Original Research Paper

General Medicine

CLINICAL STUDY OF NEUTROPHIL TO LYMPHOCYTE RATIO AS PROGNOSTIC MARKER OF SEVERITY AND MORTALITY IN COVID 19 PATIENTS ADMITTED AT A TERTIARY HEALTHCARE CENTER DURING SECOND WAVE OF EPIDEMIC.

Priya Waghmare	Junior Resident, Department of Medicine , A.C.P.M. Medical College, Dhule.
Puneet Patil*	Professor, Department of Medicine , A.C.P.M. Medical College, Dhule.*Corresponding Author
Pranjali Shinde	Professor, Department of Physiology, A.C.P.M. Medical College, Dhule.
Swapnil Sarode	Junior Resident, Department of Medicine , A.C.P.M. Medical College, Dhule.

ABSTRACT
Background: A high neutrophil to lymphocyte ratio (NLR) indicates an imbalance of the inflammatory response and can be used as a surrogate marker of disease severity in infectious diseases. Present study was aimed to study the prognostic value of Neutrophil to lymphocyte ratio as a marker of severity and mortality in covid 19 patients during second wave. **Material and Methods:** Present study was single-center, Retrospective Cross-sectional study, conducted from case records of patients with clinical manifestations like fever and pulmonary symptoms and RT-PCR positive for SARS-CoV-2. NLR ratio was calculated using as absolute count of Neutrophils by absolute count of Lymphocytes. **Results:** 88 case-records satisfying study criteria were considered for this study. Majority cases were male (59.09 %), from 50-59 years (28.41 %) & 60-69 years (23.86 %) age group. Clinically & radiologically majority cases had moderate COVID (50 %) as compared to severe COVID (31.82 %) & mild COVID (18.18 %). Mortality was 14.77 %. Neutrophil to Lymphocyte ratio (NLR) was significantly raised in Severe COVID cases (7.09 ± 3.64) as compared to Moderate (5.21 ± 2.24) & Mild (3.96 ± 1.52) COVID disease severity, difference was statistically significant (p<0.05). Hematological parameters (absolute neutrophil count, absolute lymphocyte count, total WBC count & absolute neutrophil count/ absolute lymphocyte count ratio) were significantly raised in COVID cases died during treatment as compared to those who survived, difference was statistically significant (p<0.05). **Conclusion:** NLR is a useful parameter which may be used along with regular parameters to predict severity, progression of disease & mortality in COVID 19 patients.

INTRODUCTION

COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), a novel virus in the same cluster as the SARS-CoV-1 and MERS-CoV, that previously caused outbreaks in 2003 and 2012 [1,2]. It is a highly communicable infectious disease. It has rapidly and widely spread worldwide[3] and was declared as pandemic in March 2020 by World Health Organization[4]. COVID -19 presents with a wide range of symptoms from being mild and self-limiting to severe life threatening acute respiratory distress syndrome (ARDS) and multi-organ failure.[5]

Inflammation plays a major role in development and progression of COVID-19 disease and people infected with COVID-19 are known to have an immune system that is dysregulated and can cause abnormal immune response.[6,7] Neutrophilia and lymphocytopenia are physiological responses of the innate immune system to systemic inflammation. The increase in proinflammatory cytokines with T cell lymphopenia predisposes Covid 19 patients to cytokine storm resulting in severe Covid 19 pneumonia progressing to ARDS and eventually multi organ dysfunction.[8,9,10,11]

Therefore, a high NLR indicates an imbalance of the inflammatory response and can be used as a surrogate marker of disease severity in infectious diseases, such as sepsis[12]. Different immunity parameters in the human body have been identified to combat the disease[13,14]which can be employed to assess the severity of disease but these tests are often expensive and most of them are time consuming resulting in time delay, jeopardising the patient's treatment. Present study was aimed to study the prognostic value of Neutrophil to lymphocyte ratio as a marker of severity and mortality in covid 19 patients during second wave.

MATERIAL AND METHODS

Present study was single-center, Retrospective Cross-sectional study, conducted in department of general medicine, at ACPM Medical College and Hospital, Dhule, India. Case records of patients of Covid 19 hospitalized in covid ward from April 2021 to June 2021 were studied. Study approval was obtained from institutional ethical committee.

