Efficiencies of Laboratory Parameters in Covid-19 Patients Follow up

Asuman ORCUN[1] [ID], Ozlem CAKIR MADENCI[1] [ID], Idris ONEM[1] [ID], Zeynep YILDIZ[1] [ID],
Ayse BATIREL[2] [ID], Kemal Tolga SARACOGLU[3] [ID]

[1]Dr Lütfi Kırdar Kartal City Hospital, Biochemistry Laboratory, İstanbul, Turkey

[2]Dr Lütfi Kırdar Kartal City Hospital, Infectious Diseases and Clinical Microbiology, İstanbul, Turkey

[3]Dr Lütfi Kırdar City Hospital, Anesthesiology & Reanimation, İstanbul, Turkey

Corresponding Author: Ozlem Cakir Madenci

E mail: ocmadenci@hotmail.com

ABSTRACT

Introduction: Diagnostic efficiencies of laboratory parameters used in COVID-19 patients and their association with disease severity were evaluated.

Materials and Methods: Laboratory parameters of COVID-19 patients hospitalized in Dr. Lütfi Kırdar Kartal City Hospital between March and August 2020 were evaluated. The patients were grouped as non-severe and severe according to the interim guidance of the World Health Organization (WHO). The diagnostic performances of NLR, D-dimer, CRP, procalcitonin, IL-6, LDH, and ferritin in discrimination of severe cases were evaluated by Receiver operator's characteristics (ROC) analysis. Generalized lineer model Analysis (GLM) was performed with mortality as a dependent variable and age, gender, NLR, D-dimer, CRP, Procalcitonin, IL-6, LDH, and ferritin as an independent variables.

Results: A total of 257 patients were evaluated and there was a significant difference between non-severe and severe cases in terms of NLR, D-dimer, CRP, Procalcitonin, IL-6, LDH, and Ferritin values. All the parameters showed comparable performances in discriminating severe disease; D-dimer with the least (AUC 73.5%), and NLR with the highest (AUC 80.7%) efficiency. Values above 4.5 for NLR, 930 ug/L for D-dimer, 64 mg/L for CRP, 0.136 ug/L for procalcitonin, 44.3 pg/mL for IL-6, 304 IU for LDH, and 312 ug/L for ferritin were associated with severe disease. Contribution of age, NLR, D-dimer, and CRP were found significant on the model.

Conclusions: NLR, D-dimer, CRP, procalcitonin, IL-6, LDH, and ferritin showed comparable performances in discriminating severe cases with predefined cut-offs. Age, NLR, D-dimer, and CRP may be considered as predictors of mortality in COVID-19 patients.

Keywords: COVID-19, ROC Curve, Predictive Value of Tests, Mortality, Laboratory markers, Disease Severity

Introduction

The world is demolished by a pandemic disease caused by a novel coronavirus which arise in Wuhan, Hubei, China at the end of December 2019 first (1). The epidemic has gradually expanded to 217 countries worldwide with almost 128 million infected people and more than 2.8 million deaths, both of which are still increasing (2).

New coronavirus 2019 disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and ıt produces a respiratory and systemic illness that progresses to a severe form of pneumonia in 10-15% of patients (1). Severe COVID-19 can lead to critical illness, with acute respiratory distress (ARDS) and multi-organ failure (MOF) as its primary complications, finally followed by intravascular coagulopathy (3).

Amplification of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) ıs accepted as the Gold Standard diagnostic method for confirmation of infection, but there are some disadvantages of this test like long turnaround time (3–4 h to generate results) high false-negative rates as much as 15%–20%, need of certified laboratories, expensive equipment, and trained personnel (4). Besides many mutations have been described that alter diagnostic performances of currently used PCR techniques. Recognition of the pathogen is of major importance both for the patient and public health. After diagnosis anyway, the clinical course of the disease is complicated (5). A wide range of variability in disease severity is observed in COVID-19 ranging from asymptomatic to critical systemic and multiorgan pathologies (1). Thus the description of biochemical biomarkers that could predict disease severity and prognosis in systemic and multiorgan disease cases are essential

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to guide clinical practice (6). Complete blood count (CBC), assays investigating coagulation and fibrinolysis; prothrombin time (PT), activated partial prothrombin time (aPTT), and D-dimers, and inflammation-related parameters; neutrophile/lymphocyte ratio (NLR), interleukin-6 (IL-6), C-reactive protein (CRP), ferritin, and procalcitonin are the commonly requested tests in COVID-19 patients (7). SARS-CoV-2 can severely damage several vital organs such as the heart, liver, and kidneys. Thus analyzing the biochemical parameters alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK), creatinine, and albumin are essential to evaluate the functional capacity of these organs (8). The lower respiratory tract is the primary site of the SARS-CoV-2 attack and lactate dehydrogenase (LDH) is an important marker of lung damage thus this enzyme's level is elevated in most COVID-19 patients (9,10).

