C-reactive Protein and Neutrophil Lymphocyte Ratio Levels as Predictive Biomarkers for Severity of COVID-19 Infection

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ABSTRACT

Introduction: COVID-19 is a highly contagious viral disease and spectrum of disease presentation range from asymptomatic to severe disease. Early diagnosis and assessment of disease severity is upmost priority to limit the morbidity, mortality and spread of disease.

Aims: The aim of present study was to assess predictive values of C-reactive protein (CRP) levels and neutrophil-to-lymphocyte ratio (NLR), to disease severity to provide reference values for clinical treatment.

Methodology: It was a prospective observational study in which 143 COVID-19 positive patients categorized into non-severe and severe groups. Clinical parameters, CRP values and NLR of all patients were recorded and analyzed. The receiver operating characteristic (ROC) curve was applied to determine the thresholds for both biomarkers.

Result: Out of 143, total 85(59.44%) were males and 58(40.56%) female in our study. 114(79.72%) patients were non-severe and 29(20.27%) patients in severe group. Mean CRP and NLR of non severe group were 12.24(±8.5) mg/l, 2.97(±1.12) and in severe group 58.8(±43.16) mg/l, 7.85(±5.51) respectively. ROC curves analysis showed area under the curve (AUC) of 0.884 (95% CI 0.800-0.967; p value<0.0001) for CRP and 0.817 (95% CI 0.707-0.927; p<0.0001) for NLR. The cutoff value for CRP and NLR was 24.4mg/L and 3.6 respectively. At cutoff point, sensitivity and specificity for CRP were 82.76% and 88.6% and for NLR 75.86% and 75.44%.

Conclusions: CRP and NLR are moderate accuracy diagnostic biomarkers to assess the severity of disease and of them CRP has better overall diagnostic performance than NLR.

Key Words: CRP, NLR, COVID-19 infection, Predictive biomarker

INTRODUCTION

The current outbreak of pneumonia which is caused by corona virus was first reported in Wuhan, China, in December 2019.¹⁻⁵ Later this disease has been officially named as "COVID-19" by World Health Organization (WHO).^{6,7} Within few months COVID-19 disease spread globally, resulting in a worldwide pandemic.⁸ Corona virus belongs to the family *Coronaviridae*, subfamily *Ortho-coronavirinae* and Order *Nidovirales*. SARS-CoV had also caused the outbreak of severe acute respiratory syndrome in 2003.⁹ The nature of this disease is rapidly progressive, and severely ill patients can develop acute respiratory distress syndrome, sepsis, and multiple organ dysfunction syndromes in very short period of time.¹⁰

To improve the patient outcome early diagnosis, clinico-pathological monitoring and appropriate treatment protocol are essential. Chest CT scan has an important role in assessing the disease.¹¹ Chest CT scan is expensive diagnostic modality and not readily available all places. So we have to consider some laboratory markers that must be inexpensive, within reach for all people and simultaneously sensitive and specific. Two biomarkers CRP and NLR both are immune-inflammatory parameters in COVID-19 infection and associated with the progression of the infection.

In 1930s Tillet and Francis discovered CRP as an acute phase reactant. It is synthesized by liver by the action of cytokine interleukin 6 (IL-6). Not only in bacterial infections even in other pathological processes like injuries, cardiovas-

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cular events and other inflammatory states CRP rise to very high levels. The high level of CRP is a biomarker of pro-inflammatory state and it can be used as a prognostic marker for the underlying disease processes.¹² For the early diagnosis of pneumonia C-reactive protein (CRP) levels can play important role and patients suffering with severe pneumonia have high CRP levels.¹³

Few studies suggest that neutrophil/lymphocyte ratio (NLR) is associated with progression of the infection,¹⁴ or an early warning signal of severe COVID-19 infection.¹⁵ It can be considered as an independent biomarker for poor clinical outcomes and mortality in COVID-19 infection.¹⁶⁻²⁰ We assessed the predictive ability of CRP levels, NLR to assess disease severity to provide reference for clinical treatment.

MATERIALS AND METHODS

This prospective observational study was started after being reviewed and approved by the institutional ethics committee (ethical committee clearance letter no. RMRI/EC/2021/54 dated on 02/04/21). We enrolled those cases that had clinical manifestations of upper respiratory tract infections like cough, shortness of breath and chest pain, which tested positive for COVID-19 by real-time reverse transcription-polymerase chain reaction (RT-PCR) using nasopharyngeal and/or oropharyngeal swabs and radiological findings of consolidation, ground-glass opacities on high-resolution computed tomography (HRCT).

