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ORIGINAL ARTICLE

Laboratory Biomarkers Associated with Severity and Mortality of COVID-19

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SUMMARY

Background: **Corona Virus Disease 2019 (COVID-19) emerged late 2019 and has become a global pandemic. There is an urgent need for identification of biomarkers to predict severity of the disease for early treatment and to avoid mortality especially in high-risk population. Therefore, the aim of this study is to investigate laboratory results in COVID-19 patients in Saudi Arabia to identify potential biomarkers correlated with disease severity.**
Methods: **Clinical records of 200 patients diagnosed with COVID-19 from July to August 2020 at Jeddah East Hospital were retrospectively analyzed. Laboratory tests including coagulation parameters, D-dimer, kidney, cardiac, and liver enzymes were statistically investigated in patients admitted to wards and intensive care units (ICU).**
Results: **The majority of patients admitted to ward (156/200) were young (mean 47 years old) compared to those admitted to ICU (mean 60 years old), 14/44 passed away in the ICU. Magnesium was significantly ($p < 0.05$) elevated in the ICU group while blood urea nitrogen and creatinine level was significantly higher in deceased patients ($p < 0.05$). Lactate dehydrogenase results were high among all groups, compared to normal range, although its level significantly increased ($p > 0.05$) in ICU and death groups.**
Conclusions: **Elevated lactate dehydrogenase, blood urea nitrogen and creatinine levels may increase the risk of ICU admission and death from COVID-19, which can be used as potential biomarkers for disease severity. Using these markers could help physicians choose the optimal therapeutical option and provide patients with better treatment.**
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KEY WORDS

COVID-19, biomarkers, intensive care unit, disease severity, SARS-CoV-2

INTRODUCTION

The novel coronavirus is a new virus that has not been previously identified. The virus was later called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The coronavirus disease 2019 (COVID-19) was first identified in Wuhan, China, and has spread throughout the country and received worldwide attention [1]. On 30 January 2020, the World Health Organization (WHO) officially declared the COVID-19 pandemic as a public health emergency of international concern [2,3]. Regarding the transmission, the disease is transmitted by inhalation or contact with infected droplets and the incubation period ranges from 2 to 14 days [2]. To date (April 5, 2021), COVID-19 has infected over 130 million people worldwide and has been responsible for about 3 million deaths (https://covid19.who.int). In Saudi Arabia, there are approximately 400,000 and over 6,000 confirmed cases and deaths, respectively (https://covid19.moh.gov.sa).

Symptoms are usually fever, cough, sore throat, breathlessness, and fatigue. In most infected people, the presentation of the disease will be mild or asymptomatic [2]. However, vulnerable population such as elderly and those with comorbidities are at higher risk for the severe illness of COVID-19 including pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ dysfunction (MOD) [4]. Moreover, those with comorbidities such as chronic lung disease, severe asthma, cardiovascular disease, diabetes, and immunocompromised patients are more likely to suffer from COVID-19 complications which may lead to hospital mortality [5].

Patients with severe COVID-19 tend to develop coagulation abnormalities that mimic other systemic coagulopathies associated with severe infections, such as disseminated intravascular coagulation (DIC) or thrombotic microangiopathy, but COVID-19 has distinct features [6]. The coagulopathies in COVID-19 patients were correlated with severe disease and increased risk of death [7]. The study observed an increased D-dimer, a relatively modest decrease in platelet count, and a prolongation of the prothrombin time among COVID-19 severe cases [7]. These conditions are markers of DIC, which is distinctively different from DIC seen in sepsis [7]. Furthermore, many studies have jumped to the same conclusion that a high level of D- dimer is correlated with mortality rate [8,9]. Venous thromboembolism tends to be more common than arterial thromboembolism in hospitalized COVID-19 patients [8]. Both pulmonary thrombosis and microvascular thrombosis are observed in autopsy studies, and this might be the reason for developing severe hypoxia in COVID- 19 patients [8]. Overall, intensive care unit (ICU) patients tend to have high levels of venous and arterial thrombotic events [10]. Moreover, severe cases of COVID-19 might affect other body systems leading to organ dysfunctions such as kidney and liver [11]. Studies showed increased prevalence of kidney impairment (hematuria, proteinuria, and kidney dysfunction) among hospitalized COVID-19 patients, and it is positively correlated with death [12]. Liver dysfunction is also common in COVID-19 severe cases [13]. Patients will have high aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and this might be associated with high risk of mortality [13]. Although there have been reports on increased levels of D-dimer, abnormal kidney and liver function tests in COVID-19 patients, there is insufficient data correlating these laboratory results to the severity of COVID-19 in Saudi Arabia. Therefore, the aim of the current study is to investigate coagulation factors, liver and kidney enzymes among COVID-19 patients in Saudi Arabia to identify potential biomarkers correlated with disease severity.

