

A combination of inflammatory and hematological markers is strongly associated with the risk of death in both mild and severe initial disease in unvaccinated individuals with COVID-19 infection

Parul Chopra¹, Tushar Sehgal¹, Ranjan Yadav¹, Suneeta Meena¹, Souvik Maitra², Kapil Dev Soni², Arulselvi Subramanian³, Shyam Prakash¹, Purva Mathur³, Sandeep Mittan⁴, Sooyun Tavolacci⁵, Ajeet Kaushik⁶, Kiran Gulia⁷, Ebrahim Mostafavi^{8,9}, Abhishek Gupta¹⁰, Anjan Trikha², Ritu Gupta¹¹, Kunzang Chosdol¹², Anant Mohan¹³, Kalaivani Mani¹⁴, Subrata Sinha^{1,12}, Sudip Kumar Datta¹

¹ Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India

² Department of Anaesthesiology, Pain Medicine and Critical Care, All India Institute of Medical Sciences, New Delhi, India

³ Department of Laboratory Medicine, JPNATC, All India Institute of Medical Sciences, New Delhi, India

⁴ Department of Obstetrics, Gynecology and Medical Oncology, Montefiore Medical Center, The University Hospital for Albert Einstein College of Medicine, New York, USA

⁵ Department of Hematology and Medical Oncology, Center for Thoracic Oncology, Tisch Cancer Institute and Icahn School of Medicine at Mount Sinai, New York, USA

⁶ Nano Biotech Laboratory, Department of Environmental Engineering, Florida Polytechnic University, Florida, USA

⁷ Department of Materials and Manufacturing, School of Engineering, University of Wolverhampton, England, UK

⁸ Stanford Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA, USA

⁹ Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

¹⁰ School of Allied Health and Midwifery, Faculty of Education, Health and Wellbeing, University of Wolverhampton, UK

¹¹ Laboratory Oncology Unit, Dr. BRAIRCH, All India Institute of Medical Sciences, New Delhi, India

¹² Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India

¹³ Department of Pulmonary, Critical Care & Sleep Medicine, All India Institute of Medical Sciences, New Delhi, India

¹⁴ Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India

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Corresponding author:

Sudip Kumar Datta
Department of Laboratory Medicine
All India Institute of Medical Sciences
New Delhi
India
Phone: +919350179698
E-mail: dr.sudipdatta@gmail.com

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ABSTRACT

Background

Inflammatory and hematological markers are used extensively for early prognostication and monitoring in COVID-19.

We aimed to determine whether routinely prescribed laboratory markers can predict adverse outcome at presentation in COVID-19.

Methods

This retrospective observational study was performed on 401 samples collected between July to December 2020 from COVID-19 positive subjects, admitted at All India Institute of Medical Sciences, Delhi, India. Clinical details and laboratory investigations within 3 days of COVID-19 positivity were obtained. Clinical outcomes were noted from patient medical records, till discharge or death. Laboratory parameters, with individually defined cut-offs, were used, either singly or in combination to distinguish survival and death for those having severe and non-severe disease at initial presentation.

Findings

Total Leukocyte count, Absolute neutrophil count, Neutrophil to Lymphocyte ratio, C-Reactive Protein (CRP), Interleukin-6 (IL-6), Lactate Dehydrogenase, Ferritin and Lymphocyte to CRP ratio (LCR) were significantly altered at presentation in severe COVID-19 as compared to non-severe cases; and, also in those who died due to COVID-19 compared to those who survived. A combination of four markers, CRP (≥ 3.9 mg/dL); IL-6 (≥ 45.37 pg/ml); Ferritin (≥ 373 ng/mL); $1/LCR \geq 0.405$ was found to strongly predict mortality in cases with non-severe presentation as also in severe cases.

Conclusion and Interpretation

The combination of routinely used markers, CRP, IL-6, Ferritin and $1/LCR$ can be used to predict adverse

outcomes, even in those presenting with mild to moderate disease. This would identify subset of patients who would benefit from closer monitoring than usual for non-severe disease.



INTRODUCTION

Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 500 million people worldwide and has caused more than 6 million deaths (1). The clinical presentation of COVID-19 varies from asymptomatic cases to mild flu-like symptoms, to high fever and severe respiratory illness (2). Often, cases have rapid respiratory deterioration, shock, and/or multiple organ dysfunction or failure. Timely diagnosis of impending complications has become most important to contain the severity of disease as well as prevent fatality or any other adverse outcomes. Real-time polymerase chain reaction (RT PCR) has been the mainstay for COVID-19 diagnosis (3), however, routine tests such as hemogram and inflammatory marker levels have been used extensively to facilitate the assessment of disease severity and prognostication (4,5).

The serum levels of acute phase reactants (APRs) including serum C-reactive protein (CRP), ferritin and Interleukin 6 (IL-6), are known to exhibit significant changes due to infectious and non-infectious conditions including COVID-19 (4-6). Studies have also investigated serial evaluation of ferritin and other markers to help in prognostication (7). Other markers like d-dimer, lactate dehydrogenase (LDH), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to CRP ratio (LCR) have also been studied. Amongst these, CRP, although a non-specific marker of inflammation, has emerged as the most widely used single marker

in COVID-19 patients (8,9). Recent studies have even advocated the use of multiple markers like ferritin-transferrin ratio, NLR and WBC counts to be used together (10,11). However, no objective criteria for prediction of mortality could be evolved till date and no study till date explored the idea of assessing simultaneous derangement of multiple inflammatory and hematological markers for prognostication of adverse outcomes in mild and severe COVID-19 disease. The novelty of this study is to use a combination of regularly prescribed laboratory parameters in combination, with individually defined cut-offs, to define the risk of death at initial presentation, in both non-severe and severe COVID-19 disease.

