RESEARCH ARTICLE

CORRELATION OF NEUTROPHIL TO LYMPHOCYTE RATIO AND PLATELET TO LYMPHOCYTE RATIO WITH HIGH RESOLUTION COMPUTED TOMOGRAPHY IN COVID-19 PATIENTS

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ABSTRACT

Objective: *To correlate the Platelet-to-Lymphocyte Ratio (PLR) and Neutrophil-to-Lymphocyte Ratio (NLR) with Computed Tomography Severity Score (CT-SS) in COVID-19 patients.*

Study design: *Cross sectional analytical study.*

Place of study*: Collaborative study between departments of Physiology, Pathology and Radiology at HITEC-IMS.*

Duration of study: *From June 2020 to December 2021.*

Patients and methods: *A total of 83 (N=83) COVID-19 patients were included in the study through Non-Probability Purposive sampling. They were grouped into mild disease(n=44) and severe disease (n= 39) based on their HRCT severity scores. Their blood samples were obtained and NLR and PLR were correlated with CT-SS using the Spearman's correlation.*

Results: *Data were analyzed by SPSS version 25. Both NLR and PLR showed strong positive correlation with the HRCT severity score ($r = 0.471$, $p < 0.05$ for NLR and $r = 0.347$, $p < 0.05$ for PLR). Mann Whitney test scores showed that NLR & PLR were significantly different in both the mild and severe disease groups based on HRCT severity scores ($p< 0.05$).*

Conclusion: *NLR and PLR values could serve as prognostic markers in patients with COVID-19 in place of HRCT.*

Keywords: *COVID-19, CT-SS, HRCT, NLR, Prognostic markers, PLR.*

INTRODUCTION

The novel corona virus outbreak was declared as a global pandemic by WHO on March 11, 2020, which created an urgency to study the diagnostic, treatments and prognostic markers of COVID-19.[1]

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) is most reliable in diagnosing COVID-19 and is usually performed on nasal swabs.

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Received: 30 Nov 2023; revision received: 28 Dec 2023; accepted: 29 Dec 2023

Different hematological parameters like Blood Complete Picture, C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), Serum Ferritin, and Procalcitonin (PCT), and radiological imaging like chest X-ray were used as diagnostic tools for monitoring the progression of lung involvement in COVID-19 disease.[2] Among these, the most effective diagnostic method to detect lung involvement at relatively early stage and estimate the progression of disease from initial diagnosis to discharge from hospital, is Computed tomography (CT) scan.[3]

Chest High Resolution Computed Tomography (HRCT) is a helpful mean in the early discovery and evaluation of disease gravity in COVID-19 patients. There are multiple CT-scoring systems like Total Severity Score (TSS),

Computed Tomography Severity Score (CT-SS) and Computed tomography Score (CTS) to categorize the degree of COVID-19 disease severity. The CT-SS score is a modification of a technique that was first applied in the 2005 SARS pandemic.[3] Lung opacification is used in this scale as a stand-in for lung disease progression. An area of increased attenuation in the lungs, is labelled as Ground Glass Opacity (GGOs). The characteristic radiological pattern of COVID-19 disease is multiple GGOs in the periphery of lungs. . Follow up HRCT can also help in evaluation of disease progression.

The main pathophysiology of COVID-19 revolves around inflammation. The indices of Neutrophil to Lymphocyte Ratio (NLR) and Platelet to Lymphocyte Ratio (PLR) indirectly depicts the inflammatory status of the patient. Recent research has validated NLR and PLR as prognostic markers in number of illnesses, including Acute Respiratory Distress Syndrome (ARDS), malignancies, sepsis, pneumonia and cardiac diseases.[4,5]

Both the NLR and PLR consistently demonstrate greater levels across all observed time points in serious cases of COVID-19. Further, the disparity between NLR and PLR readings becomes wider as the disease severity increases. Both NLR and PLR reflects an increasing trend beginning with admission and culminating at the 7-day point.[6]

For developing countries like Pakistan, where resources are limited and out of reach for most of the patients, CT scan is a costly option. Alternatively, Serial CT scans expose the patients to a very high dose of radiation, which itself is a health hazard. Therefore, there is a need to look for alternatives as prognostic markers for COVID disease which should be safe, cost effective and at the same time as useful as HRCT for predicting prognosis of this disease. For developing countries like Pakistan, where there is dearth of tertiary care facilities lodging CT scan machines, this further limits the patient care.

Purpose of this study was to find correlation between PLR and NLR with HRCT scoring and then establishing these ratios as significant prognostic markers for devising and modifying the treatment plans and discharge criteria in COVID-19 disease patients.

