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Dynamics changes of hematological and in predicting mortality in severe COVID-19

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***Abstract*---**Introduction: COVID-19 pandemic has been a challenge. Biomarkers have always played an important role in clinical decision making in various infectious diseases. It is crucial to assess the role of biomarkers in evaluating severity of disease and appropriate allocation of resources. The aim of study is to evaluate association between biomarkers and outcomes in COVID-19 hospitalized patients. Methods: We conducted a retrospective study of 100 patients admitted with severe COVID-19 in Dar-Alsalam hospital, Baghdad, from January to July 2021. We obtained their clinical, and biochemical characteristics at baseline and we fallow up for 20 days after admission with 4 days' interval. The data were analyzed to determine the prognostic significance of these markers on the final outcome. Results: the mean age(years) was 56.79 ± 11.717 years with range (29-77) years. Gender: 39(39%) patients were female and 61(61%) of them were male. 50(50%) patients survived and 50(50%) of them not survived. WBC (10"3/ul) was significantly higher in day8, 12,16 and 20, Lymphocytes (10"3/ul) was significantly lower in non-survived patients during all follow-up days, Neutrophils (10"3/ul) was significantly higher in day8, 12,16 and 20, N/L Ratio was significantly higher in day1, 4,16 and 20 (15.42, 17.96, 42.48 and 59.64) respectively. PLT(10"3/ul): was significantly lower in non-survived patients, PT(sec.): was significantly higher in non-survived patients, PTT(sec.): was significantly higher in non-survived patients, P/L Ratio: was significantly lower in non-survived patients, D. Dimer(ug/ml): was significantly higher in non-survived patients, Fibrinogen: was significantly higher in non-survived patients. Conclusion: Our study found a significant association between lymphopenia, neutrophilia

and elevated levels of CRP, LDH, D-dimer and COVID-19 severity and mortality.

***Keywords*---**hematological markers, predicting, mortality, severe COVID-19.

Introduction

In December 2019, a new strain of coronavirus, severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, and starting in March of 2020, the world was plunged into a pandemic due to the disease (COVID-19) caused by this new coronavirus. As of March 11th, COVID-19 had been detected in 221 countries, with 117,799,584 confirmed cases and more than 2,615,018 deaths [1]. The clinical spectrum of this disease ranges from asymptomatic cases to severe pulmonary involvement with respiratory failure, systemic involvement with cytokine storm, sepsis, septic shock, and multiple organ failure [2]. Anomalies in several inflammatory, hematological and biochemical biomarkers have been observed in severe COVID-19 patients which can be utilized further for earlier identification of COVID-19 severity as well as to monitor adverse outcomes, mortality and prognosis of COVID-19 patients. The vital role of abnormal laboratory parameters in patients with COVID-19 has lately become apparent, and published studies recommend that particular clinical laboratory parameters may help in risk stratification and prognosis of these patients, ultimately leading to earlier interventions and the achievement of desired clinical outcomes [3]. Hence, early recognition and timely intervention of COVID-19 are crucial factors to prevent adverse clinical outcomes and burden on the scarce health resources due to the admission of a large number of patients in intensive care units. By incorporating these clinical laboratory biomarkers in routine testing, limited medical resources can be allocated to those COVID-19 patients who require urgent and timely treatment especially in the areas of epidemic origin [4]. Coronavirus represents the large family of positive-sense ssRNA viruses belonging to the order Nidovirales. These usually cause diseases of the upper respiratory tract in birds and mammals, including humans. Seven coronaviruses have been identified as causing diseases in humans thus far. Among them, SARS-CoV and MERS-CoV have caused outbreaks in the past. SARS-CoV-2 is the cause of the ongoing pandemic, while other coronaviruses (like the 229E, NL63, OC43, and HKU1) are known to cause the common cold and a few mild respiratory infections. Coronaviruses can be divided into 4 genera, namely: alpha, beta, gamma, and delta [5]. These viruses can result in anything from mild illnesses (like common cold) to severe diseases, like SARS and MERS, both of which have caused outbreaks in the last two decades. SARS-CoV-2, previously known as the 2019-novel coronavirus, is classified as beta-CoV by the WHO [6]. The COVID-19 infection is highly contagious and transmissible due to its affinity for binding to the ACE2 receptors, and this is why it has rapidly spread from its epicenter in Wuhan, China, to more than 200 countries around the world [7, 8] On 11 March 2020, WHO categorized COVID-19 as a pandemic. The origin of the virus is still a topic of debate. The symptoms of COVID-19 range from mild respiratory tract illness to severe pneumonia, hypoxia, multiorgan failure, and

death [9]. The aim of study is to identify laboratory biomarkers that predict disease severity and outcome among severe COVID-19 hospitalized patients.

