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Original Article

Pathology Section

Neutrophil-to-Lymphocyte Ratio as an Early Predictor of Severe COVID-19: A Retrospective Study

DEEPA KRISHNAN[1], JAYANTHI RANGARAJAN[2], PUSHPA BALAMURUGESAN[3], SELVI PALANISAMY[4], SUDHA SUBANANDAM[5], PREMALATHA SUNDERASAN[6]

ABSTRACT

Introduction: The ongoing pandemic Coronavirus disease-2019 (COVID-19) is posing a great challenge to the medical fraternity across the world. The disease has an unpredictable complex clinical course. Despite intermittent lockdowns of varying lengths and several containment procedures including personal behavioural changes observed, the virus persists with continuing mutations and surges in infection being reported in many countries. With existing limited knowledge about post infection immunity and efficacy of the newer vaccines currently, there is a need to identify risk factors and early predictors of severe COVID-19 that could help timely risk stratification and prompt initiation of appropriate treatment to reduce morbidity and mortality.

Aim: To identify the risk factors associated with severe COVID-19 and to analyse the significance of neutrophil-to-lymphocyte ratio (NLR) in detecting severe COVID-19 early.

Materials and Methods: This was a retrospective observational study conducted in Government Medical College Hospital, Omandurar Government Estate, Chennai, a tertiary care hospital in Tamil Nadu, India from July 2020-September 2020. The study included 300 COVID-19 patients admitted during the study time period. The clinical, demographic and laboratory profile were compared between the patients with severe and non severe disease to analyse the role of baseline NLR and other risk factors associated with severe COVID-19.

Results: Majority of the patients were males (69%), with median age of 55 years. Older age and co-morbidities, diabetes mellitus and hypertension showed increased association with severe COVID-19. Complete Blood Count (CBC) examination at admission demonstrated significant elevation of total count, neutrophil count, NLR and reduction of lymphocyte count, platelet count in patients with severe and critical illness. Logistic regression identified NLR at admission, age, co-existing diabetes mellitus, serum ferritin and Computed Tomography (CT) chest lung involvement as independent risk factors for severe COVID-19. NLR had the largest Area Under the Curve (AUC) of 0.901; 95% Confidence Interval (CI), 0.867-0.935 followed by CT chest lung involvement with AUC of 0.887; 95% CI, 0.851-0.923 and serum ferritin with AUC of 0.818; 95% CI, 0.762-0.874 in Receiver Operating Characteristic (ROC) curve analysis.

Conclusion: This study demonstrated NLR at admission as an early predictor of severe COVID-19. Increased baseline NLR, older age, co-existing diabetes mellitus, elevated serum ferritin and higher CT chest lung involvement percentage were independent risk factors associated with severe and critical illness.

Keywords: Complete blood count, Coronavirus disease-2019, Pandemic, Risk factors, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The pandemic COVID-19 is caused by a novel coronavirus, named Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) by International Committee on Taxonomy of Viruses on February 11' 2020 [1]. The disease originated from the city of Wuhan, China where the virus was identified in the human airway epithelial cells of a cluster of patients with pneumonia of unknown aetiology in the months of December' 2019 and January' 2020 [2]. World Health Organisation (WHO) declared COVID-19, a global pandemic on March 11' 2020 following alarming levels of spread of the disease outside China and in many countries [3]. The disease has a wide range of complex clinical course from asymptomatic infection, mild and moderate illness in majority of the infected people who recover with appropriate treatment, to severe, critical illness occurring in a small proportion of patients, with acute, fatal complications including severe pneumonia, Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, coagulopathy and multiorgan failure [4].

Research studies have reported the association of elderly age, male gender and pre-existing co-morbidities with severe disease and poor clinical outcome [5-7]. Significant haematological abnormalities including raised Neutropil-to-Lymphocyte Ratio (NLR), lymphopenia, thrombocytopenia and raised inflammatory parameters including Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), Lactate Dehydrogenase (LDH), ferritin and Interleukin-6 (IL-6), and coagulation abnormalities such as prolonged Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), and elevated D-dimer, fibrin degradation products are observed to be associated with increasing clinical severity of COVID-19 [8]. The identification of risk factors that predict severe COVID-19 early, may help to reduce mortality and improve the treatment outcome. The present study was done to identify the risk factors associated with severe COVID-19 and to analyse the role of baseline NLR in predicting severe COVID-19 early.

MATERIALS AND METHODS

The present study was a retrospective observational study conducted in Government Medical College Hospital, Omandurar Government Estate, Chennai, a tertiary care centre in Tamil Nadu, India from July 2020-September 2020, analysis of the data was done from December 2020-February 2021. Study population included 300 COVID-19 patients admitted in the tertiary care centre during the study period. The study was approved by the Institutional Ethics Committee ECR/1492/Inst/TN/2021 of the hospital.

Inclusion criteria: The inclusion criteria were patients: i) ≥18 years of age; and ii) with confirmed SARS-CoV-2 infection by Reverse Transcription Polymerase Chain Reaction (RT-PCR) test done using nasopharyngeal and oropharyngeal swabs.

