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Biomarkers of severity and outcome among adult COVID-19 positive patients admitted to a tertiary referral hospital

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RESEARCH

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ABSTRACT

Background
Several biomarkers were found to predict the severity and outcome of COVID-19 infection.

Aims
To determine the associations between laboratory biomarkers with COVID-19 severity and outcome.

Methods
A prospective study among confirmed COVID-19 patients aged 18 years old and above for biomarkers, and correlated with severity and mortality.

Results
Of 347 patients, 245 (70.6 per cent) were RT-PCR COVID-19 positive. Patients who were admitted to the ICU had higher levels of WBC count, lymphocytes, neutrophils, N/L ratio, D-dimer, potassium, phosphates, ferritin, CRP, ESR and cortisol, whereas patients who died were older, and had higher levels of N/L ratio, D-dimer, potassium, phosphates, ferritin, CRP, procalcitonin, cortisol, FBS, HbA1c, PTH and number of comorbidities compared to those who survived. Cortisol, FT3, and FBS were the most significant predictor for mortality and ferritin for ICU admission.

Conclusion
High cortisol and blood sugar and low FT3 were associated with higher mortality whereas low haemoglobin and lymphocytes and high N/L ratio, ferritin and D-dimer were associated with ICU admission among COVID-19 (+) patients. The first day biomarkers may provide clues for the eventual outcome and severity among COVID-19 patients. This will facilitate a more focused and aggressive intervention and early ICU admission.

Key Words
COVID-19, biomarkers, outcome, severity, mortality

What this study adds:

1. What is known about this subject?
Several biomarkers were suggested to predict the severity and outcome among patients with COVID-19 infection.

2. What new information is offered in this study?
Biomarkers including cortisol, FBS and FT3 were associated with higher mortality whereas haemoglobin, lymphocytes, N/L ratio, ferritin and D-Dimer were associated with ICU admission.

3. What are the implications for research, policy, or practice?
The findings of this research will facilitate a more focused and aggressive intervention and early ICU admission.

Background

Studies have suggested several biomarkers related to disease progression, severity and outcome of COVID-19 infection including lymphocyte count, neutrophil count, neutrophil lymphocyte ration (NLR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet count, procalcitonin (PCT), interleukin 6 (IL-6), D-dimer, troponin,

creatine kinase (CK), aspartate aminotransferase (AST) and many others.[1] These biomarkers were found to have significant roles in the monitoring among COVID-19 patients and will help clinicians in the management protocols.

Lymphopenia was found to be associated with poor outcome and predicts disease severity among patients with COVID-19.[2] These studies have reported that more severe outcomes and disease progression of COVID-19 infection occur with lymphopenic patients particularly among younger patients.[2] Patients with lymphopenia were reported to have a 3-fold higher risk of developing severe COVID-19.[3] Higher neutrophil count and NLR have been observed among severe COVID-19 cases as much as 5.9-fold compared to patients with mild and moderate infection.[4] Marked increase in the CRP level and its association with IL-6 was also reported to predict disease severity in its early stages especially among critical patients who need more aggressive management.[5] Studies have also shown the association between elevated ESR, D-dimer and IL-6 with severe COVID-19 infection among older patients aged >60 years old.[6] Coagulopathies were also reported among critical COVID-19 patients and the association between thrombocytopenia and COVID-19 severity and mortality was suggested.[7]

The multitude of studies that were conducted to determine useful laboratory markers to predict severe outcomes and mortality form COVID-19 was exorbitant. The plethora of studies have suggested these biomarkers' potential use in the early management of COVID-19 patients at the time of admission and claimed that these biomarkers have the potential to predict severe outcomes and even mortality. In this regard, we aimed to determine the associations between anthropometric and laboratory biomarkers with COVID-19 severity and outcome and compare it with the findings from previous studies.