METHODS

This study is a retrospective, observational analysis of hospital and laboratory reports of 401 COVID-19 positive patients, confirmed by RT-PCR, admitted in COVID-19 care facility of a tertiary teaching hospital in New Delhi, India between July 2020 to December 2020 and includes unvaccinated individuals. The patient records were compiled as a part of a sample repository to facilitate retrospective research. (Institutional Ethics Committee approval: Ref No. IEC-578/19.06.2020, RP-03/2020). Blood samples for baseline laboratory assessment as per clinicians' requests were collected within three days of positive RT-PCR report. The clinical details regarding the severity of the disease at initial presentation and outcome in terms of survival (S) or death (D) were noted from the patient medical records comprising their entire hospital stay till discharge or death. The patients were categorized into mild to moderate or non-severe (NSD), and severe (SD) groups as per the Indian Council of Medical Research (ICMR) and Ministry of Health and Family Welfare guidelines (12). Patients with history of recent surgery or hematological malignancies

were excluded from the study. All the patients were treated as per institutional protocol.

Complete blood count (CBC) parameters were run on Sysmex hematology analyzer (XN-9000) Kobe, Japan. Total leukocyte count (TLC), absolute lymphocytes (ALC) and absolute neutrophil count (ANC), platelet count (PC) were noted. NLR, PLR and LCR were calculated using absolute neutrophil and lymphocyte counts. Serum samples were analyzed on Roche Cobas 8000 series c702 and e801 analyzers for CRP (Tinaquant C-Reactive Protein IV - Roche Diagnostics), ferritin (Elecsys® Ferritin - Roche Diagnostics) and LDH (Lactate Dehydrogenase - Roche Diagnostics). IL-6 was estimated using Beckman DXI CLIA system.

For analysis, patients were divided into 4 subgroups based on their initial disease severity and final outcome: (a) Mild to moderate COVID-19 illness who survived (NSD-S); (b) Mild to moderate COVID-19 illness who succumbed to death (NSD-D); (c) Severe COVID-19 illness who survived (SD-S) and (d) severe COVID-19 illness who succumbed to death (SD-D). The Spearman correlation was calculated among the inflammatory markers and represented using correlation matrix graph. The comparison of laboratory parameters between NSD and SD, and between S and D was analyzed using t-test for parametric data and the Mann-Whitney U test for non-parametric data. Laboratory parameters were compared between all four subgroups (a to d) using analysis of variance (ANOVA) or Kruskal-Wallis test. Continuous data were reported as mean \pm standard deviation (SD) and categorical data were reported as number (percentage). Non-parametric data were reported as median (range). A receiver operating characteristics (ROC) curve analysis was carried out to assess the discriminative ability of various laboratory parameters between survival and death in both, severe and non-severe disease. The cut-off and its sensitivity and specificity along

with area under the ROC curve was reported. Further, with a sensitivity of 80% the cut-offs for four parameters which had maximum AUC in the ROC analysis were decided. An unadjusted and adjusted (for age and gender) likelihood of death for patients presenting with any three or all four out of the four elevated inflammatory markers (CRP \geq 3.9 mg/dL, IL-6 \geq 45.37 pg/mL, ferritin \geq 373 ng/mL and 1/LCR \geq 0.4052), and clinical severity was calculated using univariate and multivariate logistic regression models respectively. The results were reported as odds ratio and 95% CI. A p-value (two-sided) of less than 0.05 was considered statistically significant. All the statistical analyses were carried out using Stata 16.0 (StataCorp LLC, Texas, USA) and GraphPad ver.9.

RESULTS

In total, 401 adults (267 males and 134 females) were recruited in the study. Table 1(a) represents the distribution of patients as per severity and outcome whereas Table 1(b) summarizes the laboratory results amongst all patient subgroups: COVID-19 patients having severe (SD) and non-severe disease (NSD) at presentation, as well as, among those who survived (S) and those who succumbed to COVID-19 (D). TLC, ANC, NLR, CRP, IL-6, LDH, and ferritin were seen to be significantly higher in SD as compared to NSD. A similar trend was also obtained for D v/s S patients. In contrast, LCR was significantly lower in SD compared to NSD; and also in D with respect to S patients. However, PLR was not significantly different amongst subgroups of severity or mortality.

The median (range) of laboratory parameters is shown as box plots (Figures 1a for SD and NSD patients and 1b for outcome of patients with S and D). Table 2 compares the different laboratory parameters amongst the 4 subgroups (a-d) as described in Methods section and several

Table 1a Clinical characteristics of patients on basis of severity and mortality due to COVID-19 (n=401)

Severity	Overall (n= 401)	Non-severe disease (NSD, n=234, 58.3%)		Severe disease (SD, n=167, 41.7%)	
		M (154)	F (80)	M (113)	F (54)
Gender		M (154)	F (80)	M (113)	F (54)
Age, years (mean \pm SD)	52.2 \pm 15.6	51.03 \pm 15.6	54.95 \pm 15.5	51.3 \pm 15.7	53.6 \pm 15.7
Survivors (S)	277(69.1%)	145	77	36	19
Non-survivors (D)	124 (30.9%)	9	3	77	35
Total No. (%)	401 (100%)	154 (38.4%)	80 (19.9%)	113 (28.1%)	54 (13.6%)

Data presented as n (%) and mean \pm SD.

Table 1b Laboratory parameters of COVID-19 patients (n=401)

Parameter (unit)	Non-severe (NSD, n=234)	Severe (SD, n=167)	p- value	Survived (S, n=276)	Death (D, n=124)	p- value
Hemoglobin (g/dL)	11.25 (3.60-18.8)	10.5 (5.3-20)	0.160	10.5 (3.60-18.8)	11.3 (5.6-20)	0.29
TLC ($\times 10^3/\mu\text{L}$)	9.67 (0.90-40.8)	12.91 (0.34-47.5)	<0.001	10.04 (0.71-40.8)	13.05 (0.34-47.57)	<0.001
Platelet Count ($\times 10^3/\mu\text{L}$)	196.5 (14-905)	180 (10-591)	0.067	201.5 (11-905)	169.5 (10-591)	0.002
ANC ($\times 10^3/\mu\text{L}$)	7.91 (0.05-36.68)	11.31 (0.03-43.91)	<0.001	8.1 (0.05-36.6)	11.47 (0.03-43.91)	<0.001
ALC ($\times 10^3/\mu\text{L}$)	0.94 (0.01-6.01)	0.90 (0.10-9.8)	0.88	0.94 (0.01-9.8)	0.091 (0.12-6.84)	0.057
NLR	7.82 (0.53-79.58)	10.5(0.09- 157.17)	0.006	7.7 (0.5-120.12)	12.30 (0.09-157.17)	<0.001
PLR	217.15 (8.62-12400.79)	184.26 (4.9-2026.5)	0.256	200.7 (6.1-12400.7)	201.07 (4.93-2026.5)	0.861