Exclusion criteria: Patients with haematological malignancies and antenatal mothers were excluded from the study.

Study Procedure

The study had 150 in-patients with mild or moderate disease who recovered with no disease progression in one group categorised as non severe group and 150 in-patients who had severe or critical disease at admission or moderate disease and progressed to severe illness later, in the other group categorised as severe group. The disease classification as mild, moderate, severe and critical disease was based on the guidelines in the Interim Guidance by WHO on Clinical Management of COVID-19 issued in May 2020 [4]. The demographic data (age and gender), the clinical manifestations, associated co-morbidities, and the laboratory data were retrieved from the electronic medical records in the hospital. The laboratory data collected include the Complete Blood Count (CBC) analysis, serum ferritin and the percentage of lung involvement evaluated by CT chest. CBC and CT chest were done for all patients at admission and repeated appropriately, as decided by the treating physician. Estimation of serum ferritin was done in patients who had an increase in NLR and other inflammatory markers, CRP and LDH. The baseline CBC parameters at admission {Total Leukocyte Count (TLC), lymphocyte count, neutrophil count, NLR, haemoglobin, platelet count}, serum ferritin and the maximum degree of lung injury (lung involvement percentage evaluated by CT chest) observed with disease progression were included for analysis. The clinical, demographic and laboratory profile were reviewed and compared between the two groups.

STATISTICAL ANALYSIS

Continuous data were expressed as median with Interquartile Range (IQR) and categorical variables as frequency and percentage. The statistical significance of the differences between the two groups was evaluated using Mann-Whitney U test and Chi-square test, where appropriate. The relationship of baseline NLR with CT chest lung involvement percentage and serum ferritin was evaluated using Pearson correlation analysis. Univariable and multivariable logistic regression analysis were done to identify the risk factors associated with severe disease. ROC curve analysis was performed to assess the predictive accuracy of the risk factors and determine the cut-off value, sensitivity and specificity. Statistical analysis of all the data was done using statistical software Statistical Package for the Social Sciences (SPSS) version 16.0. All the tests were bilateral and a p-value <0.05 was considered statistically significant.

RESULTS

Of the 300 patients enrolled in the study, the median age was 55 years with IQR of 46-64 years and 207 patients (69%) were males. Patients in the severe group were significantly older {median age (IQR), 59 years (50-67 years)} compared to those in the non severe group {median age (IQR), 53 years (40-61 years)}. There was no significant gender difference between the severe and non severe patients, with males constituting the majority (72% in severe and 66% in non severe group). Regarding clinical features [Table/Fig-1], 289 patients (96%) were symptomatic with fever (53.3% in severe group; 56.7% in non severe group), cough (52% in severe group; 43.3% in non severe group) and breathlessness (48% in severe group; 22.7% in non severe group) being the commonly observed clinical symptoms. The other less commonly reported clinical features included myalgia, fatigue, diarrhoea, sore throat, rhinorrhea, loss of smell and loss of taste. Of the total 300 patients,

Clinical features	Severe [No.(%)]	Non severe [No.(%)]
Fever	80 (53.3)	85 (56.7)
Cough	78 (52)	65 (43.3)
Breathlessness	72 (48)	34 (22.7)
Myalgia	2 (1.3)	4 (2.7)
Fatigue	1 (0.7)	1 (0.7)
Sore throat	1 (0.7)	3 (2)
Head ache	1 (0.7)	0
Diarrhoea	2 (1.3)	2 (1.3)
Loss of smell	2 (1.3)	1 (0.7)
Loss of taste	1 (0.7)	0

[Table/Fig-1]: Clinical features of the study population.

141 patients (47%) had pre-existing chronic diseases. Diabetes mellitus (33.7%), hypertension (26%), coronary artery disease (3.3%) and chronic obstructive pulmonary disease (2.3%) were the commonly encountered diseases. The co-morbidities were more frequently seen among the patients in severe group (63.3% in severe group vs 30.7% in non severe group). The median time to admission since illness onset was four days for severe patients and three days for non severe patients showing statistical significance. The demographic and clinical characteristics of the study population are shown in [Table/Fig-2].

Parameters	Total n (%)	Severe n (%)	Non severe n (%)	p-value
Age (years)	55 (46-64)	59 (50-67)	53 (40-61)	<0.001
<50	94 (31.3)	33 (22)	61 (40.7)	<0.001
≥50	206 (68.7)	117 (78)	89 (59.3)	<0.001
Gender				
Male	207 (69)	108 (72)	99 (66)	0.261
Female	93 (31)	42 (28)	51 (34)	
Co-morbidities*				
Total	141 (47)	95 (63.3)	46 (30.7)	
Diabetes mellitus	101 (33.7)	69 (46)	32 (21.3)	<0.001
Hypertension	78 (26)	52 (34.7)	26 (17.3)	0.001
Coronary artery disease	10 (3.3)	6 (4)	4 (2.7)	0.513
Chronic obstructive lung disease	7 (2.3)	5 (3.3)	2 (1.3)	0.251
Others	16 (5.3)	10 (6.7)	6 (4)	0.304
Time to admission since illness onset (No. of days)	3 (2-5)	4 (3-5)	3 (2-4)	<0.001

