(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 5, Issue 1, Page No: 1019-1024
January-February 2022

IJMSCR

Study of Role of Neutrophil/lymphocyte Ratio (NLR) in the Diagnosis of Corona Virus 2019 Disease (COVID-19) in Tertiary Care Hospital Of MadhyPradesh

[1]Dr. Thakur Dilbagh singh,[2]Dr.Ateeq Ur Rehman ,[3]Dr.Mohd Mustufa Malik,[4]Dr.Saumin Dhaneriya[5]Dr.Mahendra Chauhan[6]Dr.Sandeep Patel

[1]Senior Resident GMC&Hamidia Hospital Bhopal, Medicine Department , [2]Associate Professor PCMS&RC Bhopal , Medicine Department ,[3] Assistant ProfessorLNMC&JK Hospital Bhopal, Medicine Department ,[4] Assistant Professor RDGMC Ujjain, Medicine Department[5]Senior Resident LNMC& JK Hospital Bhopal,Medicine Deptt.[6]Post Graduate Resident LNMC&RC Bhopal

***Corresponding Author:**
Dr. Thakur Dilbagh Singh
HIG 2, Flat No 308 ,Rachna Nagar ,Bhopal

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Introduction: The present study aimed to investigate the role of neutrophil/lymphocyte ratio (NLR), an inflammation marker, complete blood count, and biochemical parameters in the diagnosis of COVID-19.

Material& Methods: A total of 80 patients who had been hospitalized in the internal medicine clinic were enrolled in the study. The cases were allocated into two groups, i.e., COVID (+) and (-), based on real-time reverse transcription-polymerase chain reaction. The demographic, clinical, and laboratory [NLR, platelet/lymphocyte ratio (PLR), complete blood count, biochemistry, and serology] data of the patients were retrospectively obtained from the hospital data management system.

Results: NLR and fever levels were found to be higher in COVID-19 (+) cases (P=0.021, P=0.001, respectively). There was no difference between males and females with regard to COVID-19 positivity (P=0.527). Total bilirubin levels were found to be lower in COVID-19 (+) cases (P=0.040). When the ROC analysis was carried out for NLR in COVID-19 (+) cases, the AUC value was found to be 0.660 (P=0.021), sensitivity as 69.01 %, specificity as 65.40 %, LR+: 1.98 and LR- : 0.48, PPV: 80.43, and NPV: 50.00, when the NLR was ≥2.4. The risk of COVID-19 was found to be 20.3-fold greater when NLR was ≥ 2.4 in the logistic regression (P=0.007).

Conclusion: NLR is an independent predictor for the diagnosis of COVID-19. We also found that fever and total bilirubin measurements could be useful for the diagnosis of COVID-19 in this population.

Keywords: Coronavirus Infections. Coronavirus. Fever. Lymphocytes. Neutrophils.3+q

Introduction

Coronaviruses (CoVs) are single-chain, enveloped RNA viruses. They do not contain the RNA poly-merase enzyme; however, they encode this enzyme in their genome. They are defined as CoV due to the protrusions on their surface (Latin: corona=crown)1.

Coronaviruses belong to the Orthocorona-virinae sub-family and are classified as four types (alpha, beta, gamma, and delta CoVs) and multiple subspe-cies. Coronavirus 2019 is within the beta-coronavirus 2b strain. The genomes of the beta-coronaviridae were shown to be closely related to the bat SARS-like coro- navirus[2]. This type of virus may be found in humans, bats, pigs, cats, dogs, remnants, and winged animals[3]. Coronaviruses are a large virus family that may lead to self-limited, mild, and common infections like the common cold, and also more severe infections like Severe Acute Respiratory Syndrome (SARS)4 and Mid- dle East Respiratory Syndrome (MERS)5. These viruses may lead to clinical conditions with various degrees of

respiratory, enteric, hepatic, nephritic, and neuro-logical involvement.

Pneumonia cases with unknown etiology and of suggested viral origin were reported in Wuhan, Hubei, China on 31 December 2019. The virus was shown in workers of the seafood wholesale market where dif- ferent animal types are sold. The patients exhibited fever, dyspnea, cough, and, radiologically, bilateral pneumonic infiltrations6. Death usually occurred in individuals who were elderly or who had comorbid systemic diseases (hypertension, diabetes mellitus, cardiovascular diseases, cancer, chronic pulmonary diseases, and other immune-suppressive conditions)7,8.