Method

We conducted a prospective cohort observational study among adult patients aged 18 years old and above who presented with signs and symptoms of COVID-19 infection (cough, fever, difficulty/shortness of breath and malaise) and history of exposure to COVID-19 at the Emergency Department (ED) of King Saud University Medical City in Riyadh, Saudi Arabia. Patients were screened and swabbed for real-time polymerase chain reaction (RT-PCR) on admission. Patients aged <18 years old and those who were not admitted to the hospital were excluded from the study. Sample size was calculated based on 95 per cent confidence level, 80 per cent power and 5 per cent confidence interval, the calculated sample size was 278.

Demographic data including age and gender, vital signs, anthropometric measurements were recorded, History of comorbid conditions including endocrine diseases, diabetes, hypertension, pulmonary diseases, cancer, and cardiac diseases were noted. COVID-19 RT-PCR was done using the Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics Corporation, Indianapolis, USA) and QuantStudio7 Flex Real-time PCR system with software version 1.3 in a singleplex format (Applied Biosystems, Beverly, Massachusetts, USA). COVID-19 RT-PCR were performed for the 2nd time within 48-72 hours from the 1st sample if it was negative to confirm the COVID-19 status. Blood extraction for all laboratory tests were measured within the 24 hours from presentation to the hospital. Admission to the ICU, mechanical ventilation, death and discharge were noted. Severity of the disease was noted if patient was admitted to the ICU, intubated or died. We also calculated the length of hospital stay for each patient.

Statistical analysis was performed using the Statistical Package for Social Sciences version 23.0 (SPSS Inc., IBM, Armonk, New York, USA). Data were reported as numbers and percentages for categorical variables and mean, standard deviation (SD), and median for continuous variables. Correlation was done using the chi-square test and Pearson correlation test for categorical and continuous variables, respectively. Test of significance between means (SD) for normally distributed variables was performed using the independent t-test. A logistic regression analysis was conducted to determine the most significant factors associated with disease severity and mortality among patients positive for COVID-19. A P-value <0.05 was considered statistically significant.

Results

A total of 347 patients were included in the study. There were 236 (68.0 per cent) males and 111 (32.0 per cent) females with a mean age of 55.6±16.3 years old (range: 19-96 years old). There were 162 patients (46.7 per cent) with hypertension, 149 (42.9 per cent) with diabetes, 67 (19.3 per cent) with heart disease, 40 (11.5 per cent) with respiratory diseases, 21 (6.1 per cent) with history of stroke, 54 (15.6 per cent) with dyslipidaemia, 39 (11.2 per cent) with chronic kidney disease, and 6 (1.7 per cent) with chronic liver disease. Two hundred and forty-five patients (70.6 per cent) were positive and 102 (29.4 per cent) were RT-PCR negative for COVID-19.

Table 1 shows the comparison of the anthropometric and laboratory results for all patients and a comparison of results between COVID-19 positive and COVID-19 negative patients. The 102 COVID-19 negative patients had a final diagnosis of pneumonia (n=89, 87.3 per cent) and lower respiratory tract infection with ARDS (n=13, 12.7 per cent). Significant differences were observed between the laboratory results between COVID-19 (+) and COVID-19 (-) patients. COVID-19 (+) patients had significantly higher mean values for haemoglobin (p<0.001), ferritin (p=0.010), CRP (p<0.001), FT4 (p=0.013) and HbA1c (p=0.001), whereas COVID-19 (-) patients had significantly higher mean values for lymphocytes (p=0.001), D-dimer (p=0.007), phosphates (p<0.001) and cortisol (p=0.007). Pearson correlation test showed significant positive correlation between COVID-19 positivity with gender (r=0.141, p=0.009), haemoglobin (r=0.287, p<0.001), ferritin (r=0.155, p=0.005), CRP (r=0.217, p<0.001), FT4 (r=0.140, p=0.013), and HbA1c (r=0.185, p=0.001). On the other hand, COVID-19 positivity had significant negative correlations with age (r=-0.171, p=0.001), WBC count (r=-0.154,p=0.004), lymphocytes (r=-0.186, p=0.001), D-dimer (r=-0.152, p=0.006), potassium (r=-0.164, p=0.002), phosphates (r=-0.213, p<0.001),cortisol (r=-0.149, p=0.010 and PTH (r=-0.256, p<0.001).