CRP (mg/dL)	6.6 (0.10-132)	11.4 (0.10-46.7)	<0.001	6.65 (0.10-132)	13.4 (0.1-46.7)	<0.001
IL-6 (pg/mL)	49.09 (0.61-1624)	111.87 (1.15-1624)	<0.001	51.5 (0.61-1624)	165.03 (4.34-1624)	<0.001
LDH (U/L)	548.5 (44-6705)	616 (243-3742)	<0.001	545.5 (44-6705)	665.5 (243-3742)	<0.001
Ferritin (ng/mL)	585 (19.7-13638)	856 (8-25755)	0.004	553 (7.9-13638)	1099 (18.9-25755)	<0.001
LCR	1.72 (0.01-300)	0.73 (0.04-91)	<0.001	1.66 (0.01-300)	0.58 (0.04-38)	<0.001

Data presented as median (range). TLC: total leukocyte count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; CRP: C-Reactive protein, IL-6: Interleukin 6; LDH: lactate dehydrogenase; LCR: lymphocyte CRP ratio.

Figure 1 Levels of laboratory parameters in: (a) severe v/s non-severe disease; (b) survival v/s death

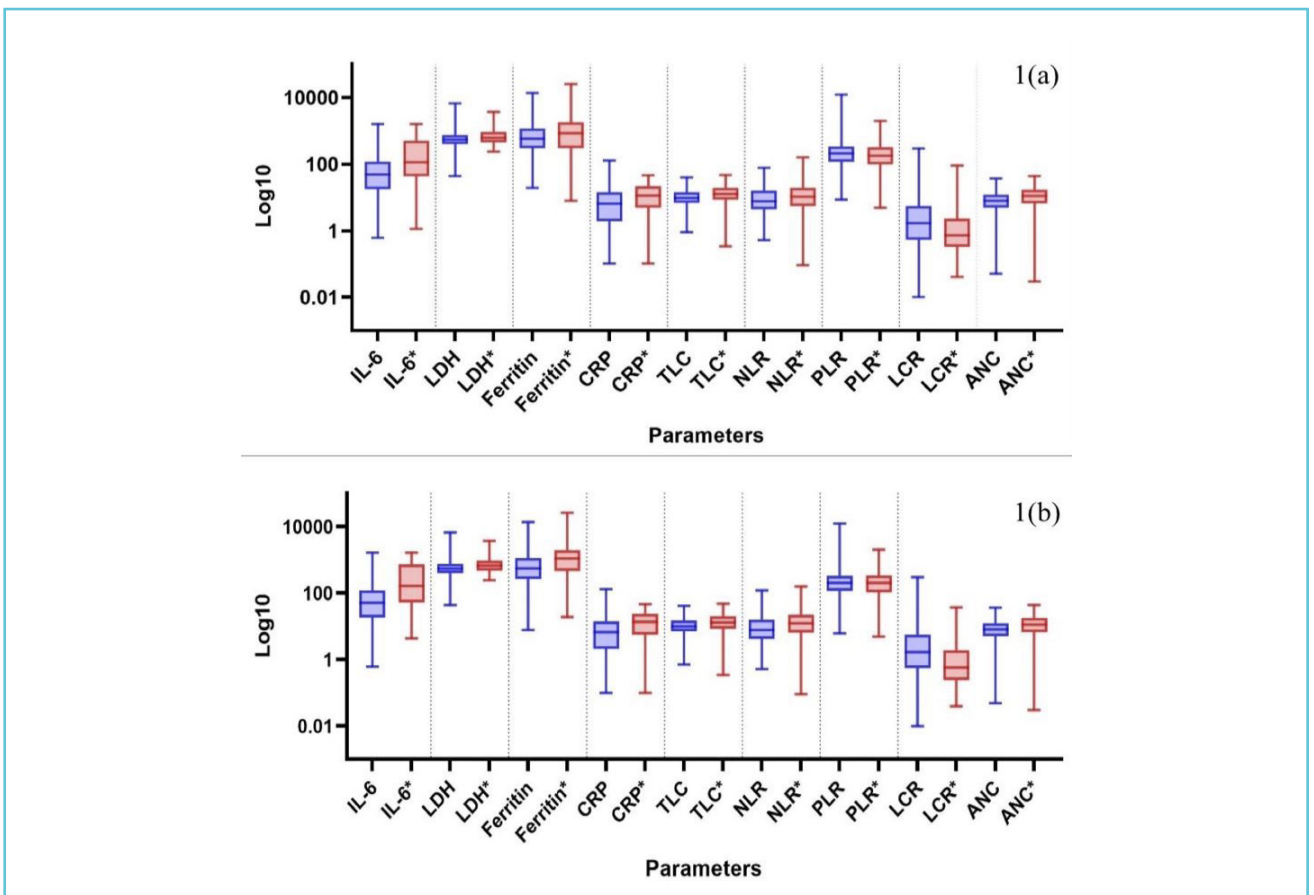


Table 2 Laboratory parameters by subgroups

	a (NSD-S)	b (NSD-D)	c (SD-S)	d (SD-D)	p-value
Number (%)	222 (55.4)	12 (3)	55 (13.7)	112 (27.9)	-
Age*, years	52.2±15.6	54.7±16.1	48.5±17.5	53.7±14.5	0.22
Hb (g/dL)	11.13±2.7	11.2±1.9	9.9±2.6	11.2±2.9	0.024
TLC (x10 ³ /μL)	9.6 (0.9-40.8)	9.2 (5.8-26.8)	12.4 (0.7-35.5)	13.1 (0.3-47.5)	<0.001
PC (x10 ³ /μL)	199 (14-905)	172.5 (15-298)	221 (11-570)	169.5 (10-591)	0.015
ANC (x10 ³ /μL)	7.9 (0.05-36.7)	8.09 (4.8-23.8)	10.2 (0.4-32.9)	11.5 (0.03-43.9)	<0.001
ALC (x10 ³ /μL)	0.9 (0.01-6.0)	0.83 (0.3-1.4)	1.02 (0.1-9.8)	0.9 (0.12-6.8)	0.03
NLR	7.7 (0.5-79.5)	9.5 (5.7-28.2)	8.5 (0.7-120.12)	12.5 (0.09-157.2)	0.001
PLR	217.2 (8.6-12400)	219.1 (21.6-562.6)	169.9 (6.1-1855.9)	196.5 (4.9-2026.5)	0.49
CRP (mg/dL)	6.4 (0.1-132)	20.85 (1.8-37.4)	10 (0.1-41.1)	13.15 (0.1-46.7)	<0.001
IL-6 (pg/mL)	46 (0.6-1624)	213 (13-1528)	73.6 (1.1-1528)	153.9 (4.3-1624)	<0.001
LDH (U/L)	537.5 (44-6705)	644 (421-934)	578 (241-3490)	667 (243-3742)	0.002
Ferritin ng/mL)	558 (20-13638)	1068 (428-2000)	500 (8-5787)	1099 (19-25755)	<0.001