[Table/Fig-2]: Demographic and clinical characteristics.
Continuous data expressed as median (interquartile range) and categorical data (count) as number (percentage). p value calculated by Mann-Whitney U test, Chi-square test, where appropriate;
*Total number of patients with co-morbid disease, single or multiple entered in the first row; Patients with multiple diseases are included under each disease category

Of the baseline CBC analysis done, the Total Leukocyte Count (TLC), Absolute Lymphocyte Count (ALC), Absolute Neutrophil Count (ANC) and NLR were found to be significantly different between the patients in severe and non severe group. The haematological and CT chest findings of the patients are presented in [Table/Fig-3]. The significant haematological abnormalities noted at admission among the patients in severe group were leukocytosis (TC >11×10^9/L) in 43.3%, lymphopenia (ALC <1×10^9/L) in 56%, neutrophilia (ANC >7.5×10^9/L) in 58.7% and thrombocytopenia (platelet count <150×10^9/L) in 20.7% of patients. Compared with patients in non severe group, patients with severe and critical disease had significant higher NLR {median(IQR); 2.4 (1.6-4.4) vs 9.6 (6.2-15.1)}. Serum ferritin was available for only 75 patients in the non severe group since it was not done as a routine test for all in-patients, unless clinically indicated. Serum ferritin levels (ng/mL) were significantly elevated among the patients with severe and critical illness {median (IQR); severe: 847.3 (501.25-1431.75) vs non severe: 300 (104-581)}. CT chest examination showed a

Parameters	Total	Severe	Non severe	p-value
Total count (*10^9/L)	8.20 (5.90-11.50)	10.10 (7.07-4.30)	6.70 (5.37-9)	0.024
<4×10^9/L	23 (7.7)	11 (7.3)	12 (8)	<0.001
4-11×10^9/L	196 (65.3)	74 (49.3)	122 (81.3)	<0.001
>11×10^9/L	81 (27)	65 (43.3)	16 (10.7)	<0.001
ALC (*10^9/L)	1.2 (0.8-1.8)	0.9 (0.6 1.2)	1.7 (1.2-2.3)	<0.001
<1×10^9/L	106 (35.3)	84 (56)	22 (14.7)	<0.001
ANC (*10^9/L)	5.95 (2.30-9.70)	8.50 (5.78-12.27)	4.10 (3.10-6.20)	0.005
>7.5×10^9/L	109 (36.3)	88 (58.7)	21 (14)	<0.001
NLR	5.35 (2.30-9.70)	9.55 (6.20-15.05)	2.4 (1.60-4.35)	<0.001
>3.1	194 (64.7)	141 (94)	53 (35.3)	<0.001
Hemoglobin (g/dL)	12.9 (11.5-13.6)	13 (11.7-13.6)	12.8 (11.5-3.6)	0.490
Platelet count (*10^9/L)	239 (185-310)	229 (169-294)	248 (196-328)	0.019
<150×10^9/L	40 (13.3)	31 (20.7)	9 (6)	<0.001
Serum ferrittin (ng/mL)	618 (323.5-1079.5)	847.3 (501.25-1431.75)	300 (104-581)	<0.001
CT chest-lung involvement (%)	35 (15-57.5)	55 (40-70)	17.5 (5-30)	<0.001
<25%	108 (36)	13 (8.7)	95 (63.3)	<0.001
25-75%	166 (55.3)	111 (74)	55 (36.7)	<0.001
>75%	26 (8.7)	26 (17.3)	0	<0.001

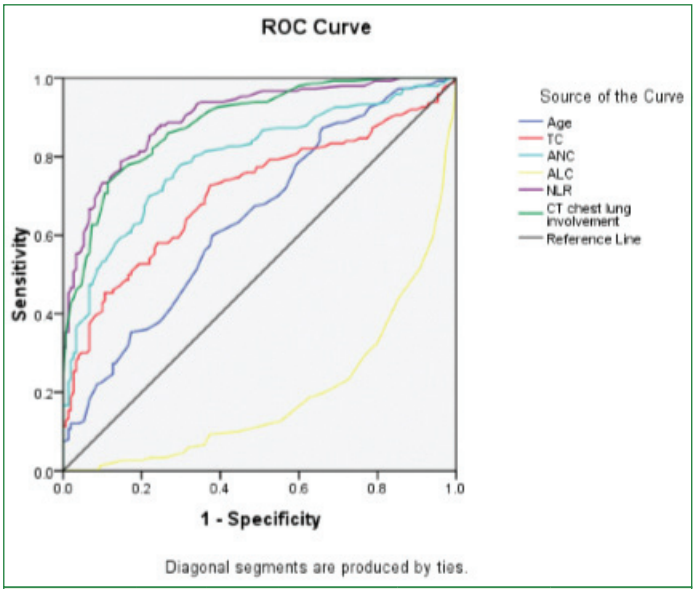
[Table/Fig-3]: Haematological and CT chest findings.
Continuous data expressed as median (interquartile range) and categorical data (count) as number (percentage); p-value calculated by Mann-Whitney U test, Chi square test, where appropriate; CT: Computed tomography; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; NLR: Neutrophil-to-lymphocyte ratio

greater percentage of lung involvement in patients with severe and critical disease {55% (40%-70%)} compared to those with non severe disease {17.5% (5%-30%)}. Among the patients in non severe group, 18 patients (12%) had no lung involvement.

