

Utility of Neutrophil-Lymphocyte Ratio (NLR) as an Indicator of Disease Severity and Prognostic Marker among Patients with Covid-19 Infection in a Tertiary Care Centre in Bangalore – A Retrospective Study

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ABSTRACT

BACKGROUND

The infection caused due to novel coronavirus 2 can cause wide spectrum of disease from asymptomatic mild disease to life threatening disease. The widespread inflammation is most likely the cause of the adverse outcomes. There are numerous markers of the inflammation which are used to identify the severity and prognosis of the disease. Neutrophil lymphocyte ratio (NLR) is one such marker which is easily available and feasible in all the hospital settings. This study intended to evaluate the NLR as a marker of disease severity and prognosis, in those with Covid-19.

METHODS

This was a retrospective study to determine the utility of NLR as a marker of severity and prognosis among patients with Covid disease. Medical records of 60 patients admitted with mild to moderate Covid-19 disease were reviewed and relevant data was retrieved. The NLR at admission and 72 hours later was noted. High resolution computerised tomography was done and computerised tomography severity score (CT-SS) was calculated. The outcomes of these patients were noted.

RESULTS

Mean NLR at admission in mild disease was 5.6 and in moderate disease was 9.2. This difference was found to be statistically significant. It was also seen that NLR had a positive co-relation with CT severity score, duration of hospital stay, C-reactive protein (CRP) and D-dimer. For predicting mortality, NLR with cut off of 6.6 had a sensitivity of 100 % and a specificity of 66.07 %. CRP with cut off of 6.8 had sensitivity of 100 %, specificity of 76.79 %. D-dimer with cut off of 1.6 had specificity of 78.58 %. For predicting severity, NLR with cut off of 6.6 had sensitivity of 75 % and specificity of 80 %. CRP with cut off of 7.9 had sensitivity of 65 % and specificity of 100 %. D-dimer with cut off of 1.3, had sensitivity of 90 % and specificity of 97.5 %.

CONCLUSIONS

The study shows that neutrophil-lymphocyte ratio is a good indicator of disease severity and has prognostic significance in Covid-19. There is a positive correlation with high-resolution computed tomography (HRCT) chest score and other markers of inflammation among patients with Covid-19. Serial monitoring of NLR can be utilised as a surrogate to HRCT chest to determine disease severity whenever the latter is not available. This can ensure early intervention and help prevent mortality.

KEYWORDS

Neutrophil-Lymphocyte Ratio, Severity of Covid

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BACKGROUND

From the first recorded case of Covid-19 pneumonia in 2019 to present day, millions around the world have fallen victim to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). On one hand, it can be mild while on the other, it can be severe and life threatening. 1.5 million lives have been lost. Those who survived continue to endure a wide variety of complaints which are now termed as the post Covid syndrome.¹

The inflammatory cytokine storm has been recognised as the primary cause of death, which is defined by the uncontrolled release of pro-inflammatory cytokines.² Circulating biomarkers that can represent inflammation and immune status are potential predictors for the prognosis of Covid-19 patients. CRP, serum ferritin and lactate dehydrogenase (LDH) are widely used to monitor the disease activity in Covid-19.³

High resolution CT scan of chest is highly specific and sensitive in Covid 19 diagnosis and has revolutionised the management of Covid infection. CT severity scores (CT-SS) are also employed to predict the severity of Covid-19.⁴

Peripheral white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR) are indicators of the systematic inflammatory response, that are widely investigated as useful predictors for the prognosis of patients with viral pneumonia. Hence, these may be adopted in the present scenario for prognostication in Covid-19.

Currently, the Indian peninsula is the second worst hit area. In densely populated developing nations, resources and health care infrastructure are sparse and their judicious utilisation is paramount. HRCT thorax and inflammatory markers may not be readily available or affordable in remote areas. The neutrophil lymphocyte ratio derived from this may be utilised and hence this study intended to evaluate the NLR as a marker of disease severity and prognosis in those with Covid-19.¹

METHODS

This was a retrospective study conducted at Ramaiah Medical College Hospital, Bangalore. Data from 60 patients admitted to the wards with mild and moderate Covid-19 infection confirmed by real-time reverse transcription-polymerase chain reaction (RT-PCR) during 1st August 2020 to 1st September 2020 for Covid-19 was retrieved from the medical records while ensuring confidentiality. Those with known haematological illnesses, with known HIV positive status, those on chemotherapeutic drugs, long term glucocorticoids and pregnant women were excluded as these conditions affect the NLR.