In the course of highly pathogenic coronavirus infections, such as SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV) lymphopenia and inflammatory cytokine storm are observed and these pathologies are accepted to be related with disease severities (11). Circulating biomarkers showing the status of inflammation and immunity are acknowledged as potential predictors for the prognosis of COVID-19 patients and will help the physicians for initiation of urgent clinical support (12). Various coagulopathies including disseminated intravascular coagulation (DIC), venous and arterial thromboembolism, or sepsis-induced coagulopathy is commonly observed in severe COVID-19 disease (13). Monitorization of PT, D-dimer, platelet, and fibrinogen levels is recommended in the determination of prognosis and markedly elevated D-dimers are accepted as one of the predictors of mortality (14).

In this study, we evaluated NLR, D-dimer, CRP, procalcitonin, IL-6, LDH, and ferritin in COVID-19 patients and investigated their diagnostic performances in discriminating severe disease and predicting mortality. We focused on inflammatory and commonly tested biomarkers in COVID-19 follow up which are readily available even low-resource settings.

Materials and Methods

In this retrospective study, a total of 257 COVID-19 patients were diagnosed according to the current guidelines and hospitalized in Dr Lütfi Kırdar Kartal City Hospital between March and August 2020 with COVID-19 were evaluated (15). Severe and non-severe patients were identified according to the interim guidance of the World Health Organization (WHO) (16). Patients included in this study were all positive in (RT-PCR) for SARS-CoV-2 RNA. Patients with the following criteria: 1) epidemiological history 2) fever or other respiratory symptoms 3) typical computed tomography (CT) images of abnormities consistent with viral pneumonia were interned and followed in infectious diseases clinics. In this study, this group is called non-severe. Severe patients additionally met at least one of the following conditions: 1) Shortness of breath, RR ≥ 30 times/min 2) Oxygen saturation (resting state) ≤93% (3) PaO2/FiO2 ≤300 mmHg (17,18). These patients were followed in the intensive care unit (ICU). Laboratory assessments consisted of white blood cell

(WBC), NLR, platelet, fibrinogen, D-dimer, CRP, procalcitonin, IL-6, CK, LDH, and ferritin levels. For non-severe cases first day of hospitalization, for severe cases the first day of admission to the ICU test results were taken into evaluation. CBC was measured in an LH750 hematology analyzer (Beckman Coulter, Brea, CA, USA). PCT was measured in E 170 (Roche Diagnostics, Penzberg, Germany) and ferritin was measured in UniCelDxl 800 (Beckman Coulter, Brea, CA, USA) analyzer. Clinical chemistry parameters were measured in AU 5800 (Beckman Coulter, Brea, CA, USA) and CRP were measured in BN II analyzer (Siemens, Germany). The rRT-PCR was performed on Roche Cobas Z480 analyzer. The study was approved by our institute's scientific committee. Decision number and date:514/190/16 (25.11.2020).

Statistical Analyses

Statistical analysis was carried out using the SPSS program (Statistical Package for Social Science, version 11.7; Chicago, IL). The distribution of data was assessed by the Kolmogorov–Smirnov test and results were expressed as median and interquartile range. Categorical variables were presented as n (%). Comparison of 2 groups was performed using the Mann-Whitney U test and P <0.05 was accepted as statistically significant. The AUC-ROC analysis was performed to evaluate diagnostic performances of tests in discrimination of severe COVID-19 patients. The optimal cut-off values of NLR, D-dimer, CRP, procalcitonin, IL-6, LDH, and ferritin for the severe disease were calculated. GLM was performed with mortality as a dependent variable and age, gender, NLR, D-dimer, CRP, Procalcitonin, IL-6, LDH, and ferritin independent variables.

