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Analysis of neutrophil to lymphocytes ratio (NLR), platelet to lymphocytes ratio (PLR) and Ct value as a prognostic tool in Covid-19 patients-A cross sectional study in southern Rajasthan

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Abstract
Introduction: Covid 19 is the ongoing pandemic which has affected millions of people across the world. Till date the number of confirmed cases in India has crossed 70 lacs. The clinical symptoms vary from a mild flu like illness to a severe from leading to ARDS, CBC analysis is a widely available test which can prove helpful in stratifying patients with Covid 19 infection. Neutrophil to Lymphocyte Ratio (NLR), Platelet to Lymphocyte Ratio (PLR) and Ct values have been studied across the world as a screening tool for severity among patients with Covid 19 infection.
Aim: This Study aims to analyze NLR, PLR ratio among Covid 19 patients and co-relate the severity of illness with viral load.
Type of Study: Retrospective Study
Material and Methods: Records of 80 Positive patients of Covid 19 were analyzed. Demographic data was recorded on a pre designed proforma, along with laboratory parameters like CBC, NLR, PLR and Ct values.
Results: Out of 80 patients analyzed, 61 were males and 19 were females. Hemoglobin ranged between 6.7 gm/dl to 15.7 gm/dl with a mean of 12.27 gm/dl. Total leucocytes count ranged from 2.9x 10^3/cumm to 22.0x 10^3/cumm with mean of 7.4 x10^3/cumm. 5 out of 80(6.25%) patients had Leucopenia and 6 out of 80 (7.5%) patients had Leucocytosis. Platelet count ranged from 65000 to 6.39 lacs/cu mm. Thrombocytopenia was observed in 13 patients (16.0%), while thrombocytosis was seen in 2 patients (2.5%). Cycle threshold value ranged between 19.18 and 34.0 with a mean of 26.59. NLR was calculated and it ranged from 0.55 to 27.0 with 17 patients having NLR >3.3, 63 patients had a NLR less than 3.3. All 17 patients who had a NLR > 3.3 were males, 14 of them were > 40 years of age(14/17) and rest 3 were between 20-40 years of age.11 patients had PLR > 180(i.e. 11 out of 80) and all of them also had NLR >3.3.
Most of the patient had mild to moderate symptoms, only 14 out of 80 patients(17.5%) required ICU care during the course of illness and 11 of these (78.57%) patients had NLR >3.3 and PLR >180.
Cycle threshold was < 24 in 13 out of 80 patients and only 4 of them (30.75%) required ICU care. Sensitivity of NLR, PLR & Ct value in predicting ICU care in our study is 78.57%, 64.28% and 35.75% respectively while specificity is 90.90%, 83.3% and 77.27% respectively.
Conclusion: NLR value of > 3.3 and PLR value of > 180 are sensitive markers in assessing the progression of disease. Ct value should not be considered as an important tool in monitoring the disease

Keywords: Covid-19, NLR, PLR, Ct value

Introduction
Analysis of Neutrophil to lymphocyte Ratio (NLR), Platelet to lymphocyte Ratio (PLR) and Ct value as prognostic tool in COVID-19 patients: A Cross sectional study in Southern Rajasthan.
Covid 19 is the ongoing pandemic declared by WHO on March 11, 2020. It is an infectious disease caused by SARS-CoV2 virus. The outbreak of this novel virus started in Wuhan, China in November 2019 and since then it has spread in more than 200 countries. The number of confirmed cases worldwide is more than 6.0 million and death toll has reached around 7.5 lacs, with 3.6 million cases in the United States and 2 million in Brazil [1]. The number of cases in India is more than 20 lacs till date The pandemic has surged in the

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country in recent weeks as it spread beyond the biggest cities, pushing India past Russia as the third-most-infected country. Compared to seasonal influenza Covid-19 has is more contagious, has a longer incubation period and is associated with higher hospitalization and mortality rates [2-4].

The clinical symptoms vary from a mild flu like illness to a severe pneumonia leading to Acute respiratory failure and Multi organ dysfunction Syndrome. Older age, associated co-morbidities are likely to be associated with worse outcomes [5, 6]. In India only 4.16% of patients have required ventilation. However the rapid person-to-person spread necessitates need of biomarkers that will help to categorize patients so optimal utilization of resources can be done.

CBC analysis is a simple and easily available test that can predict inflammatory response caused by various infections. Severe inflammatory response contribute to weak adaptive immune response, thereby resulting in immune response imbalance, therefore circulating biomarkers that can represent inflammation and immune status are important predictors of severity of illness. Studies across the world have shown that total WBC count, Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are reliable indicators of systemic inflammatory response going on in body as a consequence of Covid -19 virus [7, 8]. The NLR is calculated as the absolute neutrophil count divided by absolute lymphocyte count while the PLR is calculated as the absolute platelet count divided by absolute lymphocyte count. NLR, PLR along with cycle threshold (CT value) can be used as a part of diagnostic panel. The cycle threshold value is an indicator of viral load. Lower the cycle threshold value higher the viral load.

