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ORIGINAL ARTICLE

Eosinopenia and Neutrophil to Lymphocyte Ratio in COVID-19 Infection, a Prospective Study from Tertiary Care Hospital in Pakistan

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ABSTRACT

OBJECTIVE: To assess eosinopenia and neutrophil to lymphocyte ratio in patients admitted with COVID-19 infection and correlate with pneumonia severity.
METHODOLOGY: This prospective observational study was conducted at Covid HDU of Dr. Ruth KM Pfau Civil Hospital, Karachi, from October - December 2021. Data was collected using convenience sampling. The inclusion criteria were patients between 18-70 years with symptoms suggestive of COVID-19 and positive RT-PCR. Patients with negative RT-PCR with alternate diagnosis, history of using a corticosteroid, pancreatic or esophageal malignancy, and recent burn were excluded. Data analysis was done using SPSS version 23.
RESULTS: Out of 141 patients, 66(46%) were classified as non-severe covid and 75(53.9%) as severe covid pneumonia. A significant association between eosinopenia and COVID severity was observed. The NLR was significantly increased in severe COVID patients compared to non-severe patients. Similarly, eosinophil was decreased considerably in severe covid compared to non-severe patients (p-value<0.05). The area under the eosinopenia ROC curve is 0.71 and 0.69 on days 7 and 3, respectively. Thus, eosinopenia on day 7 was fair in predicting severe COVID-19 pneumonia compared with increased NLR, which was poor in predicting severe COVID-19. Using the area under the ROC curve, an eosinophil count cutoff of < 100 cells/mm3 produced a sensitivity of 75% for severe COVID-19 pneumonia.
CONCLUSION: Eosinopenia and elevated NLR were found with increased frequency in patients with severe COVID-19 pneumonia; however, persistent eosinopenia was a better marker than NLR in predicting COVID-19 infection severity.

KEYWORDS: Eosinopenia, Neutrophil to lymphocyte ratio, Covid-19, Pneumonia.

INTRODUCTION

NLR is the ratio to assess subclinical inflammation and is measured by dividing the neutrophils by the no. of lymphocytes while measuring the complete blood count. A higher NLR is a predictor of cardiovascular events and all-cause death[1]. The NLR can be used in many clinical scenarios as a prognostic marker, and presently, the NLR ratio is significantly observed as a prognostic marker in patients admitted with COVID-19 infection in which a significant difference in the NRL ratio is found in recovered and dead patients[2]. The NLR ratio is 0.78 to 3.53 in 95% of healthy adult subjects[3].

Eosinopenia is a form of granulocytopenia with lower counts than expected. Eosinopenia with leukocytosis is the predictor of bacterial infection, while eosinopenia with lymphopenia is observed as a marker to assess the severity in patients with COVID-19 infection[4,5]. Increased absolute neutrophil count with leukocytosis is found in severely ill patients of COVID-19 and is a separate risk factor for inpatient death[6].

Eosinopenia $<0.02 \times 10^9$ cells/L was found in more than half of the admitted patients on admission[7]. It is observed that Eosinopenia had high sensitivity and specificity to diagnose COVID-19 infection, and the Eosinophil/PMN ratio is a helpful measure for COVID-19 diagnosis[8]. A study by Roser Terradas found the significance of Eosinopenia (0.0454×10^3/ul) and NRL ratio > 7 as poor prognostic indicators in bacteremia patients. Survivors in this study tended to raise eosinophil count and reduce NRL <7 on days 2 and 3 of admission[9]. Eosinophil-related ratios were also found as markers of survival in patients with endometrial carcinoma[10]. Eosinophil to lymphocyte ratio is also observed in many hypersensitivity reactions to NSAIDs[11]. SARS-CoV-2 infection is found to alter many blood parameters, which have prognostic significance, and their timely assessment helps in better management. Severe COVID-19 infection is observed by marked eosinopenia, and higher NLR has been seen in patients known to have diabetes and hypertension[12]. Eosinopenia and raised NLR are essential tools to diagnose COVID-19 infection in patients having symptoms suggestive of COVID-19 even with negative PCR. A study by Vasiliki E Georgakopoulou found statistically significant eosinopenia and eosinophil to lymphocyte count in patients admitted with moderate to severe COVID-19[13].