There were 140 patients who were admitted to the ICU. ICU admission was significantly higher among COVID-19 (+) patients (n=104/140 (74.3 per cent) versus 36/140 (25.7 per cent), p<0.001) and mortality was also significantly higher among COVID-19 (+) patients (n=46/62 (74.2 per cent) versus 16/62 (25.8 per cent) COVID-19 (-) patients (p<0.001).

Sub analysis of 245 COVID (+) patients with severity and mortality showed that patients who were admitted to the ICU had significantly higher levels of WBC count (p=0.030), neutrophils (p<0.001), N/L ratio (p<0.001), D-dimer (p=0.006), potassium (p=0.009), ferritin (p<0.001), CRP (p<0.001), ESR (p=0.024), cortisol (p=0.037) and longer hospital stay (p<0.001) but significantly lower levels of haemoglobin (p=0.027) and FT3 (p<0.001) compared to patients who were not admitted to the ICU. On the other hand, patients who died were significantly older in age (p=0.012), had higher levels of D-dimer (p<0.001), potassium (p=0.002), ferritin (p=0.007), cortisol (p=0.002), FBS (p=0.002), HbA1c (p=0.046), PTH (p=0.006), had more comorbidities (p=0.011) and longer hospital stay (p=0.006) compared to those who survived. However, the levels of haemoglobin, FT4 and FT3 were significantly lower among those who died than those who survived (p<0.001, p=0.004, and p<0.001, respectively) (Table 2).

Significant positive correlates of ICU admission included WBC count (p=0.030), lymphocytes (p=0.047), neutrophils (p<0.001), N/L ration (p<0.001), D-dimer (p=0.004), potassium (p=0.009), ferritin (p<0.001), CRP (p<0.001), ESR (p=0.024), and cortisol (p=0.026). Significant negative correlates of ICU admission included haemoglobin (o=0.027) and FT3 (p<0.001). For mortality, the significant positive correlates included age (p=0.012), N/L ratio (p=0.001), D-dimer (p<0.001), potassium (p=0.002), ferritin (p<0.001), CRP (p=0.001), procalcitonin (p=0.030), cortisol (p<0.001), FBS (p=0.002), HbA1c (p=0.046), PTH (p<0.001), and number of comorbidities (p=0.002). Significant negative correlates of mortality included haemoglobin (p<0.001), FT4 (p=0.004) and FT3 (p<0.001) (Table 3).

A stepwise regression analysis was performed with death as the dependent variable and predictors including age, haemoglobin, N/L ratio, D-dimer, potassium, ferritin, CRP, procalcitonin, FT4, FT3, cortisol, FBS, HbA1c, PTH and number of comorbidities entered into the equation. Significant predictors for mortality included serum cortisol (beta=0.339, t=3.813, p<0.001, 95 per cent CI: 0.000-0.001), FT3 (beta=-0.284, t=-3.173, p=0.002, 95 per cent CI: -0.201—0.046) and FBS (beta=0.252, t=2.798, p=0.006, 95 per cent CI=0.007-0.040). For prediction of ICU admission as the dependent variable with WBC, haemoglobin, lymphocytes, neutrophils, N/L ratio, D-dimer, potassium, ferritin, CRP, ESR, FT3 and cortisol as predictors in the model revealed serum ferritin as the most significant variable (beta=0.389, t=3.933, p<0.001, 95 per cent CI:0.00-0.00).

Discussion

In the hope to understand more about the laboratory and anthropometric biomarkers among hospitalized COVID-19 patients, we have undertaken this research to help clinicians in the prognosis for severity and mortality. Understandably, COVID-19 patients who present with elevated levels of biomarkers can give us a better idea on how aggressive management we can institute.