LCR	1.76 (0.01-300)	0.52 (0.09-5.3)	1.05 (0.08-91)	0.56 (0.04-38)	<0.001

Data represented as median (range) and mean ± SD*.

a: (NSD-S) Mild to moderate COVID-19 who survived; b: (NSD-D) Mild to moderate COVID-19 who succumbed to death; c: (SD-S) Severe COVID-19 who survived; d: (SD-D) severe COVID-19 who succumbed to death.

Hb: Hemoglobin; TLC: total leukocyte count; PC: Platelet count; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; NLR: neutrophil to lymphocyte ratio; PLR: platelet to lymphocyte ratio; CRP: C-Reactive protein, IL-6: Interleukin 6; LDH: lactate dehydrogenase and LCR: lymphocyte CRP ratio.

parameters showed significant differences between the subgroups. The correlations between some of the laboratory parameters were statistically significant namely: IL-6 vs LDH; Ferritin vs CRP; TLC vs NLR; NLR vs PLR; PLR vs LCR; IL-6 vs CRP; IL-6 vs LCR; LDH vs TLC; Ferritin vs LCR; CRP vs LCR; TLC vs LCR; NLR vs LCR, and is as depicted using scatter plot matrix in Figure 2.

Table 3 summarizes the ROC analysis with individual parameter-wise cut-offs, specificity, and AUC for a fixed sensitivity of 80.6%. It was observed that the AUC of individual parameters to distinguish between death and survival varied between 0.710 and 0.614 which are generally

considered to be poorly discriminatory. To improve the discriminatory power, we tried a combination approach with two, three and four parameters together. The parameters which had the highest point estimates of AUC amongst all parameters (CRP, IL-6, ferritin and 1/LCR with AUC of 0.659, 0.710, 0.654 and 0.674 respectively), were chosen. We derived the individual parameter-wise cut-offs keeping a sensitivity of 80.6%. Next we defined subsets of patients who had these parameters elevated as per our derived cut-offs (as shown in Table 3). It is evident that the percentage of patients succumbing to death increased with the increased number of