The pathophysiology of the high pathogenicity of this unusual highly contagious SARS-CoV2 could not be fully understood yet. Inflammation plays an important role in infectious diseases. Accumulating evidence has shown the importance of inflammation in the progression of viral pneumonia, including in coronavirus disease 2019 (COVID-19) cases8. Pro-in- flammatory cytokines have been shown to increase in sera of patients with pulmonary inflammation9. The white blood cell (WBC) count, neutrophil, lymphocyte count, neutrophil/lymphocyte ratio (NLR), and plate- let/lymphocyte ratio (PLR) are markers of systemic inflammation10,11. These markers are useful predic- tors for the prognosis and follow-up of patients with viral pneumonia. NLR is a very useful, rapid, and inex- pensive indicator, the significance of which has been shown in bacterial pneumonia12 and viral infections13. This retrospective, single-center study was con- ducted to investigate the complete blood count param- eters, NLR, PLR, C-reactive protein (CRP), and the other infection bio-markers and biochemical data of a total of 80 COVID-19 positive and negative cases.

METHODS

A total of 80 patients who had been hospitalized at the medical clinic between 01 April 2020 and 25 April 2020 and tested for COVID-19 with real-time reverse transcription-polymerase chain reaction (rRT-PCR) were enrolled in the study. The nasal and pharyngeal swabs of all patients were obtained. Iso-lated patient samples that were obtained with VNAT viral transport and brought to the molecular virol-ogy laboratory were examined using the Biospedy (Bioeksen, Turkey) rRT-PCR kit. The patients whose rRT- PCR results were positive were regarded as COVID-19 (+), and those whose rRT-PCR results were negative twice with a 48-hour interval were regarded as COVID-19 (-). Hospital records (demographic, clinical, and laboratory data) of the cases above 18 years were analyzed retrospectively. The patients were divided into two groups, i.e., COVID-19 (+) and COVID-19 (-). Neutrophil, lymphocyte, platelets, MPV, hemoglobin, and CRP values of all patients were recorded, and the NLR and PLR values were calculated. The reports from the thoracic computed tomographies were obtained from the data management system. Serum urea, creatinine, total cholesterol, triglyceride, low-density lipoprotein (LDL)-cholesterol, high-den- sity lipoprotein (HDL)-cholesterol, aspartate amino- transferase (AST), alanine aminotransferase (ALT), and albumin were analyzed using the kinetic alkaline picrate method with the Architect C 16000 (Abbott) device at the biochemistry laboratory of the hospital. The complete blood count parameters were exam- ined with the Celldyn 3700 device. Ethics committee approval was obtaind from GMC & Hamidia Hospital Bhopal.

STATISTICAL ANALYSIS

Data analysis was performed by using statisti- cal software (SPSS, version28.0 [SPSS Inc, Chicago, IL]. Normally distributed data were compared by one-way analysis of variance, and non–normally dis-tributed data were compared via the Mann-Whitney U test. Categorical associations were evaluated by using the □2 test and multiple logistic regression. The goodness of fit was determined by using the Nagelkerke R2 and Hosmer-Lemeshow goodness- of-fit test. The performance of NLR was assessed using receiver operating characteristic (ROC) curve analysis and by calculating the area under the curve (AUC) of the ROC curves. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Of the total 80 patients, 39 (49%) were females and 41 (51%) were males. COVID-19 was determined to be positive in 54 out of the 80 cases (67.5%). The mean age (SD) was 53 (18) years for COVID-19 (+) patients and 60

(14) for COVID-19 (-) patients, and the difference was not statistically significant (F=3.029; P=0.086). Simi- larly, there was no difference between the

Volume 5, Issue 1; January-February 2022; Page No 1019-1024

groups con- cerning gender (□2 =0.400 P=0.527). Fever was present in 41% of COVID-19 (+) cases. There was a significant difference between the groups concerning HDL-cho- lesterol values (F=4.984; P=0.031). The rates of fever, lactate, and ferritin levels were significantly higher in COVID-19 (+) cases compared to COVID-19 (-) cases (Mann-Whitney U=390.0, P<0.001; 152.0, P=0.040; 202.5, P=0.046; 396.0, P=0.008, respectively). The rates of total bilirubin level were significantly lower in COVID-19 (+) cases (Mann-Whitney U=152.0, P=0.040). While the NLR, PLR, and CRP values were significantly higher (Mann-Whitney U 477.5, P=0.021; 508.0, P=0.046; 448.5, P=0.012, respectively), the lym- phocyte count was significantly lower (Mann-Whitney U 419.0, P=0.004) in COVID-19 (+) cases compared to COVID-19 (-) cases. There was no difference between COVID-19 (+) and (-) cases concerning WBC, neu- trophil, platelet count, MPV, and procalcitonin. The demographic and laboratory characteristics of patients infected with and without COVİD-19 are shown in Table - I. The mean neutrophil/lymphocyte ratio and fever in COVID-19 (+) and (-) cases are displayed in Fig- ure 1. The effect of NLR on the diagnosis of COVİD-19 was analyzed by ROC curve and AUC and was found to be significant (AUC:0.660; P=0.021, 95% CI 0.538 to 0.783) (Fig.2). Sensitivity, specificity, positive pre- dictive value, negative NPV, LR+, LR- values, and the disease prevalence for NLR ≥ 2.4 were 69.01%, 65.40%, 80%, 50%, 1.98, 0.48 and 67.5%, respectively. The effect of fever on the diagnosis of COVİD-19 was analyzed by

