storm that gradually attracted the attention of the medical community.[20] The SARS-CoV-2 virus is extremely closely related to the SARS-CoV virus. In the virus spectrum, both belong to β-CoV coronavirus family,[21]in terms of genetic characteristics, both have 79.5% sequence similarity,[22] and with regards to clinical manifestations, it is easy to progress to ARDS and multiple-organ dysfunction in infections caused by these viruses. Therefore, based on the close similarities between the two viruses, COVID-19 patients' clinical condition changes from mild to critical, which may be related to the inflammatory factor storm. Li *et al* found in their study that the patients with COVID-19 pneumonia admitted to ICU had higher levels of inflammatory markers such as IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNFα in the plasma, reflecting severe inflammation.[23] This is due to an apparent inflammatory response in the patient's body, which is consistent with our findings.

In summary, our study conducted a prospective analysis of common clinical risk factors or parameters that can be easily obtained from the laboratory. Our study found that there was a significant difference in NLR

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between general and heavy group COVID-19 patients with heavy group patients tending to have significantly higher NLR. In addition to NLR being an independent risk factor for severe COVID-19 infection, the ROC curve calculation of AUC showed a high predictive value of NLR for severe COVID-19 infection. As per these results, we believe that increasing NLR can be used as a warning signal for rapidly deteriorating COVID-19 infection and can provide us with a certain objective basis for the early identification of severe COVID-19 pneumonia. This can further allow us to be prepared for the exploration of subsequent treatment mechanisms. However, this study had certain limitations, such as having a small sample size and being a single-center study. For more accurate and precise results, and wider generalizability of the findings, multicentred and larger sample size clinical studies are required to validate our results.

5. CONCLUSION

NLR can be used as an early warning signal for deteriorating severe COVID-19 infection and can provide an objective basis for early identification and management of severe COVID-19 pneumonia. This marker is especially important in our setting, where lack of resources often prevents costly testing.

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TABLE 1 Comorbidities in the two groups of patients

Comorbidities	**General group (n=32)**	**Heavy group (n=31)**	χ^2	P value
Hypertension	9	15	2.741	0.098
Diabetes	3	9	3.946	0.047
Coronary heart disease	0	2	NA	0.238
Tumor	1	2	0.001	0.978
Lung Disease	2	1	0	1

NA: Not applicable.

χ^2: Chi-square value.

TABLE 2 Clinical manifestations in the two groups of patients

Symptoms	**General group (n=32)**	**Heavy group (n=31)**	χ^2	P value
Fever	*26*	*25*	0.004	0.951
Cough	22	21	0.007	0.932
Chest tightness	9	12	0.794	0.373
Dyspnea	14	20	2.733	0.098
Chest Pain	19	16	0.384	0.535

χ^2: Chi-square value.

TABLE 3 Mean laboratory test results in the two groups of patients

Blood Tests	**General group (n =32)**	**Heavy group (n =31)**	**t / Z**	P value
NLR	2.88 (1.77-5.55)	8.78 (5.76-25.10)	-4.51	<0.001

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Blood Tests	**General group (n =32)**	**Heavy group (n =31)**	**t / Z**	P value
CRP (μg/L)	26.25 (7.40-75.15)	81.49 (19.40-107.42)	-2.104	0.035
FIB (g/L)	3.58 (3.08-3.99)	3.74 (2.91-4.42)	-0.653	0.514
ALB (mg/L)	36.16±4.64	33.23±3.98	-2.688	0.009

NLR: Neutrophil/lymphocyte ratio; CRP: C-reactive protein; ALB: Albumin.

t: t score

Z: Z score

TABLE 4 Correlation of diabetes, NLR, CRP, ALB with severe COVID-19

Statistics	**Diabetes**	**NLR**	**CRP**	**ALB**
R value	0.25	0.5	0.27	-0.33
P value	0.048	<0.001	0.04	0.009

NLR: Neutrophil/lymphocyte ratio;

CRP: C-reactive protein; ALB: Albumin.

TABLE 5 Regression analysis of risk factors for severe COVID-19

Risk factors	P value	**OR**	**OR (95% CI)**	**OR (95% CI)**
			Lower limit	Upper limit
Diabetes	0.191	0.305	0.052	1.804
NLR	0.015	1.264	1.046	1.526
CRP	0.891	0.999	0.981	1.017
ALB	0.868	0.987	0.841	1.157

OR: Odds ratio; NLR: Neutrophil/lymphocyte ratio;

CRP: C-reactive protein; ALB: Albumin

FIGURE 1 NLR prediction of severe COVID-19

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