The patients were grouped into mild and moderate categories as per the existing Government of India Guidelines on Covid-19.

Mild category included patients having symptoms of Covid-19 who were clinically stable and whose finger pulse oximetry revealed oxygen saturation more than 95 percent on room air. Moderate category were the patients with symptoms and having oxygen saturation between 90 - 94

percent on room air or who had respiratory rate more than or equal to 24 cycles per min. Clinical details such as complete history & examination and investigations including complete blood count, C-reactive protein (CRP), D-Dimer, serum ferritin, renal function tests, liver function test and high resolution CT scan of chest (HRCT thorax) were noted.

According to the lung anatomical structure, 18 segments of both lungs were divided into 20 regions, in which the posterior apical segment of the left upper lobe was subdivided into apical and posterior segmental regions, while the anteromedial basal segment of the left lower lobe was subdivided into anterior and basal segmental regions. The lung opacities in all of these 20 regions were subjectively evaluated on chest CT using a system attributing scores of 0, 1 and 2 if parenchymal opacification involved 0 %, less than 50 % or equal or more than 50 % of each region. The CT-SS was defined as the sum of the individual scored in the 20 lung segment regions, which may range from 0 to 40 points. A study was conducted by Rang et al. regarding CT scan finding in Covid-19 infection and the severity. According to this study, it was determined that the CT severity score threshold of 19.5 could identify severe Covid-19, with a sensitivity of 83.3 percent and a specificity of 94 percent, resulting in a negative predictive value of 96.3 percent.⁴

The sample size was calculated based on the study conducted by Jingyuan Lie et al. Neutrophil-Lymphocyte ratio predicts severe illness with Novel Coronavirus in early stage. Journal of Translational Medicine volume 18, Article number: 206 (2020). The present study could find the correlation between NLR with CT severity score, clinical profile and the outcome. Considering the effect size of 0.20 (medium), the power of the study (1-beta) as 80 %, type 1 error of 5 %, the sample size was calculated using G*Power software version 3.1.9.2. F tests – linear multiple regression, R² deviation from zero. Analysis – A priori: Compute required sample size. Input: Effect size $f^2 = 0.2$, alpha error = 0.05, power (1-betaprob) = 0.80, number of predictors = 6. Output: Noncentrality parameter lambda = 14.000000, critical F = 2.3583183. Numerator df = n5, denominator df = 64. Total sample size = 60, actual power = 0.8008234.

Statistical Analysis

Inferential and descriptive analysis was done. Continuous data was presented as mean + / - SD and categorical data as frequency (%). P value of less than 0.05 was considered as statically significant. Student's t-test was used for continuous scale on metric parameters. The significance of study parameters on categorical scale between two groups in non-parametric setting for qualitative data were analysed using chi square test. Median and interquartile range was calculated for the inflammatory markers and test of significance used was Mann-Whitney U test.

ROC curve was done to identify cut-off value, area under the curve (AUC), sensitivity and specificity of haematological parameters in predicting severity and mortality was analysed. Correlation between parameters was done using Pearson correlation coefficient. The statistical analysis were performed using Statistical Package for the Social Sciences (SPSS) 18.

RESULTS

Out of 60 patients, 40 were males and 20 were female patients. 40 (66.6 %) patients were having mild Covid illness (28 males and 12 females). 20 (33.4 %) were having moderate Covid illness (12 males and 8 females).