Eosinopenia and NLR are efficient and effective measures in diagnosing and evaluating the severity and have prognostic significance. This study assesses the eosinopenia and NLR in patients admitted with SARS-CoV-2 infection and correlates them with the severity of pneumonia and length of hospital stay.

METHODOLOGY

This observational prospective study was conducted at medical units 1 and 5, designated as Covid HDU of Dr. Ruth KM Pfau, Civil Hospital Karachi, from October - December 2021 with a convenience sampling technique. This study was reviewed and accepted by the institutional review board committee of Dow University of Health Sciences.

Using the reported prevalence of COVID-19 diseases in different phases, it was found between 8.5% and 15% were affected by the population of Karachi. A sample size of 140 achieves 80% power to detect a 95% confidence interval and an odd ratio of 4 with a prevalence ratio of 3.5 and a prevalence difference of 12. The sample size was calculated using openEPI.

Inclusion Criteria

· All patients are between 18-70 years.

· Patients with symptoms suggestive of COVID-19 and positive RT-PCR SARS-Co-V-2.

· Negative SARS-CoV-2 detection by RT-PCR but having prominent features such as clinical, biochemical, and radiological evidence supporting SARSCoV-2.

Exclusion Criteria

· Negative RT-PCR SARS-Co-V-2 with alternate diagnosis, history of using corticosteroid.

· Exacerbation of COPD, pancreatic cancer, esophageal cancer, Cushing's syndrome, history of recent burn, and stress inducer

All patients between 18 and 70 years who presented to the emergency room with suspected symptoms suggestive of COVID-19 were included. Symptoms favouring COVID-19 include unexplained fever of >38 °C, flu-like symptoms, cough, shortness of breath, loose motion in those older than 65, and a history of exposure to infected patients[8].

All the participants who were admitted to the COVID-19 treatment centre with positive covid-19 detection by RT-PCR, or in case negative covid-19 detection by RT-PCR but having prominent features such as clinical, biochemical, and radiological evidence supporting SARS-CoV-2 were included in the study after taking proper consent.

Before the study started, permission from the ethical committee was requested. Patients received adequate information regarding the study, and patient confidentiality was upheld. Each patient's demographics, brief history, clinical examinations, and comorbidities, like DM and HTN, were documented on proforma.

PCR for RNA COVID-19 virus was done through a nasopharyngeal swab. CBC, CRP, LDH, ferritin, D-DIMER, TSH, procalcitonin, LFT, PT/INR, and chest x-rays were done at the time of admission and during a stay in the hospital. The complete blood count of all patients included the no. of eosinophils, lymphocytes, PMN (with the calculation of neutrophil to lymphocyte ratio × 1000), platelets, and the haemoglobin (Hb) concentration. Repeated labs were done on days 1, 3, and 7 to compare biochemical parameters with patients' conditions and radiology.

Patients were categorized as non-severe and severe COVID pneumonia according to the clinical management guidelines issued by the Ministry of National Health Services, Govt of Pakistan.

Descriptive data were collected as frequencies and percentages, using mean and median values for variables such as differential white cell counts, frequencies of the gender, and comorbidities like DM and HTN, which will be reported and compared with gender by Student's t-test. Means ±SD will be reported for age and COVID-19 severity assessed. Using the chi-square test and independent t-test, baseline demographic, clinical, and laboratory data were compared between disease severity categories. Each haematological parameter was compared with various severity

groups using one-way analysis of variance (ANOVA) as the statistical significance test. P-value 0.05 was the threshold for statistical significance. All tests for hypotheses had two tails. The analysis was done with the help of SPSS software version 23.0. COVID-19 severity and the results were categorized as per the outcome achieved.