Results

A total of 257 patients was evaluated. Eighty-four (32.7%) were classified as severe COVID-19 cases and followed in the ICU. A total of 63 patients (24.5%) were died during hospitalization and 55 of them (87.3%) were discharged from the ICU while 8 (12.7%) were followed in the infectious diseases clinic. Length of stay median (2.5-97.5 percentile) of survivors and non-survivors were 9(3-64) and 19(3-139) days respectively. The demographics and laboratory parameters in non-severe and severe COVID-19 were shown in Table 1. There was a significant difference between non-severe and severe cases in terms of gender, hospital length of stay, WBC, lymphocytes, neutrophils, NLR, monocytes, hemoglobin, fibrinogen, D-dimer, CRP, procalcitonin, IL-6, CK, LDH, and ferritin values. Severe COVID-19 cases showed higher INR results compared with non-severe cases and median (2.5-97.5) values were 1.27 (1-3.58) and 1 (0.85-2.0) respectively. There was no significant difference in thrombocyte levels between non-severe and severe cases. The severe patients showed significantly high frequencies of cardiovascular (p= 0.021), and central nervous system disease (p= 0.017) compared with non-severe cases (Table 2). The optimal cut-off values for discriminating severe COVID-19 were 4.5 for NLR, 930 ug/L for D-dimer, 64 mg/L for CRP, 0.136 ug/L for procalcitonin, 44.3 pg/mL for IL-6, 304 IU for LDH, and 312 ug/L for ferritin. Results were shown in Table 3. Using GLM analyses with mortality as a dependent variable and age, gender, NLR, D-dimer, CRP, Procalcitonin, IL-6, LDH, and

Table 1. Laboratory Parameters in Non-severe and Severe COVID-19 Disease

Analyte	Unit	Reference Range	Non-Severe COVID-19 (n=173)	Severe COVID-19 (n=84)	p
			(Median; 2.5-97.5 percentile)		
Age			64(31.6-91)	69(30.4-89.8)	0.079
Gender (F/M)			84/89	31/53	0.014*
Duration of Hospitalization	Days		8 (2.8-42)	20(6.1-130)	0.001*
Hematological Parameters					
WBC	X10^9 cells/L	4.8-10.8	7.1(3-17.6)	9.4(2-30.5)	0.001*
Lymphocytes	X10^9 cells/L	1.3-2.0	1.35(0.3-4.08)	0.8(0.1-3.54)	0.001*
Neutrophils	X10^9 cells/L	2.2-4.8	4.6(1.16-13.24)	8(1.32-25.7)	0.001*
NLR			3.3(0.6-22)	9.3(1.3-87.7)	0.001*
Platelet	X10^9 cells/L	130-400	240(80.4-529)	240(32.2-441)	0.696
Hemoglobin	g/dL	12-16	11.6(7.5-15.7)	10.3(6.4-15.7)	0.001*
Coagulation Parameters					
Fibrinojen	mg/dL	200-400	504(249-807)	595(236-1042)	0.006*
D-dimer	ug/L(FEU)	0-500	840(200-4985)	1930(505-13341)	0.001*
PT	Second	9.7 - 14.8	13.5(11.3-26.6)	16.8(13.2-35.4)	0.001*
INR		0.8 - 1.2	1(0.85-2.0)	1.27(1-3.58)	0.001*
aPTT	Second	24.8 - 35	28.9(21.2-37.8)	30.7(20.6-54.4)	0.048*
Inflamatory Parameters					
CRP	mg/L	0-3.5	30(3-191)	110(12.6-326)	0.001*
Procalcitonin	µg/L	< 0.1	0.09(0.02-11.5)	0.81(0.059-65)	0.001*
IL-6	pg/mL	<7	16.7(1.51-193)	30.2(20.1-46.8)	0.001*
Biochemical parameters					
Albumin	g/L	35-52	34(22-45)	26(18.6-34.4)	0.001*
Creatinine	mg/dL	0.51-0.95	0.81(0.34-5.52)	0.89(0.28-4.09)	0.290
ALT	U/L	<35	21(4-92.4)	26.5(5.4-361)	0.027*
AST	U/L	<35	24(10-92)	34(12.6-934)	0.001*
CK	U/L	0-145	64(16-449)	99.5(14-1490)	0.006*
LDH	U/L	135-224	221(147-503)	342(155-1412)	0.001*
Ferritin	µg/L	5.8-274	154(16.7-1500)	487(76-1500)	0.001*

*Significant difference; p<0.05

Table 2. Comorbidities in Non-Severe and Severe Covid Disease

Comorbidities	Non Severe COVID-19 N=173 n(%)	Severe COVID-19 N=84 n(%)	p
Hypertension	40.4%	50%	0.149
Diabetes Mellitus	23.1%	32.1%	0.123
Chronic Renal Failure	11.6%	9.8%	0.105
Malignancy	12.7%	7.5%	0.272
Cardiovasculer Disease	23.1%	38%	0.021*
Immunodeficiency	3.5%	7.14%	0.115
Chronic Obstructive Lung Disease	16.8%	15.5%	0.794
Central Nervous System Disease	8.7%	19%	0.017*

*Significant difference; p<0.05

ferritin as independent variables were performed. Contribution of age, NLR, D-dimer, and CRP were found significant on the model. (All p's<0.005) Table 4.