After considering the inclusion and exclusion criteria we collected data of 143 patients admitted in our hospital from mid of April to May 2021 from medical records of patients. Since this was a time bound observational study, no formal sample size calculation was done. Patients were clinically categorized into mild, moderate and severe disease according to ICMR guideline.²¹ Then we sub-categorized the patients into two groups: non-severe and severe. Patients who had mild and moderate symptoms like fever, upper respiratory tract symptoms without breathlessness, $\text{SpO}_2 > 90\%$ at room air were grouped into non-severe group and severe group had those patients who had severe symptoms like breathlessness and $\text{SpO}_2 < 90\%$ at room air.

The venous blood was collected at the time of admission and along with other investigations, CRP and NLR were also detected. The level of CRP was determined by the immunoturbidimetry method using Liquid Microexpress Turbilyte CRP on the Benesphera C61 semiautomatic biochemical analyzer. Complete blood count was determined by five part analyzer Mindray BC-5150. NLR ratio was calculated using the simple formula of- Absolute numbers of neutrophils/ Absolute no of lymphocytes (ANC/ALC).

STATISTICAL ANALYSIS

SPSS (*Statistical Package for Social Sciences*, version 16.0.1 of IBM, USA) and Excel were adopted for data analysis. All study variables depending on the data type were summarized using appropriate measures of central tendency (mean, median) and dispersion - standard deviation (SD) or inter-quartile range (IQR). Categorical variables were expressed as frequencies and percentages. Receiver operation curve (ROC) was used, computation of areas under curve (AUCs; with 95% confidence intervals) and cut-off values of CRP and NLR were done. $\text{AUC} > 0.70$ were considered to be clinically significant or relevant for good predictive score. For optimal cut-off values of CRP and NLR respective sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. $p \text{ value} \leq 0.05$ were considered statistically significant along with the 95% confidence interval for the test statistic computed.

RESULTS

A total of 143 patients with age above 18 years and confirmed diagnosis of COVID-19 were included in during the study period. Patients were divided into two comparison groups according to their clinical profile. Among the enrolled study subjects 114(79.72%) patients were in non-severe group and, 29(20.27%) patients in severe group. There were 85(59.44%) males and 58(40.56%) females in our study. As per laboratory findings mean CRP and NLR of non-severe group was $12.24(\pm 8.5)$ mg/L, $2.97 (\pm 1.12)$ and $58.8(\pm 43.16)$ mg/L, $7.85(\pm 5.51)$ in severe group respectively. (Table 1)

ROC curves analysis revealed moderate accuracy, with an area under the curve (AUC) of 0.884 (95% CI -0.800-0.967; $p \text{ value} < 0.0001$) for CRP (Figure 1) and 0.817 (95% CI -0.707-0.927; $p < 0.0001$) for NLR (Figure 2). Defined by the ROC curve, the optimal threshold value was 24.4 mg/L for CRP and 3.6 for NLR (Table 2). At optimal cut-off values, sensitivity and specificity for CRP were 82.76% and 88.6% and for NLR 75.86% and 75.44% respectively (Table 3). Table 3 also illustrates Positive predictive value and negative predictive value of respective biomarkers.

Comparative analysis of AUCs of CRP and NLR shows that CRP is a better diagnostic biomarker for patients with severe COVID-19 disease. (Figure 3)

DISCUSSION

COVID-19 is a pandemic and caused by the SARS-CoV-2 virus infection. The SARS-CoV-2 virus is closely related to the SARS-CoV virus and both virus belong to the β -CoV corona virus family,²² with 79.5% similarity in genetic

characteristic.²³ The spectrum of disease varies from asymptomatic individual to severe disease and death. Clinical observations have found that some patients with mild disease progress to severe disease within a short period of time. In view of this, an opportune assessment of the severity of patient's condition by means of early monitoring of different laboratory parameters is valuable for modifying the treatment.²⁴ The pathological changes of COVID-19 are clearly evident chiefly in respiratory system and immune system damage.²⁵ Acute inflammatory changes like congestion, edema, exudation and clear membrane formation appear in the lungs.²⁶ Later development of multiple-organ dysfunction occurs which may be related to the "cytokine release storm".²⁷

The concentration of CRP in serum fairly correlate with the severity of inflammation, and is unaffected by age, gender, and physical condition.²⁸ Therefore in clinical practice CRP is one of the common inflammatory biomarker used for identifying infection. CRP released by injured tissues and cells and activates the complement system and facilitates clearing of microorganism by enhancing phagocytic process. CRP has high specificity for identifying bacterial or viral infections and it can be used for early diagnosis of pneumonia.^{13,29} Patients with severe pneumonia have high CRP levels, and it is used not only for diagnosis, even for the assessment of severity of pulmonary infection.³⁰ Similar to our study, a study by L. Wang et al. suggest that CRP levels can reflect disease severity and may be a valuable biomarker for monitoring disease.³¹ Study conducted by G. Wang et al. also suggest that CRP level can be a worthy marker to predict the possibility of aggravation of nonsevere COVID-19 patients.³² Zhou et al. also observed similar findings in their study.³³