This study aims to analyze NLR, PLR among COVID-19 positive patients and Correlating the severity of illness with NLR, PLR & viral load.

Material and Methods
The present study is a retrospective analysis of RT-PCR confirmed Covid 19 patients with mild to severe symptoms admitted in dedicated COVID hospital in South Rajasthan from 5th July to 5th August. Prior permission from institution committee was taken from institutional ethical committee. Data was analyzed from the available indoor records.

Demographic data, clinical history, lab investigation were recorded on a predesigned performa. RT-PCR test was used to define Covid-19 positive cases. The isolated RNA target is transcribed generating complementary DNA by reverse transcriptase which is followed by the amplification of a conserved region of ORF1ab and N genes for SARS CoV-2 using specific primers and a fluorescent labeled probe. The cycle threshold < 35 was taken as a cut off for Covid positivity. CBC analysis on day of admission was done and NLR, PLR was calculated.

A total of 80 patients formed the study group.

Results
Of the 80 patients analyzed, 61 were males (76.25%) and 19 were females (23.75%) [Table-01]. Mean age was 45.73 years, 17 patients were less then 30 years of age(20.98%) while 46 patients were in 31-60 years of age (57.5%),18 were of > 60 years of age (21.25%), so the incidence was more in middle age population i.e. in 30-60 years of age [Diag-1]. Hb of patients ranged from 6.7 gm/dl to 15.7 gm/dl with a mean of 12.27 gm/dl. Total leucocytes count ranged from 2.9x 10^3/cumm to 22.0x 10^3/cumm with mean of 7.4 x10^3/cumm. 5 out of 80(6.25%) patients had Leucopenia and 6 out of 80 (7.5%) patients had Leucocytosis. Platelet count ranged from 65000 to 6.39 lacs/cu mm. Thrombocytopenia was observed in 13 patients (16.0%), while thrombocytosis was seen in 2 patients (2.5%). Mean platelet count was 223x10^6 /cumm. Cycle threshold value ranged between 19.18 and 34.0 with a mean of 26.59. [TABLE-2]

NLR was calculated and it ranged from 0.55 to 27.0 with 17 patients having NLR >3.3, 63 patients had a NLR less than 3.3. All 17 patients who had a NLR > 3.3 were males, 14 of them were > 40 years of age(14/17) and rest 3 were between 20-40 years of age.11 patients had PLR > 180(i.e. 11 out of 80) and all of them also had NLR >3.3.

Most of the patient had mild to moderate symptoms, only 14 out of 80 patients(17.5%) required ICU care during the course of illness and 11 of these (78.57%) patients had NLR >3.3 and PLR >180.

Cycle threshold was < 24 in 13 out of 80 patients and only 4 of them (30.75%) required ICU care. Sensitivity of NLR, PLR & Ct value in predicting ICU care in our study is 78.57%,64.28% and 35.75% respectively while specificity is 90.90%,83.3% and 77.27% respectively.

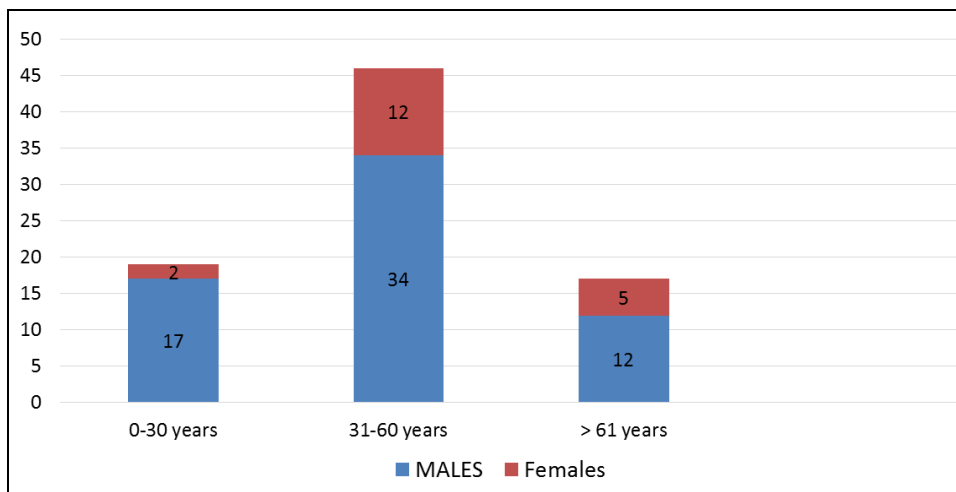**Table 1:** Distribution of cases according to sex