There was no statistically significant difference in the mean age of presentation in the two different categories of illness with the mean age among those with mild and moderate Covid-19 being 56 years and 59 years respectively. In the mild category, 47.5 % patients (N = 19) were diabetics whereas in the moderate category 60 % patients (N = 12) were diabetics. This difference was statistically significant. Systemic hypertension was observed in 17 patients (47.5 %) with mild disease and 13 patients (65 %) with moderate disease. This difference was statistically significant. (Table 1). There was a statistically significant difference found between mild Covid and moderate Covid with respect to NLR, CRP, d-Dimer and CT severity score. There was a positive correlation between NLR and CT score (0.133). There was a positive correlation between NLR and duration of hospital stay (0.025) but it was not statistically significant. There was a positive correlation between NLR and d-Dimer (0.419) and it was statistically significant. There was a positive correlation between NLR and CRP (0.324) and it was statistically significant (Table 3). For predicting mortality, AUC for NLR was 0.792, for CRP it was 0.897, for D-dimer it was 0.897 (Figure 1). For predicting severity, AUC for NLR was 0.781, CPR was 0.875, D-dimer was 0.941 (Figure 2). For predicting mortality, NLR with cut off of 6.6 sensitivity of 100 %, specificity of 66.07 %. CRP with cut off of 6.8 sensitivity of 100 % and specificity of 76.79 %. D-dimer with cut off of 1.6, specificity of 78.58 %. (Table 4)

For predicting severity, NLR with cut off of 6.6 sensitivity of 75 % and specificity of 80 %. CRP with cut off of 7.9 sensitivity of 65 % and specificity of 100 %. D-dimer with cut off of 1.3, sensitivity of 90 % and specificity of 97.5 %. (Table 4).

It was found that among 20 patients admitted with moderate disease, 4 patients did not survive. It was found that among these patients, mean NLR at admission was 15.2. Whereas, mean NLR at admission among patients who survived was 6.5. This difference was found to be statistically significant.

NLR at 72 hours post admission was compared among patients who survived and patients who did not survive. It was seen that among patients who survived mean NLR after 72 hours was 7.2. Mean NLR after 72 hours among patients who did not survive was 18.5. This difference was statistically significant (P < 0.00). (Table 5).

Characteristics	Mild Covid-19 Disease	Moderate Covid-19 Disease	P-Value
Age (years)	56 (SD + / - 15)	59 (SD + / - 12)	P = 0.12
Diabetes	19 (47.5 %)	12 (60 %)	P = 0.002
Hypertension	17 (42.5 %)	13 (65 %)	P = 0.003
Hypothyroidism	6 (15 %)	3 (15 %)	P = 0.42

Table 1. Demographic Details, Comorbidities among the Patients

Test of significance for age – students t-test
Test of significance for diabetes, hypertension, hypothyroidism – Z test

	Mild Covid-19		Moderate Covid-19		P Value
	Median	Interquartile Range (IQR)	Median	Interquartile Range (IQR)	
NLR	4.01	7.5 - 1.2	9.59	14.2 - 7.5	0.031
CT score	17.53	24 - 10	31.55	34 - 27	0.002
CRP	3.82	7.43 - 1.5	10.10	24.5 - 5.6	0.001
D-DIMER	0.75	1.15 - 0.23	2.42	3.85 - 1.42	< 0.001

Table 2. Comparison of Median and Interquartile Range for NLR, D-Dimer, CRP, CT Severity Score, according to Severity of Covid (Test of Significance - Mann Whitney U Test)

Parameters		NLR
CT score	Pearson correlation	0.133
	P-value	0.312
Duration of hospital stay	Pearson correlation	0.025
	P-value	0.855
D-DIMER	Pearson correlation	0.419
	P-value	0.001
CRP	Pearson correlation	0.324
	P-value	0.011

Table 3. Correlation of NLR with CT Severity Score, Duration of Hospital Stay, d-Dimer, and CRP

Parameters	AUC	95 % CI
NLR	0.792	0.668 to 0.886
CRP	0.897	0.792 to 0.961
D-DIMER	0.897	0.792 to 0.961

Table 4. ROC Curve Comparison of NLR, CRP, D-Dimer, in Predicting Mortality of Covid-19 Disease

Parameters	AUC	95 CI
NLR	0.781	0.656 to 0.878
CRP	0.875	0.764 to 0.946
D-DIMER	0.941	0.849 to 0.986

Table 5. ROC Curve Comparison of NLR, CRP, D-Dimer in Predicting Severity of Covid-19 Disease