Figure 2 Correlations between laboratory parameters using scatter plot matrix

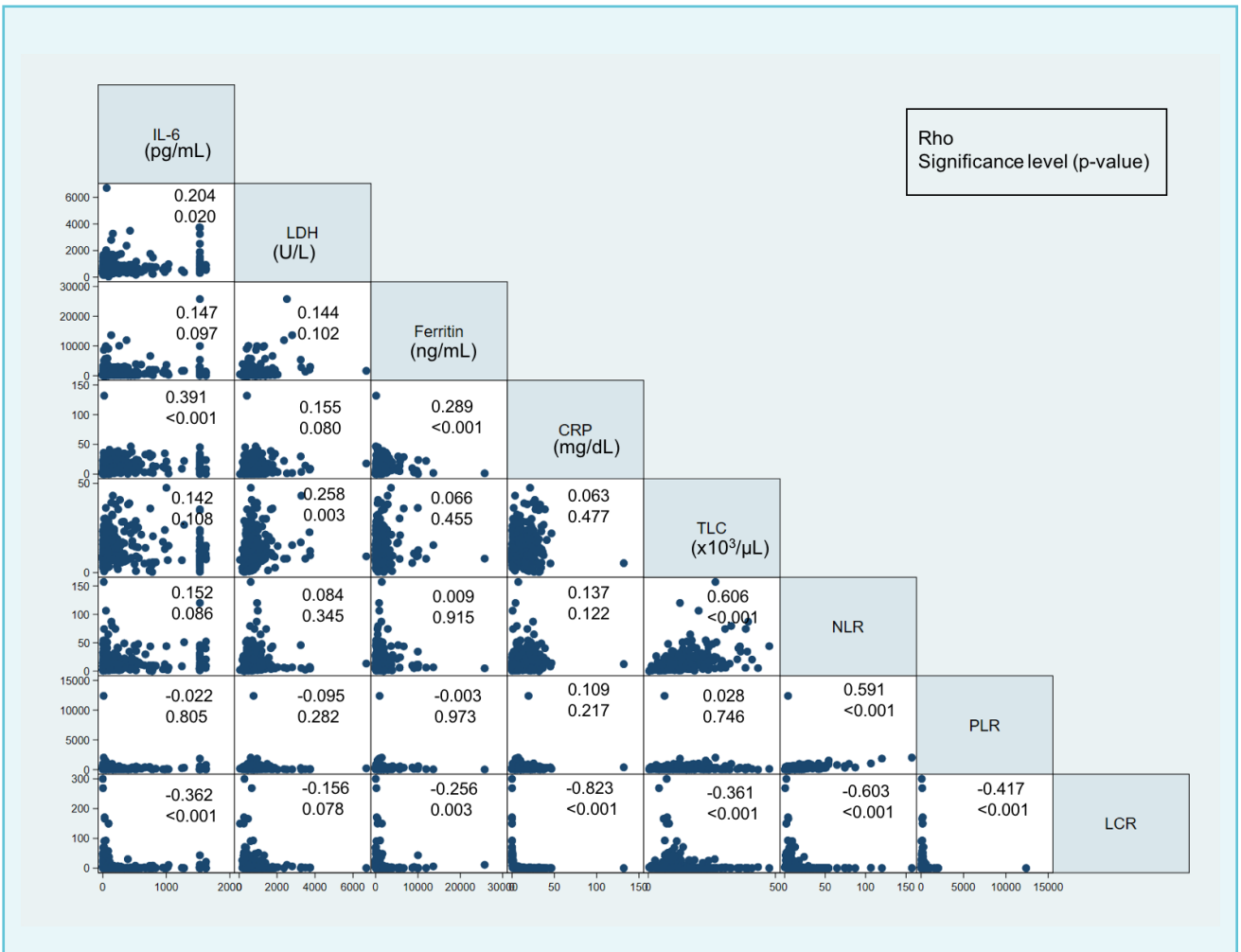
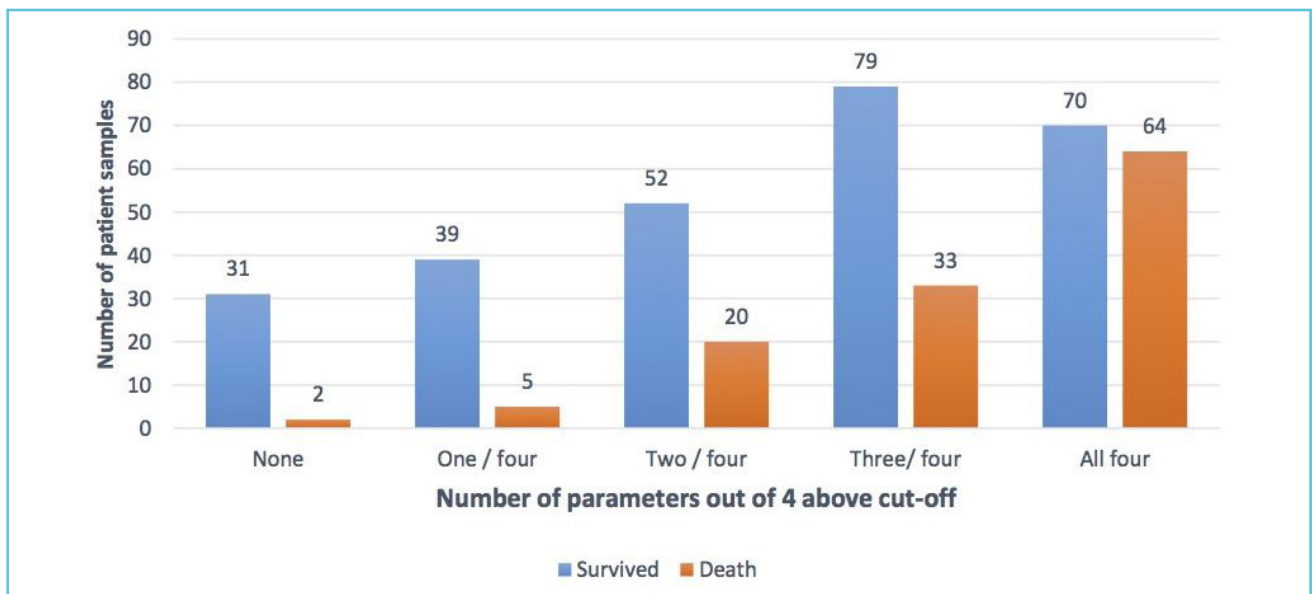


Table 3 Receiver Operating Characteristics curve for different laboratory parameters against death (n= 395#)

Parameter	Cut-off	Specificity [§]	Area under curve [§]
IL-6 (pg/ml)	≥45.37	46.13%	0.710
LCR1*	≥0.405	42.07%	0.674
CRP (mg/dL)	≥3.9	33.21%	0.659
Ferritin (ng/mL)	≥373	35.06%	0.654
ANC (x10 ³ /μL)	≥6.15	30.87%	0.627
NLR	≥5.76	35.06%	0.624
LDH (U/L)	≥421	31.00%	0.616
TLC (x10 ³ /μL)	≥7.55	29.89%	0.614

*LCR 1 is inverse of LCR (1/LCR), [§] Specificity and AUC given keeping the sensitivity fixed at 80.6% for all parameters, #Samples from patients with age≥18 years included.

Figure 3 Distribution of patients with abnormal laboratory parameters among death and survival groups

Legend: Bar diagram shows the number of patients with one or more abnormal laboratory parameters amongst the four selected using designated cut-offs: CRP (≥3.9 mg/dL); IL-6 (≥45.37 pg/ml); Ferritin (≥373 ng/mL); LCR1 ≥0.405 (LCR1 is 1/LCR).

parameters (out of our four defined parameters) having values above the decided cutoff by the ROC analysis (Figure 3).