One of the major findings of this study is that COVID-19 positive patients in contrast to those who tested negative by RT-PCR but had shown COVID-19 like symptoms had significantly elevated levels of several biomarkers including. Haemoglobin and lower levels of lymphocyte count among COVID-19 (+) patients. This is in contrast to leucocytosis, thrombocytopenia and lymphocytopenia which have been reportedly associated with poor prognosis.[7] Lymphocytopenia occurs with COVID-19, however its association with poor prognosis still remains to be further

elucidated. The elevation of other several other biomarkers is inconclusive to state that these markers have significant roles in the severity and outcome of COVID-19 at this point by just comparing between these two groups.

Several biomarkers were found to be associated with ICU admission and/or ventilation and mortality. Biomarkers associated with ICU admission and mortality has been reported including leucocytosis, and increased N/L ratio.[8] Furthermore, we found lower haemoglobin levels associated with mortality which contradicts findings from previous studies.[8,9] However, haemoglobin levels remain low among patients who required mechanical ventilation and ICU admission.[10] It has been reported that the prompt increase in the N/R ratio driven by an increase in the endogenous cortisol, catecholamines and a rise in CRP, is a better reflection of acute physiologic stress that occurs in COVID-19 infection compared to other markers such as WBC count.[11,12] This is reflected in the significant rise in both N/L ratio in both our ICU admitted patients and also among our patients who died. High D-dimer level was reported to predict both severity and mortality and may improve the management of patients with COVID-19 infection.[13] One study suggested that an optimum cut-off value of D-dimer to predict mortality among COVID-19 patients was 2.0ug/mL with a sensitivity of 92.3 per cent and specificity of 83.3 per cent.[13] Hypokalaemia, hyponatremia and hypocalcaemia were reported among patients with severe COVID-19 infection.[14,15] However, we found that COVID-19 (+) patients had lower potassium and calcium levels. than COVID-19 (-) patients. Increased procalcitonin was also suggested to predict severe COVID-19 infection similar to our study.[16,17] However, there were limited literatures that profoundly established the role of procalcitonin in the evolution of a more severe COVID-19 disease but may be helpful in the management of COVID-19 patients who require mechanical ventilation.[17,18]

This study reconfirms the role of serum cortisol, FT3, ferritin and highlights the role of FBS as the most significant predictors for severity and mortality among COVID-19 positive patients. Serum cortisol plays an important role to reduce the aggressive inflammatory response (cytokine storm)and organ damage specifically the lungs brought about by COVID-19 infection.[19-21] One study showed that doubling of the cortisol concentration was associated with a 42 per cent increase in the mortality of COVID-19 patients.[22] Dysregulated thyroid function particularly elevations of FT3 levels of 2.4–4.0pmol/L had a higher mortality rate of 40 per cent, higher rates of mechanical ventilation (45 per cent) and higher rates of ICU admission (55 per cent). [23,24], This study also confirms the role of high blood sugar in the fatal outcome among COVID-19 positive patients. [25,26] Hyperferritinemia were observed among patients who were admitted to the ICU and for patients who died. This study confirms that COVID-19 positive patients may have extremely high levels of serum ferritin (ferritin >3000ng/ml) and is indeed associated with poor prognosis and even death.[27,28]

The author acknowledges several limitations including the non-inclusion of medication and management of the patients that may have an effect on the progression, severity and outcome of the disease. Furthermore, the laboratory values reported in this study were taken at the time of admission. It would be more beneficial if a series of laboratory results were taken at the time of admission and even up to the time of discharge or prior to death. In this regard, we continue to collect the data and follow-up these patients to further understand the dynamics and relationship between these biomarkers and the outcome of COVID-19 infection including those who survived and discharged, as well as those patients who were readmitted.

Conclusion

High serum cortisol, high blood sugar and low FT3 were associated with higher mortality. The propensity for ICU admission among COVID-19 (+) patients with low haemoglobin, low lymphocytes, a high N/L ratio, high ferritin and D-dimer, is higher. Identifying high risk patients based on the initial biomarkers on the first day of admission may anticipate the outcome and severity for the disease and give us a more focused direction in the management plan including aggressive intervention and early ICU admission.