MATERIALS AND METHODS

Study design and participants

This is a retrospective review of medical records and laboratory results of COVID-19 patients admitted to East Jeddah General Hospital in Jeddah, in western Saudi Arabia from 1 July to 30 August, 2020. All patients were adults and diagnosed with COVID-19 based on positive real-time reverse transcriptase polymerase chain reaction (RT-PCR) of nasopharyngeal samples according to the World Health Organization protocol [14].

Data collection

Demographic data, body mass index, admission information and laboratory results including coagulation parameter platelet count (PLT), activated partial thromboplastin time (APTT), prothrombin time (PT), D-dimer, international normalized ratio (INR), troponin, creatine kinase (CK), renal function tests; calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), blood urea nitrogen (Bun), and creatinine (Creat), liver function tests; total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH). All data were collected from electronic medical records using a customized data sheet form, although bilirubin results (total and direct) were not found for those who passed away. Patients were categorized into three groups based on admission and outcome; those admitted and discharged from the isolation ward, those admitted and discharged from the intensive care unit (ICU), and those who died in ICU because of COVID-19 complications.

Statistical analysis

Data was expressed as the mean (± STD) with 95% confidence interval (CI). The statistical analysis was performed using GraphPad Prism program (Version 6.01); it was used to determine whether there were statistically significant differences in biomarker parameter patterns between the three groups. The analysis test used in this study was one way analysis of variance (ANOVA) with Bartlett's test ($p < 0.05$).

Table 1. Number, age and BMI of patients included in the study.

	Ward	ICU	Death
Number of participants	156	30	14
Age average (year ± SD)	47.33 (13.2)	60 (12.7)	60.13 (10.7)
BMI (mean ± SD)	27.4 (3.3)	26.8 (4.6)	26.9 (4.2)

