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Original Research Article

Evaluation and comparison of utility of neutrophil-to-lymphocyte ratio (NLR) as a diagnostic marker in covid-19 infection

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Abstract

Introduction: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first described during a pneumonia outbreak in Wuhan, has attracted tremendous attention in a short period of time as the death toll and the number of confirmed cases is growing unceasingly. Although molecular testing is the gold standard method of SARS-CoV-2 detection, the existence of the false-negative results presents a major limitation to this method.
Materials and Methods: Our present study aimed to determine the relationship between NLR and COVID-19 patients underwent treatment. The study was an analytical observational with a cross-sectional approach from May 2021 to January 2022 at the SMMH Medical College, Saharanpur, Uttar Pradesh.
Results: COVID-19 infection is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Diabetes mellitus and heart disease comorbid have high morbidity and mortality. Increased Neutrophils to Lymphocyte Ratio (NLR) assist in early screening of disease severity, especially patients admitted in the Intensive Care Unit (ICU). There was a correlation between NLR in COVID-19 patients who were severely ill and admitted in the ICU with p=0.012.
Conclusion: Increased NLR of COVID-19 patients occurs due to infiltration of the innate and adaptive immune system in infected tissue, resulting in decreased circulating lymphocytes. This subsequently increases NLR in COVID-19 patients. This study found a moderate positive correlation between NLR in COVID-19 patients who were severely ill.

Keywords: COVID, NLR, PLR, Haematological parameters

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first described during a pneumonia outbreak in Wuhan, has attracted tremendous attention in a short period of time as the death toll and the number of confirmed cases is growing unceasingly [1]. Despite being lower in its mortality rate compared to SARS (severe acute

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respiratory syndrome) and MERS (Middle East respiratory syndrome), its long incubation period together with the relatively low pathogenicity increases the risk of contagion of SARS-CoV-2 and facilitates its spread [2]. Taking advantage of this, rapid identification of healthy carriers looks to be a great deal not only to diminish viral spread also to impede disease progression [3].

Although the containment measures carried out in China have, at least for the moment, decreased the risk of contagion significantly, most countries have not taken the first and the last steps properly, both steps requiring truthful diagnostic testing [4]. Even though molecular testing of pharyngeal swab specimens is the gold standard method for the etiological detection of SARS-CoV-2, false-negative results missing 30% to 50% of infected cases present a major limitation to this method [5, 6]. In a scramble to fix this challenge before it is too late, an urgent need to find an alternative approach is felt much more than before; one that would identify COVID-19 cases in a simple as well as a rapid way. Earlier studies showed that the alteration in the hematologic parameters not only may help to identify patients with SARS-CoV-2 infection but also may assist clinicians to economically discriminate between the severe and non-severe cases in a timely fashion [7].

The primary objective of the study is to see the Neutrophil-Lymphocyte Ratio, Platelet Lymphocyte Ratio, and other haematological parameters (C-reactive protein (CRP), ferritin, interleukin 6 (IL-6) and plasma D-dimer) alteration of COVID 19 Patients in the Indian scenario. The secondary objective of the study is to assess the follow up haematological parameters of the patients in order to obtain key indicators of disease progression and outcome that will provide guidance for subsequent clinical practice.

Materials and Method

Subject selection: Our present study was a single center prospective study of 80 patients with laboratory-confirmed COVID-19 admitted to ICU in SMMH Medical College, Saharanpur, Uttar Pradesh, were enrolled for the study. The study was an analytical observational with a cross-sectional approach from May 2021 to January 2022.

The diagnosis of COVID-19 was according to the Ministry of Health and Family Welfare (MOHFW) Government of India (GOI) guidelines and confirmed by RTPCR performed on respiratory samples of the patient. All adult cases were included in the study. Patients with chronic lung diseases, haematological disorders, liver disease and malignancy on treatment were excluded from the study.

Patients were divided into asymptomatic (mild) patient and symptomatic (severe) patients based on the Ministry of Health and Family Welfare (MOHFW) Government of India (GOI) Revised Guidelines on clinical management of COVID-19.

Baseline data Collection: The demographic date, the clinical status of the patients during admission, and follow up and haematological findings were recorded. The samples for complete blood count (CBC) and peripheral smears for microscopy examination were collected on the day of admission. The samples were tested for complete blood count on 5-part haematology analyser, and values were noted. Peripheral blood smears were stained by Romanowsky stains, and findings were noted.

Measurement of serum CRP, ferritin, IL-6 and plasma D-dimer levels

Estimation of serum CRP, D-dimer, ferritin and IL-6 were also done in the present study, Estimation of serum ferritin was based on electro chemiluminescence immunoassay (ECLIA) and its biological reference interval was considered as 30-400 μg/L in 20-60 years of male and 13-150 μg/L in 17-60 years of female.

Estimation of serum IL-6 was also based on ELISA and its biological reference interval was

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considered as <6.4 pg/mL. Whereas, Plasma D-dimer was estimated by ECLIA with biological reference interval was considered as <0.5 μg/mL.