Pearson correlation analysis showed positive association of baseline NLR with serum ferritin (r=0.279, p<0.001) and CT chest lung involvement percentage (r=0.473, p<0.001) observed with increasing disease severity. Univariable logistic regression analysis was done for the variables with significant between-group difference to identify the risk factors associated with severe COVID-19. Age, pre-existing diseases--diabetes mellitus and hypertension, NLR, TC, ANC, ALC <1×10^9/L, platelet count <150×10^9/L, serum ferritin and CT chest lung involvement percentage emerged as statistically significant risk factors. Variables with significant higher odds of developing severe disease {factors with Odds Ratio (OR) >1, with statistical significance and with a higher level of precision of OR i.e., smaller 95% confidence interval (95% CI)} were subjected to multivariable logistic regression. Since NLR reflects neutrophil and lymphocyte count, they were excluded. The results of univariable and multivariable regression are presented in [Table/Fig-4]. Baseline NLR at admission {OR (95% CI), 1.264 (1.069-1.496)}, age {OR (95% CI), 1.067 (1.025-1.111)}, CT chest lung involvement percentage {OR (95% CI), 1.064 (1.033-1.096)} and serum ferritin {OR(95% CI), 1.003 (1.002-1.004)} were found to be significant independent risk factors of severe COVID-19. Patients with diabetes mellitus also had significant increased odds {OR(95%CI), 3.298 (1.105-9.839)} of developing severe disease. The variables with potential risk for severe illness in univariable regression (age, TC, ANC, ALC, NLR, serum ferritin, CT chest lung involvement percentage) were also evaluated by ROC curve analysis to assess the predictive ability [Table/Fig-5a,b]. NLR at admission had the largest area under the curve (AUC-0.901, 95% CI 0.867-0.935, p<0.001) followed by CT chest lung involvement percentage (AUC-0.887, 95% CI 0.851-0.923, p<0.001) and serum ferritin (AUC-0.818, 95%CI 0.761-0.874, p<0.001). The optimal cut-off value, sensitivity and specificity of the risk factors were calculated using Youden index [Table/Fig-6]. The cut-off values for baseline NLR at admission, age, serum ferritin and CT chest lung involvement percentage were 5.85, 54.5 years, 376.15 ng/mL and 41%, respectively.

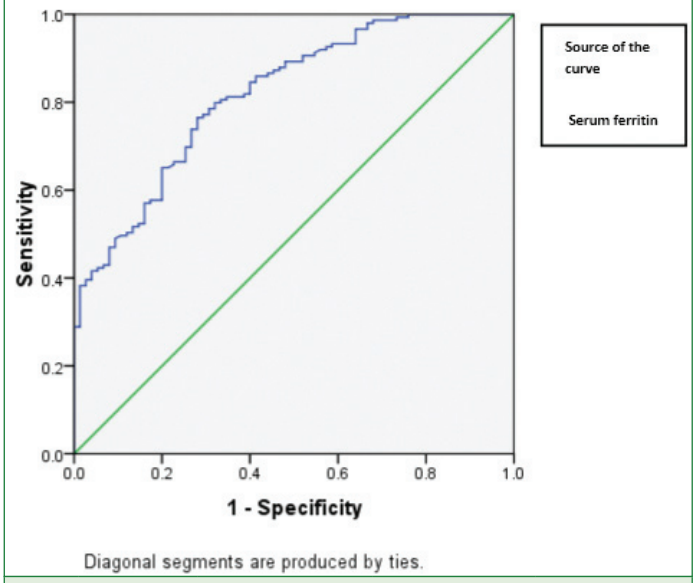
Variables	Univariable regression		Multivariable regression	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.043 (1.024-1.062)	<0.001	1.067 (1.025-1.111)	0.002
Gender	1.325 (0.811-2.165)	0.262		
Diabetes mellitus	3.141 (1.894-5.208)	<0.001	3.298 (1.105-9.839)	0.032
Hypertension	2.531 (1.474-4.343)	0.001	2.339 (0.686-7.178)	0.175
Total count	1.228 (1.148-1.313)	<0.001	1.087 (0.944-1.251)	0.246
ANC	1.408 (1.287-1.541)	<0.001		
ALC	0.165 (0.103-0.265)	<0.001		
Hemoglobin	1.039 (0.905-1.193)	0.590		
Platelet count	0.997 (0.995-0.999)	0.015		
NLR	1.638 (1.463-1.833)	<0.001	1.264 (1.069-1.496)	0.006
ALC <1×10^9/L	3.818 (2.531-5.760)	<0.001		
Platelet count <150×10^9/L	3.444 (1.699-6.983)	0.001		
Serum ferritin	1.003 (1.002-1.004)	<0.001	1.003 (1.002-1.004)	<0.001
CT chest lung involvement percentage	1.083 (1.065-1.102)	<0.001	1.064 (1.033-1.096)	<0.001