Next, we analyzed the different subgroups based on clinical severity at onset, with reference to the presence of number of abnormal laboratory parameters as per our defined criteria. These were assessed to determine the likelihood of death with adjustment for age and gender (Table 4). NSD patients with three or more elevated markers, had an adjusted risk of death

(95% CI) of 2.62 (0.69-10.00) times when compared to NSD with two or less markers. For SD with three or more elevated markers, the adjusted risk of death was 88.76 (26.25-300.08), compared to SD with two or less markers having, the adjusted risk of death of 47.64 (12.91-175.77). Further, the combination of all four of the chosen inflammatory or hematological parameters versus three or less of these parameters were also analyzed similarly. The striking feature of this analysis is that when all four

Table 4 Age and sex adjusted Odds ratios (OR) for death v/s survival using clinical and laboratory characteristics (n= 395#)

Severity of disease at onset	No. of parameters selected [§]	Survived (S)	Death (D)	Total Number	Unadjusted OR (95% CI)	P-value	Adjusted OR* (95% CI)	P-value
Non severe (NSD)	2 or less [@]	104 (97.2)	3 (2.8)	107	1.0	-	1.0	-
	3 or more	114 (92.7)	9 (7.3)	123	2.73 (0.72-10.38)	0.139	2.62 (0.69-10.0)	0.157
Severe (SD)	2 or less	18 (42.8)	24 (57.2)	42	46.22 (12.59-169.6)	<0.001	47.64 (12.91-175.77)	<0.001
	3 or more	35 (28.5)	88 (71.5)	123	87.16 (25.91-293.1)	<0.001	88.76 (26.25-300.08)	<0.001
Non severe (NSD)	3 or less [@]	167 (97.7)	4 (2.3)	171	1.0	-	1.0	-
	All 4	51 (86.4)	8 (13.6)	59	6.54 (1.89-22.6)	0.003	6.26 (1.80-21.71)	0.004
Severe (SD)	3 or less	34 (37.8)	56 (62.2)	90	68.76 (23.36-202.3)	<0.001	70.20 (23.75-207.47)	<0.001
	All 4	19 (25.3)	56 (74.7)	75	123.05 (40.15-377.1)	<0.001	128.33 (41.62-395.64)	<0.001

Data presented as n (%), Odds Ratio (OR) with 95% Confidence Interval (CI), [§] No. of parameters selected out of four having values above cut-off, * Adjusted for age and gender, [@] Reference category, # Samples from patients with age ≥ 18 years included.

parameters are deranged (as per our cut-offs) in the NSD group, there is a 6.26 (1.80-21.71) fold increase in the likelihood of death (95%CI) when compared with those having three or less deranged parameters. In SD patients with all 4 elevated markers, the adjusted risk of death was 128.33 (41.62-395.64), compared to SD with 3 or less markers having, the adjusted risk of death of 70.20 (23.75-207.47).

The results were further analyzed to find the independent effect of severity of disease and elevation of all four laboratory parameters adjusting for age and sex. The patients having SD were having 37.97 (19.15-75.27) times higher adjusted risk of death compared to NSD and all four elevated parameters had 2.48 (1.36-4.51) times higher adjusted risk of death as compared to 3 or less elevated parameters. The predictive ability of this model as assessed by AUC of the ROC for this model was 0.89.

DISCUSSION

It has been widely reported that in COVID-19, despite the presence of mild to moderate symptoms initially, many patients go on to develop complications such as acute respiratory distress syndrome (ARDS) and may succumb to death (13,14). Several laboratory markers like, TLC, ANC, NLR, CRP, IL-6, LDH, Ferritin, d-dimer etc. have been studied for prognostication and the importance of these markers used individually, has been reported. However, not enough literature is available on the combined use of these markers to facilitate prognostication in the initial phase of the disease. The novelty of our approach lies in using routinely prescribed inflammatory and hematological parameters, that were assessed and compared between patients presenting with varying severity of COVID-19 disease.

In our study, TLC, ANC, NLR, CRP, IL-6, LDH, and Ferritin were found significantly higher and LCR

significantly lower in those with SD compared to NSD and in those who succumbed to death due to COVID-19 compared to them who survived. These markers are routinely done as a part of COVID-19 workup but are produced as a result of different pathophysiological processes and work independently. Their serum levels do not necessarily correlate strongly with each other as has been observed in the correlation matrix. Even the ROC analysis revealed poor discriminatory performance of individual parameters. We then used a combination of four markers (CRP, IL-6, Ferritin and 1/LCR) with cut-offs defined at 80.6% sensitivity and we found that these together were valuable in predicting mortality even in the cases that were non-severe at presentation. Next we took a novel combination approach, where, to improve the discriminatory power, we evaluated two, three and four parameters together. Further evaluation was done on the subset of patients who had these parameters (CRP, IL-6, ferritin and 1/LCR) elevated as per our derived cut-offs.

Studies on inflammatory markers including serum ferritin, procalcitonin (PCT), CRP, and IL-6 have reported these markers to be significantly associated with the high risks of the poor prognosis and development of severe disease with COVID-19 (5,15). There is a higher risk of ARDS and lung damage in those having elevated CRP levels (16,17). Recent literature has shown that IL-6 levels are significantly higher in the initial phase of the disease or at admission in those who develop more severe symptoms or succumb to the disease or have interstitial lung involvement (13,14).

A few studies including the current one showed the association of increased ferritin levels with severity and mortality in COVID-19 (18,19), however, some studies report higher ferritin to be a bystander rather than being a true characteristic of COVID-19 (20).

In a meta-analysis of 28 studies LDH levels reported in severe vs. non-severe groups showed mean difference of 154.49 (95% CI 121.24 - 191.73, $p < 0.001$) (21). On comparison a statistically significant and raised level of LDH was also seen in patients admitted to ICU vs. non-ICU patients (mean difference = 272.98; 95% CI: 195.46, 350.51; $p < 0.001$), and in patients who could not survive vs. survived patients (mean difference = 259.21; 95% CI: 166.91, 351.51; $p < 0.001$) (21).