Follow up: The patients whose clinical status remained unchanged were re-examined for laboratory indexes. The composite endpoint of the study was a clinical improvement, stability, or deterioration.

Cut-off for Neutrophil to lymphocytes ratio was defined as 4, and patient with NLR >4 would be considered as significant of severity.

Cut-off for Albumin to Globulin ratio was defined as 1, and value less than 1 would be considered as significant of severity.

Oxygen saturation at room air less than 90% at the time of admission would be considered as significant of severity.

Statistical analysis: Data are presented on means and standard deviation (SD). Differences in values between tested confidence intervals were calculated. Correlation analysis used a simple linear correlation. All statistical analyses were carried out by SPSS 15.0 (SPSS Inc., Chicago, USA).

Results

A total of 80 patients that were admitted to the COVID general ward and ICU (those who had saturation levels less than 94% on room air) were included in the study. The outcome was decided based on whether the patient was discharged or succumbed to the disease. Out of the 80 patients, 41 were males and 39 were females. The average age was 41.4 years, the range being 23-65 years. The study had 63 patients who were discharged and 17 patients who succumbed either due to COVID-19 or due to its complications.

Table 1: Comparison of demographics characteristics of among COVID infected patients

Characteristics		**COVID Mild (n=40)**	**COVID Severe (n=40)**	**p Value**
Age (Years)		38 ± 14	39 ± 17	0.12
Gender	Male	18	23	1.02
	Female	22	17	0.62
Smoking		5	6	0.74
Alcohol Drinking		4	4	0.68
Co-morbidities	Diabetes	2	3	-
	Hypertension	1	4	1.08
	Cardiovascular Disease	0	1	-
	Respiratory Disease	0	1	-

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.05).

A total of 80 patients diagnosed with COVID-19 were enrolled in the study and followed up during hospitalization. The average hospitalization duration was 10.24 days. The overall number of in-hospital deaths was 17. Compared to patients in the survivor cohort, in-hospital dead patients were significantly older, but no significant difference was observed in terms of gender. The most common comorbidity was hypertension, followed by diabetes. Except for hypertension, there was no statistically significant difference in the comorbidity frequency between surviving and dead patients. The sign and symptoms data of all patients on admission are shown in Table 2. Several variables were significantly associated with poor outcomes.

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Table 2: Comparison of sign and symptoms of among COVID infected patients

Sign and Symptoms	COVID Mild (n=40)	COVID Severe (n=40)	p Value
Cold and Runny Nose	21	27	0.94
Sore throat	19	22	1.02
Headache	4	6	0.37
Fever	24	26	0.48
Chill	3	5	0.21
Cough	16	21	1.08
Sputum production	9	20	0.89
Chest tightness	8	18	0.46
Mild shortness of breath	10	21	0.38
Dyspnoea	9	23	1.04
Mild Shortness of Breath	16	12	0.51
CT evidence of pneumonia	22	34	0.46

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.05).

Table 3: Presents the laboratory finding in mild and severe group of patients on admission. There were many significant differences in the parameters of laboratory findings between the mild and severe group of patients.

Table 3: Comparison of haematological parameters among COVID infected patients

Haematological Parameters	COVID Mild (n=40)	COVID Severe (n=40)	p Value
Haemoglobin (g/dL)	12.4 ± 3.01	12.2 ± 1.7	0.87
Leukocyte ($\times 10^9$/L)	4.1 ± 1.25	2.9 ± 1.54	0.07
Neutrophil ($\times 10^9$/L)	2.5 ± 0.82	2.7 ± 0.91	0.98
Lymphocyte ($\times 10^9$/L)	1.02 ± 0.48	1.3 ± 0.72	0.42
Monocyte ($\times 10^9$/L)	0.2 ± 0.05	0.3 ± 0.09	0.37
Platelets ($\times 10^9$/L)	1.28 ± 0.22	1.23 ± 0.53	0.56
Prothrombin time (Second)	12 ± 3.7	17 ± 4.1	0.08
NLR	2.3 ±1.53	2.8 ±1.74	0.61
PLR	0.58 ± 0.07	1.59 ± 0.08	0.052
International Normalized Ratio (INR)	1.31 ± 0.58	3.80 ± 0.72	0.072
C-reactive protein (mg/L)	17.48 ± 4.9	22.62 ± 6.4	1.47
Ferritin, ng/mL	273±12.98	735±18.41	0.05
IL-6, pg/mL	19.71±3.16	56.24±9.02	0.01
D-dimer, μg/mL	238.0±11.89	902.5±43.38	0.01

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.05).

Association of NLR, and PLR results with the risk of COVID-19 pneumonia to identify the factors that may affect COVID-19 progression, we obtained the crude odds ratio (OR) after conducting the logistic regression analysis (Table 4). Given that the blood test results were influenced by age and gender, we excluded the possible effects of age and gender and obtained the adjusted OR after the adjustment of gender and age. The results showed that NLR was positively correlated with the risk of COVID-19 (Table 4).