TABLE 1. Demographic & laboratory Characteristics of patients infected with & without Covid-19.

Indicators	COVİD-19 (+) n=54		COVİD -19
Mean age (SD), year	53(18)	60(14)	0.086
Men	29	12	0.635
Women	25	14	0.347
lymphocyte (Ir),K/ul	1.3(0.7)	2.0(1.0)	0.004
Nlr median (Ir)	4.7(2.8)	2.9(1.7)	0.021
platelet (Ir), K/ul	183(21)	221(43)	0.681
Mpv (SD), fl	9(1.3)	9.2(1.1)	0.987
hemoglobin (SD), gr/dl	12.4(1.7)	11.5(1.8)	0.033
plr median (Ir)	141(22)	104(14)	0.046
Kreatinin mg/dl	0.8(0.6)	0.6(0.4)	0.703
Total Cholesterol (SD), mg/dl	151(34)	158(49)	0.197
Triglycerides (Ir), mg/dl	115(45)	80(23)	0.120
low-density lipoprotein (SD), mg/dl	95(24)	109(29)	0.099
high-density lipoprotein (SD), mg/dl	30(9)	38(14)	0.031
Alanine aminotransferase (Ir), U/l	33(27)	25(22)	0.170
Aspartate (Ir), U/l	33(9)	28(8)	0.015
Albumin (SD), mg/dl	3.4(0.5)	3.4(0.6)	0.934
protrombin zamanı (SD), s	12.7(1.4)	12.3(1.2)	0.304
INr (Ir)	1.2(0.3)	1.0(0.2	0.016
Activated partial thromboplastin time (SD)	25(2.8)	26(3.4)	0.567
ldh (Ir), U/l	322(17)	211(15)	0.016
Creatine kinase (Ir), U/l	64(14)	71(18)	0.039
Ferritin(Ir) µg/l	503(131)	108(51)	0.008
Total bilirubin (Ir)mg/dl	0.64(0.1)	0.9(0.2)	0.040
d-Dimer (Ir), ugFEU/l	633(176)	570(150)	0.934
Crp (Ir) mg/l	89(88)	3.1(1.1)	0.012
procalcitonin (Ir)	0.09(0.2)	0.05(0.1)	0.945
lactate(Ir) mmol/l	1.7(1.5)	1.4(1.1)	0.046
Thorax computorize tomography (typical viral	10	2	0.204

Volume 5, Issue 1; January-February 2022; Page No 1019-1024

ROC curve and AUC and was found to be significant (AUC:0.722; P=0.001, 95% CI 0.606 to 0.838). Sensi-

tivity, specificity, positive predictive value, negative NPV, LR+, LR- values, and the disease prevalence for fever ≥ 36.8 were 66.67%,76.92%, 86%, 43%, 2.98, 0.43

and 67.5%, respectively.

We built a logistic regression model including NLR ≥ 2.4, temperature≥ 36.8, and serum total bilirubin as free predictors of a Covid-19 positive diagnosis. According to our model, the odds ratio for a covid-19-positive result was 20.3 and 10.5 when NLR was >2.4 and temperature was >36.8 (B=3.011, Standart Error=1.324, Wald=5.170, Odds ratio=20.3, P=0.023 for NLR; B=2.356, Standart Error=1.079, Wald=4.768, Odds ratio=10.5, P=0.029 for fever and B=-7.726, Standart Error=3.141, Wald=6.049, Odds ratio=0.0, P=0.014 for serum total bilirubin). The decrease of total serum bilirubin was significant for a covid-19-positive result, but without affecting the odds ratio. Nagelkerke R^2 was 65%.

DISCUSSION

In the present study, we reported the cohort of 54 COVID-19 (+) cases and 26 COVID-19 (-) cases con- firmed with laboratory tests. NLR and fever were found tobe significantly higher in COVİD-19 cases. Total bilirubin levels were found to be lower in COVİD- 19 cases. There was no difference between COVID-10 (+) and (-) cases concerning age and gender.