Lymphopenia is caused by multiple factors including direct viral injury due to expression of ACE 2 on surface of lymphocytes (15), cytokine storm causing lymphocyte apoptosis induced by interleukins, as well as atrophy of lymphoid organs leading to reduced turn over. In the initial part of the disease, peripheral blood leukocyte and lymphocyte counts are normal or slightly reduced (22). With progression, significant lymphopenia occurs along with deterioration in medical condition and increase in inflammatory mediators in the blood. In our study, samples were collected within 3 days of positive RT-PCR for COVID-19, hence, significant difference in ALC between SD and NSD ($p=0.88$), and between S and D ($p=0.057$) was not observed. However, TLC and ANR were significantly increased in those with SD compared to NSD as seen in other studies. It has been reported that lymphopenia on admission (lymphocyte count $< 1,100$ cells/ μ l) is associated with a three times higher risk of poor outcomes, in younger vs older patients (22). Lymphocyte counts were lower in patients with ARDS, severe disease requiring ICU care, and in non-survivors (23). Interestingly, NLR, PLR, and LCR (all having Lymphocyte count as a component) have been reported extensively as prognostic biomarkers in COVID-19 studies (24-31). The increased mobilization of neutrophils due to increased release of pro-inflammatory cytokines leads to higher neutrophils in peripheral blood and relative lymphopenia, as a

manifestation of severe infection. Lymphopenia along with stress-mediated neutrophilia, leads to a high NLR value and increased NLR was found to be associated with poor clinical outcome in severe COVID-19 (24). Another similar biomarker, LCR, which has previously been studied and used as a prognostic marker for various cancers, including colon and gastric carcinomas (25,26) serves as a marker for the systemic inflammatory process due to interaction of tumor and host immune cells (27). Since COVID-19 also leads to a systemic inflammatory response, LCR helps in prognostication and management of patients. NLR and LCR have thus been studied as inflammatory markers that reflect systemic inflammatory response, and both are easily available in almost all the laboratories (24,26,28). In our study, NLR was found to be higher and LCR was found to be significantly lower in SD compared to NSD and in D compared to S. Yang et al, 2020 reported that 46.1% of their COVID-19 patients with age ≥ 49.5 years and $NLR \geq 3.3$, were likely to become severe, within a mean duration of 6.3 days; thus, close monitoring for progression of the disease is required (29).

From all the previous studies no single predictive laboratory parameter has emerged for prognostication. In our study we report that if four markers with decided cut-offs: CRP (≥ 3.9 mg/dL); IL-6 (≥ 45.37 pg/mL); Ferritin (≥ 373 ng/mL); $1/LCR \geq 0.405$ are used together it acts as a significant predictor of adverse outcome even in cases of clinically non-severe disease as well as severe disease. The novelty, of this study is the use of routinely prescribed laboratory parameters being used to do so. Thus, it provides a cost-effective solution for prognostication in COVID-19 cases.

The outcome of COVID-19 may depend upon multiple factors like strain of virus, host factors, comorbidities, vaccination status etc. This study was carried out during the first wave of COVID-19 in India when all the individuals

were non-vaccinated and had no previous exposure to the virus. Thus the findings of the study though interesting need to be validated in the current scenario. Another limitation of the study was non availability of data regarding the co-morbidities of the patients, if any. Overall, this study presents an interesting outlook for prognostication using routinely available and prescribed diagnostics.

CONCLUSION

The combination of routinely used markers, CRP (≥ 3.9 mg/dL), IL-6 (≥ 45.37 pg/mL), Ferritin (≥ 373 ng/mL) and $1/LCR \geq 0.405$ can be used to predict adverse outcomes, even in those presenting with mild to moderate disease. This would identify a subset of patients who would benefit from closer monitoring than usual for non-severe disease. The authors however feel, that at this juncture it is difficult to predict which patients would have derangements of which parameters, hence routine screening for all the above-mentioned tests would be the appropriate strategy. However, endeavors to use machine-learning tools to develop artificial intelligence-based algorithms using larger data sets could be taken up to develop more cost-effective and accurate predictors for prognostication.



Corresponding author

Sudip Kumar Datta, MD, DHM, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India, by email: dr.sudipdatta@gmail.com.

Author contributions

Concept and design: Chopra, Datta, Sehgal, Maitra, Sinha.

Investigation/Methodology/Acquisition, analysis, or interpretation of data: Chopra, Datta, Mani, Sehgal, Yadav, Meena, Soni, Subramanian, Mathur, Prakash, Chosdol, Maitra, Trikha, Gupta R, Gupta A, Mohan, Lee, Gulia.

Drafting of the manuscript: Chopra, Datta, Mani, Sinha, Sehgal, Lee, Kaushik, Mostafavi.

Critical revision of the manuscript for important intellectual content: All authors.

Accessed or Verified Data/Statistical analysis: Mani, Chopra, Datta, Sehgal, Lee.

Project administration/Supervision: Datta, Sinha, Mohan, Trikha, Mittan.

Conflict of interest disclosures

None declared.



REFERENCE

1. WHO Coronavirus (COVID-19) Dashboard WHO Coronavirus (COVID-19) Dashboard With Vaccination Data. Accessed August 4, 2022. <https://covid19.who.int/>
2. Vetter P, Vu DL, L'Huillier AG, Schibler M, Kaiser L, Jacqueroiz F. Clinical features of covid-19. *BMJ*. 2020;369. doi:10.1136/BMJ.M1470
3. Patel A, Jernigan DB, 2019-nCoV CDC Response Team. Initial public health response and interim clinical guidance for the 2019 novel coronavirus outbreak - United States, December 31, 2019-February 4, 2020. *Am J Transplant*. 2020;20(3):889-895. doi:10.1111/ajt.15805
4. Mardani R, Ahmadi Vasmehjani A, Zali F, Gholami A, Mousavi Nasab SD, Kaghazian H, et al. Laboratory Parameters in Detection of COVID-19 Patients with Positive RT-PCR; a Diagnostic Accuracy Study. *Arch Acad Emerg Med*. 2020;8(1):e43. doi:10.22037/aaem.v8i1.632
5. Zeng F, Huang Y, Guo Y, Yin M, Chen X, Xiao L, et al. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *International Journal of Infectious Diseases*. 2020;96:467-474. doi:10.1016/J.IJID.2020.05.055
6. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerg Microbes Infect*.

