Value of biochemical markers in predicting outcome of COVID-19 infection in University Hospital, Alexandria, Egypt

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Abstract

This paper aims to examine the value of different biochemical markers in predicting the outcome of COVID-19 infection. A total of 140 patients with confirmed COVID-19 infection by polymerase chain reaction (PCR), different biochemical markers were tested, their relation to the outcome of the disease was monitored, and the most reliable tests were determined. The study found a significant correlation between all evaluated biochemical markers and severity of the disease, including C-reactive protein (CRP), D-dimer, alanine aminotransferase (ALT), Aspartate aminotransferase (AST), prothrombin time (PT), activated partial thromboplastin time (aPTT). In addition, ferritin, lactate dehydrogenase (LDH), procalcitonin (PCT) and Pro- Brain natriuretic peptide (proBNP) demonstrated highly sensitivity and specificity as well as significant prognostic performance. These markers were also independently significant in predicting mortality. Early assessment of biochemical markers in patients with COVID-19 can help clinicians in tailoring treatment and providing more intensive care to those with greater mortality risk. In particular, the assessment of ferritin, LDH, procalcitonin and proBNP can independently predict mortality.

Introduction

In late 2019, an outbreak of atypical pneumonia cases emerged in Wuhan, Hubei province, China, and SARS-Cov2 was identified as the causative organism. This atypical pneumonia was called Corona virus disease 2019 (COVID-19), with the primary target of the virus being the lung, although it can infect other organs that express angiotensin converting enzyme 2 receptors (ACE2).[1]

SARS-CoV-2 is a single stranded RNA virus, belonging to Coronaviridae family, and has a characteristic corona when viewed under and electron microscope due to its spike like surface glycoproteins, which attach to target cell receptors in the host.[1]

The World Health Organization (WHO) has classified COVID-19 patients into four categories: ordinary, mild, severe and critically ill types. Patient may progress from one category to another within 7-10 days, particularly if the virus replicates rapidly and causes a cytokine storm.[2]

Clinical presentation of COVID-19 includes fever, cough, muscle pain and fatigue, with the patient may also show additional symptoms such as olfactory and gustatory dysfunction. Progression to severe disease may be affected by comorbidities of the patient such as diabetes mellitus, dyslipidemia, pulmonary disease, and cardiovascular disorders; therefore, good history taking

is mandatory in those patients.[3] A hyper-inflammatory state has been identified during COVID-19 infection, and several biochemical markers can differentiate between severe and non-severe outcomes. These markers can also predict the likelihood of complications and the course of disease, thus helping in clinical decision-making.[4] To ensure early and effective management of the disease, various biochemical markers have been used to predict the course of the COVID-19, differentiate between severe and non-severe cases, and determine the need for more advanced care.[5] Recent research has focused on identifying the most specific biochemical markers that are implicated in evolution of the disease.

Materials and Methods

This prospective cohort study was conducted on patients admitted to Alexandria student university hospital, Egypt from June 2021 to December 2021. The study was approved by the Ethics Committee of Alexandria Student University Hospital in Egypt and was carried in accordance with the Helsinki Declaration. Written informed consent was obtained from all the participants.

Inclusion criteria

A total of 140 adult patients with confirmed COVID-19 infection by PCR throat swab testing[6] were enrolled in the study.

Exclusion criteria

Bacterial pneumonia, bacterial sepsis, asymptomatic patients who tested positive for the infection but did not exhibit symptoms consistent with COVID-19.

Clinical data included: i) Demographic data: age and gender; ii) Clinical data: time passed from the onset of symptoms till arriving to the hospital, clinical symptoms including cough, fever, headache, muscle aches, vomiting or diarrhea, with or without respiratory symptoms; iii) Past medical history including any cardiac, renal, hepatic, or thyroid diseases, and presence of diabetes mellitus, hypertension or any autoimmune diseases.

Biochemical markers:[7] i) Routine blood tests were done including CBC, Renal function, Liver function tests, and electrolytes; ii) Inflammatory markers including CRP, Ferritin, LDH, and procalcitonin; iii) Coagulation markers including D-dimer, PT, aPTT; iv) Cardiovascular markers including Pro-BNP.