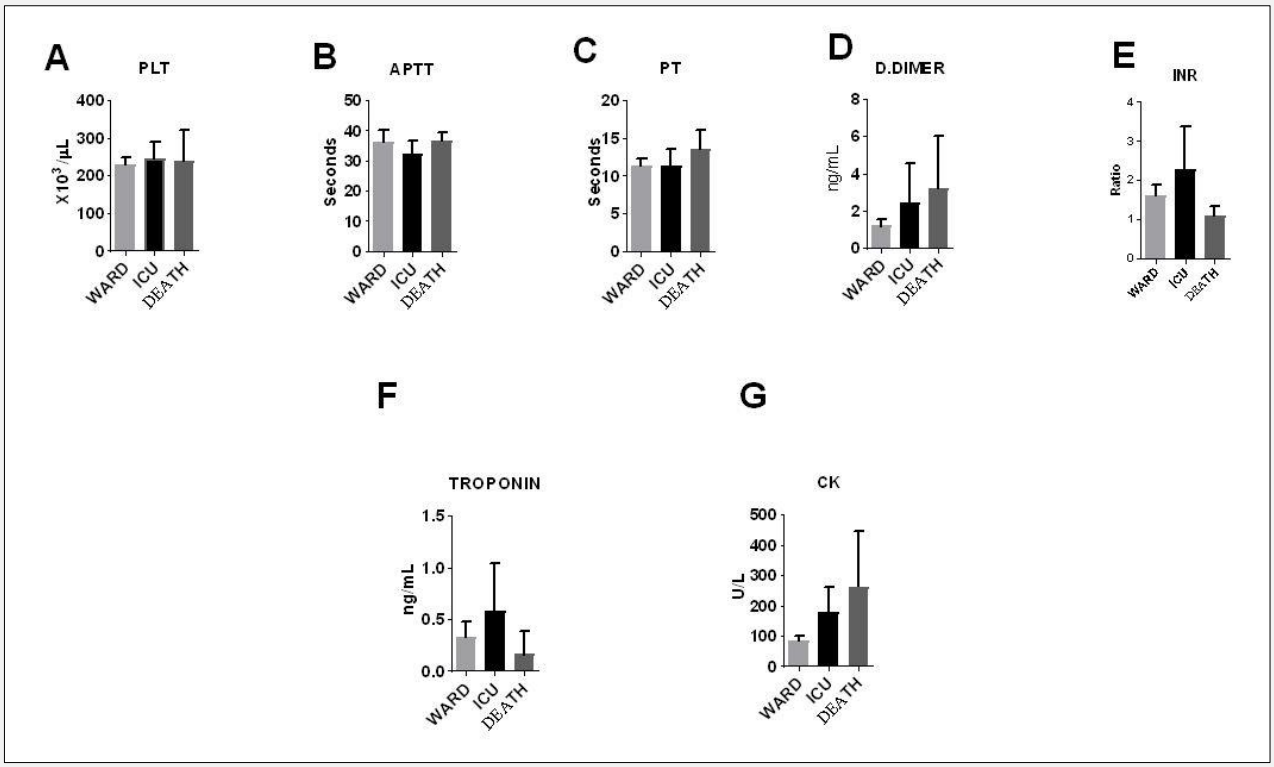

Figure 1. Coagulation profile among COVID-19 patients admitted to Ward, ICU, and Death groups.

In the bar graphs, coagulation parameters include: (A) PLT, (B) APTT, (C) PT, (D) D-dimer, and (E) INR among COVID-19 patients (n = 200) admitted to Ward (n = 156, light grey), ICU (n = 30, black), and Death (n = 14, dark grey) and then compared. Cardiac enzymes (F) troponin and (G) CK play a role in a defective coagulation process. Data are represented as mean with 95% confidence interval (CI).

Ethical approval

This study was approved by the Jeddah Health Affaires ethics board at the Ministry of Health in Saudi Arabia (No. H-02-J-002-01285). As this is a retrospective study, the patient consent form was waived by the ethics committee.

RESULTS

A total of two hundred patients of confirmed COVID-19 were included in this study. These were categorized according to the severity of the health condition which was manifested by the admission to either ward or intensive care unit (ICU) (Table 1). Accordingly, they were grouped into (Ward n = 156, ICU n = 30, and Deceased n = 14), with an age average of the 47, 60, and 60 years, respectively (Table 1). Those who died had all