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PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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None

ETHICS COMMITTEE APPROVAL

Approval for the conduct of the study was issued by the Institutional Review Board of the College of Medicine, King Saud University, Riyadh, Saudi Arabia.

Table 1: Anthropometric and laboratory results for all 347 patients, 245 COVID (+) and 104 COVID (-) patients

Variables	All patients N=347	COVID (-) N=102	COVID (+) N=245	P values
Age, years	55.6±16.3	59.9±17.9	53.8±15.2	0.001
BMI, kg/m^2	29.4±7.4	28.6±9.0	29.7±6.6	0.195
WBC	8.2±4.0	9.2±4.2	7.8±3.9	0.004
Hemoglobin	12.7±2.5	11.6±2.7	13.2±2.2	<0.001
Lymphocytes	1.5±1.5	1.9±2.1	1.3±1.1	0.001
Neutrophils	6.4±4.7	7.2±5.0	6.1±4.5	0.060
N/L ratio	8.4±11.9	9.7±15.5	7.9±10.0	0.196
Platelets	262.1±113.1	271.5±112.9	258.2±113.1	0.319
D-dimer	2.1±2.9	2.91±3.2	1.9±2.8	0.006
Sodium	136.9±6.2	136.4±7.9	137.2±5.3	0.242
Potassium	4.4±0.8	4.55±0.9	4.3±0.7	0.002
Corrected calcium	2.3±0.2	2.4±0.2	2.3±0.2	0.240
Vitamin D	42.6±28.3	40.6±27.1	43.6±28.9	0.481
Ferritin	1020.9±1328.4	672.4±1474.8	1142.4±1254.1	0.010
CRP	101.6±81.3	70.7±80.6	111.7±79.1	<0.001
Procalcitonin	2.1±7.6	2.5±7.7	2.0±7.5	0.660
ESR	71.2±34.4	68.8±33.9	72.2±34.7	0.458
TSH	1.9±2.1	2.1±1.9	1.8±2.1	0.380
FT4	15.9±4.0	15.0±3.1	16.3±4.3	0.013
FT3	3.2±0.9	3.2±0.8	3.2±0.9	0.929
Cortisol	505.8±422.6	601.6±384.4	464.3±432.4	0.007
FBS	8.8±4.3	8.6±4.1	8.9±4.4	0.448
HbA1c	7.5±2.2	6.9±1.7	7.8±2.4	0.001
PTH	8.9±10.8	13.0±14.0	7.0±8.3	<0.001
IL-6	219.4±552.1	258.2±509.6	213.6±559.8	0.728
Number of comorbidities	1.6±1.6	2.3±1.6	1.2±1.4	<0.001
Length of hospital stay	11.3±9.9	14.9±14.7	10.0±7.4	0.002

Table 2: Anthropometric and laboratory analysis of 245 COVID (+) patients according to ICU admission and mortality

Variables	Alive N=199	Dead N=46	p value	Not admitted to ICU N=141	Admitted to ICU N=104	p value
Gender Male Female	145/177 (81.9% 54/68 (79.4%)	32/177 (18.1%) 14/68 (20.6%)	0.652	101/177 (57.1%) 40/68 (58.8%)	76/177 (42.9%) 28/68 (41.2%)	0.803
Age, years	52.7±15.2	58.9±14.5	0.012	52.8±15.9	55.2±14.1	0.217
BMI, kg/m^2	29.5±6.2	30.6±8.2	0.312	29.6±6.9	29.9±6.2	0.698
WBC	7.9±3.9	7.2±3.7	0.240	7.4±3.5	8.4±4.2	0.030
Hemoglobin	13.4±2.1	12.0±2.4	<0.001	13.4±2.1	12.8±2.4	0.027
Lymphocytes	1.3±1.1	1.1±1.4	0.182	1.4±1.1	1.1±1.3	0.047