Definition of clinical outcome

In this study, clinical outcome was defined as discharge of the patient after recovery or death. Discharge was considered when the patient was free of symptoms and had no fever for at least 3 days, and had two consecutive negative PCR test results.[8]

Statistical analysis of the data

Data were entered into IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) and analyzed using appropriate statistical tests. Categorical data were presented as numbers and percentages. Chi-square test was applied to investigate the association between the categorical variables. Alternatively, Fisher Exact correction test was applied when more than 20% of the cells have expected count less than 5. For continuous data, they were tested for normality by the Kolmogorov- Smirnov test and Shapiro-Wilk test. Quantitative data were expressed mean, standard deviation, median and range (minimum and maximum). For normally distributed quantitative variables Student t-test was used to compare two groups. On the other hand for not normally distributed quantitative variables Mann Whitney test was used to compare two groups. Logistic Regression was used to detect the most independent factor for affecting deceased patients. The obtained results were judged at the 5% level of significance.

Results

According to the inclusion/exclusion criteria mentioned earlier, a total of 140 patients with confirmed COVID-19 infection were involved in the study, out of these patients, 100 patients were discharged and 40 patients passed away. The mean age patients with COVID019 was 65.2±15.3 years. The mean age of the discharged patients was 63.5±15.3 years, whereas the mean age of the deceased patients was 69.7±14.8 years. The age of the deceased patients was significantly higher than that of the discharged patients, p=0.031(Table 1).

Male patients infected with COVID-19 were 86 (61.4%) of the study sample, the discharged males were 57 (57.0%) patients, and the deceased males were 29 (72.5%) patients. Females infected with COVID-19 were 54 (38.6%) patients of the study sample, the discharged females were 43 (43.0%) patients, and the deceased females were 11 (27.5%) patients, no significant effect of gender on mortality in COVID-19 infection, p=0.089.

Table 1. Comparison between the two studied groups according to demographic data and co-morbidity.

	Total n=140 (%)	Discharged n=100 (%)	Deceased n=40 (%)	Test of Sig.	p
Age (years)					
Mean±SD.	65.2±15.3	63.5±15.3	69.7±14.8	t= 2.179*	0.031*
Median (Min.–Max.)	65 (30–98)	64 (30–91)	73 (36–98)		
Gender					
Male	86 (61.4)	57 (57.0)	29 (72.5)	$\chi\chi^2$= 2.897	0.089
Female	54 (38.6)	43 (43.0)	11 (27.5)		
DM	63 (45.0)	44 (44.0)	19 (47.5)	χ^2=0.141	0.707
Dyslipidemia	48 (34.3)	33 (33)	15 (37.5)	χ^2=0.257	0.612
HTN	55 (39.3)	41 (41)	14 (35)	$\chi\chi^2$=0.431	0.511
Anemia	14 (10)	7 (7)	7 (17.5)	$\chi\chi^2$=3.500	^{FE}p=0.114
Ischemic heart disease	13 (9.3)	10 (10)	3 (7.5)	χ^2=0.212	^{FE}p=0.758
Thyroid	13 (9.3)	9 (9)	4 (10)	χ^2=0.034	^{FE}p=1.000

SD, standard deviation; t, student t-test; χ^2, Chi square test; FE, Fisher Exact; p, p value for comparing between the studied groups. *Statistically significant at p≤0.05.

Patients infected with COVID-19 had multiple comorbidities, including DM in 63 (45.0%) patients, 44 (44.0%) patients were discharged and 19 (47.5%) patients were deceased, p=0.707. (Table 1).

Dyslipidemia in 48 (34.3%) patients, 33 (33%) patients were discharged and 15 (37.5%) patients were deceased, p=0.612. HTN in 55 (39.3%) patients, 41 (41%) patients were discharged and 14 (35%) patients were deceased, p=0.511. Anemia in 14 (10%) patients, 7 (7%) patients were discharged and 7 (17.5%) patients were deceased, p=0.114. Ischemic heart disease in 13 (9.3%) patients, 10 (10%) patients were discharged and 3 (7.5%) patients were deceased, p=0.758. Thyroid disease in 13 (9.3%) patients, 9 (9%) patients were discharged and 4 (10%) patients were deceased, p=1.000. None of them significantly affects the outcome of the disease.

Many laboratory investigations were conducted and their relation to the outcome of the disease were recorded. CRP showed mean value of 17.7±16.3 mg/L in all COVID-19 infected patients, with a mean of 11.4±8.3 mg/L in discharged patients, and a mean of 33.4±20.4 mg/L in deceased patients. CRP also showed high significant increase in deceased patients more than discharged patients, p<0.001 (Table 2).

Ferritin showed mean value of 1308±2006 ng/mL in all COVID-19 infected patients, with a mean of 715.8±1188.6 ng/mL in discharged patients, and a mean of 2788.8±2758.5 ng/ml in deceased patients, the increase in ferritin level was highly significant in deceased patients in comparison to discharged patients, p<0.001 (Table 2, Figure 1).