For Predicting Severity	Cut off	Sensitivity	Specificity	PPV	NPV
NLR	> 6.6	100.00	66.07	17.4	100.0
CRP	> 6.8	100.00	76.79	23.5	100.0
D-Dimer	> 1.6	100.00	78.58	25.0	100.0
For predicting mortality	Cut off	Sensitivity	Specificity	PPV	NPV
NLR	> 6.6	75	80	65.2	86.5
CRP	> 7.9	65	100	100	85.1
D-Dimer	> 1.3	90	97.5	94.7	95.1

Table 4. NLR, CRP, D-Dimer for Predicting Severity and Mortality

	Survivors (N = 56)	Non-Survivors (N = 4)	P-Value
Neutrophil lymphocyte ration at admission	6.5 (SD + / - 2.5)	15.2 (SD + / - 4.5)	P < 0.001
Neutrophil lymphocyte ratio after 72 hours	7.6 (SD + / - 3.2)	18.5 (SD + / - 3.2)	P < 0.001

Table 5. Neutrophil Lymphocyte Ratio among Survivors and Non-Survivors (Mann Whitney U Test)

DISCUSSION

The present study included 60 Covid-19 patients admitted to the isolation wards. It was observed that patients having diabetes and hypertension are more likely to have severe disease than those who did not have these comorbidities. There was a significant difference in the mean NLR of mild and moderate Covid-19 patients with mean NLR being higher in the moderate Covid-19 group. Similar to this are the results of a meta-analysis by Abigail. S.Y Chan et al. with 20 studies and 3508 patients which identified that severe Covid-19 patients had a higher NLR than non-severe patients.⁵ This suggests that higher the NLR, the more severe the disease.

Further more information was attained via serial assessment of this parameter. Patients in whom NLR increased during the course of illness are more likely to worsen than the patients in whom it did not. This study found that the mean NLR at admission as well as the mean NLR after 72 hours were significantly higher in non survivors when compared to survivors. Wenjing Ye et al. reported that when compared to survivors, the deceased Covid-19 patients had a significantly higher NLR and D-dimer values at admission. They also described a significant increasing trend in NLR and D-Dimer values during hospital stay in the deceased. Hence, serial monitoring of NLR can alarm the treating physicians regarding the need for escalation of the treatment and heightened surveillance measures to recognise complications.⁶

CT severity scores are good indicators of severity of the disease. In this study, it was seen that NLR had a positive correlation with CT severity score. In many places where high resolution CT chest facilities are not available, NLR can act as a surrogate marker of CT severity scores. Similarly, Yang Zhang et al. in their study on Covid-19 patients in China concluded that NLR and CT severity score positively correlated and postulated that NLR reflected the extent of lung injury in Covid-19.⁷

According to the present study, there is a positive correlation between NLR and duration of hospital stay. This was comparable to the study by Jin Hu et al. who found that length of hospital stay was significantly associated with increasing NLR quartiles.⁸

There are several studies which denote NLR to be a good early warning factor for identifying severe disease or progression to critical illness and highlight its prognostic significance. According to a study conducted by Ai Ping et al. increased age and NLR can be considered independent biomarkers for indicating poor clinical outcomes. Elevated NLR and increased age were significantly associated with illness severity.² A meta-analysis by Furong Zeng et al. highlights the association of inflammatory markers with the severity of Covid-19 and concludes that measurement of inflammatory markers might assist clinicians to monitor and evaluate the severity and prognosis of Covid-19.⁹ Gustavo D et al. documented that NLR is an affordable alternative to cytokines because in routine clinical practice, every admitted patient is ordered a complete haemogram. NLR is a useful measure of systemic inflammation for screening Covid-19 patients and may be used as a useful indicator of a poor prognosis at the initial moment of hospitalisation.¹ A study conducted in Italy by Arturo Ciccullo et al. shows that NLR may be a rapid, widely available, useful prognostic factor in the early screening of critical illness in patients with confirmed Covid-19. This study also showed that clinical improvement was predicted by younger age and NLR of less than 3.¹⁰ Jingyuan Liu et al. conducted a study in China for screening predictive factors useful for identifying critical Covid-19 illness. They found that NLR was the most important prognostic factor for disease progression followed by the age of the patient.¹¹

Neutrophils are peripheral white blood cells which are increased during acute infection and inflammation. The role of these cells in the pathogenesis of Covid-19 disease and

its complications can be explained by the phenomenon of NETosis. Neutrophil extracellular traps (NETs) are extracellular webs of deoxyribonucleic acid (DNA) or histones released by neutrophils to control infections. The process of generation of NETs is called NETosis which is a type of cell death.