Male	61	76.25%
Female	19	23.75%
Total	80	100%

Fig 1: Demographic data of Study group

Table 2: Laboratory parameters of the study group

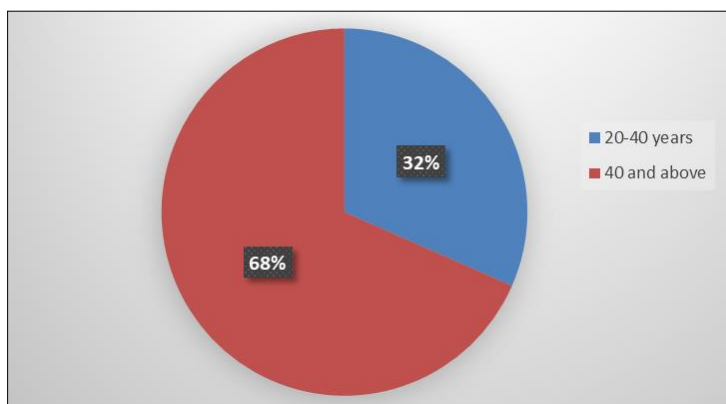| Parameter	Mean	Range
Age	45.73years	03-85 years
Hb	12.27gm/dl	6.7-15.7 gm/dl
Total leucocyte count	7.4 x10^3/cumm.	2.9x 10^3/ cumm to 22.0x 10^3/cumm
Platelet count	223x10^6/cumm	65000 to 6.39 lacs/cumm
NLR	3.15	0.55 to 27.0
PLR	136.01	23.84-395.0
Ct value	26.59	19.18 to 34.0

Fig 2: Age wise distribution of Patients with NLR >3.3

Table 3: Sensitivity and specificity of NLR, PLR &Ct value in predicting disease progression

	Mild to moderate symptoms	ICU CARE	Total	sensitivity	Specificity
NLR >3.3	06 (35.29%)	11(64.71%)	17	78.57%	90.90%
NLR <3.3	60 (95.23%)	03 (4.77%)	63		
PLR >180	11 (55.0%)	09 (45.0%)	20	64.28%	83.3%
PLR <180	55 (91.66%)	05 (8.33%)	60		
Ct value <24	15(75.0%)	05(25.0%)	20	35.75%	77.27%
Ct value >24	51 (83.60%)	09 (16.39%)	60		

Discussion

Covid-19 has demonstrated a rapid spread of outbreak with human- to- human transmission, with a median incubation period of 3-5 days and a relatively low fatality rate. As of August 5th 2020 more than 22 lac cases were confirmed in India with approximately 45000 deaths and mortality was less than 2.0% according to ICMR data. The clinical features of Covid-19 were similar to those seen in SARS-CoV infection. Fever and Cough were the predominant symptoms however symptoms like loss of taste, smell and GIT symptoms like loose motions were also seen in a quite a few of patients.

A pattern of hematological, biochemical, inflammatory and immune biomarker abnormalities has been identified in patients with severe disease compared to mild systemic disease [9-15].

Hematological biomarkers that can be used to stratify COVID-19 patients include WBC count, Lymphocyte count, Neutrophil count, Neutrophil-to- Lymphocyte ratio(NLR), ESR, Platelet count, Platelet-to- Lymphocyte ratio(PLR) and Hemoglobin [16-18].

Various studies have analyzed hemoglobin values in COVID-19 patients with or without severe disease (i.e., those needing mechanical ventilation, intensive care unit (ICU) admission or those who died), and some of them have found an association with low hemoglobin value at admission to severity of disease [19]. However we could not find any significant association between low hemoglobin value and severity of infection or correlation of low Hb value with NLR & PLR values.

NLR is an important biomarker for sepsis and it can predict the probability of death in patients with various cardiovascular diseases and stroke.

Patients with severe COVID-19 disease present with increased leukocytosis, neutrophilia, lymphopenia, and thrombocytopenia than those with non-severe disease. These patients were more likely to develop ARDS and required intensive care unit (ICU) level of care. NLR and PLR are easily obtained from a serum complete blood count with a differential profile. Thus NLR & PLR serve as a function of relative neutrophilia, thrombocytosis, and lymphopenia. The different mechanisms of lymphopenia in COVID-19 patients have been linked to the virus's ability to infect T cells through the angiotensin- converting enzyme 2 (ACE2) receptors and cluster of differentiation (CD) 147-spike proteins.

In our study the NLR ranged between 0.55 to 27.0. Those patients having NLR higher than 3.3 developed more severe clinical symptoms as compared to those with NLR less than 3.3. Our findings are in conjunction with studies across the world which have shown that a higher NLR is associated with greater severity of infection. In cases of other viral and bacterial pneumonia, NLR was more sensitive than individual levels of neutrophils and lymphocytes.