LDH showed mean value of 585±590 IU/L in all COVID-19 infected patients, with a mean of 380.7±246.7 IU/L in discharged patients, and a mean of 1095.9±843.5 IU/L in deceased patients, there was a high significant increase of LDH in deceased patients

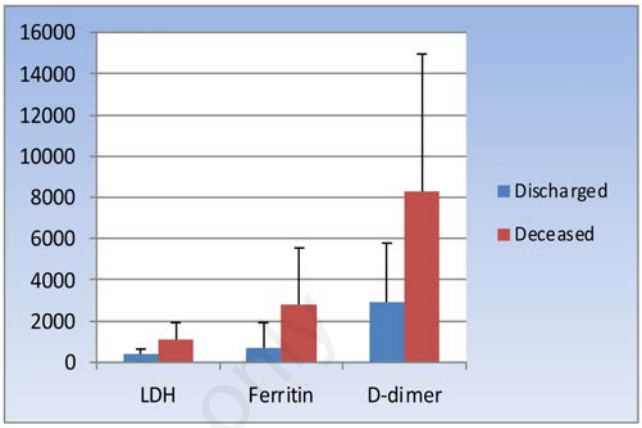

Figure 1. Error Bar showing differences in three markers between discharged and deceased patients.

Table 2. Comparison between the two studied groups according to laboratory investigations.

	Total n=140 (%)	Discharged n=100 (%)	Deceased n=40 (%)	U	p
CRP (mg/L)					
Mean±SD.	17.7±16.3	11.4±8.3	33.4±20.4	543.0*	<0.001*
Median (Min.–Max.)	14.5 (0.3–71)	8.6 (0.3–36)	28 (10–71)		
Ferritin (ng/mL)					
Mean±SD.	1308±2006.1	715.8±1188.6	2788.8±2758.5	774.0*	<0.001*
Median (Min.–Max.)	642.5 (14–10105)	354.5 (14–7006)	1588 (21–10105)		
LDH (IU/L)					
Mean±SD.	585±590	380.7±246.7	1095.9±843.5	336.50*	<0.001*
Median (Min.–Max.)	444 (134–3894)	290.5 (134–966)	874.5 (500–3894)		
D-dimer (ng/mL)					
Mean±SD.	4442±4943.2	2902.5±2873.8	8290.8±6697.2	677.0*	<0.001*
Median (Min.–Max.)	2427 (331–28809)	1581.8 (331–10177)	8524.5(1255–28809)		
ALT (IU/L)					
Mean±SD.	91.2±230.2	42.5±32.7	212.9±406	1258.50*	0.001*
Median (Min.–Max.)	41 (9–1577)	35.5 (9–148)	91 (11–1577)		
AST (IU/L)					
Mean±SD.	129.5±276.2	54.2±51	318±462.9	727.0*	<0.001*
Median (Min.–Max.)	45.5 (15–1692)	34 (15–284)	98.5 (30–1692)		
PT (seconds)					
Mean±SD.	17.6±5.5	16.4±5.2	20.5±5.2	1042.0*	<0.001*
Median (Min.–Max.)	16 (1–33)	15.7 (1–33)	18.1 (15–32)		
aPTT					
Mean±SD.	41.4±13.5	39.7±13.7	45.5±12.2	1376.50*	0.004*
Median (Min.–Max.)	37.4 (20–78)	35 (20–78)	43 (25–77)		
Procalcitonin (ng/mL)					
Mean±SD.	4.4±8.3	1.7±3.8	11.0±12.2	457.0*	<0.001*
Median (Min.–Max.)	1.2 (0.03–45.0)	0.6 (0.03–23.0)	5.9 (0.2–45.0)		
Pro-BNP (pg/mL)					
Mean±SD.	4219.3±9446	1121.8±1601.1	11963.1±15013.3	486.50*	<0.001*
Median (Min.–Max.)	784.5 (43–45908)	556.5 (43–8084)	6547.5 (300–45908)		

SD, standard deviation; U, Mann Whitney test; p, value for comparing between the studied groups. *Statistically significant at p≤0.05.

more than discharged patients, p<0.001.

D-dimer showed mean value of 4442±4943.2 ng/mL in all COVID-19 infected patients, with a mean of 2902.5±2873.8 ng/mL in discharged patients, and a mean of 8290.8±6697.2 ng/mL in deceased patients, the increase in D-dimer was highly significant in deceased patients, p<0.001.

ALT showed mean value of 91.2±230.2 IU/L in all COVID-19 infected patients, with a mean of 42.5±32.7 IU/L in discharged patients, and a mean of 212.9±406 IU/L in deceased patients, there was a significant increase of ALT in deceased patients more than discharged patients, p=0.001.