Inclusion criteria
* Case records of patients with clinical manifestations like fever and pulmonary symptoms and Real-time fluorescent reverse

transcription-polymerase chain reaction (RT-PCR) of respiratory samples (nasal/oropharyngeal swab or tracheal secretions) positive for SARS-CoV-2 were studied

Exclusion criteria
* Patients age less than 18 yrs.
* Pregnant females.
* Patients with negative detection of novel coronavirus nucleic acid.
* Patients with previous history of lung disorders.
* Patients on immunosuppressive drugs (including long-term steroids).
* Patients with haematological disorders.

Patient's parameters such as demographic data (gender and age), comorbidities (Diabetes mellitus, Hypertension, Coronary Artery Disease), clinical manifestations of Covid 19 (fever, chest pain, cough, shortness of breath) investigations like complete blood count, differential cell count, neutrophil to lymphocyte ratio, Renal function tests, Liver function tests, Serum electrolytes, C-reactive proteins, D-dimer, HRCT Severity index were recorded. NLR ratio is calculated using as absolute count of Neutrophils by absolute count of Lymphocytes. Normal value of NLR is in range of 1.0-2.3.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables were tested using chi- square test or Fisher exact test as applicable. P value less than 0.05 was considered as statistically significant.

RESULTS

88 case-records satisfying study criteria were considered for this study. Majority cases were male (59.09 %), from 50-59 years (28.41 %) & 60-69 years (23.86 %) age group. Clinically & radiologically majority cases had moderate COVID (50 %) as compared to severe COVID (31.82 %) & mild COVID (18.18 %). Mortality was 14.77 %.

Table 1- General characteristics

Characteristics	No. of patients	Percentage
Age groups (in years)		
30-39	5	5.68%

40-49	20	22.73%
50-59	25	28.41%
60-69	21	23.86%
>70	17	19.32%
Mean age (mean±SD)	59.25 ± 13.63	
Gender		
Male	52	59.09%
Female	36	40.91%
COVID severity		
Mild	16	18.18%
Moderate	44	50.00%
Severe	28	31.82%
Clinical outcome		
Discharged	75	85.23%
Death	13	14.77%

Neutrophil to lymphocyte ratio (NLR) was significantly raised in Severe COVID cases (7.09 ± 3.64) as compared to Moderate (5.21 ± 2.24) & Mild (3.96 ± 1.52) COVID disease severity, difference was statistically significant (p<0.05).

Table 2: NLR as per disease severity (severity correlation).

Outcome	NLR	p value
Mild	3.96 ± 1.52	0.001
Moderate	5.21 ± 2.24	
Severe	7.09 ± 3.64	

Hematological parameters (absolute neutrophil count, absolute lymphocyte count, total WBC count & absolute neutrophil count/ absolute lymphocyte count ratio) were significantly raised in COVID cases died during treatment as compared to those who survived, difference was statistically significant (p<0.05).

Table 3. Comparison of hematological parameters among survived & died

Study Parameter	Survived cases	Died cases	p value
Absolute Neutrophil Count (X103/µl)	5.75 ± 3.22	9.32 ± 5.50	<0.001
Absolute Lymphocyte Count (X103/µl)	1.96 ± 1.5	1.12 ± 0.42	<0.001
Total WBC count (X103/µl)	7.28 ± 3.60	11.28 ± 5.43	<0.001
Absolute Neutrophil count/ Absolute Lymphocyte count ratio	4.97 ± 2.80	11.93 ± 4.72	<0.001

DISCUSSION

Second wave of Covid 19 started in middle of March 2021 and was supposed to be caused by several mutants of SARS-COV2 virus and its presentation was slightly different from the first wave with newer symptoms involving gastrointestinal system, with more cases of sudden onset breathlessness, with predominant involvement of younger age group, with less comorbidities, more oxygen requirement and ICU admissions.[15]

Shetty BA et al.,[16] studied 1977 patients with a male: female ratio of 1:0.62. Fever, dyspnoea, and cough were noted as the major symptoms prevalent among patients who succumbed to death when compared to those who survived (P < 0.00001). Statistically significant variables noted between the groups were age, total leukocytes count (TLC), neutrophil, lymphocytes, all the comorbidity variables, and the asymptomatic status. Variables identified as significant predictors of disease outcomes were TLC, neutrophils, lymphocytes, and N-L ratio. Most of the subjects belonging to the mortality group required oxygen and other intensive care unit facilities when compared to the survival group (P < 0.00001)

Rathod BD et al.,[17] noted that mean NLR was significantly higher in the severe COVID-19 group as compared to the mild/moderate group and in deceased as compared to discharged cases. ROC curve analysis revealed NLR to be an excellent predictor of disease severity as well as a prognostic parameter for risk of death. NLR was found to be a significant independent positive predictor for contracting the severe disease (Odd's ratio 1.396, 95% CI=1.112-1.753, p=0.004) and mortality (Odd's ratio 1.276, 95% CI=1.085-1.499, p=0.003).