In the study by Yang *et al*. [20] NLR was found to be the most important prognostic factor among multiple variables in determining the severity of illness.

Ciccullio *et al*. [21] analysed 74 COVID positive patients and found that patients having NLR > 4 at time of admission had a more severe clinical course as compared to those with NLR < 4 at time of admission. Qin *et al*. [22] studied a cohort of 452 hospitalised patients with COVID 19 infection and found that a high NLR value was seen in patients with severe disease in particular a NLR value of > 4 at admission was a predictor to admission to ICU. Patients with higher NLR at time of admission have had a more severe form of disease as compared to those with low NLR. Both the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) indirectly reflect a patient's inflammatory state.

The NLR is calculated as the absolute neutrophil count divided by the absolute lymphocyte count, while the PLR is calculated by platelet count divided by absolute lymphocyte count. Platelets are important immune cells in the human body, which play an important role in hemostasis, coagulation, vascular integrity maintenance, angiogenesis, innate immunity, inflammatory response, tumor biology and so on. Changes in its number and activity are closely related to a variety of diseases. Platelets are produced by mature megakaryocytes in the bone marrow, and current studies have shown that a variety of cytokines, including TPO, IL- 3, IL- 6, IL- 9, IL- 11, and stem cell factor (SCF), can promote the production of megakaryocytes. *In vitro* experiments, the addition of mixed cytokines can stimulate the generation of megakaryocytes. SCF plays an important role in promoting the proliferation of megakaryocytes at the early stage of differentiation, while IL- 3 and TP0 play a synergistic role in the differentiation of megakaryocytes. In state of inflammation, IL- 6 can promote the generation of megakaryocytes by stimulating the increase of TPO level.12- 15 The absolute value of lymphocytes and platelet levels can be used as sensitive indicators to reflect the body's infection and inflammation control. Studies across the world have shown a significant association between high PLR and severity of infection [17]. In our study 11 patients out of 80 had a higher PLR value(> 180), also had a NLR > 3.3 on day of admission and required admission to ICU. Studies across the world have reported similar findings.

Currently, RT-PCR is the most widely used diagnostic method for COVID-19. The SARS-CoV-2 genome encodes four structural proteins. The spike surface glycoprotein (S) mediates specific binding to the host cell receptors, the nucleocapsid (N) protein binds to the coronavirus RNA genome to make the nucleocapsid, the membrane (M) protein is the main structural protein that connects between the membrane and the capsid, and the small envelope (E) protein which is involved in the assembly and budding process of the coronavirus. Among them, the genes for the N and E proteins are used as the targets for amplification in the RT-PCR assay combined with the open reading frame 1 (ORF1) ab, and the RNA-dependent RNA polymerase (RdRP) gene. The cycle threshold or Ct value of a RT-PCR reaction is the number of cycles at which fluorescence of the PCR product is detectable over and above the background signal. However, the problem with a Ct-based diagnosis is that there is no absolute or constant Ct cut-off value, and Ct cut-off values are different for each diagnostic reagent even for the same gene. For example, although there are differences according to diagnostic reagents, a sample is usually judged positive for COVID-19 based on a Ct value of 35. Studies have reported that a lower Ct value on admission is associated with progression to severe clinical course [23]. Out of the 80 patients in our study 41 patients had Ct value < 30 at time of admission but their clinical course varied from mild to severe infection, similarly 13 patients had Ct values <24 but only 4 of them required ICU care. So we could not conclude any significant association of lower Ct values with the progression of disease. Our findings are in consistence with the recent guidelines of ICMR which point out that patients in early symptomatic stage may show a high Ct value which may subsequently change [24]. Ct values of RT-PCR reported by laboratories has several limitations [25] in light of this scenario a high Ct values will give a false sense of security, so severity of COVID-19 disease largely depends on host factors besides the viral load.

Conclusion
COVID-19 is a rapidly spreading infectious disease caused by SARS-CoV-2, a novel coronavirus. NLR >3.3 and PLR >180 could help in predicting the severity of illness among Covid 19 patients. Higher NLR and PLR at time of hospital admission was associated with a more severe outcome. In particular, a NLR of > 3. 3 is an important, predictor of admission to the ICU On the other hand cycle threshold values which serves as an indicator of viral load is not a very specific predictor of progression or severity of illness.

A small sample size and non-assessment of comorbid conditions among the study group are the limitations of our study.

Although further studies with a larger sample size and detailed clinical workup will be needed to properly assess this matter, the current study shows that NLR & PLR may be an easily and widely available, useful prognostic factor in the early screening of critical illness among COVID-19 patients in resource limited settings.

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