AST showed mean value of 129.5±276.2 IU/L in all COVID-19 infected patients, with a mean of 54.2±51 IU/L in discharged patients, and a mean of 318±462.9 IU/L in deceased patients, there was a significant increase of AST in deceased patients more than discharged patients, p<0.001.

PT showed mean value of 17.6±5.5 seconds in all COVID-19 infected patients, with a mean of 16.4±5.2 seconds in discharged patients, and a mean of 20.5±5.2 seconds in deceased patients, PT showed high significant increase in deceased patients more than discharged patients, p<0.001.

aPTT showed mean value of 41.4±13.5 seconds in all COVID-19 infected patients, with a mean of 39.7±13.7 seconds in discharged patients, and a mean of 45.5±12.2 seconds in deceased patients, aPTT showed significant increase in deceased patients more than discharged patients, p<0.004.

PCT showed mean value of 4.4±8.3 ng/mL in all COVID-19 infected patients, with a mean of 1.7±3.8 ng/mL in discharged patients, and a mean of 11.0±12.2 ng/mL in deceased patients, PCT showed highly significant increase in deceased patients more than discharged patients, p<0.001.

Pro-BNP showed mean value of 4219.3±9446 pg/mL in all COVID-19 infected patients, with a mean of 1121.8±1601.1 pg/mL in discharged patients, and a mean of 11963.1±15013.3 pg/mL in deceased patients, there was a high significant increase of Pro-BNP in deceased patients more than discharged patients, p<0.001. To detect if the previous laboratory investigations were accurate in predicting mortality, we used the area under the curve (AUC) and 95% CI of the receiver operator characteristic (ROC) curve. We found that there was significant prognostic performance of CRP, ferritin, LDH, D-dimer, PCCT and pro-BNP with mortality, and the highest performance of variables were, LDH > PCT > pro-BNP > CRP, and the least was D-dimer and ferritin. The cut off value for LDH was > 500 IU/L, for PCT was >2.09 ng/mL, for pro-BNP was >1755 pg/mL, and for CRP > 15.4 mg/L. (Table 3, Figure 2). Univariate analysis showed that each one of the variables was associated with mortality after adjustment with ALT, AST, PT,

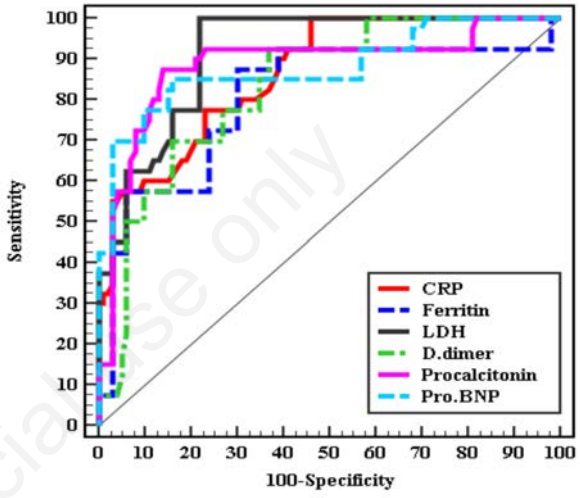

Figure 2. ROC for CRP, Ferritin, LDH, D. dimer, PCT and Pro-BNP to predict mortality.

Table 3. Prognostic performance for CRP, Ferritin, LDH, D. dimer, Procalcitonin and Pro-BNP to predict mortality (n=40) from discharged (n=100).

	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
CRP	0.864	<0.001*	0.803–0.926	>15.4	80.0	69.0	50.8	89.6
Ferritin	0.807	<0.001*	0.718–0.895	>677#	87.50	70.0	53.8	93.3
LDH	0.916	<0.001*	0.872–0.960	>500	97.50	78.0	63.9	98.7
D. dimer	0.831	<0.001*	0.761–0.900	>2188#	92.50	63.0	50.0	95.5
Procalcitonin	0.886	<0.001*	0.815–0.957	>2.09	90.0	79.0	63.2	95.2
Pro-BNP	0.878	<0.001*	0.806–0.950	>1755#	85.0	84.0	68.0	93.3

AUC, area under a curve; p value: probability value; CI, confidence intervals; NPV, negative predictive value; PPV, positive predictive value. *Statistically significant at p≤0.05. #Cut off was choose according to Youden index.

Table 4. Univariate and multivariate Logistic regression analysis for the parameters affecting deceased patients (n=40 *vs*. 100).