Method

An institution based statistical analysis study was conducted at DAR- Al Salam COVID-19 Care Center,Baghdad. The follow up was made from January to July, 2021. The source population was all cases of COVID-19 admitted to the hospital during first 7 days after onset of symptoms, with one of the fallowing SPO2 less than 93% on room air, respiratory rate more than 30/min, CT scan and chest x-ray with more than 50% lung involvement, confirmed diagnosis with RT-PCR, admission last at least 3 weeks. follow up to 20 days after admission, with 4 days' interval with CBC, coagulation profile, CRP, LDH, S. Ferritin. All COVID-19 patients were on treatment guidelines by Iraqi ministry of health, follow up at the canter from January to July, 2021.

COVID-19 severity score: was determined based on the WHO classification as follows [10]:

* Mild Disease: Characterized by fever, malaise, cough, upper respiratory symptoms, and/or less common features of COVID-19 (headache, loss of taste or smell etc.)
* Moderate Disease: Patients with lower respiratory symptom/s. They may have infiltrates on chest X-ray. These patients are able to maintain oxygenation on room air.
* Severe Disease: These patients have developed complications. The following features can define severe illness:
  1. Hypoxia: SPO2 ≤ 93% on atmospheric air or PaO2:FiO2 < 300mmHg 2.
  2. 2.Tachypnea: in respiratory distress or RR>30 breaths/minutes
  3. More than 50% involvement seen on chest imaging.

Exclusion criteria

The patients with history of thromboembolic disorders, cardiovascular disease, inflammatory bowel disease, hematological disorders, trauma or surgeries in last six months or bedridden patients, pregnant females or drugs known to affect the coagulation profile/platelets, kidney or liver disease, neoplasms, were excluded from the study. Statistical analysis done by SPSS 22, frequency and percentage used for categorical data, mean, median and SD for continuous data. Chi-square used for assessed association between variables, person correlation shows the correlation between continuous data. T test used for evaluation differences between mean and median of continues variables. ROC curve also used to show more specific and sensitive cutoff point. P-value less or equal to 0.05 is consider significant.

Results

Age: the mean age(years) was 56.79 ± 11.717 years with range (29-77) years. Gender: 39(39%) patients were female and 61(61%) of them were male.

Study groups: 50(50%) patients survived and 50(50%) of them not survived.

Distribution of study sample according to CBC and survival status

1. WBC (10"3/ul) was significantly higher in day8, 12,16 and 20 (15.81, 17.51 19.83 and 21.88) respectively.
2. Lymphocytes (10"3/ul) was significantly lower in non-survived patients during all follow-up days of the study.
3. Neutrophils (10"3/ul) was significantly higher in day8, 12,16 and 20 (14.45, 16.13, 18.70 and 20.40) respectively.
4. N/L Ratio was significantly higher in day1, 4,16 and 20 (15.42, 17.96, 42.48 and 59.64) respectively. As show in table 1.

Table 1: distribution of study sample(N=100) according to CBC and survival status

	Survival Status				
	Survived		Non survived		
	Mean	SD	Mean	SD	P-Value
WBC(10"3/ul) day 1	8.45	2.96	12.79	3.16	0.651
WBC(10"3/ul) day 4	9.89	2.88	13.61	3.72	0.057
WBC(10"3/ul) day 8	11.43	2.92	15.81	4.42	0.012
WBC(10"3/ul) day 12	12.78	3.01	17.51	5.91	0.001
WBC(10"3/ul) day 16	13.73	2.99	19.83	7.09	0.001
WBC(10"3/ul) day 20	14.18	2.67	21.88	8.95	0.001
Lymphocytes(10"3/ul) day 1	1.84	0.78	0.83	0.27	0.001
Lymphocytes(10"3/ul) day 4	1.52	0.72	0.76	0.26	0.001
Lymphocytes(10"3/ul) day 8	0.72	0.35	0.65	0.19	0.010
Lymphocytes(10"3/ul) day 12	0.74	0.47	0.61	0.23	0.000
Lymphocytes(10"3/ul) day 16	1.28	1.72	0.51	0.24	0.001
Lymphocytes(10"3/ul) day 20	1.34	1.07	0.48	0.36	0.001
neutrophils(10"3/ul) day 1	6.29	2.88	11.71	3.07	0.581
neutrophils(10"3/ul) day 4	7.95	2.84	12.45	3.60	0.059
neutrophils(10"3/ul) day 8	10.27	2.87	14.45	4.44	0.005
neutrophils10"3/ul) day 12	11.60	2.93	16.13	5.97	0.001
neutrophils(10"3/ul) day 16	12.06	2.92	18.70	7.37	0.001
neutrophils(10"3/ul) day 20	12.45	2.61	20.40	8.86	0.001
N/L Ratio day 1	4.39	2.96	15.42	6.45	0.002
N/L Ratio day 4	6.84	4.43	17.96	7.79	0.005
N/L Ratio day 8	17.58	10.65	23.99	9.50	0.754
N/L Ratio day 12	21.32	12.31	29.19	13.44	0.336
N/L Ratio day 16	19.47	14.63	42.48	23.33	0.003
N/L Ratio day 20	25.59	25.08	59.64	41.69	0.004
Significant P value < 0.05					