- 2020;9(1):1123-1130. doi:10.1080/22221751.2020.1770129/SUPPL_FILE/TEMI_A_1770129_SM3242.DOCX
7. Yadav D, Pvsn KK, Tomo S, Sankanagoudar S, Charan J, Purohit A, et al. Association of iron-related biomarkers with severity and mortality in COVID-19 patients. *J Trace Elem Med Biol Organ Soc Miner Trace Elem GMS*. 2022 Dec;74:127075.
 8. Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z. Plasma CRP level is positively associated with the severity of COVID-19. *Ann Clin Microbiol Antimicrob*. 2020;19(1):1-7. doi:10.1186/S12941-020-00362-2/FIGURES/4
 9. Stringer D, Braude P, Myint PK, Evans L, Collins JT, Verduri A, et al. The role of C-reactive protein as a prognostic marker in COVID-19. *Int J Epidemiol*. 2021;50(2):420-429. doi:10.1093/IJE/DYAB012
 10. Zali F, Mardani R, Farahmand M, Ahmadi N, Shahali M, Salehi-Vaziri M, et al. Predictive Value Alteration of Laboratory Routine and Ferritin/Transferrin Ratio in Monitoring of COVID-19 Patients. *Clin Lab*. 2022 Oct 1;68(10).
 11. Yousaf MN, Sarwar S, Tarique S, Ahmed M, Tahir H. Mortality in Patients of COVID-19 Infection: Biochemical Markers and its Cut-off Values for Predicting Outcome. *J Coll Physicians Surg--Pak JCPSP*. 2022 Jan;32(1):37-41.
 12. Revised Guidelines on Clinical Management of COVID-19. Govt of India, Ministry of Health & Family Welfare, DGHS. March 2020. - Google Search. Accessed August 22, 2022. <https://dghs.gov.in/>
 13. Li G, de Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov*. 2020; 19(3):149-150. doi:10.1038/D41573-020-00016-0
 14. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *Lancet*. 2020;395(10223):473-475. doi:10.1016/S0140-6736(20)30317-2
 15. Samprathi M, Jayashree M. Biomarkers in COVID-19: An Up-To-Date Review. *Front Pediatr*. 2021;8. doi:10.3389/FPED.2020.607647
 16. Xie J, Covassin N, Fan Z, Singh P, Gao W, Li G, et al. Association Between Hypoxemia and Mortality in Patients With COVID-19. *Mayo Clin Proc*. 2020;95(6):1138-1147. doi:10.1016/J.MAYOCP.2020.04.006
 17. Sadeghi-Haddad-Zavareh M, Bayani M, Shokri M, Ebrahimpour S, Babazadeh A, Mehraeen R, et al. C-Reactive Protein as a Prognostic Indicator in COVID-19 Patients. *Interdiscip Perspect Infect Dis*. 2021;2021. doi:10.1155/2021/5557582
 18. Huang I, Pranata R, Lim MA, Oehadian A, Alisjahbana B. C-reactive protein, procalcitonin, D-dimer, and ferritin in severe coronavirus disease-2019: a meta-analysis. *Ther Adv Respir Dis*. 2020;14. doi:10.1177/1753466620937175
 19. Lin Z, Long F, Yang Y, Chen X, Xu L, Yang M. Serum ferritin as an independent risk factor for severity in COVID-19 patients. *J Infect*. 2020;81(4):647-679. doi:10.1016/J.JINF.2020.06.053
 20. Kappert K, Jahić A, Tauber R. Assessment of serum ferritin as a biomarker in COVID-19: bystander or participant? Insights by comparison with other infectious and non-infectious diseases. *Biomarkers*. 2020;25(8):616-625. doi:10.1080/1354750X.2020.1797880
 21. Szarpak L, Ruetzler K, Safiejko K, Hampel M, Pruc M, Kanczuga-Koda L, et al. Lactate dehydrogenase level as a COVID-19 severity marker. *The American Journal of Emergency Medicine*. 2021 Jul;45:638-639. DOI: 10.1016/j.ajem.2020.11.025.
 22. Terpos E, Ntanasis-Stathopoulos I, Elalamy I, Kastritis E, Sergentanis TN, Politou M, et al. Hematological findings and complications of COVID-19. *Am J Hematol*. 2020;95(7):834-847. doi:10.1002/AJH.25829
 23. Huang I, Pranata R. Lymphopenia in severe coronavirus disease-2019 (COVID-19): Systematic review and meta-analysis. *J Intensive Care*. 2020;8(1):1-10. doi:10.1186/S40560-020-00453-4/FIGURES/4
 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. doi:10.1002/JMV.25819
 25. Aoyama T, Nakazano M, Nagasawa S, Hara K, Komori K, Tamagawa H, et al. The Association of the Lymphocyte-to-C-Reactive-Protein Ratio With Gastric Cancer Patients Who Receive Curative Treatment. *In Vivo (Brooklyn)*. 2022;36(1):482. doi:10.21873/INVIVO.12728
 26. Okugawa Y, Toiyama Y, Yamamoto A, Shigemori T, Ide S, Kitajima T, et al. Lymphocyte-C-reactive Protein Ratio as Promising New Marker for Predicting Surgical and Oncological Outcomes in Colorectal Cancer. *Ann Surg*. 2020; 272(2):342-351. doi:10.1097/SLA.0000000000003239
 27. Bacha S, Sghaier A, Habibech S, Cheikhrouhou S, Racil H, Chaouch N, et al. Combined C-reactive protein and Neutrophil to Lymphocyte ratio use predict survival in non-small-cell lung cancer. *Tunis Med*. 2017 Dec;95(12): 229-235. PMID: 29878284.
 28. Meng LB, Yu ZM, Guo P, Wang QQ, Qi RM, Shan MJ, et al. Neutrophils and neutrophil-lymphocyte ratio: Inflammatory markers associated with intimal-media thickness of atherosclerosis. *Thromb Res*. 2018;170:45-52. doi: 10.1016/J.THROMRES.2018.08.002
 29. Yang AP, Liu J ping, Tao W qiang, Li H ming. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol*. 2020;84. doi: 10.1016/J.INTIMP.2020.106504

30. Qu R, Ling Y, Zhang YH, Wei LY, Chen X, Li XM, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with coronavirus disease-19. *J Med Virol.* 2020; 92(9):1533-1541. doi:10.1002/JMV.25767

31. Sarkar S, Kannan S, Khanna P, Singh AK. Role of platelet-to-lymphocyte count ratio (PLR), as a prognostic indicator in COVID-19: A systematic review and meta-analysis. *J Med Virol.* 2022;94(1):211-221. doi:10.1002/JMV.27297