	Univariate		Model 1		Model 2	
	p	OR (95%C.I) (LL-UL)	p	OR (95%C.I) (LL-UL)	p	OR (95%C.I) (LL-UL)
CRP (>15.4)	<0.001*	8.90 (3.68–21.53)	<0.001*	11.41(3.11–41.86)	0.073	3.96 (0.88–17.79)
Ferritin (>677)	<0.001*	16.33 (5.83–45.75)	<0.001*	18.40 (4.60–73.72)	0.018*	10.95 (1.51–79.25)
LDH (>500)	<0.001*	138.3 (17.97–106)	0.005*	608(14.3–2588727)	0.003*	34.72 (3.23–373.7)
D. dimer (>2188)	<0.001*	21.0 (6.05–72.91)	<0.001*	15.27(3.53–66.09)	0.573	2.64 (0.09–77.02)
Procalcitonin(>2.09)	<0.001*	33.86 (10.83–105.8)	<0.001*	27.12(6.95–105.9)	0.003*	11.82 (2.26–61.86)
Pro-BNP (>1755)	<0.001*	29.75 (10.74–82.45)	<0.001*	28.21 (7.07–112.6)	0.014*	7.73 (1.50–39.80)

OR, odd's ratio; CI: confidence interval; LL, lower limit; UL, upper limit; Model 1, Each marker was adjusted by other significant variables (ALT, AST, PT, aPPT and age); Model 2, Multivariate Regression for the six markers. *Statistically significant at p ≤ 0.05.

aPTT, and age, model I showed that they were statistically significant, and after performing multivariate analysis (model II) we found that ferritin > 677 ng/ml, LDH >500 IU/L, PCT >2.09 ng/mL, and pro-BNP > 1755 pg/mL were still independently significant for predicting mortality (Table 4, Figure 3).

Discussion

Numerous studies have confirmed that increasing age and presence of chronic illness are significant risk factors in COVID-19 infection, leading to extended hospitalization periods and increase mortality. For instance, a retrospective study conducted on older patients infected with COVID-19 at Zhongnan Hospital of Wuhan University found that the mortality rate was significantly higher among patients aged over 65 years (34.5%) than in younger patients (4.7%).[9] Another study conducted in Hyderabad, Telangana, India revealed the impact of preexisting comorbidities on disease outcome, including diabetes mellitus, hypertension, coronary artery disease, and chronic kidney disease, either individually or in combination. The study found that preexisting comorbidities were significant contributing factor in increasing mortality, especially when diabetes mellitus and hypertension occurred together.[10] Moreover, a multivariate retrospective cohort conducted in Bangladesh assessed the effect of sociodemographic factors, comorbidities, symptoms, Charlson comorbidity index, and access to health facilities on disease outcomes. The study reported that increased age, the presence of more than 3 symptoms, and multiple comorbidities, were associated with higher morbidity and mortality in COVID-19 patients.[11] In our study, we also observed that the age had a significant impact on COVID-19 mortality rates, with higher mortality rates observed in patients aged over 65 years. However, we did not observe any significant effect of other comorbidities on COVID-19 mortality rates.

One of the frequently studied aspects of COVID-19 infection is its relation to patient sex, and its effect on the rate of infection and the outcome of the disease. According to data reported on 239,709 patients in Italy, mortality is 17.7% in men and 10.8% in women, with 59% of total deaths were in males. Even though the rate of infection was lower in males than in females, with 45.8% and 54.2% respectively, indicating that evolution of the disease may be affected by gender.[12] In contrast, another study found higher infection risks among females than males at working ages, but the opposite trend was observed at older age, and across all age groups, mortality rate in males was double that in females.[13] However, in our study, there was no significant effect of gender on mortality in COVID-19 infection.

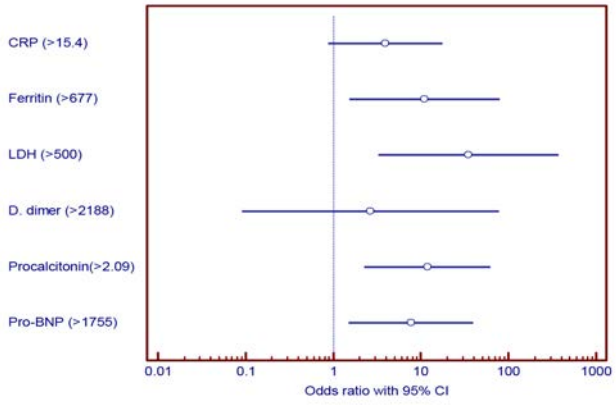

Figure 3. Graph showing odds ratio of different markers with 95% CI after a multivariate logistic regression for COVID-19 mortality.