Neutrophils	5.9±3.3	7.0±7.7	0.129	5.2±3.2	7.3±5.6	<0.001
N/L ratio	6.8±7.4	12.4±16.7	0.001	5.0±5.0	11.7±13.4	<0.001
Platelets	263.5±113.1	235.5±111.7	0.131	263.6±117.7	250.9±106.8	0.389
D-dimer	1.5±2.4	3.2±3.8	<0.001	1.4±2.3	2.5±3.3	0.006
Sodium	137.2±5.2	137.2±5.9	0.980	137.8±5.0	136.5±5.7	0.062
Potassium	4.2±0.6	4.6±0.9	0.002	4.2±0.5	4.4±0.8	0.009
Corrected calcium	2.3±0.2	2.3±0.2	0.581	2.4±0.2	2.3±0.2	0.257
Vitamin D	41.4±25.6	54.6±40.9	0.056	41.6±21.9	46.4±36.6	0.359
Ferritin	979.8±929.9	1831.6±2014.9	0.007	734.9±739.8	1679.2±1558.9	<0.001
CRP	102.8±72.5	148.7±94.3	0.001	90.3±65.1	137.9±86.9	<0.001
Procalcitonin	1.3±6.6	4.2±9.8	0.080	0.9±3.2	2.9±9.8	0.059
ESR	70.5±35.3	81.4±30.4	0.180	65.6±34.7	79.2±33.6	0.024
TSH	1.8±1.9	2.0±2.6	0.654	1.9±1.8	1.8±2.5	0.863
FT4	16.7±4.3	14.5±3.8	0.004	16.1±3.6	16.5±4.9	0.509
FT3	3.4±0.9	2.5±0.9	<0.001	3.5±0.8	2.9±1.0	<0.001
Cortisol	398.1±333.1	788.2±665.5	0.002	404.3±294.9	538.8±550.6	0.037
FBS	8.5±3.9	11.1±6.1	0.002	8.6±4.3	9.5±4.5	0.157
HbA1c	7.7±2.3	8.5±2.7	0.046	7.6±2.1	8.1±2.6	0.102
PTH	5.8±6.2	12.9±13.6	0.006	6.0±6.7	8.3±9.9	0.070
IL-6	223.1±627.9	187.4±306.7	0.669	238.1±769.0	195.2±329.2	0.699
Number of comorbidities	1.1±1.3	1.8±1.8	0.011	1.2±1.4	1.3±1.5	0.383
Length of hospital stay	9.3±6.5	13.6±10.2	0.006	7.8±5.1	15.7±8.9	<0.001

Table 3: Correlates of mortality and ICU admission among 245 COVID (+) patients

	Mortality		ICU admission	
	r	p	r	p
Gender	-0.029	0.654	0.016	0.804
Age, years	0.159	0.012	0.079	0.217
BMI, kg/m^2	0.065	0.312	0.025	0.698
WBC	-0.075	0.240	0.139	0.030
Hemoglobin	-0.242	<0.001	-0.140	0.027
Lymphocytes	-0.086	0.182	-0.128	0.047
Neutrophils	0.098	0.129	0.225	<0.001
N/L ratio	0.218	0.001	0.330	<0.001
Platelets	-0.097	0.131	-0.055	0.389
D-dimer	0.236	<0.001	0.185	0.004
Sodium	0.002	0.980	-0.120	0.062
Potassium	0.198	0.002	0.166	0.009
Corrected calcium	-0.036	0.581	-0.074	0.257
Vitamin D	0.170	0.056	0.082	0.359
Ferritin	0.267	<0.001	0.374	<0.001
CRP	0.229	0.001	0.300	<0.001

Procalcitonin	0.163	0.030	0.136	0.073
ESR	0.115	0.188	0.197	0.024
TSH	0.037	0.594	-0.012	0.859
FT4	-0.196	0.004	0.045	0.509
FT3	-0.350	<0.001	-0.304	<0.001
Cortisol	0.340	<0.001	0.155	0.026
FBS	0.217	0.002	0.098	0.155
HbA1c	0.139	0.046	0.117	0.092
PTH	0.323	<0.001	0.137	0.060
IL-6	-0.028	0.751	-0.038	0.669
Number of comorbidities	0.199	0.002	0.056	0.380