MATERIAL AND METHODS

This was a one and a half year long cross-sectional analytical study conducted from June 2020 to December 2021, after ethical approval from IRB (Ref # HITEC-IRB-29-2022) at Physiology department of HITEC-Institute of Medical Sciences in collaboration with the Pathology and Radiology department of HIT hospital. Sampling technique used was non-probability purposive sampling. The sample size was calculated using the WHO calculator by taking a confidence interval, 80% power of study with an anticipated NLR mean + SD of group I is 3.76+4.5 and group II is 8.44+8.86.[7]

All patients were Covid positive (N=83) both males and females between 18 to 70 years of age.[8] Data were collected retrospectively, in the form of Blood CP and HRCT reports. Blood CP reports were collected from Pathology laboratory of HIT hospital. Blood CP was performed on hematology Analyzer Sysmex (Sysmex Corporation, Kobe, Japan) KX21 3-Part Differential. HRCT severity scores were calculated by using Chest Severity Score System (CT-SS). The method used here was published in Radiol Cardiothoracic imaging and was proposed by Yang et al.in March 2020.[9] The 18 segments of both lungs were divided into 20 regions for this study. The left upper lobe's posterior apical segment was further divided into apical and posterior segmental regions, while the left lower lobe's antero-medial basal segment was split into anterior and basal segmental regions. After wards, the lung opacities were intuitively evaluated on chest CT scan by Radiologist. Depending upon the parenchymal opacification, the regions were scored as 0, 1 or 2 showing involvement of 0%, 1-50% or 51-100% respectively. The overall score ranges from 0-40 depending upon the sum of point scored in all 20 lung segments.[3]

HRCT reports were prepared by consultant radiologist of HIT hospital by using this score. HRCT was performed on Toshiba (Japan) Alexion-16 slice CT scan machine. These patients (N=83) were divided into mild disease group (n=44) with CT-SS of 1-19 and the severe disease group (n=39) with CT-SS of 20-40. PLR was calculated by dividing Absolute Platelets Count APC (150,000-450,000/ul) by Absolute Lymphocyte count ALC (1000-4800/ul).[10] NLR was calculated by dividing Absolute Neutrophils Count ANC (2500-6000/ul) by Absolute Lymphocyte Count ALC (1000-4800/ul) using QxMD calculator.[8,11] Data were analyzed by SPSS version 25 (SPSS Inc., Chicago, IL, USA).

RESULTS

This study included 52 (62.7%) males & 31 (37.3%) females, with an average age of 47.97 years (SD + 13.6). A total of 83 COVID-19 patients were grouped into mild disease (N=44) with CT-SS 1-19 and in the severe

disease group (N=39) with CT-SS 20-40.

We studied the correlation between the Computed Tomography Severity Score (CT-SS) and two inflammatory markers: PLR and NLR.

The median Neutrophil to Lymphocyte Ratio (NLR) was seen to be significantly higher in severe disease group. In the mild disease group having CT-SS between 1-19, it was 2.91 (IQR:1.60 - 4.69), while in the severe disease group having CT-SS between 20-40, the median NLR was 8.00 (IQR: 4.81 -14.67).

The median PLR was seen to be significantly raised in severe disease group. In the mild disease group (CT-SS 1-19), it was 148.67 (IQR: 97.47-251.82), while in the severe disease group (CT-SS 20-40), the median PLR was 210.89 (IQR: 150.0-324.9).

According to the table II, Mann Whitney test scores showed that NLR & PLR were significantly different in both the mild and severe disease groups based on CT-SS ($p< 0.05$) *.

Spearman correlation score was obtained for correlating the HRCT severity score with the inflammatory markers; NLR and PLR. Both showed strong positive correlation with the CT-SS ($r = 0.471$, $p < 0.05$ for NLR and $r = 0.347$, $p < 0.05$ for PLR).

DISCUSSION

According to the findings of this study, COVID-19 pneumonia patients with high HRCT scores had considerably higher NLR and PLR ratios. Explosive and unrestrained production of cytokines is the central pathophysiology of COVID-19 pneumonia.[12]. The virus reaches alveolar cells via Angiotensin Converting Enzyme 2 receptors, prompting the cells to release inflammatory chemicals that activate alveolar macrophages. The stimulation factors and chemokines produced by macrophages trigger an increase in mononuclear cells in lung tissue. The severe consequences of COVID-19 pneumonia are caused by a cytokine storm caused by extreme inflammatory cell infiltration. In our study, the NLR and PLR ratios were significantly higher in the severe illness group.[12,13]