Discussion

In this study, there was a significant difference between non-severe and severe cases in terms of gender, hospital length of stay, WBC, NLR, monocytes, hemoglobin, fibrinogen, D-dimer, CRP, procalcitonin, IL-6, CK, LDH, and ferritin values. Values greater than 4.5 for NLR, 930 ug/L for D-dimer, 64 mg/L for CRP, 0.136 ug/L for procalcitonin, 44.3 pg/mL for IL-6, 304 IU for LDH, and 312 ug/L for ferritin were associated with severe disease. Age, NLR, D-dimer, and CRP were found predictive variables for mortality.

Several studies reported that inflammatory parameters such as NLR, CRP, procalcitonin, IL-6, and ferritin are closely linked to the COVID-19 severity and mortality (19-21). According to Ai-Ping

Table 3. Performance of NLR, D-Dimer, CRP, procalcitonin, IL-6, LDH and ferritin in predicting severity, using ROC curves

Test	NLR	D dimer (ug/L)	CRP (mg/L)	Procalcitonin (ug/L)	IL-6 (pg/mL)	LDH (IU/L)	Ferritin (ug/L)
Optimum cut-off value	4.5	930	64	0.136	44.3	304	312
AUC-ROC (95% CI)	0.807 (0.74-0.85)	0.735 (0.66-0.79)	0.793 (0.73-0.85)	0.789 (0.72-0.84)	0.798 (0.73-0.85)	0.738 (0.67-0.79)	0.785 (0.72-0.83)
Sensitivity(%)	84.5	84.8	76.2	84.3	68.7	59.0	80.2
Specificity(%)	65.1	55	67.5	63.3	79.7	82.5	75.5
PPV(%)	54.05	47.79	53.25	52.74	21.21	63.4	62.3
NPV(%)	89.63	88.16	85.37	89.24	96.97	79.6	88.04
p	*0.0001	*0.0001	*0.0001	*0.0001	*0.0001	*0.0001	*0.0001

NLR:Neutrophile lymphocyte ratio,CI: Confidence Intervals; PPV: Positive predictive value; NPV: Negative predictive value
*Significant difference; p<0.05

Table 4. Generalized Lineer Model analyses using mortality as dependent variable and Age, Gender, NLR, D Dimer, CRP, Procalcitonin and IL-6 as independent variables

Variables	B	Std Error	95 % Wald Confidence Interval		Wald Ci Square	p
			Lower	Upper		
Constant	-5.965	1.372	-8.655	-3.275	18.895	*0.001
Age	0.034	0.016	0.002	0.067	4.244	*0.039
Gender	0.045	0.469	-0.876	0.966	0.009	0.924
NLR	0.106	0.025	0.056	0.156	17.436	*0.001
D-Dimer	0.001	7.3882E-5	2.929E-6	0.001	3.998	*0.046
CRP	0.010	0.003	0.003	0.018	7.980	*0.005
Procalcitonin	0.021	0.025	-0.029	0.070	0.678	0.410
IL-6	0.001	0.001	-0.001	0.003	0.567	0.451
LDH	0.001	0.001	-0.003	0.002	0.111	0.739
Ferritin	0.001	0.001	-0.001	0.001	0.394	0.530

Significant difference; p<0.05

Yang's study, severe COVID-19 patients had higher concentrations of IL-6, IL-8, IL-2, tumor necrosis factor- α (TNF-α), and ınterferon-γ(IFN-γ) than mild COVID-19 cases, which indicated that the significance of cytokine storm was related to the disease severity (20). In another study by Ai-Ping Yang, the optimal cut-off value for NLR was found as ≥ 3.3 with an AUC value of 0.841 (0.737- 0.945) with the highest sensitivity and specificity. It was shown that 46.1% of the patients with NLR concentrations ≥ 3.3 and older than 49.5 years would become severe, at a mean time of 6.3 days while the patients who didn't meet these criteria would be discharged at 13.5 days. Nevertheless, WBC and CRP weren't found associated with the disease progression in this study (21). In a study by Chen et al, plasma CRP level was found to be positively correlated with the severity of COVID-19 and CT results, and a higher level of CRP (≥ 20.44 mg/L) showed a longer hospitalization (22). In another study; increased procalcitonin levels were found to be associated with a higher risk of severe COVID-19 infection (odds ratio (OR), 4.76; 95% CI, 2.74–8.29). They concluded that serial monitorization of procalcitonin levels might to severe COVID-19 (19).