Distribution of study sample according to Coagulation profile and survival: status

1. PLT(10"3/ul): was significantly lower in non-survived patients in days 1 and 4 (165.02 and 180.02, P=0.001) respectively.
2. PT(sec.): was significantly higher in non-survived patients in days 8, 12, 16 and 20 (12.26, 12.58, 12.72 and 12.78) respectively.
3. PTT(sec.): was significantly higher in non-survived patients in days 12 and 20 (4.87 and 5.72) respectively.
4. P/L Ratio: was significantly lower in non-survived patients in days 8, 12, 16 and 20 (303.99, 333.99, 409.33 and 474.81) respectively.
5. D. Dimer(ug/ml): was significantly higher in non-survived patients in all follow-up days (1.23, 1.54, 1.96, 2.54, 3.16 and 4.06) respectively.
6. Fibrinogen: was significantly higher in non-survived patients in days 4 and 12 (175.75 and 167.35) respectively. As show in table 2.

Table 2: distribution of study sample(N=100) according to CBC and survival status

	Survival Status				
	Survived		Non survived		P-Value
	Mean	SD	Mean	SD	
PLT(10"3/ul) day 1	267.80	115.27	165.02	66.92	0.001
PLT(10"3/ul) day 4	290.50	127.99	180.02	79.33	0.001
PLT(10"3/ul) day 8	310.82	117.85	180.22	88.66	0.109
PLT(10"3/ul) day 12	331.38	124.65	188.20	116.13	0.169
PLT(10"3/ul) day 16	310.86	124.30	185.58	109.80	0.073
PLT(10"3/ul) day 20	295.72	106.24	177.00	101.16	0.460
PT(sec.) day 1	11.30	1.64	11.64	1.83	0.277
PT(sec.) day 4	11.88	.94	12.26	1.29	0.109
PT(sec.) day 8	12.24	1.04	12.26	1.85	0.001
PT(sec.) day 12	12.28	1.05	12.58	2.18	0.001
PT(sec.) day 16	12.28	1.20	12.72	2.62	0.001
PT(sec.) day 20	12.04	1.21	12.78	2.48	0.002
PTT(sec.) day 1	27.98	4.57	31.10	4.32	0.120
PTT(sec.) day 4	28.14	4.11	33.38	3.80	0.132
PTT(sec.) day 8	28.04	3.96	32.90	3.89	0.833
PTT(sec.) day 12	27.74	3.56	34.04	4.87	0.015
PTT(sec.) day 16	27.76	3.73	34.40	5.65	0.039
PTT(sec.) day 20	27.74	3.50	34.74	5.72	0.001
P/L Ratio day 1	177.03	113.82	215.38	113.80	0.521
P/L Ratio day 4	236.58	152.35	259.48	139.81	0.669
P/L Ratio day 8	512.18	269.96	303.99	187.99	0.005
P/L Ratio day 12	596.52	385.54	333.99	252.64	0.002
P/L Ratio day 16	492.32	442.02	409.33	319.86	0.014
P/L Ratio day 20	573.20	578.51	474.81	363.85	0.001
D.Dimer(ug/ml) day 1	0.56	0.26	1.23	0.46	0.001
D.Dimer(ug/ml) day 4	0.69	0.43	1.54	0.80	0.001