Previous research shows that pneumonic COVID-19 patients had lower lymphocyte, monocyte, and eosinophil counts, as well as greater neutrophil and CRP levels, than non-pneumonic individuals.[14]WBC and their varying quantities, which include lymphocytes, neutrophils, eosinophils, and monocytes, are associated with inflammation and the immune system [12] Platelets, which are anucleate blood cells derived from megakaryocytes in the bone marrow, play an important role in the host's inflammatory and immunological responses, as well as the regulation of hemostasis and thrombosis.[15]

Yang et al. and Sun et al. studied certain haematological indices in COVID-19 patients and discovered that NLR, PLR, and MLR values were significantly higher in severe patients compared to non-severe patients.[15]

Our findings were consistent with previous research on the relationship between NLR and the prognosis of a variety of infectious diseases.[16] The likely explanation for this association is that neutrophils are a constituent of the leukocyte population that activates and travels from the venous system to the immunological organ or system, creating a large number of reactive oxygen species that can cause DNA injury in cells and release the virus. Consequently, antibody-dependent cell mediated cytotoxicity (ADCC) has the property to directly terminate the virus, expose virus antigen, and trigger cell-specific and humoral immunity.[17] Additionally, neutrophils interact with countless additional cell types then yield a widespread array of cytokines and effector chemicals, as well as circulating vascular endothelial growth factor. [16]

One more study found that the absolute value of lymphocytes was significantly lower in the severe ICU group, whereas neutrophil count was significantly more.[18]

In the current study, the HRCT severity score was associated with these inflammatory indicators. Together, these parameters displayed a strong positive association with the HRCT severity score. In COVID-19 patients, PLR has some links with coagulation disturbances, even though it does not have a substantial link with other inflammatory markers in other inflammatory disorders like myocardial infarction.[14]

Ding et al discovered that the NLR index is absolutely correlated with the degree of hospital stay and can forecast illness outcome.[19]

According to Le Qiu et al, in burn patients, NLR was a strong predictor for 3 months mortality.[20] A recent study found that patients with COVID-19 may have a worse prognosis if they have high neutrophil/lymphocyte ratios (NLR) and low lymphocyte/CRP ratios (LCR).[20]

Milena et al, correlated NLR, PLR, and eosinophils with HRCT severity scores; they included 149 COVID positive patients and 149 healthy age-matched subjects.

In our study, we used CT-SS to assess COVID severity and divided our sample population into two groups based on severity scores. In contrast, they divided COVID patients into three groups based on international standards established by the Fleischner Society Glossary of thoracic imaging.

Stage 1, categorized as mild severity including less than three areas of GGOs with a maximum diameter of 3cm. For Stage 2- Moderate severity, the criteria encompass up to three GGOs area or multiple lung areas with GGO linked to a propensity for lung consolidation (<50% lung parenchyma). Stage 3-High severity is categorized by diffuse GGO or lung consolidation covering more than 50% of lung parenchyma, accompanied by signs of distortion of lung architecture. Their results showed median NLR 2.56 (IQR=1.72-3.79) in COVID-19 patients vs 2.11 (IQR=1.65-2.57) in healthy controls. And median PLR 151.85 (IQR=112.86-211.59) in COVID-19 patients vs 125.84 (IQR=99.02-155.36) in healthy controls.[14] They didn't compare median NLR and PLR in three severity groups, as we did in our study. Our results displayed median NLR in mild disease was 2.91 (IQR=1.6-4.69) vs 8.00 (IQR=4.81-14.67) in severe disease and median PLR in mild disease was 148.0 (IQR=97.47-251.82) vs 210.0 (IQR=150.0-324.9) in severe disease.

In 2020 Yang et al, explored the association of NLR with COVID-19 disease severity in five centers of China. They graded the disease severity by symptomatology and imaging (TSS) into mild, moderate and severe disease.[21] Their results are analogous to our study, showing a mean NLR 2.38+1.10 in mild disease, 3.74+1.49 in moderate and 9.26+2.76 in severe disease.

A retrospective study conducted on 100 patients in India in 2021 classified the patients into mild and severe based on their clinical features. Their results showed that PLR was far lesser (141.4+82.9) in mild patients than in severe ones (252.6 +198.8). NLR was also calculated to be 3.76+4.5 in mild disease while it was 8.44+8.8 in severe disease.[7] Our study differed from theirs in that we compared the NLR and PLR ratios with the CTseverity score, whereas their study compared these ratios with clinical symptoms.

Not only do NLR and PLR values positively correlate with the severity of COVID-19 disease, but they can also be used as a more practical, safe, and cost-effective, that can take the place of a CTscan to predict severity and influence the treatment plan. Additionally, they can assist the clinician in determining when and if to prescribe a chest CTscan, which can help avoid repeat Ctscans.

Limitations of the study

The major limitation in our study was that it were conducted in single center.

CONCLUSION

Overall, our results demonstrate a significant positive correlation between the HRCTseverity score and two inflammatory markers, NLR and PLR, in COVID-19 patients.