RESULTS

A total of 141 patients were included in our study. Out of 141 patients, 65(46%) were males and 76(53.9%) were females. 66(46.8%) patients were classified as non-severe covid and 75(53.1%) were classified as severe covid. In our study, the mean age of the non-severe covid patients was 39 years, whereas that of severe covid patients was 46 years. The most common symptom in the non-severe COVID group was abdominal pain, loose stool, and vomiting. In contrast, respiratory symptoms, i.e., dry cough and dyspnea, were significantly more present in the severe cohort, as shown in **Table I**. **Table I** shows the clinical characteristics of our sample. According to Table 2, the haematological parameters were compared between the two cohorts. The NLR on Days 1, 3 and 7 was significantly increased in severe covid patients compared to non-severe patients (10.9 vs 7.3 p-values = 0.002), (11.7 vs 6.3 p-values = <0.001), (11.8 vs 6.1 p-values = 0.009) respectively. Similarly, eosinophil counts on Days 3 and 7 were significantly decreased in severe COVID patients compared to non-severe patients (123 vs 71 p-values =<0.001), (121vs 57 p-values =<0.001) respectively. According to Table 3, more patients in the severe group (87.1% vs. 12.9% p value=0.001) required non-invasive ventilation than in the non-severe group (12.9%).

Table I: Baseline Clinical Features Comorbid illness and vital characteristics of COVID-19 patients concerning disease severity

Disease severity	Non-severe n =66		Severe n =75		Pearson Chi-Square	
Statistic	**n**	**%**	**n**	**%**	**Value**	**P-value**
Age (years) mean±SD	39±7.39		46.9±12.34			
Gender					0.284	0.594
Male	32	49.2	33	50.8		
Female	34	44.7	42	55.3		
Clinical features						
Abdominal pain	27	64.3%	15	35.7%	7.339	0.007
Vomiting	27	61.4%	17	38.6%	5.442	0.020
Diarrhea	18	62.1%	11	37.9%	3.415	0.065
Fever	56	45.5%	67	54.5%	0.634	0.426
Dry cough	36	38.7%	57	61.3%	7.197	0.007
Loose motions	20	69.0%	9	31.0%	7.199	0.007
Dyspnea	34	35.4%	62	64.6%	15.678	<0.001
Comorbid illness						
HTN	27	51.9%	25	48.1%	0.866	0.352
DM	23	46.9%	26	53.1%	0.001	0.982
CLD	8	66.7%	4	33.3%	2.077	0.149
COPD	27	51.9%	25	48.1%	0.866	0.352
CVD	23	46.9%	26	53.1%	0.001	0.982
HIV	8	66.7%	4	33.3%	2.077	0.149
Vitals						
Systolic (mmHg)	122.6818	15.17605	124.8400	14.07993	-0.876	0.383
Diastolic (mmHg)	76.4848	10.80063	78.5600	9.66079	-1.204	0.231
Pulse (beats/minute)	85.0000	10.01998	81.8267	10.54126	1.825	0.070
PaCO2	35.7348	8.38663	38.3840	7.71440	-1.953	0.053