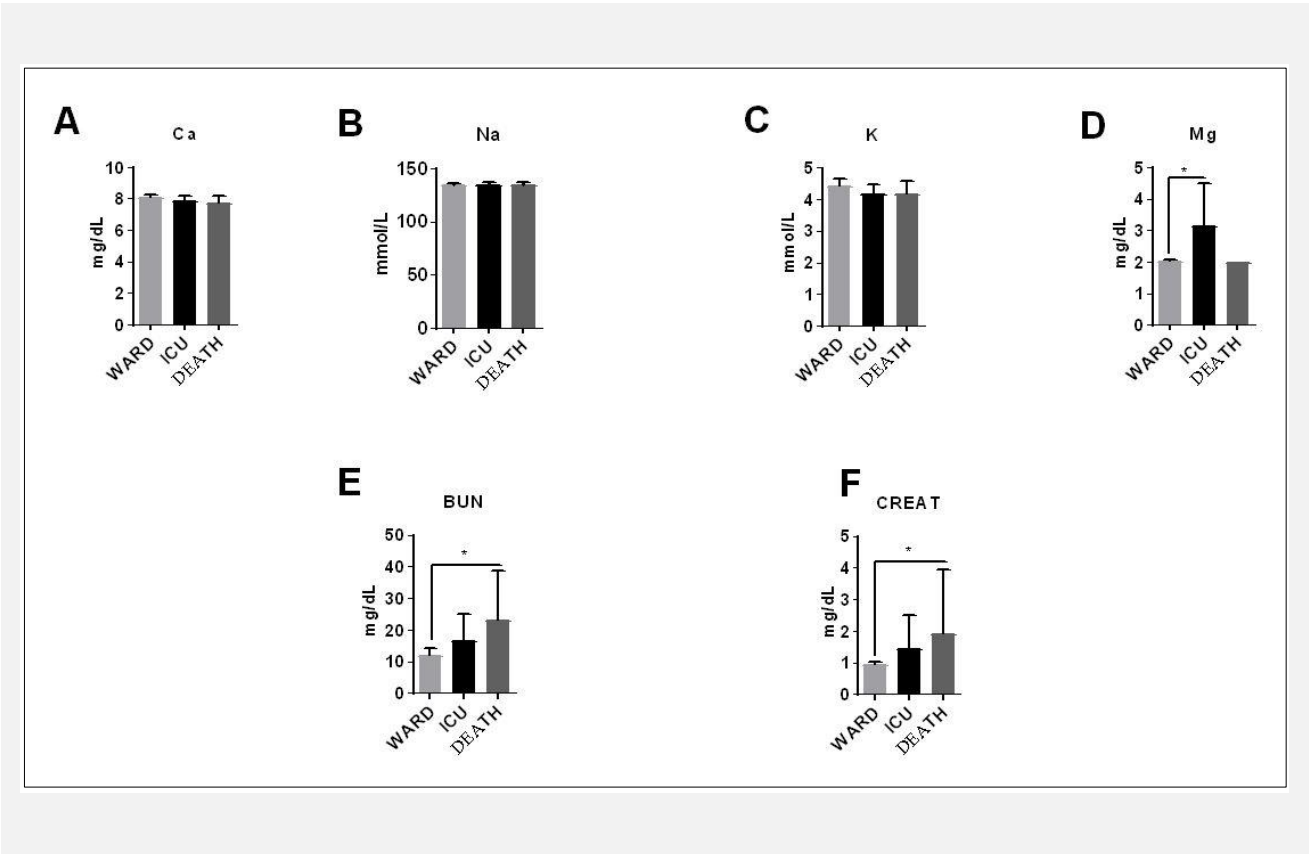

Figure 2. Renal function tests among COVID-19 patients admitted to Ward, ICU, and Death.

In the bar graphs, renal function tests include, A: calcium (Ca), B: sodium (Na), C: potassium (K), D: magnesium (Mg), E: blood urea nitrogen (Bun) and F: creatinine (Creat) among COVID-19 patients *(*n = 200) admitted to Ward (n = 156, light grey), ICU (n = 30, black), and death (n: 14, dark grey) are compared. Data are represented as mean with 95% confidence interval (CI). Statistical analysis was performed using a one-way ANOVA analysis of variance with Bartlett's test. * $p < 0.05$.

been admitted to ICU. The average BMI showed that majority of COVID-19 patients were obese (BMI over 25). The blood coagulation profile with parameters: PLT, APTT, PT, D-dimer, and INR were investigated (Figure 1). A small variation in PLT, APTT, and PT among the three groups with a slight increase illustrated in those in the ICU including the deceased. With regards to the D-dimer and INR, the trend was a noticeable increase in the ICU admitted patients. The cardiac enzymes, namely troponin and CK were elevated in the ICU group with an average of 0.6 ng/mL and 263.29 U/L, respectively.

As displayed in Figure 2, renal function tests including calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), blood urea nitrogen (Bun), and creatinine (Creat) were investigated. Ca, Na, and K remained within the normal range among the studied groups whereas mean Mg level in the ICU patients was significantly higher (3.2 mg/dL, $p < 0.05$) compared to average Mg level (2 mg/dL) in Ward admitted patients, which was within the normal range (1.7 - 2.3 mg/dL). Likewise, BUN average level was within the normal range in both Ward and ICU admitted individuals (12 and 17 mg/dL, respectively). However, this was significantly increased in Death samples (23.4 mg/dL, $p < 0.05$) compared to normal range (8 - 20 mg/dL). This trend was also applied to creatinine level among the groups, which is seen in the significant rise of the average (1.9 mg/dL, $p < 0.05$) in deceased patients. In addition, the average level was higher (1.5 mg/dL) in ICU patients compared to that of Ward admitted patients (1 mg/dL), which is within the normal range (0.6 - 1.2 mg/dL).