Laboratory investigations are crucial for the detection of COVID-19 infection and monitoring evolution of the disease. CRP is an acute-phase protein synthesized by the liver, and elevated in response to bacterial infection, which is usually used in the diagnosis of pneumonia.[14] A retrospective study conducted in China involving 76 patients with confirmed COVID-19 infection, found that CRP ≥ 52.14mg/L was correlated with the severity of infection, and had prognostic value for mortality.[15] Additionally, another study conducted in China found that CRP was strongly correlated with Murray score which was originally used to determine the severity of lung injury in patients developing acute respiratory distress syndrome.[3] In our study, CRP showed high significant increase in deceased patients more than in discharged patients, and CRP >15.4 mg/L had a significant prognostic performance with mortality, with sensitivity 80% and specificity 69% after adjustment with ALT, AST, PT, aPTT, and age.

Ferritin, although known primarily as an iron storage protein; has multiple functions, including serving as a signaling molecule and direct mediator of the immune system. Its expression can be induced by cytokines, and it may also plays role in induction of pro- and anti-inflammatory cytokines.[16] It was observed that hyperferritinemia in patients with severe COVID-19 infection was between 1.5 and 5.3 times higher in patients with severe disease usually reaching >800 µg/L and less than theses values in patients with moderate disease.[17] A Study conducted in Wuhan, China on patients survived the infection and patients died during their stay in the hospital found that ferritin levels on admission was around 1400 µg/L in non-survivors, which is between 3 and 4 times higher than that observed in survivors.[18] In our study, ferritin showed significant increase in deceased patients more than discharged patients. Ferritin levels above 677 ng/mL were significantly predictive of mortality, with a sensitivity of 87.5% and specificity 70% after adjusting for ALT, AST, PT, aPTT, and age. Ferritin was also found to be independent significant predictor of mortality.

LDH is a glycolytic enzyme that catalyzes the conversion between L-lactate and pyruvate and conversion between NADH and NAD+. It is present in the cytoplasm of all tissues especially the heart, liver and skeletal muscles. In COVID-19 infection, there is tissue injury and low oxygenation of the cells leading to up-regulation of glycolytic pathway and increased LDH levels especially in severe lung injury which leads to release of large amounts of LDH isoenzyme 3.[19] A Meta-analysis performed by Martha J. showed that increased levels of LDH was associated with poor prognosis and high mortality rate, with a sensitivity of 74 % and specificity of 69%. Positive likelihood ratio was 2.4, negative likelihood ratio was 0.38 and area under curve of 0.77, independently from age, male sex, hypertension and diabetes.[20] Another meta-analysis conducted including twenty eight study showed that high levels of LDH were observed in ICU patients versus non-ICU patients and in non-survivors compared with survivors and concluded that LDH is an important severity marker for COVID-19 infection and can be used as a predictor of survival.[21] In our study, LDH showed highly significant increase in deceased patients more than discharged patients, as LDH levels > 500 IU/L had a significant prognostic performance with mortality, with a sensitivity 97.5% and specificity of 78% after adjusting for ALT, AST, PT, aPTT, and age. LDH was also found to be independent significant

predictor of mortality. D-dimers are fragments resulted from cleavage of fibrin to break down clots. Therefore, any increase in production or degradation of fibrin will elevate plasma D-dimer levels. In severe COVID-19 infection, proinflammatory and prothrombotic events are prominent leading to D-dimer production.[22] Studies also suggest that in SARS-CoV-2 infection there is dysregulation of the coagulation cascade with diffuse alveolar damage and infiltration with mononuclear inflammatory cells in the interstitium, This promotes prothrombotic activity. Furthermore, proinflammatory cytokines cause endothelial injury, which enhances coagulation and inhibit fibrinolysis in those patients. These high levels of D-dimer that indicate increased hypercoagulability increase severity and contribute to mortality.[23]

Reports from Wuhan hospital showed that from the patients requiring ICU admission, 26% had increased D-dimer levels. The main differences between non-survivors compared to survivors is markedly elevated D-dimer which progress on day 5 of infection, also lymphopenia, and renal dysfunction.[24] In a multivariable logistic regression model of 171 patients in another Wuhan hospital, an initial D-dimer level >1.0 µg/mL was associated with poor prognosis and high mortality with an odds ratio of 18.42 (2.64-128.55; p=0.003).[25] In our study, there was highly significant difference in D- dimer level between deceased and discharged patients, as D-dimer levels > 2188 ng/mL had a significant prognostic performance with mortality, with sensitivity 92.5% and specificity 63% after adjustment with ALT, AST, PT, aPTT, and age.