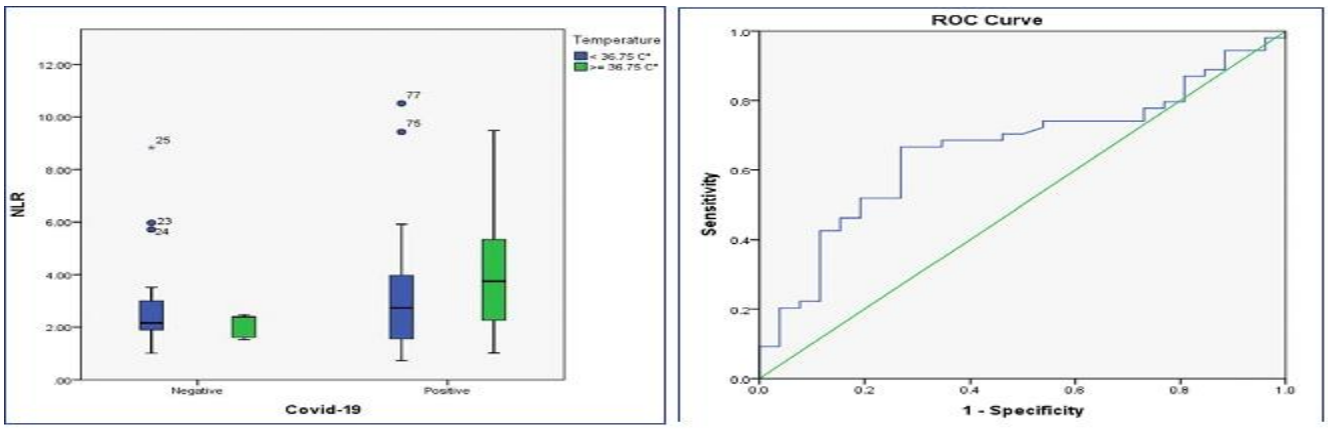
Fever is among the most important clinical mani-festations of CoV infections. In a study reported in the Lancet, fever was detected in 83% of the cases with COVID-19 pneumonia14; this rate was found to be 43.8% in another study7. The rate of fever was found to be 41% in our study. If the definition of case surveillance is mainly focused on the detection of fever, patients may be overlooked in the absence of fever, since fever was not detected in about half of the patients at the beginning. We determined a significant difference between COVID-19 (+) and (-) cases concerning fever. The risk of COVID-19 was found to be 10.5-fold greater when the fever was ≥36.8 degrees. Fever and CRP are not only systemic markers of inflammation but also mediators of inflammatory factors15. CRP was found to be high in COVID-19 patients in a previous study16. We also found CRP to be significantly high in our study.

The decrease of total serum bilirubin was signif-icant for covid-19-positive results but without affect-ing the odds ratio. Also, AST levels were significantly higher in COVİD-19 cases; however, this increase was not observed for ALT. This was compatible with a study in China17,18. This elevation may be related to viral load and changes in the liver synthesis capac- ity. Other causes of changes in liver function include ACE2-mediated direct viral infection of hepatocytes or critically-ill status and immune-mediated injury.

Thrombocytopenia is another pathological finding that could be detected in a complete blood count19.

FIGURE 1. Mean Netrophil To lymphocyte ratio temperature in patients with & without Covid-19.

FIGURE 2. receiver operating Characteristic curve for neutrophil To lymphocyte ratio in patients with or without Covid-19.



Volume 5, Issue 1; January-February 2022; Page No 1019-1024

Thrombocytopenia was detected in our study, consistent with the previous study; furthermore, PLR was significantly high. The platelet count, dynamic changes during treatment, and PLR were a source of concern in severe COVID-19 pneumonia cases. It was interpreted that PLR could serve as a novel indicator of the degree of cytokine storm20.

In a study conducted in China, no difference was found between severe and moderate cases concerning the WBC count in the correlation analysis, and lymph- openia was reported to develop when the WBC count was normal21. Consistent with the previous study, we detected lymphopenia when the WBC and the neu- trophil count were normal in hospitalized COVID- 19 cases.

A decrease was determined in the peripheral blood lymphocyte count of critically ill COVID-19 patients9,14,16. Immune cells infiltrate the lungs and lead to unexplained severe lung infections4. In a study, the lymphocyte count was found to be <1.0x109/L (9). We found the lymphocyte value as 1.3x109/L, consis- tent with the previous studies.