These findings suggest that elevated NLR and PLR values could serve as an indicator of the systemic inflammatory response seen in COVID patients, as reflected by HRCTseverity score. They can be used as prognostic marker for COVID-19 pneumonia in settings where HRCTfacilities are not available.

Authors' contributions:

Prof. Dr Farhat Abbas Bhatti, Dr Rabia Waseem Butt, Dr Sumera Mumtaz, Dr Radia Amir: Substantial contributions to the acquisition of data

Dr Sumera Mumtaz: Data entry, Analysis, and interpretation for the work

Dr Sumera Mumtaz, Dr Radia Amir: Drafting the work

Prof. Dr Zubia Razzaq, Prof. Dr Aneeqa Shahid: Reviewing work draft critically for important intellectual content

Prof. Dr Zubia Razzaq: Final approval of the version to be published

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Acknowledgement:

We would like to acknowledge Prof. Dr. Wajiha, Incharge Research Cell, HITEC-IMS for helping in statistical analysis of our study.

Disclaimer:

Conflict of Interest:

The authors of this manuscript declare no relationship with any company, whose products or services may be related to the subject matter of this article.

Ethical statement:

Ethical approval was obtained from ERB of HITEC-IMS.

Funding Disclosure:

The authors received no financial support for the research, authorship, and/or publication of this article.

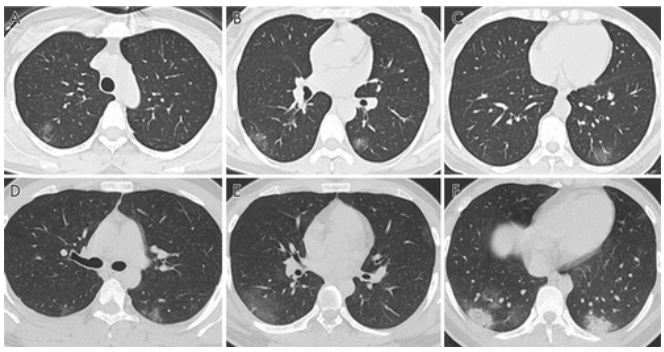

Fig. 1: MILD DISEASE: Faint glass opacities and sporadic consolidation signifying mild COVID disease.

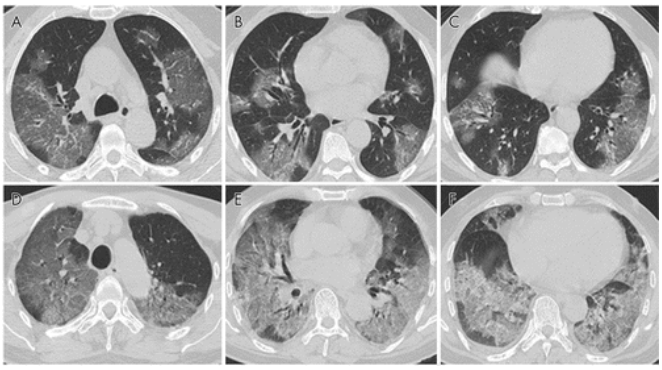

Fig. 2: SEVERE DISEASE: Dense areas of consolidation (GGOs) involving major lung parenchyma signifying severe COVID disease.

Table I: Median NLR & PLR for mild & severe COVID disease.

Parameter		Mild Disease (CT-SS 1-19)	Severe Disease (CT-SS 20-40)
NLR	Median	2.91	8.00
	IQR	1.6-4.69	4.81-14.67
PLR	Median	148.67	210.89
	IQR	97.47-251.82	150.0-324.9

Table II: Comparison of NLR & PLR in mild and severe disease using Mann Whitney Test.

Ratios	Groups	Total Number (N)	Mean rank	Sum of Ranks	*p*-Value
NLR	Mild Disease (CT-SS 1-19)	44	31.03	1365.5	0.000*
	Severe Disease (CT-SS 20-40)	39	54.37	2120.5	
PLR	Mild Disease (CT-SS 1-19)	44	34.43	1515.0	0.002*
	Severe Disease (CT-SS 20-40)	39	50.54	1971.0	

*p-value less than 0.05 is considered significant

Table III: Spearman's correlation between CT-SS and NLR & PLR (N=83)

			PLR	NLR	CT-SS
Spearman's rho	PLR	Correlation Coefficient	1.000	0.729	0.347
		Significance (2-tailed)	-	0.000	0.001
	NLR	Correlation Coefficient	0.729	1.000	0.471
		Significance (2-tailed)	0.000	-	0.000
	CT-SS	Correlation Coefficient	0.347	0.471	1.000
		Significance (2-tailed)	0.001	0.001	-