D.Dimer(ug/ml) day 8	0.79	0.44	1.96	1.04	0.001
D.Dimer(ug/ml) day 12	0.91	0.68	2.54	1.26	0.001
D.Dimer(ug/ml) day 16	0.93	0.83	3.16	1.59	0.001
D.Dimer(ug/ml) day 20	0.85	0.77	4.06	2.17	0.001
Fibrinogen day 1	389.78	118.57	513.98	148.76	0.235
Fibrinogen day 4	461.62	92.37	589.14	175.75	0.001
Fibrinogen day 8	511.34	120.66	719.40	737.93	0.082
Fibrinogen day 12	518.02	118.41	686.64	167.35	0.026
Fibrinogen day 16	509.46	155.49	714.96	180.48	0.230
Fibrinogen day 20	485.36	175.55	730.18	188.04	0.947
Significant P value < 0.05					

Prediction analysis (ROC curve) was used to assess the accuracy of the previous biomarkers which showed significant difference between survival status groups through calculating area under curve (AUC) for each as follows:

CBC: WBC (10"3/ul): day 8, 12, 16 and 20 showed good accuracy 70% - 80% (AUC 0.7- 0.8) in predicting non survival status of study patients as shown in figure (1). Lymphocyte (10"3/ul): day1,4, 8, 12, 16 and 20 showed very poor accuracy 10% - 50% (AUC 0.1-0.5) in predicting non survival status of study patients as shown in figure (2). Neutrophils (10"3/ul): day 8, 12, 16 and 20 showed good accuracy 70% - 80% (AUC 0.7-0.8) in predicting non survival status of study patients as shown in figure (3). N/L Ratio: Day 1, 4, 16 and 20 showed very good accuracy 70% - 100% (AUC 0.7-1) in predicting non survival status of study patients as shown in figure (4).

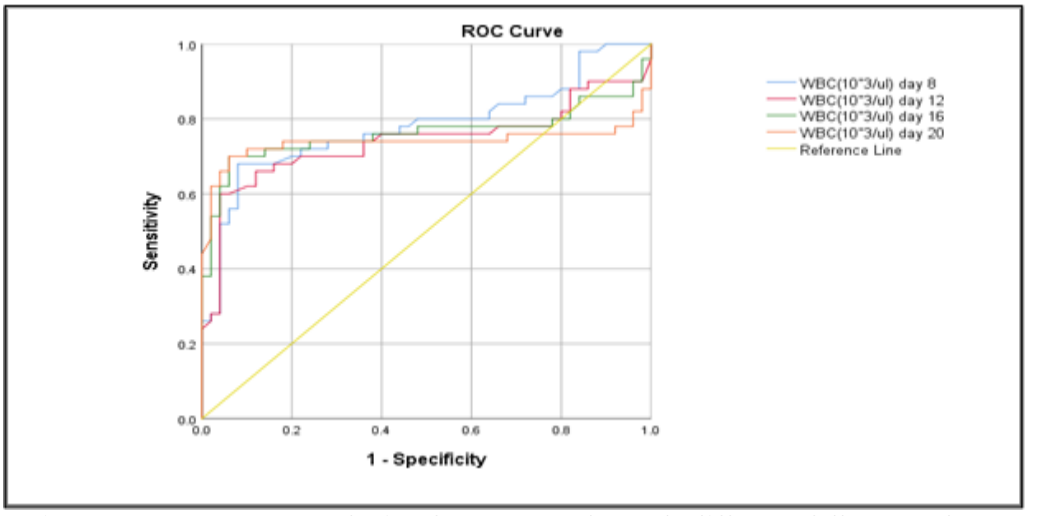

Figure 1: ROC curve analysis of WBC test through different follow-up days.