Neutrophils are major component of the leukocyte population and after invasion of microorganisms in the body, it gets activated and migrates at infection site and play role in host defense and immune regulation.³⁴ Neutrophils release a large amount of reactive oxygen species to kill the virus infected cells and interact with different cells and produces numerous cytokines and effector molecules. One of them is Vascular endothelial growth factor (VEGF) which stimulates tumor angiogenesis, growth, and metastasis.³⁵ It is observed that the expression of VEGF-A and VEGF-C are significantly higher in COVID-19 patients as compared to normal individuals.³⁶ Severe reduction of neutrophils count is associated with compromised immunity and increased risk of infection.³⁷ Considering the other side of coin, human immune response to viral infections mainly taken care by lymphocytes.³⁸ The systemic inflammation causes depression of cellular immunity resulting into significant decrease in CD4+ T lymphocytes and increase CD8+ suppressor T lymphocyte.³⁹ NLR is increased in viral infections and associated with worsening of clinical profile in COVID-19 patients. Ai-Ping Yang et al found in their study that NLR may be related to the severity

of infection and may also stipulate the outcome.⁴⁰ Study by M. Imran et al. also suggest that NLR can be used as an early alarming signal for clinician to assess the progression of severity in COVID-19 infection.^{41, 42} Findings of the study,⁴¹ are very much in alignment with our study.

Our study is not free from limitations. First, stronger evidences can be reported by conducting multicentric studies with larger sample size. Secondly, we classified the patients into two groups on the basis of clinical findings. It could have been better if we should have included some other radiological parameter like CT score. Thirdly, patients with mild and moderate symptoms may deteriorate to severe clinical condition at any point of time where investigating other biochemical parameters may be necessitated. Exacerbation of nonsevere disease to severe requires meticulous follow up of patients which was not included in our study design. Lastly, severe disease can also be associated with some co-morbid conditions which might require multivariate analysis for confirmation.

CONCLUSION

To summarize with, we observed that both laboratory parameters CRP and NLR are moderate accuracy diagnostic biomarkers to assess the severity of disease and of them CRP has better overall diagnostic performance than NLR. Elevated CRP and NLR can be used early warning indicators of deterioration of non-severe to severe COVID-19 disease irrespective radiological findings and can help clinicians to identify severe COVID-19 cases promptly and intervene accordingly. However, further more studies are needed to strengthen these facts.

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Authors contributions

Dr Prashant Bhardwaj- Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – Original Draft, Writing – Review & Editing, Visualization, Project administration

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Table 1: Baseline characteristics of COVID-19 patients

		Age (years) (Mean±SD)	CRP (mg/L) (Mean±SD)	NLR (Mean±SD)
Non-Severe Patients(114)	Male (64)	43.39 ±10.60	12.26 ±8.3	2.97 ±1.16
	Female (50)	42.2 ±9.53	12.22 ±8.86	2.98 ±1.07
	Total (114)	42.87 ±10.12	12.24 ±8.50	2.97 ±1.12
Severe Patients(29)	Male (21)	60.38 ±11.66	58.68 ±42.97	7.84 ±5.46
	Female (8)	51.75 ±12.11	59.14 ±46.64	7.87 ±6.04
	Total (29)	58.0 ±12.22	58.8 ±43.16	7.85 ±5.51
p value		<0.0001	<0.0001	<0.0001

CRP-C-Reactive Protein; NLR- Neutrophils Lymphocytes Ratio

Table 2: Area Under Curve (AUC) of CRP and NLR

	Area under curve(AUC)	Cutoff Value	Standard Error	95% Confidence Interval
CRP	0.884	24.4mg/L	0.043	0.800- 0.967
NLR	0.817	3.6	0.056	0.707-0.927

CRP-C-Reactive Protein; NLR- Neutrophils Lymphocytes Ratio

Table 3: Indices of Performances of biomarkers

		CRP	NLR	
			95%CI	95%CI
Sensitivity	82.76%	64.23%-94.15%	75.86%	56.46%- 89.70%
Specificity	88.6%	81.29%-93.79%	75.44%	66.49%-83.02%
PPV	64.86%	51.88%-75.97%	44%	34.91%-53.51%
NPV	95.28%	90.07%-97.82%	92.47%	86.47%-95.94%

PPV-Positive Predictive Value; NPV- Negative Predictive Value

Figure 1: Receiver operating characteristic (ROC) curve of CRP for diagnosis of severe COVID-19 patients.

Figure 3: ROC curve of CRP v/s NLR.

Figure 2: Receiver operating characteristic (ROC) curve of NLR for diagnosis of severe COVID-19 patients.