[Table/Fig-4]: Risk factors associated with severe COVID-19.
CT: Computed tomography; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; NLR: Neutrophil-to-lymphocyte ratio



Area under the curve					
Test result variable (s)	Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Age	0.643	0.032	<0.001	0.581	0.705
TC	0.709	0.030	<0.001	0.650	0.768
ANC	0.796	0.026	<0.001	0.746	0.847
ALC	0.194	0.025	<0.001	0.145	0.243
NLR	0.901	0.017	<0.001	0.867	0.935
CT chest lung involvement	0.887	0.018	<0.001	0.851	0.923

[Table/Fig-5a]: The test result variable(s): Age, TC, ANC, ALC, NLR, CT chest lung involvement have atleast one tie between the positive actual state group and the negative actual state group. Statistics may be biased; a) Under the non parametric assumption b. Null hypothesis: true area=0.5.
*ROC curve for serum ferritin done separately as serum ferritin levels were available for 225/300 patients only



Area under the curve				
Test result variable (s): Sr. ferritin (ng/mL)			Asymptotic 95% confidence interval	
Area	Std. error[a]	Asymptotic Sig.[b]	Lower bound	Upper bound
0.818	0.029	<0.001	0.762	0.874

[Table/Fig-5b]: The test result variable(s): Serum Ferritin has atleast one tie between the positive actual state group and the negative actual state group. Statistics may be biased; b) Null hypothesis: true area=0.5.
*ROC curve for serum ferritin done separately as serum ferritin levels were available for 225/300 patients only

S. No.	Variable	AUC (95%CI)	Sensitivity (%)	Specificity (%)	Cut-off value	p-value
1.	Age	0.643 (0.581-0.705)	63.3	55.3	54.5	<0.001
2.	TC	0.709 (0.650-0.768)	58	74.7	8.95	<0.001
3.	ANC	0.796 (0.746-0.847)	68.7	78.7	6.55	<0.001
4.	ALC	0.194 (0.145-0.243)	80	66.7	1.35	<0.001
5.	NLR	0.901 (0.867-0.935)	78.7	85.3	5.85	<0.001
6.	Serum ferritin	0.818 (0.762-0.874)	82	60	376.15	<0.001
7.	CT chest lung involvement	0.887 (0.851-0.923)	73.3	88.7	41	<0.001

[Table/Fig-6]: Results of ROC curve analysis.
ROC curve: Receiver operating characteristic curve; AUC: Area under the curve; CT: Computed tomography; ALC: Absolute lymphocyte count; ANC: Absolute neutrophil count; NLR: Neutrophil-to-lymphocyte ratio

DISCUSSION

The COVID-19 has a wide spectrum of clinical severity. More than the viremia, it is the infection associated hyperinflammation and aberrant cytokine production developing in a subset of patients, complicate the disease course with challenging unprecedented lethal complications, difficult to be treated effectively. Studies on the immunological characteristics of COVID-19 suggested the possible association of exuberant inflammatory response and cytokine storm with increasing disease severity [6,7]. The observations include marked elevation of serum cytokines, Interleukin-2 Receptor (IL-2R), IL-6, IL-8, IL-10 and Tumour Necrosis Factor α (TNFα), more pronounced in severe cases and significant reduction in the absolute number of T lymphocytes, in particular CD4+ T cells, also CD8+ T cells and decreased Interferon γ (IFN-γ) expression by CD4+ T cells, again more evident in severe cases [6,7]. Hence, dysregulation of immune response was reported to have a major role in COVID-19 pathogenesis. The laboratory assessment of inflammatory and immunological parameters in blood, thus play a vital role in monitoring the disease progression and guide prompt initiation of appropriate treatment and improve treatment outcome.

In the present study, hospitalised COVID-19 patients were more of older age (≥50 years-68.7%) showing a male predominance (69%), and more so among the patients with severe disease (patients ≥50 years-78%; males-72%). Patients with pre-existing co-morbidities (63.3%) were observed to develop progressive disease more frequently, diabetes mellitus (46%) and hypertension (34.7%) being the common diseases encountered. In particular, patients with diabetes mellitus were found to have a significant higher odds of developing severe disease {OR (95%CI); 3.298 (1.105-9.839)} probably due to weaker immune functions. Studies done elsewhere have also shown a male predominance in COVID-19 infected patients and a significant association of patients with older age, and pre-existing co-morbidities with severe COVID-19 [2,5-7]. Consistent with other studies, fever and cough were the common presenting clinical features observed, followed by breathlessness more common in the severe group than in the non severe group [5,7,9].