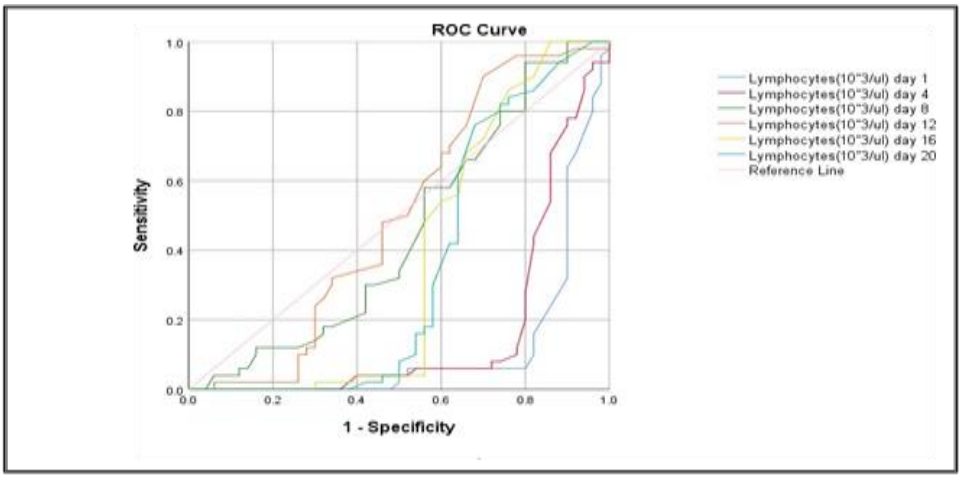

Figure 2: ROC curve analysis of Lymphocyte test through different follow-up days

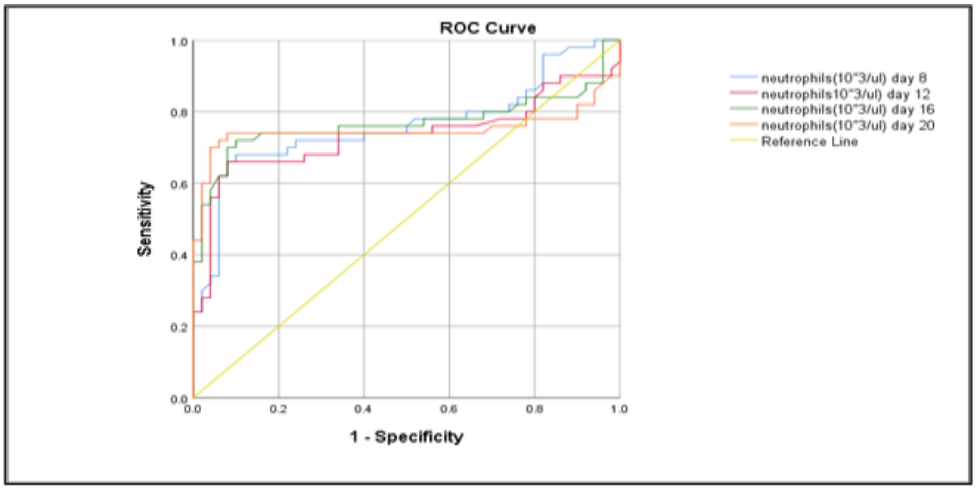

Figure 3: ROC curve analysis of Neutrophils test through different follow-up days.