The analysis of the liver function tests including total bilirubin, direct bilirubin, aspartate aminotransferase, alanine transaminase, and lactate dehydrogenase were also investigated (Figure 3). Regarding the analysis of the total bilirubin and direct bilirubin levels, a number of noteworthy points were encountered. The total bilirubin was found within the normal range (0.1 - 1.2 mg/dL) [15] in both tested groups (Ward and ICU) although the level was seen to be higher in those admitted in the ICU. On the other hand, the level of direct bilirubin exceeded the normal range (0.02 - 0.3 mg/dL) in Ward and ICU Individuals. The level of direct bilirubin

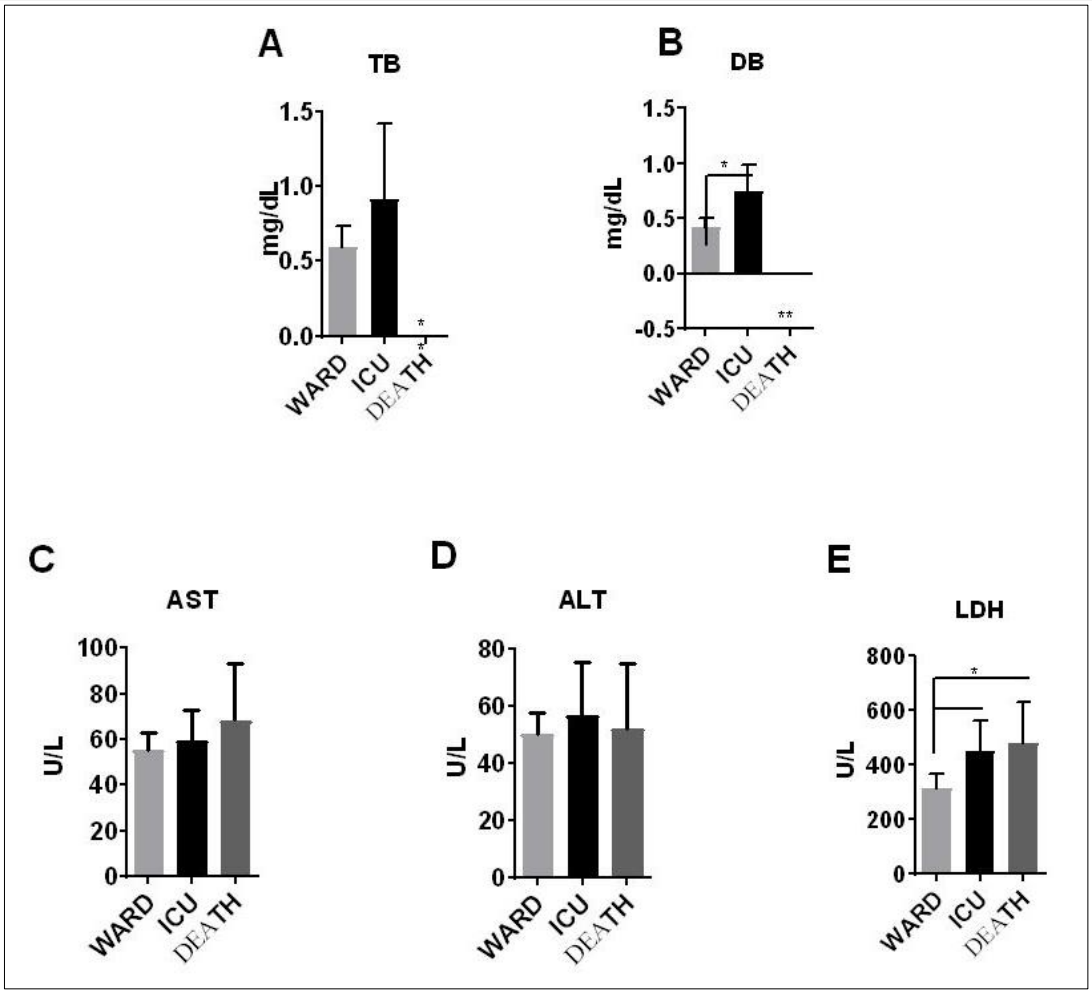

Figure 3. Liver function tests among COVID-19 patients admitted to Ward, ICU, and Death groups.