Table II: Laboratory parameters of COVID-19 patients concerning Disease Severity

Disease severity	Non-severe n =66		Severe n =75		Independent Samples Test	
Statistic	Mean	SD	Mean	SD	Value	P-value
Hematological markers						
HbA1C	5.63	1.15	5.84	1.34967	-0.941	0.348
Haemoglobin (g/dL)	11.35	1.89	11.98	1.79	-2.024	0.045
White Blood Cell Count (x $10^3/\mu L$)	13.21	8.303	14.62	9.60	-0.927	0.356
Platelets	258.07	120.43	279.85	130.27	-1.026	0.307
Neutrophils (%)	77.89	10.26	82.90	11.96	-2.647	0.009
Lymphocytes (%)	16.31	10.74	11.17	8.11	3.230	0.002
Eosinophils (x $10^3/\mu L$) Day1	183.9	107	157	127	1.348	0.18
Eosinophils (x $10^3/\mu L$) Day 3	123	79.6	71.2	80.4	3.855	<0.001
Eosinophils (x $10^3/\mu L$) Day 7	121	89.9	57.2	76.3	4.583	<0.001
N/L Ratio on Day 1	7.38	5.83	10.95	7.31	-3.175	0.002
N/L Ratio on Day3	6.36	4.99	11.79	10.55	-3.816	<0.001
N/L Ratio on Day 7	6.10	6.41	11.84	16.53	-2.633	0.009
Procalcitonin (ng/mL)	0.48	0.55	0.55	0.62	-0.786	0.433
CRP (mg/dl)	96.00	73.47	73.47	82.61	0.637	0.525
D-Dimer (ng/mL)	4.10	6.06	4.01	5.45	0.099	0.921

Table III: Outcome of COVID-19 patients concerning Disease Severity

Disease severity	Non-severe n =66		Severe n =75		Pearson Chi-Square	
Non-Invasive Ventilation					18.347	<0.001
Yes	4	12.9%	27	87.1%		
No	62	56.4%	48	43.6%		
Outcome					3.927	0.048
Discharge or Referred to ICU	64	49.2%	66	50.8%		
Death	2	18.2%	9	81.8%		

Figure I: ROC curve showing sensitivity in Eosinopenia

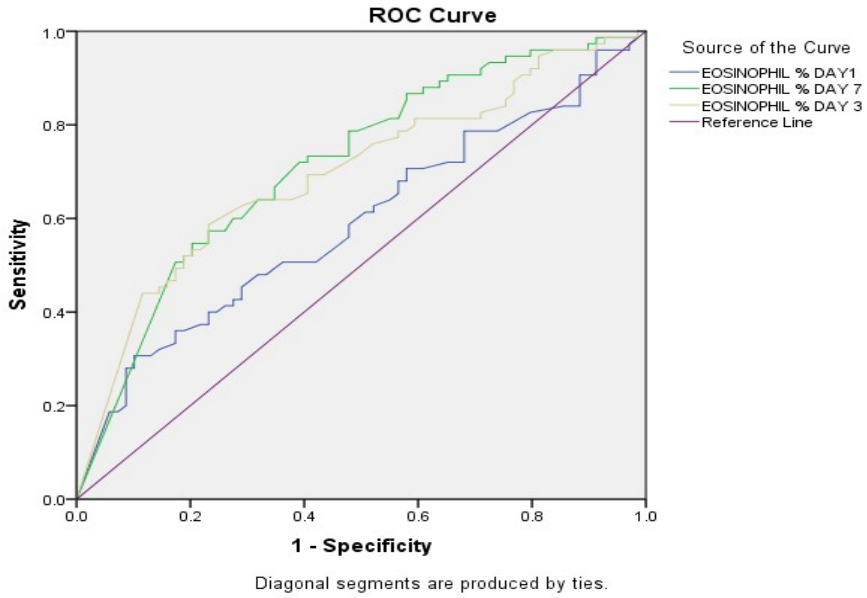
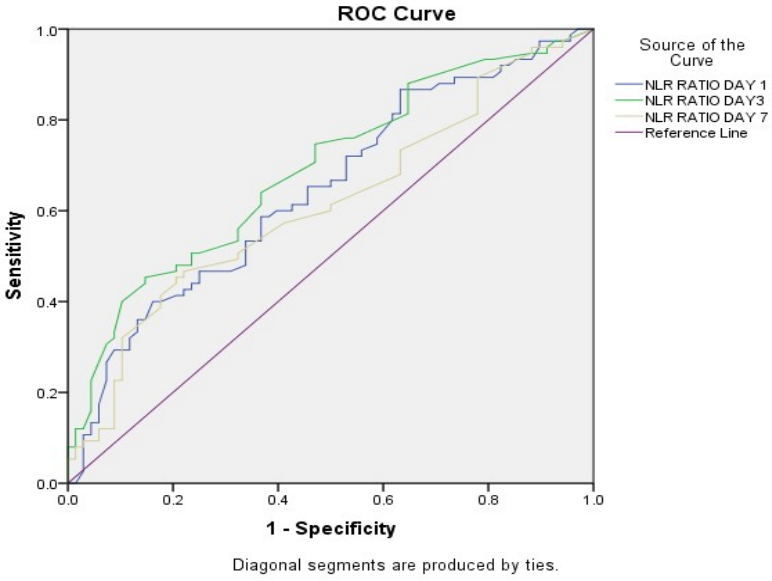