Cytokine storm and hypoxia associated with COVID-19 infection, may also contribute to liver injury in seriously ill patients, as shock and hypoxia leading to hypoperfusion of the liver, this inturn leads to hepatic dysfunction. Moreover, liver damage may occur due the use of medications especially lopinavir and ritonavir.[26]

Chen *et al*.[27] evaluated 99 confirmed cases of COVID-19 patients. He found that 43 patients presented with liver dysfunction, as ALT and/or AST were elevated especially in severe cases. In a multicenter retrospective cohort study conducted in Hubei province, liver injury indicators were evaluated and their relation to death risk was recorded. The study found that AST was elevated before ALT and both were highly increased in severe group of patients, and that AST is in particular was associated with mortality risk.[28] In our study, ALT and AST were significantly elevated in deceased patients more than discharged patients. However, we did not find any significant prognostic performance with mortality.

Viral, bacterial, or fungal infection leads to activation of host defense mechanisms results in activation of coagulation pathways as a part of communication between humoral and cellular components of the immune response in what is called thromboinflammation or immunothrombosis.[29] Evidence of coagulopathy has been reported with appearance of COVID-19 infection in China. Reports of the first 99 patients hospitalized in Wuhan demonstrated elevated aPTT in 6% and elevated PT in 5%.[27] Another report from another Wuhan hospital showed mild elevation in PT but normal aPTT in the first 138 patients admitted to the hospital.[24] In our study, PT and aPTT were significantly elevated in deceased patients more than discharged patients. However, we did not find any significant prognostic performance with mortality.

PCT is a product of calcitonin-related gene, produced by epithelial cells during bacterial infection; it is considered as biomarker of blood infection, and usually used to monitor antibiotic therapy.[30] In a recent study, elevated serum level of PCT in COVID-19 infected patients was associated with high mortality with high sensitivity, and reported that serum level of PCT (≥ 0.10 ng/mL) was independent risk factor for mortality specially in old patients (age ≥60 y) and severe COVID-19 infection.[15] In our study, PCT showed significant increase in deceased patients more than discharged patients, as PCT levels > 2.09 ng/mL had a significant prognostic performance with mortality with, sensitivity 90% and specificity 79% after adjustment with ALT, AST, PT, aPTT, and age, it was independently significant for predicting mortality.

Cardiac complications during the COVID-19 infection are predisposed by old age, prior cardiovascular disease and severe disease presentation. The pathogenic mechanisms include pro-inflammatory cytokines (IL-6, IL-7, IL-22, CXCL10) which contribute to plaque rupture, activation of pro-coagulation factors; and hemodynamic changes leading to ischemia and thrombosis. These cardiac events lead to production of the N-terminal pro B type natriuretic peptide (NT-proBNP) in those patients.[29] A meta-analysis involving 4,189 patients enrolled in 28 studies reported that patients with severe COVID-19 infection had significant high level NT-proBNP, and its level rises progressively in non-survivors.[5] In another study involving 138 patients, the average levels of NT-proBNP (301.2 ng/L vs 2887.5 ng/L; p<0.01) were maximum in ICU patients who stayed on mechanical ventilation, or extracorporeal membrane oxygenation or deceased patients.[24] In our study, proBNP showed significant increase in deceased patients more than discharged patients, as proBNP levels > 1755 pg/mL had a significant prognostic performance with mortality, with sensitivity 85% and specificity 84% after adjustment with ALT, AST, PT, aPTT, and age, and it was independently significant for predicting mortality.

After assessment of many biochemical markers during COVID-19 infection, we found that there was significant prognostic performance of CRP, ferritin, LDH, D-dimer, PCT and pro-BNP with mortality, and the highest performance of variables were LDH > PCT > pro-BNP > CRP, and the least was D-dimer and ferritin. The cut off value for LDH was > 500 IU/L, for PCT was >2.09 ng/mL, for pro-BNP was >1755 pg/ml, and for CRP > 15.4 mg/L. Univariat analysis showed that each one of the variables was associated with mortality after adjustment with ALT, AST, PT, aPTT, and age, multivariate analysis showed that ferritin > 677 ng/ml, LDH >500 IU/L, PCT >2.09 ng/mL, and pro-BNP > 1755 pg/mL were still independently significant for predicting mortality.