The human immune response is created by lymphocytes triggered by viral infections22. Systemic infections suppress cellular immunity. The novel coronavirus may mainly act on lymphocytes, especially T lymphocytes23. The total lymphocytes, CD4+ T cells, CD8+ T cells, B cells, and NK cells decreased in COVID-19 patients, and severe cases had lower levels of these cells than mild cases22,24. Therefore, CoV-in- duced inflammation-related lymphopenia increased NLR. In the only study conducted before ours, the optimal threshold of 3.3 for NLR showed a superior prognostic possibility of clinical symptoms for change from mild to severe7. . We found NLR to be high and the likelihood of COVID-19 was 20-fold greater when NLR was ≥2.4.

The results of rRT-PCR can be obtained in hours; hence the diagnosis and treatment may be delayed.

The shortcomings of the PCR method due to false pos- itive/false negative results from insufficient sampling, insufficient laboratory facilities due to the pandemic, samples collected too early or too late, and the binding sites of primer/probe couples used in the rRT-PCR lead to some difficulties in the diagnosis25. However, NLR is a rapid, inexpensive, and useful indicator that could be estimated via the complete blood count. The clinical use of NLR has been shown in bacterial pneumonia12 and viral infections13. The surveillance of NLR and lym- phocyte subsets is helpful in the early screening of critical illness, diagnosis, and treatment of COVID-1917. The COVID-19 pandemic may spread rapidly from human-to-human. The clinical characteristics of the disease vary among patients. The severity of the condition may be related to the number of immune cells.

CONCLUSION

In conclusion, we found that NLR was significantly elevated in COVID-19 patients. We also provided a cut-off for this readily available test and showed that patients with NLR ≥2.4 were 20.5 times more likely to have COVID-19 compared to patients whose NLR was ≤2.4. Similarly, the likelihood of COVID-19 was 10.5-fold greater when fever was ≥36.8 °C. This study indicates that high fever and NLR are independent bio- markers for COVID-19 patients. Our findings may also help in the early diagnosis of COVID-19.

REFERENCES

1. Zhou y, yang y, huang J, Jiang S, Du l. Advances in MErS-Cov vaccines and therapeutics based on the receptor-binding domain. viruses. 2019;11(1):60.
2. Tan W, Zhao X, Ma X, Wang W, Niu p, Xu W, et al. A novel coronavirus genome identified in a cluster of pneumonia cases — Wuhan, China 2019−2020. China CDC Weekly. 2020;2(4):61-2. [cited 2020 May 12]. Available from: http://weekly.chinacdc.cn/fileCCDCW/journal/article/ ccdcw/2020/4/pDF/Wuhan.pdf
3. richman DD, Whitley rJ, hayden Fg. Clinical virology. 4th ed. Washington: ASM press; 2017. p.1243-7.
4. Chan JF, li KS, To KK, Cheng vC, Chen h, yuen Ky. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (hCov-EMC) the beginning of another SArS-like pandemic? J Infect. 2012;65:477-89.
5. Zaki AM, van Boheemen S, Bestebroer TM, osterhaus AD, Fouchier rA. Isolation of a novel coronavirus from a man with

Volume 5, Issue 1; January-February 2022; Page No 1019-1024

pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20.

6. World health organization. Novel coronavirus – China. geneva: World health organization; 2020. [cited 2020 May 12]. Available from: https:// www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/
7. yang Ap, liu Jp, Tao WQ, li hM. The diagnostic and predictive role of Nlr, d-Nlr and plr in CovID-19 patients. Int Immunopharmacol. 2020;84:106504.
8. Zhu N, Zhang D, Wang W, li X, yang B, Song J, et al; China Novel Corona- virus Investigating and research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
9. huang C, Wang y, li X, ren l, Zhao J, hu y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. lancet. 2020;395(10223):497-506.
10. Zahorec r. ratio of neutrophil to lymphocyte counts: rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl lek listy. 2001;102(1):5-14.
11. ying hQ, Deng QW, he BS, pan yQ, Wang F, Sun hl, et al. The prognostic value of preoperative Nlr, d-Nlr, plr and lMr for predicting clinical outcome in surgical colorectal cancer patients. Med oncol. 2014;31(12):305.
12. yoon NB, Son C, Um SJ. role of the neutrophil-lymphocyte count ratio in the differential diagnosis between pulmonary tuberculosis and bacterial community-acquired pneumonia. Ann lab Med. 2013;33(2):105-10.
13. 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;ciaa248.

Volume 5, Issue 1; January-February 2022; Page No 1019-1024