Figure II: ROC curve showing sensitivity in NLR ratio



DISCUSSION

The upper limit of the normal range for eosinophil is 350 cells/mm3 blood, which is commonly between 1% and 3% of peripheral blood leucocytes[14]. The sequestration of eosinophils in the peripheral tissues triggered by chemotactic factors generated in response to inflammation and mediated by the adrenal axis are a few of the multifactorial causes of eosinopenia in sepsis[15]. According to Cortés et al., eosinopenia may be linked to adverse illness outcomes in COVID-19 infection, and it was more frequently seen in patients who had died during the hospital course than in those who had recovered[16]. Similarly, Roca et al. and others also supported that persistent eosinopenia after admission was associated with high disease severity[17-19]. However, our results are against Soni et al., who found no prognostically significant relation between eosinopenia and COVID-19 pneumonia[20]. The results of our study showed that the two groups of patients had a significant difference in symptoms at presentation, NLR, and Eosinophil count. In our study, increasing age was an essential factor associated with poor outcomes, as reported in other studies[21,22]. Our study's median absolute eosinophil count on day 3 and 7 in severe covid pneumonia patients was 71 and 57, respectively. In our study, there was a link between persistent eosinopenia and poor outcomes, including in-hospital death. It has been hypothesized that continuous eosinopenia and increased NLR after admission is associated with poor rates of recovery[17,22,23]. Eosinophil count patterns across the course of the disease in our study between the non-severe and severe groups revealed a substantial difference; similarly, in our study, it is also observed that persistent eosinopenia, i.e. on days 3 and 7 were associated with worse outcomes and death. The area under the eosinopenia ROC curve is 0.71 and 0.69 on days 7 and 3, respectively. The eosinopenia on day 7 is fair in predicting severe COVID-19 pneumonia compared with NLR, which was poor in predicting severe COVID in our study. Eosinophil cell count (cutoff of 100 cells/mm3) revealed a sensitivity of 75% for severe COVID-19 pneumonia using the area under the receiver operating characteristics (ROC) curve **Figure I**.

These findings lead us to believe that persistent eosinopenia can serve as a warning sign for a severe COVID-19 infection and give us a solid scientific foundation for the early detection of severe COVID-19 pneumonia. Additionally, it can help us to anticipate COVID-related ARDS earlier and, thus, timely escalation of therapy.

The limitation of our study is that it's a single-centre study conducted at a high-dependency unit. More studies at multiple centres should be conducted to establish eosinopenia's role and elevated NLR in COVID-19 infection.

CONCLUSION

Eosinopenia and elevated NLR were found with increased frequency in patients with severe covid pneumonia; however, persistent eosinopenia of <100cells/mm3 after admission was a better marker than elevated NLR in predicting COVID-19 infection severity and low rates of recovery. Additional research is required to determine this marker's optimal cutoff values and assess the relationship between the severity of COVID-19 pneumonia and the advancement of eosinopenia.

ETHICAL PERMISSION: Dow University of Health Sciences IRB letter No. IRB-2254/DUHS/Approval/2021/607.
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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publically.

AUTHOR'S CONTRIBUTION

Kashif SM: Conception, design and final approval of study
Sunder WH: Analysis and interpretation of data
Qadeer R: Conception and provision of patients
Kumar D: Drafting of article, critical revision of article
Anum G: Drafting of article
Kumar R: Critical revision of article

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