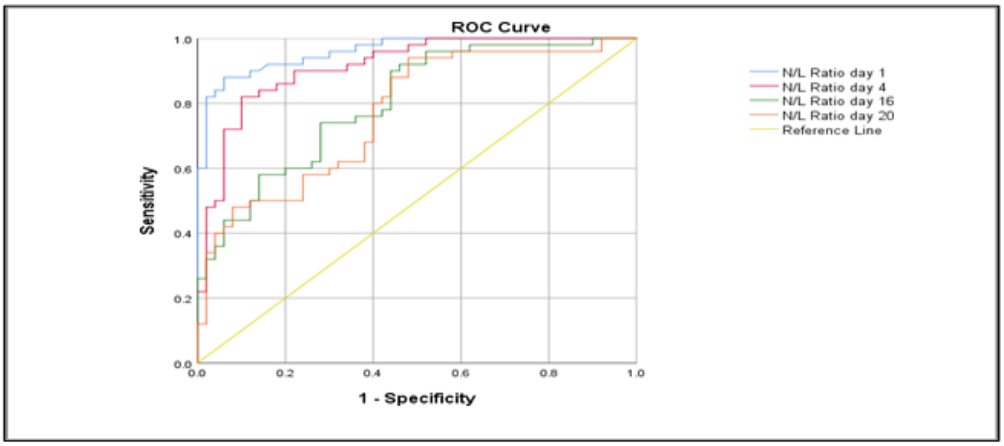

Figure 4: ROC curve analysis of N/L Ratio test through different follow-up days

Coagulation profile: PLT (10"3/ul) day 1 and 4 showed very poor accuracy 10% - 50% (AUC 0.1-0.5) in predicting non survival status of study patients as shown in figure (5). PT (sec.) day 8, 12, 16 and 20 showed poor accuracy 40% - 60% (AUC 0.4-0.6) in predicting non survival status of study patients as shown in figure (6). PTT (sec.) day 12 and 20 showed excellent accuracy 80% - 100% (AUC 0.8-1.0) in predicting non survival status of study patients as shown in figure (7). P/L Ratio day 8, 12, 16 and 20 showed poor accuracy 10% - 60% (AUC 0.1-0.6) in predicting non survival status of study patients as shown in figure (8). D. Dimer(ug/ml) through all follow-up days showed excellent accuracy 80% - 100% (AUC 0.8-1.0) in predicting non survival status of study patients as shown in figure (9). Fibrinogen day 4 and 12 showed good accuracy 70% - 90% (AUC 0.7-0.9) in predicting non survival status of study patients as shown in figure (10).

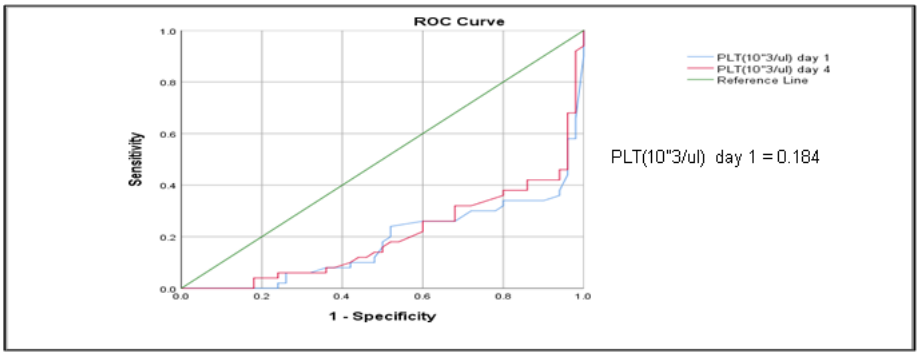

Figure 5: ROC curve analysis of PLT test through different follow-up days

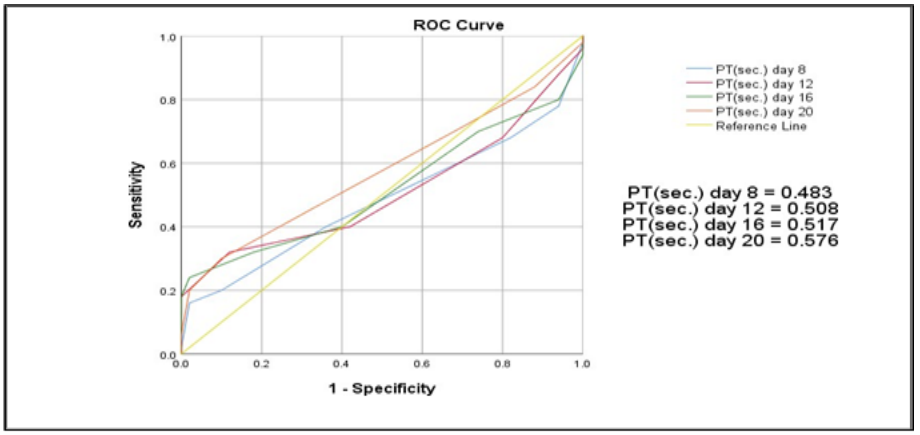

Figure 6: ROC curve analysis of PT test through different follow-up days.