Conclusions

Early assessment of biochemical markers in patients with COVID-19 infection can assist clinicians in tailoring treatment and providing more intensive care to those at a greater risk of mortality. Ferritin, LDH, PCT and proBNP are important markers to assess as they have demonstrated high sensitivity and specificity, prognostic performance, and independent significance in predicting mortality. By monitoring these markers, clinicians can better identify patients who may require more aggressive interventions or closer monitoring to improve outcomes

References

1. Lu R, Zhao X, Li J. Genomic characterization and epidemiology of 2019 novel coronavirus: implication for virus origins and receptor binding. Lancet 2020;395:565-74.
2. Chen T, Wu D, Chen H, Yan W, Yang D. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ.2020; 368: m1091.
3. Hariyanto I and Kurniawan A. Thyroid disease is associated with severe corona disease 2019 (COVID-19) infection.

Diabetes Metab Syndr 2020;14:1429-30.
4. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Science China. Life Sciences 2020;63:364–74.
5. Hariyanto T, Japar K, Kwenandar F, et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. Am J Emerg Med 2021;41:110–9.
6. Kubina R, Dziedzic A. Molecular and serological tests for COVID-19 a comparative review of SARS-CoV-2 coronavirus laboratory and point-of-care diagnostics. Diagnostics 2020;10:434.
7. Letelier P, Encina N, Morales P, et al. Role of biochemical markers in the monitoring of COVID-19 patients. J Med Biochem 2012;40:115–28.
8. Centers of disease control and prevention (2020) COVID-19 (Coronavirus disease): people with certain medical conditions. 2020. Accessed August 31, 2021. Available from: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautiona/people-with-medical-conditions.html
9. Chen T, Dai Z, Mo P, et al. Clinical Characteristics and Outcomes of Older Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective Study. J Gerontol 2020;75:1788–95.
10. Koyyada R, Nagalla B, Tummala A, et al. Prevalence and Impact of Preexisting Comorbidities on Overall Clinical Outcomes of Hospitalized COVID-19 Patients. BioMed Res Int 2022:2349890.
11. Sharif N, Opu R, Ahmed S, et al. Prevalence and impact of comorbidities on disease prognosis among patients with COVID-19 in Bangladesh: A nationwide study amid the second wave. Diabetes Metab Syndr 2021;15:102148.
12. Foresta C, Rocca S, Di Nisio A. Gender susceptibility to COVID-19: a review of the putative role of sex hormones and X chromosome. J Endocrinol Invest 2021;44:951–6.
13. Davies G, Klepac P, Liu Y, et al. Age-dependent effects in the transmission and control of COVID-19 epidemics. Nature Med 2020;26:1205-11.
14. Kalra L, Smith C, Hodsoll J, et al. Elevated C-reactive protein increases diagnostic accuracy of algorithm-defined stroke-associated pneumonia in afebrile patients. Int J Stroke 2019;14:167-73.
15. Xu J, Xu C, Zhang R, et al. Associations of procalcitonin, C-reaction protein and neutrophil-to-lymphocyte ratio with mortality in hospitalized COVID-19 patients in China. Sci Rep 2020;10:1-10.
16. Rosário C, Zandman-Goddard G, Meyron-Holtz G, et al. The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. BMC Med 2013;11:1-11.
17. Ji D, Zhang S, Chen Z, et al. Clinical characteristics predicting progression of COVID-19 (2/17/2020). Available at SSRN 3539674.
18. Hou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
19. Henry M, Aggarwal G, Wong J, et al. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med 2020;38:1722–26.
20. Martha W, Wibowo A, Pranata R. Prognostic value of elevated lactate dehydrogenase in patients with COVID-19: a systematic review and meta-analysis. Postgrad Med J 2022;98:422–7.
21. Szarpak L, Ruetzler K, Safiejko K, et al. Lactate dehydrogenase level as a COVID-19 severity marker. Am J Emerg Med 2021;45:638–9.
22. Mikacenic C, Hahn O, Price L, et al. Biomarkers of Endothelial Activation Are Associated with Poor Outcome in Critical Illness. PLoS One 2015;10:e0141251.
23. Lippi G, Favaloro E. D-dimer is Associated with Severity of Coronavirus Disease 2019: A Pooled Analysis. Thrombosis Haemostasis 2020;120:876–8.
24. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
25. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
26. Hui S, Memish A, Zumla A. Severe acute respiratory syndrome vs. the Middle East respiratory syndrome. Curr Opin Pulm Med 2014;20:233-41.
27. 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;395:507–13.
28. Lei F, Liu M, Zhou F, et al. Longitudinal Association Between Markers of Liver Injury and Mortality in COVID-19 in China. Hepatology 2020;72:389–98.
29. Jackson P, Darbousset R, Schoenwaelder M. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood 2019;133:906–18.
30. Mierzchała-Pasierb M, Lipińska-Gediga M. Sepsis diagnosis and monitoring - procalcitonin as standard, but what next? Anaesthesiol Intensive Ther 2019;51:299-305.