In study by Divyarani MN et al.,[18] majority patients (76.5%) were asymptomatic (Mild). Amongst symptoms, fever was the most common symptom. Mean NLR in mild cases were 2.75+ 1.22, moderate cases showed 13.10+6.40 and in severe cases 28.85+15.66. NLR increased in severe cases compared to mild and moderate severity and was statistically significant (p<0.0001).

Rajnish K et al,[19] studied 117 admitted patients 74 patients were male, and 43 were female, with a mean age of 49.11 ± 18.63 years. Mild patients had a mean NLR of 4.76 (2.03 to 7.77), the moderate disease had a mean NLR of 5.21 (2.00 to 9.88), and severe disease had a mean NLR of 6.19 (0.2 to 25) at admission. Our results show a strong relationship between higher NLR values with mortality (AUC = 97.4) with a sensitivity of 92.3% and specificity of 86.6% and is statistically significant.

In a systematic review and meta-analysis. Sarkar PG et al.,[20] included 24 studies involving 4,080 patients reported the prognostic value of NLR for severe COVID-19. The pooled sensitivity (SEN), specificity (SPE), and area under the curve were 0.75 (95% CI 0.69–0.80), 0.74 (95% CI 0.70–0.78), and 0.81 (95% CI 0.77–0.84). Fifteen studies involving 4,071 patients reported the prognostic value of NLR for mortality in COVID-19. The pooled sensitivity (SEN), specificity (SPE), and area under curve were 0.80 (95% CI 0.72–0.86), 0.78 (95% CI 0.69–0.85), and 0.86 (95% CI 0.83–0.89). Evaluating the NLR at admission can assist treating clinicians to identify early the cases likely to worsen. This would help to conduct early triage, identify potentially high-risk cases, and start optimal monitoring and management, thus reducing the overall mortality of COVID-19.

Ulloque-Badaracco JR et al.,[21] conducted a systematic review and meta-analysis, analysed 61 studies (n = 15 522 patients), 58 cohorts, and 3 case-control studies. An increase of one unit of NLR was associated with higher odds of severity (OR 6.22; 95%CI 4.93 to 7.84; P < .001) and higher odds of all-cause mortality (OR 12.6; 95%CI 6.88 to 23.06; P < .001). In our sensitivity analysis, we found that 41 studies with low risk of bias and moderate heterogeneity (I2 = 53% and 58%) maintained strong association between NLR values and both outcomes (severity: OR 5.36; 95% CI 4.45 to 6.45; P < .001; mortality: OR 10.42 95% CI 7.73 to 14.06; P = .005). Higher values of NLR were associated with severity and all-cause mortality in hospitalised COVID-19 patients.

NLR has been reported to prognosticate mortality, progression to severe disease, risk of intubation, risk of severe disease in intubated patients, days intubated, ICU admission, and longer intensive care unit (ICU) admission.[22,23] Furthermore, patients with higher NLR appear to have more comorbidities and, therefore, are more prone to severe COVID-19 (36). Even in patients with comorbidities, NLR might maintain its predictive ability for COVID-19 severity.[24,25] Hematological parameters, being a simple investigation and widely available, is a lesser strain on medical and financial resources and have been observed to predict outcomes in COVID 19 patients.

CONCLUSION

Early identification of the prognostic factors for severe disease can facilitate rapid access to intensive care units when required. NLR is cost-effective, readily available, and easy to calculate laboratory marker. NLR is a useful parameter which may be used along with regular parameters to predict severity, progression of disease & mortality in COVID 19 patients.

REFERENCES

1. Sun P, Lu X, Xu C, Sun W, Pan B. Understanding of COVID-19 based on current evidence Med Virol. 2020;92(6):548–51. https://doi.org/10.1002/jmv.25722.
2. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication and pathogenesis. J Med Virol. 2020;92(4):418–23.
3. Zhang J, Litvinova M, Wang W et al. Evolving epidemiology and transmission dynamics of coronavirus disease 2019 outside Hubei province, China: a descriptive and modelling study. The Lancet Infectious Diseases. 2020;20(7):793-802. doi:10.1016/s1473-3099(20)30230-9.
4. World Health Organization. Coronavirus disease (COVID-19) pandemic. 2020; https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed 19 August, 2020.
5. Ciccullo A, Borghetti A, Dal Verme LZ, Tosoni A, Lombardi F, Garcovich M, 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. doi: 10.1016/j.ijantimicag.2020.106017.
6. Rabinowich H, Cohen R, Bruderman I, Steiner Z, Klajman A. Functional analysis of

mononuclear cells infiltrating into tumors: lysis of autologous human tumor cells by cultured infiltrating lymphocytes. Cancer Res. 1987;47(1):173-7.

7. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020;71(15):762- 768.

8. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-273. pubmed

9. Wang K, Chen W, Zhou Y-S, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. bioRxiv. 2020.

10. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, Xie C, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis. 2020.doi

11. Liu J, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts severe illness patients with 2019 novel coronavirus in the early stage. medRxiv. 2020.doi

12. Song H, Kim HJ, Park KN, Kim SH, Oh SH, Youn CS. Neutrophil to lymphocyte ratio is associated with in-hospital mortality in older adults admitted to the emergency department. Am J Emerg Med. 2020; S0735-6757(20):30044-30049.

13. Qin C, Zhou L, Hu Z et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clinical Infectious Diseases. 2020;71(15):762-768. doi:10.1093/cid/ciaa248.

14. Ponti G, Maccaferri M, Ruini C, Tomasi A, Ozben T. Biomarkers associated with COVID-19 disease progression. Crit Rev Clin Lab Sci. 2020;57(6):389-399.

15. Jalali S.F., Ghassemzadeh M., Mouodi S., Javanian M., Akbari Kani M., Ghadimi R., `Bijani A. Epidemiologic comparison of the first and second waves of coronavirus disease in Babol, North of Iran. Caspian J Intern Med. 2020 Fall;11(Suppl 1):544–550.

16. Shetty BA, Shilpa TA, Rajanna AH, Ravi K, Rao M, Bhat B. Association of neutrophil-lymphocyte ratio with clinical outcome of COVID-19 patients: A prospective study. APIK J Int Med 2021;9:215-20.

17. Rathod B D, Amle D, Khot R S, et al. (March 31, 2022) Neutrophil-to-Lymphocyte Ratio as a Predictor of Disease Severity and Mortality in Coronavirus Disease 2019: Prospective Study From Central India. Cureus 14(3): e23696.

18. Divyarani MN, Sreelakshmi K, Ramya BS, Dayananda BS, ROLE OF NEUTROPHIL TO LYMPHOCYTE RATIO IN COVID 19 PATIENTS, Journal of cardiovascular disease research, VOL13, ISSUE 05, 2022

19. Rajnish Kaushik, Anubhav Gupta, Devyani Thakur, MPS Chawla, Amit Suri, Parkash Chugh Neutrophil to Lymphocyte Ratio: A Simple, Quick, and Independent Predictor of Severity and Outcome in COVID-19 Disease, JIACM 2022; 23 (3-4): 86-90

20. Sarkar PG, Pant P, Kumar J, Kumar A. Does Neutrophil-to-lymphocyte Ratio at Admission Predict Severity and Mortality in COVID-19 Patients? A Systematic Review and Meta-analysis. Indian J Crit Care Med 2022;26(3):361–375.

21. Ulloque-Badaracco JR, Ivan Salas-Tello W, Al-kassab-Córdova A, et al. Prognostic value of neutrophil-to-lymphocyte ratio in COVID-19 patients: A systematic review and meta-analysis. Int J Clin Pract. 2021;75:e14596.

22. Yang A-P, Liu J-P, Tao W-Q, Li H-M. The Diagnostic and Predictive Role of NLR, D-NLR and PLR in COVID-19 Patients. Int Immunopharmacol (2020) 84:106504.

23. Karaman M, Balta S, Ahmet AYS, Cakar M, Naharci I, Demirkol S, et al. The Comparative Effects of Valsartan and Amlodipine on vWf Levels and N/L Ratio in Patients With Newly Diagnosed Hypertension. Clin Exp Hypertens (2013) 35(7):516–22.

24. Tatum D, Taghavi S, Houghton A, Stover J, Toraih E, Duchesne JJS. Neutrophil-To-Lymphocyte Ratio and Outcomes in Louisiana Covid-19 Patients. Shock (2020) 54(5):652–8.

25. Karimi A, Shobeiri P, Kulasinghe A, Rezaei N (2021) Novel Systemic Inflammation Markers to Predict COVID-19 Prognosis. Front. Immunol. 12:741061