The haematological parameters evaluated in CBC including TC, ANC, ALC and NLR reflect the inflammatory status of the patient. In this study, patients with severe disease had significant elevation of TC, ANC, NLR and reduction of ALC, platelet count compared to those with non severe disease. The CBC analysis at disease onset, showed leukocytosis in 43.3%, neutrophilia in 58.7%, lymphopenia in 56% and thrombocytopenia in 20.7% of patients in severe group observed to be statistically significant when compared with non-severe group. With increasing disease severity, neutrophilic leukocytosis was noted in 75.3%, lymphopenia in 76%, and thrombocytopenia in 25.3% of patients in the severe group. High leukocyte count, neutrophilia and lymphopenia have been reported in association with severe COVID- 19 [6-8]. Yang AP et al., and Guan W et al., noted an increased incidence of lymphopenia in COVID-19 patients (80.6% and 83.2%, respectively) in their studies [9,10]. Another retrospective study by Liu J et al., found that a decrease in lymphocyte count was related to disease progression [11]. Huang C et al., observed lymphopenia ($<1\times10^9$/L) more frequently in patients who needed Intensive Care Unit (ICU) admission (85% in ICU patients and 54% in non ICU patients) [12]. In a retrospective study on 201 COVID-19 patients, Wu C et al., demonstrated significant association of neutrophilia and lymphopenia with a higher risk for developing ARDS in bivariate Cox regression analysis [13]. Thrombocytopenia observed in significant association with severe COVID-19 in the present study, was more frequently severe thrombocytopenia ($<50\times10^9$/L) in critically ill patients. Patients with critical illness were observed to develop a more prominent low lymphocyte count and a progressively low platelet count. A meta-analysis of nine studies (totaling 1779 COVID-19 patients with 399 (22.4%) of them having severe disease) was done by Lippi G et al., to evaluate the association of thrombocytopenia with severe COVID-19. The authors reported that low platelet count was significantly associated with increased risk for severe disease and mortality in COVID-19 patients and hence should serve as an indicator of worsening illness during hospitalisation [14].

Serum ferritin, another important inflammation related parameter, was significantly elevated in patients with severe disease (847.3 ng/mL), in the present study. Median serum ferritin level was 800.4ng/ml in severe COVID-19 patients in the study by Qin C et al., [6], and 1029.28 ng/mL in COVID-19 patients who developed ARDS in the study by Wu C et al., [13]. Significant association of elevated serum ferritin with death in COVID-19 patients was noted by Zhou F et al., in their study (1435.3 ng/mL in non survivors versus 503.2 ng/mL in survivors) [5]. The patients who developed severe and critical illness had a significant higher degree of lung injury compared to those with mild and moderate disease with no disease progression.

The NLR is a biomarker of systemic inflammation. It is a simple, inexpensive and easy to calculate parameter obtained from CBC analysis, a routine investigation performed in the evaluation of COVID-19 patients in all centres. It is calculated by dividing ANC by ALC. NLR is a promising indicator of systemic inflammatory response, whose prognostic role has been studied in patients with several infections, inflammatory diseases and cardiovascular diseases [15-18]. It has also been reported as a significant predictor of prognosis and treatment outcome in various malignancies including breast, stomach, colorectal, pancreatic, oesophageal and lung cancers [19-21]. Currently, in the ongoing pandemic COVID-19, increasing NLR is being observed as a significant indicator of disease progression. Its role in early prediction of severe disease is being evaluated. In present study, baseline NLR is significantly increased among the patients who developed severe and critical illness. A single centre retrospective study done to identify predictors of disease progression in COVID-19 pneumonia in Wuhan, China showed that, of the total 456 patients, 251 (55.04%) patients with progressive disease had elevated NLR on admission {3.37 (2.06-5.66)} compared to those (205/456, 44.96%) with non progressive disease {2.00 (1.42-3.25)} [22]. Another multicentre retrospective study, also observed that COVID-19 patients with progressive disease had raised NLR on admission {4.8 (3.1-5.1)} than those with stable disease {2.5 (1.8-3.4)} [23]. A meta-analysis of five studies by Lagunas-Rangel FA showed that NLR values increased significantly in severe COVID-19 patients (SMD=2.404, 95% CI=0.98-3.82) [24]. Increased NLR reflects an increase in neutrophil count and decrease in lymphocyte count. Various factors have been suggested for these haematological changes noted. The viral infection is associated with dysregulated immune response and resulting hyperinflammation with excess cytokines, could trigger the production of neutrophils and promote the apoptosis of lymphocytes [6,9,25,26]. Neutrophilia could also be due to the bacterial co-infections occurring in patients with severe viral infection due to low immune functions [25]. In hospitalised patients, increased neutrophils might even be linked to the treatment with corticosteroids. Apart from the virus triggered inflammation, the ability of the virus to infect T lymphocytes through Angiotensin Converting Enzyme-2 (ACE-2) receptors and viral cluster of differentiation (CD)147-spike proteins is also related to the lymphopenia seen [26-28].