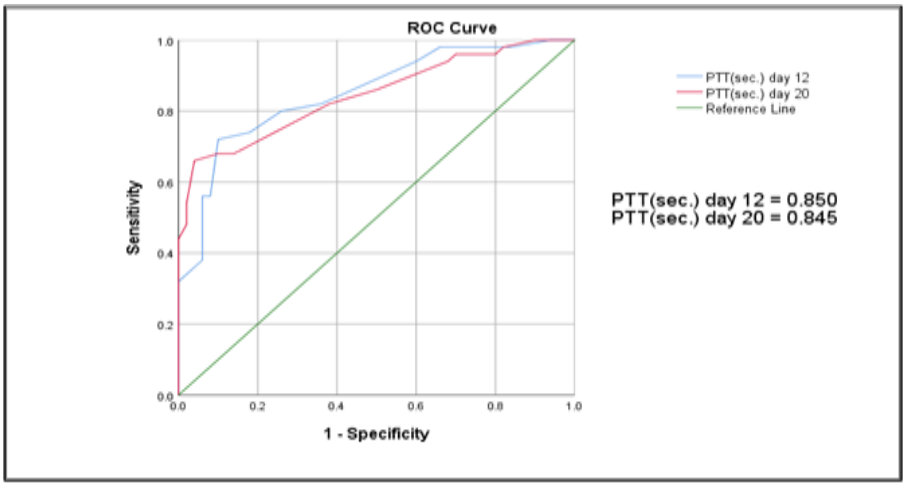

Figure 7: ROC curve analysis of PTT test through different follow-up days

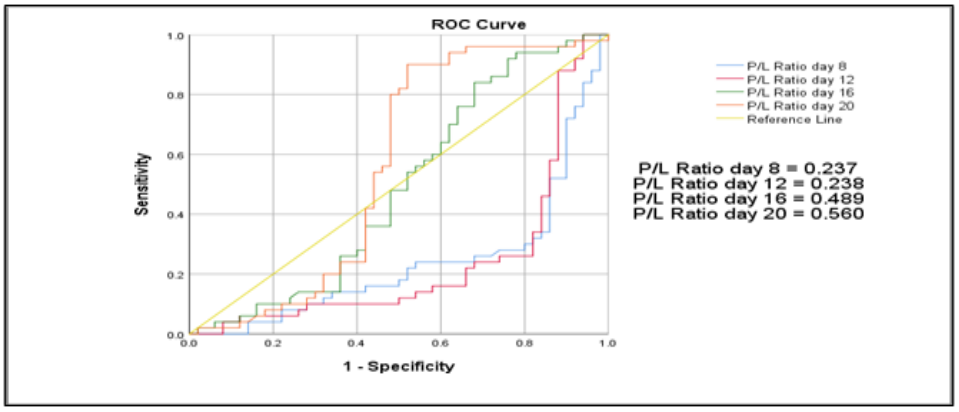

Figure 8: ROC curve analysis of P/L ratio test through different follow-up days

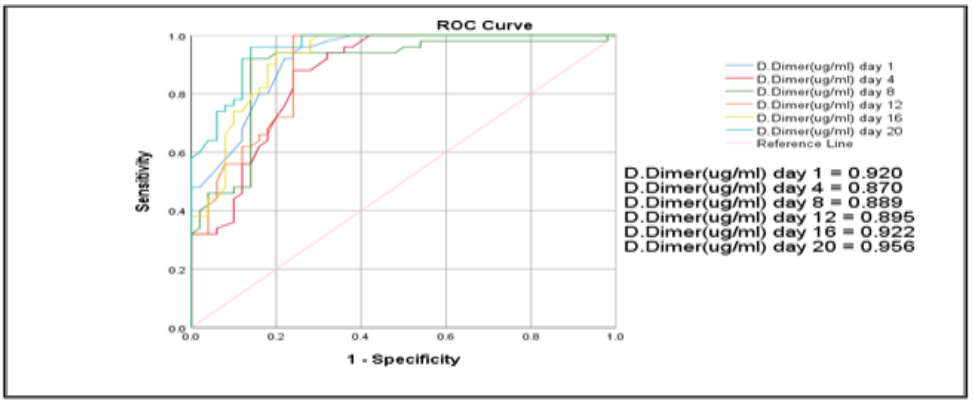

Figure 9: ROC curve analysis of D. Dimer test through different follow-up days.

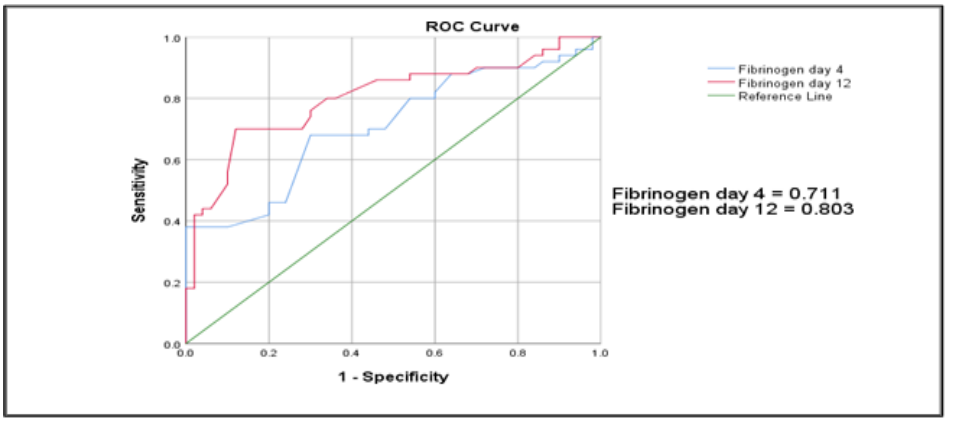

Figure 10: ROC curve analysis of Fibrinogen test through different follow-up days