In a study by Litao Zhang et al; D-dimer levels > 2000 μg/L on admission were found the independent predictor of mortality for patients with COVID-19 (23). Considerably elevated D-dimer results were determined as one of the predictors of mortality according to Tang et al study (13). They presented a D-dimer value of 2102 μg/L (range 770-5270 μg/mL) in the non-survivors while it was 610 μg/L (range 350-1290 μg/L) in survivors. We also found D-dimer levels > 930 μg/L as the predictor of severity in COVID-19 and concluded that D-dimer could be an early and helpful marker to improve the management of COVID-19 patients.

Similar to our study; significantly higher INR results were observed in severe COVID-19 patients and ıf there is impairment of INR results, more aggressive critical care support was proposed (14). Thrombocytopenia was also associated with an over five-fold increased risk of severe COVID-19 illness (OR, 5.1; 95% CI, 1.8-14.6) in previous studies (14). However, there was no significant difference in thrombocyte levels between non-severe and severe cases in our study.

Our study suffers from a few limitations like the relatively limited number of non-survivors. Besides, a longitudinal series of markers would be more reasonable instead of only one measurement identifying each case. Further investigations with the combination of different marker for predicting mortality is needed.

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Conclusions

NLR, D-dimer, CRP, procalcitonin, IL-6, LDH, and ferritin showed comparable performances in discriminating severe cases with predefined cut-offs. Age, NLR, D-dimer, and CRP may be considered as predictors of mortality in COVID-19 patients.

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References

1. WHO-China Joint Mission, Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19), (2020). https://www.who.int/docs/default-source/coronaviruse/who-chinajoint- mission-on-COVID-19-final-report.pdf (accessed March 1, 2020).

2. Pandemic C-19 C. No Title [Internet]. Available from: https://www.worldometers. info/coronavirus/.

3. Mattiuzzi C, Lippi G. Which lessons shall we learn from the 2019 novel coronavirus outbreak? Ann Transl Med. 2020;8:48.

4. Ferrari D, Motta A, Strollo M, et.al. Routine blood tests as a potential diagnostic tool for COVID-19. Clin Chem Lab Med. 2020;58:1095-1099.

5. Wang R, Hozumi Y, Yin C, et al. Mutations on COVID-19 diagnostic targets. Genomics. 2020;112:5204-5213.

6. Henry BM, de Oliveira MHS, Benoit S, et al. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. Clin Chem Lab Med. 2020;58:1021-1028.

7. Pourbagheri-Sigaroodi A, Bashash D, Fateh F, et al. Laboratory findings in COVID-19 diagnosis and prognosis. Clin Chim Acta. 2020;510:475-482.

8. Wang T, Du Z, Zhu F, et al. Comorbidities and multi-organ injuries in the treatment of COVID-19. Lancet. 2020;395(10228):e52.

9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506.

10. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci. 2020;63:364-374.

11. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 ;395:507-513.

12. Chang D, Lin M, Wei L, et al. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. JAMA. 2020;323:1092-1093.

13. Tang N, Li D, Wang X, et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-847.

14. Thachil J, Tang N, Gando S, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020;18:1023-1026.

15. World Health Organization, Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 (accessed 19 March 2020).

16. World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected: interim guidance. Interim guidance 2020. p. 21.

17. Guan WJ, Ni ZY, Hu Y, et al. China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382:1708-1720.

18. Liu W, Tao ZW, Wang L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J (Engl). 2020;133:1032-1038.

19. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. Clin Chim Acta. 2020;505:190-191.

20. Yang AP, Li HM, Tao WQ, et al. Infection with SARS-CoV-2 causes abnormal laboratory results of multiple organs in patients. Aging (Albany NY). 2020;12:10059-10069.

21. Yang AP, Liu JP, Tao WQ, et al. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. Int Immunopharmacol. 2020;84:106504.

22. Chen W, Zheng KI, Liu S, et al. Plasma CRP level is positively associated with the severity of COVID-19. Ann Clin Microbiol Antimicrob. 2020;19:18.

23. Zhang L, Yan X, Fan Q, et al. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J Thromb Haemost. 2020;18:1324-1329.