With logistic regression analysis, present study demonstrated the significance of the baseline CBC parameters, TLC, neutrophil count, ALC $<1\times10^9$/L, platelet count $<150\times10^9$/L and NLR in early prediction of severe COVID-19. Serum ferritin and CT chest lung involvement percentage noted with increasing disease severity, age and co-morbidities, diabetes mellitus and hypertension also had significant association with severe COVID-19 in logistic regression. Baseline NLR at disease onset, age, pre-existing diabetes mellitus, CT chest lung involvement percentage and serum ferritin emerged as significant independent risk factors for severe and critical illness. NLR exhibited outstanding discrimination and predictive accuracy for severe disease with largest AUC of 0.901 in ROC curve analysis. CT chest lung involvement and serum ferritin also had excellent discriminating capability with AUC of 0.887 and 0.818 respectively. The optimal cut-off value for NLR at admission, CT chest lung involvement, serum ferritin and age were 5.85, 41%, 376.15 ng/mL, and 54.5 years respectively. A review of the current scientific literature done to study the importance of laboratory medicine in COVID-19 diagnosis and prognosis, analysed the abnormal laboratory findings in COVID-19 patients in 19 studies (totaling 2988 patients with 484(16.1%) having severe disease). The authors concluded that laboratory medicine may provide essential assistance to discriminate between severe and non severe COVID-19 and predict COVID-19 prognosis [8].

The observations in present study indicate that increased NLR at admission, older age and co-existing diabetes mellitus would serve as early predictors for severe COVID-19. A retrospective study analysing the laboratory findings in 115 COVID-19 patients identified NLR as the most important prognostic factor for disease progression, followed by age. The authors found that NLR was an independent risk factor for predicting critical illness early and suggested risk stratification of patients based on NLR and age; patients aged ≥50 years and having NLR ≥3.13 were predicted to develop critical illness, and hence should have rapid access to ICU if necessary [11]. Another group, retrospectively assessed the clinical, laboratory and CT characteristics of 247 COVID-19 patients and demonstrated that CT severity score was associated with inflammatory indexes and older age, higher NLR and CT severity score on admission were independent predictors for progression to severe COVID-19 pneumonia [23]. Liu Y et al., had even reported NLR as an independent risk factor for mortality in hospitalised COVID-19 patients [25]. Further, present study, showed positive correlation of NLR at admission with the degree of lung injury (assessed by CT chest) and serum ferritin levels noted with increasing disease severity. Hence, baseline NLR, age and co-morbid disease diabetes mellitus, as early predictors of severe COVID-19 might help in early risk stratification and management and also might reduce mortality.

Limitation(s)

Though this was a single centre retrospective study with a possibility of selection bias, the study report was in accordance with studies done in different countries across the world. Hence, this emphasises the significance of laboratory parameters in the management of COVID-19 patients.

CONCLUSION(S)

In present study, NLR has shown a promising independent role in predicting severe COVID-19 early. NLR, an inexpensive and rapidly available parameter, age and co-existing diabetes mellitus would help identify patients with risk for severe COVID-19 early, even at disease onset. Further, NLR and other CBC parameters (including total count, neutrophil count, lymphocyte count, platelet count), CT chest lung examination and estimation of serum ferritin have a key role in clinical disease monitoring, enabling prompt risk stratification and appropriate management, improving clinical outcome.

REFERENCES

[1] Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-44. Doi: 10.1038/s41564-020-0695-z. Epub 2020. PMID: 32123347; PMCID: PMC7095448.

[2] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33. Doi: 10.1056/NEJMoa2001017. Epub 2020 Jan 24. PMID: 31978945; PMCID: PMC7092803.

[3] WHO Director-General's opening remarks at the media briefing on COVID-19--11 March 2020. https://www.who.int/director-general/speeches/detail.

[4] World Health Organization. (2020). Clinical management of COVID-19: Interim guidance, 27 May 2020. World Health Organization. https://apps.who.int/iris/handle/10665/332196.

[5] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet. 2020;395(10229):1054-62. Doi: 10.1016/S0140-6736(20)30566-3. Epub 2020 Mar 11. Erratum in: Lancet. 2020;395(10229):1038. Erratum in: Lancet. 2020;395(10229):1038. PMID: 32171076; PMCID: PMC7270627.

[6] Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with Coronavirus 2019 (COVID-19) in Wuhan, China. Clin Infect Dis. 2020;71(15):762-68. Doi: 10.1093/cid/ciaa248. PMID: 32161940; PMCID: PMC7108125.

[7] Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020;130(5):2620-29. Doi: 10.1172/JCI137244. PMID: 32217835; PMCID: PMC7190990.

[8] Pourbagheri-Sigaroodi A, Bashash D, Fateh F, Abolghasemi H. Laboratory findings in COVID-19 diagnosis and prognosis. Clin Chim Acta. 2020;510:475-82. Doi: 10.1016/j.cca.2020.08.019. Epub 2020 Aug 14. PMID: 32798514; PMCID: PMC7426219.