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Table 4: Comparison of clinical findings with different value of NLR

Clinical Parameters	NLR ≤ 2 (n=67)	NLR ≤ 2.1-4 (n=34)	NLR ≥ 4 (n=19)	p Value
Albumin (g/L)	42 ± 3.47	43 ± 4.07	44 ± 2.56	0.27
Sodium (mmol/L)	136 ± 3.42	141 ± 8.01	132 ± 5.77	0.45
Potassium (mmol/L)	4.1 ± 1.78	4.4 ± 2.05	5.7 ± 1.98	0.55
Serum Chlorine (mmol/L)	103 ± 3.53	105 ± 4.51	101 ± 1.24	0.27
Serum urea nitrogen (mmol/L)	3.9 ± 0.97	4.3 ± 1.57	6.01 ± 2.02	0.62
Serum glucose (mmol/L)	5.9 ± 1.51	6.4 ± 1.84	7.2 ± 1.61	0.35
Creatinine (μmol/L)	93.6 ± 6.29	97.4 ± 4.81	109 ± 7.29	1.06
Alanine aminotransferase (U/L)	20.4 ± 5.04	17.8 ± 3.79	26.5 ± 4.97	0.39

Data are presented as mean ± standard deviation (SD), P values are derived from a t test and Significant differences at (p<0.01).

Discussion

COVID-19 is a highly infectious disease which is an ongoing immense threat to global public health. It has been rapidly spreading throughout world with a second peak now evident in many countries [1]. Even though majority patients have self-limiting and mild illness, patients who develop severe or critical cases have a grave prognosis. It had been observed very early after the beginning of COVID-19 pandemic that the neutrophil-to-lymphocyte ratio (NLR) is much higher in severe or critically ill patients as compared to those with milder disease. NLR has been shown to be a reliable indicator to determine disease severity in COVID-19 [2-4].

Many mechanisms have been postulated regarding the response of neutrophils and lymphocytes to corona virus infection. Neutrophils activate the immune system and release reactive oxygen species that can induce cell DNA damage and release the virus from the cells which is then targeted by antibodies. In addition, neutrophils trigger the production of various cytokines and effector molecules. On the other hand, although the viral infection itself triggers lymphocyte response predominantly, the systemic inflammation especially high Interleukin 6 paradoxically decreases the lymphocyte count and resultant cellular immunity. Both these factors result in elevated NLR [4, 5]. Hence a higher NLR predicts the severity of inflammation. The mean age of patients developing COVID is variable.

The mean age in our population was 41.4 years which is lower as compared to other studies [6-8]. Our patients were predominantly males which is keeping with other studies as COVID-19 is known to have a predilection for male gender. The patients in our study were mostly asymptomatic which is similar to data acquired by WHO suggesting up to eighty percent patients maybe asymptomatic [9]. Amongst the symptomatic individuals, the most common clinical symptoms were fever, respiratory symptoms, and myalgias which are comparable to those described by the WHO interim guidance. Our study has shown that mean NLR value significantly increases as disease severity progresses with lowest NLR recorded in asymptomatic and mild disease. This is in line with a Cochrane Meta-analysis Review of twenty Chinese studies which established that NLR is an independent prognostic marker to differentiate severe vs non-severe COVID-19 disease [9-11].

Our results are also consistent with another study with patients in whom severe cases had an NLR of 4.15 vs 2.23 in non-severe cases [10-13]. According to this study, it was suggested that NLR is superior in early prediction of severe and critical illness when compared to widely used scoring severity criteria accepted for pneumonia.

We reviewed the difference in mean NLR between disease categories as well and found that the highest difference in mean NLR is between the asymptomatic and severe patients. This was expected and in keeping with our findings of strong association between mean NLR and severe disease. We established a link between high NLR and mortality as well in our study.

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The 17 patients that succumbed to the disease in our hospital had a mean NLR ratio of 4.15 as

compared to 2.34 in those who recovered which was significantly high. The observations from the earlier study showed that high NLR is an independent risk factor causing in hospital morality in COVID-19 patients [14-16]. Our study results are consistent with the above-mentioned studies supporting the theory that NLR is a cheap, robust and easily available predictor.

Conclusion

In our study we highlight the importance of NLR in COVID-19 patients in predicting disease severity and mortality. The NLR was the most promising predictive factor for critical illness incidence of COVID-19 pneumonia. The early application of NLR will be beneficial to patient classification, management and relief of medical resource shortage. In a developing country like ours where there are resource limited settings, NLR can be used as an effective marker to predict and stratify COVID-19 patients, which in turn would lead to efficient resource utilization. Studying the trends of NLR may also help in predicting the risk for mortality in severely ill patients.

Limitations of the study: Firstly, it was conducted at a single centre. Secondly predominantly male patients were recruited to be admitted if they tested positive for RT-PCR. On the other hand, female patients with mild disease opted to isolate at home and only admitted if they had severe disease symptoms requiring hospital management.

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