Discussion

There is a study support our finding done by N. David Yanez et al [11]. In the 16 countries examined, persons age 65 years or older had strikingly higher COVID-19 mortality rates compared to younger individuals, and men had a higher risk of COVID-19 death than women. We also found in our study that all patients with severe infection had increase neutrophil count with decrease lymphocyte count in both survival and non-survival group, neutrophil were significantly higher and lymphocyte was significantly lower in non-survival in comparison with survival group. We also found WBC count at admission is significantly correlated with death in COVID-19 patients. Higher level of WBC count should be given more attention in the treatment of COVID-19. We found the degree of lymphopenia was significantly associated with oxygen supplement requirements. Moreover, lymphopenia had a convincing impact on adverse survival outcomes, High NLR levels on admission were associated with severe COVID-19 and mortality. Neutrophils are the most abundant immune cells in human blood. They account for approximately 50–70% of all leukocytes. Besides serving as first responders to many infections, neutrophils have critical homeostatic functions being also implicated in chronic inflammatory diseases [12]. These polymorphonuclear cells play a protective role during bacterial or fungal infections; however, their role in viral infections is not fully understood [13]. Although the evidence is limited, it has been suggested that neutrophils enhance antiviral defenses by interaction with other immune cell populations, virus internalization and killing mechanism, cytokines release, degranulation, oxidative burst, and neutrophil extracellular traps (NETs) [14]. Respiratory burst from activated neutrophils induces ROS release, such as superoxide radicals and H2O2, leading to oxidative stress that contributes to the cytokine storm and alveolar damage, blood clots formation in SARS-CoV-2 infection [15]. Study done by Bin Zhu et al [16], suggest that WBC count at admission is significantly correlated with death in COVID-19 patients. Higher level of WBC count should be given more attention in the treatment of COVID-19. Study done by Jorge A. Masso-Silva et al [17], suggest that evidence of increased neutrophils in the circulation and lungs of COVID-19 patients. Importantly, neutrophil quantity and activation correlates with severity and mortality of disease. Both study agree with our finding in the research mechanism of significant lymphocyte reduction in severe COVID-19 remains unclear, there

are hypothesis other than lymphocyte infiltration and sequestration in the lungs, gastrointestinal tracts, and or lymphoid tissues: (1) lymphocytes express the ACE2 receptor and may be a direct target of SARS-CoV-2 infection [18], and (2) an increase of pro-inflammatory cytokines in COVID-19, especially IL-6, could induce further lymphocyte reduction [19]. Jongmin Lee et al [20], found that lymphopenia was significantly related to severities and mortality of patients diagnosed with COVID-19, even after adjusting for confounding factors.

Study done by Ruan, Yang, Wang et al 2020 [21], show that, these patients tended to have low lymphocyte count; the condition that is associated with increased COVID-19 severity. Therefore, individuals who died of COVID-19 are demonstrated to have had expressively lower lymphocyte level than survivors Daniel Martin Simadibrata et al [22], found High NLR levels on admission were associated with severe COVID-19 and mortality. All above studies agree with our finding in this research. Regarding to coagulation profile in our study we observed that most common pattern of coagulopathy characterized by elevations in fibrinogen and D-dimer levels, mild prolongation of PT/aPTT. This correlates with a parallel rise in markers of inflammation, in non-survival Elevated D-dimer at the time of admission and markedly increasing over time. Several cases in non-survival group have minimal prolongation of the aPTT and/or PT, and mild thrombocytopenia. Mamta Soni,a et ak [23], found that Among the measured coagulation parameters, D-dimer during hospital stay had the highest predict in-hospital mortality in COVID-19 patients. D-dimer value ≥ 2.01 μg/mL can effectively predict in- hospital mortality in patients with COVID-19. A significant association of increased D-dimer level has been found with diabetes mellitus and elderly age.

Conclusion

Our study found a significant association between lymphopenia, neutrophilia and elevated levels of CRP, LDH, D-dimer and COVID-19 severity and mortality. The results have the potential to be used as an early biomarker to improve the management of COVID-19 patients, by identification of high-risk patients and appropriate allocation of healthcare resources in the pandemic.

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