[9] 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. Doi: 10.1016/j.intimp.2020.106504. Epub 2020. PMID: 32304994; PMCID: PMC7152924.
[10] Guan W, Ni Z, Hu Yu, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-20. https://doi.org/10.1056/NEJMoa2002032.
[11] Liu J, Liu Y, Xiang P, Pu L, Xiong H, Li C, et al. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med. 2020;18(1):206. Doi: 10.1186/s12967-020-02374-0. PMID: 32434518; PMCID: PMC7237880.
[12] 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. Doi: 10.1016/S0140-6736(20)30183-5. Epub 2020 Jan 24. Erratum in: Lancet. 2020 Jan 30;: PMID: 31986264; PMCID: PMC7159299.
[13] Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020;180(7):934-43. Doi: 10.1001/jamainternmed.2020.0994. PMID: 32167524; PMCID: PMC7070509.
[14] Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. Clin Chim Acta. 2020;506:145-48. Doi: 10.1016/j.cca.2020.03.022. Epub 2020. PMID: 32178975; PMCID: PMC7102663.
[15] Ge YL, Zhang HF, Zhang Q, Zhu XY, Liu CH, Wang N et al. Neutrophil-to-Lymphocyte ratio in adult community-acquired pneumonia patients correlates with unfavorable clinical outcomes. Clin Lab. 2019;65(5). Doi: 10.7754/Clin.Lab.2018.181042. PMID: 31115235.
[16] Liu H, Zhang H, Wan G, Sang Y, Chang Y, Wang X, et al. Neutrophil-lymphocyte ratio: A novel predictor for short-term prognosis in acute-on-chronic hepatitis B liver failure. J Viral Hepat. 2014;21(7):499-507. Doi: 10.1111/jvh.12160. Epub 2013. PMID: 24750274.
[17] Yao C, Liu X, Tang Z. Prognostic role of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio for hospital mortality in patients with AECOPD. Int J Chron Obstruct Pulmon Dis. 2017;12:2285-90. Doi: 10.2147/COPD.S141760. PMID: 28814856; PMCID: PMC5546734.
[18] Angkananard T, Anothaisintawee T, McEvoy M, Attia J, Thakkinstian A. Neutrophil lymphocyte ratio and cardiovascular disease risk: A systematic review and meta-analysis. Biomed Res Int. 2018;2018:2703518. Doi: 10.1155/2018/2703518. PMID: 30534554; PMCID: PMC6252240.
[19] Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. J Natl Cancer Inst. 2014;106(6):dju124. Doi: 10.1093/jnci/dju124. PMID: 24875653.
[20] Ozyurek BA, Ozdemirel TS, Ozden SB, Erdogan Y, Kaplan B, Kaplan T. Prognostic value of the Neutrophil to Lymphocyte Ratio (NLR) in lung cancer cases. Asian Pac J Cancer Prev. 2017;18(5):1417-21. Doi: 10.22034/APJCP.2017.18.5.1417. PMID: 28612596; PMCID: PMC5555556.
[21] Yodying H, Matsuda A, Miyashita M, Matsumoto S, Sakurazawa N, Yamada M, et al. Prognostic significance of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in oncologic outcomes of esophageal cancer: A systematic review and meta-analysis. Ann Surg Oncol. 2016;23(2):646-54. Doi: 10.1245/s10434-015-4869-5. Epub 2015 Sep 28. PMID: 26416715.
[22] Cheng B, Hu J, Zuo X, Chen J, Li X, Chen Y, et al. Predictors of progression from moderate to severe coronavirus disease 2019: A retrospective cohort. Clin Microbiol Infect. 2020;26(10):1400-05. Doi: 10.1016/j.cmi.2020.06.033. Epub 2020. PMID: 32622952; PMCID: PMC7331556.
[23] Feng Z, Yu Q, Yao S, Luo L, Zhou W, Mao X, et al. Early prediction of disease progression in COVID-19 pneumonia patients with chest CT and clinical characteristics. Nat Commun. 2020;11(1):4968. Doi: 10.1038/s41467-020-18786-x.
[24] Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. J Med Virol. 2020;92(10):1733-34. Doi: 10.1002/jmv.25819. Epub 2020 Apr 8. PMID: 32242950; PMCID: PMC7228336.
[25] Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalised patients with COVID-19. J Infect. 2020;81(1):e06-12. Doi: 10.1016/j.jinf.2020.04.002. Epub 2020 Apr 10. PMID: 32283162; PMCID: PMC7195072.
[26] Chan AS, Rout A. Use of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in COVID-19. J Clin Med Res. 2020;12(7):448-53. Doi: 10.14740/jocmr4240. Epub 2020 Jun 25. PMID: 32655740; PMCID: PMC7331861.
[27] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-73. Doi: 10.1038/s41586-020-2012-7. Epub 2020 Feb 3. PMID: 32015507; PMCID: PMC7095418..
[28] Wang K, Chen W, Zhou YS, Lian JQ, Zhang Z, Du P, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. bioRxiv; 2020. Doi: 10.1101/2020.03.14.988345.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.
2. Professor and Dean, Department of General Medicine, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.
3. Professor, Department of Pathology, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.
4. Assistant Professor, Department of Pathology, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.
5. Tutor, Department of Pathology, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.
6. Associate Professor, Department of Pathology, Government Medical College, Omandurar Government Estate, Block B, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Selvi Palanisamy,
Government Medical College, Omandurar Government Estate, Block B,
Chennai-600002, Tamil Nadu, India.
E-mail: drdsomc@gmail.com

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