In the bar graphs, liver function tests include, A: total bilirubin (TB), B: direct bilirubin (DB), C: aspartate aminotransferase (AST), D: alanine transaminase (ALT), and E: lactate dehydrogenase (LDH) among COVID-19 patients *(*n: 200) admitted to Ward (n: 156, light grey), ICU (n: 30, black), and Death (n: 14, dark grey) groups are compared. Data are represented as mean with 95% confidence interval (CI). Statistical analysis was performed using a one-way ANOVA analysis of variance with Bartlett's test. * $p < 0.05$, ** Data not provided.

in the ICU group nevertheless was notably higher than the Ward admitted patients. The level of AST displayed severity dependent fashion. Accordingly, AST value in the Ward, ICU, and Death patients was elevated (55.6, 59.3, and 68.63 U/L, respectively) compared to the normal range (10 - 40 U/L). The value of ALT was found to be higher in all patients (50, 56.6, and 52 U/L, respectively) compared to the normal range (5 - 41 U/L). Likewise, severity/elevation relationship was observed in the LDH value among all groups Ward, ICU, and Death (313.3, 453.6, and 481.8 U/L, respectively) compared to the normal range (122 - 222 U/L). Furthermore, there was a significant difference in the LDH values between Ward and both ICU and Death groups ($p < 0.05$).

DISCUSSION

Since the emergence of SARS-CoV2 in China in late 2019, huge efforts have been made by researchers to identify factors associated with the disease severity in order to develop a suitable treatment protocol and reduce mortality rate [2]. However, limited literature highlighted these predictors among COVID-19 patients in Saudi Arabia. Thus, the current study aimed to inves-

tigate biomarkers, including coagulation profile, renal and liver function tests, among survival and dead Covid-19 patients in order to identify predictors associated with disease severity. To our knowledge, this set of biomarkers was not examined previously, especially in Saudi Arabia.

Patients included in this study were grouped into two groups (Ward, n = 156 and ICU, n = 44) with the intention to reflect the mentioned biomarkers linked with the severity of the disease progression. Of the patients admitted,14/44 admitted to ICU passed away (Table 1) indicating a high mortality rate among ICU patients. A number of factors contributed to the severity of the symptoms and ICU admission. Age is one example as the mean age of those admitted to the ward was about 47 years whereas it was about 60 years in severe cases (ICU patients). Obesity is a well-known risk factor for many diseases, which was recently linked to sever cases of COVID-19 [16]. Several cases in the current study died at young age (below 40 years old). This unfortunate consequence was possibly due to the high BMI which exceed 38, reflecting severe obesity. In addition, it was observed in this study that all patients were overweight according to the high BMI that may reflect the lifestyle in Saudi population [17].

The investigation of the coagulation profile parameters such as PLT, APTT, and PT remained normal which is in line with previous observation [6]. COVID-19 patients, specifically with more severe status, are suspected to have fatal hypercoagulability status that was manifested by the increased level of D-dimer, which is considered as an important biomarker of thrombosis [9] in ICU patients (Figure 1). COVID-19 patients may also be admitted to ICU due to a possible heart injury that could be observed in the elevated values of cardiac enzymes investigated in this study (troponin and CK) [18-21]. Moreover, a few other factors may lead to an increased level of cardiac enzymes, including pulmonary embolism and kidney diseases [18,20].

The increased Mg level, which was analyzed in the renal function tests, indicates an association with both cardiac and renal impairment [22,23]. Studies have shown a link between COVID-19 and organ failure [24]. This is illustrated in the possible kidney deterioration manifested by the increased level of blood urea nitrogen and creatinine (Figure 2). Moreover, the hepatic abnormalities may be pronounced in COVID-19 patients investigated in this study as supported by the elevated hepatic parameters including direct bilirubin, AST, ALT, and LDH [25,26].

Although the current study included many biomarkers, it is beneficial to explore other factors to give an extended biomarker profile in COVID-19 patients. One common factor is ABO blood group status that was investigated in a number of other studies, which illustrated that those with blood group A were associated with an increased risk of COVID-19 infection, whereas group O individuals are less susceptible to the disease [27]. Such extended sets of biomarkers are suggested to subsidize and guide in designing therapeutic protocols.

CONCLUSION

Together this data strongly suggests that several tests including magnesium, lactate dehydrogenase, blood urea nitrogen and creatinine can be used as predictors of disease severity and ICU admission. However, the severity of COVID-19 is not solely due the infection of SARS-CoV-2 virus, but rather a series of health complications. Identifying potential laboratory biomarkers could help in early therapeutic intervention and better treatments.

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Declaration of Interest:
The authors declare that there is no conflict of interest.

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