NETosis has been implicated in worsening inflammatory response as well as turning the haemostatic milieu in favour of thrombosis. On the other hand, there is a significant reduction in the circulating T and B lymphocytes in Covid-19 especially with severe and critical disease. This immunological dysregulation with neutrophilia and lymphopenia has been theorised to have a role in the occurrence of cytokine storm and resultant complications like acute respiratory distress syndrome (ARDS) and multi organ failure.^{12,13}

In this study, it was seen that NLR, CRP and D-Dimer had good ability to predict severity and mortality. NLR was comparable with CRP and D-dimer. NLR is a simple marker, economically feasible and available in all the medical centres.

Hence it can be used as a surrogate marker of severity of Covid illness. According to the study conducted by Jingyuan Liu et al. NLR had an area under receiver operating characteristic of 0.849 (95 % confidence interval [CI], 0.707 to 0.991) in the derivation cohort and 0.867 (95 % CI 0.747 to 0.944) in the validation cohort. Clinical impact curves showed that the NLR had high standardised net benefit.¹¹

CONCLUSIONS

The above study demonstrates that neutrophil-lymphocyte ratio is a good measure of disease severity in Covid-19 and has prognostic significance. Serial monitoring of NLR can be used to observe all Covid-19 patients, especially those with moderate disease, to identify patients who may require additional monitoring so that aggressive measures may be instituted early to reduce mortality. With no visible end to this pandemic, investing on already available resources including lab parameters will prove advantageous in the long run and will help save both men and money.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

Financial or other competing interests: None.

Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

REFERENCES

- [1] WHO coronavirus disease (Covid-19) dashboard. Geneva: World Health Organisation 2020. <http://Covid19.int>.
- [2] Pimentel GD, Vega MCMD, Laviano A. High neutrophil to lymphocyte ratio as a prognostic marker in Covid-19 patients. Clin Nutr ESPEN 2020;40:101-102.
- [3] Yang AP, Liu JP, Tao WQ, et al. The diagnostic and predictive role of NLR, d-NLR and PLR in Covid-19 patients. Int Immunopharmacol 2020;84:106504.

- [4] Yang R, Li X, Liu H, et al. Chest CT severity score: an imaging tool for assessing severe Covid-19. *Radiol Cardiothoracic Imaging* 2020;2(2):e200047.
- [5] Chan AS, Rout A. Use of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in Covid-19. *J Clin Med Res* 2020;12(7):448-453.
- [6] Ye W, Chen G, Li X, et al. Dynamic changes of D-dimer and neutrophil-lymphocyte count ratio as prognostic biomarkers in Covid-19. *Respir Res* 2020;21(1):169.
- [7] Zhang Y, Wu W, Du M, et al. Neutrophil-to-lymphocyte ratio may replace chest computed tomography to reflect the degree of lung injury in patients with corona virus disease 2019 (Covid-19). *Research Square* 2020.
- [8] Hu J, Zhou J, Dong F, et al. Neutrophil-to-lymphocyte ratio on admission predicts in-hospital mortality in patients with Covid-19. *Research Square* 2020.
- [9] Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of Covid-19: a meta-analysis. *Int J Infect Dis* 2020;96:467-474.
- [10] Ciccullo A, Borghetti A, Verme LZD, et al. Neutrophil-to-lymphocyte ratio and clinical outcome in Covid-19: a report from the Italian front line. *Int J Antimicrob Agents* 2020;56(2):106017.
- [11] Liu J, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *J Transl Med* 2020;18(1):206.
- [12] Wang J, Jiang M, Hen X, et al. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: review of 3939 Covid-19 patients in China and emerging pathogenesis and therapy concepts. *J Leukoc Biol* 2020;108(1):17-41.
- [13] Mozzini C, Girelli D. The role of neutrophil extracellular traps in Covid-19: only an hypothesis or a potential new field of research? *Thromb Res* 2020;191:26-27.