Infection and Drug Resistance

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ORIGINAL RESEARCH

SARS-CoV-2 Infection-Dependent Modulation in Vital Components of the Serum Profile of Severely SARS-CoV-2 Infected Patients

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Background: COVID-19 modulates many serological biomarkers during the progress of disease severity. The study aimed to determine COVID-19 severity-associated perturbance in the serum profile.

Methods: A retrospective study including COVID-19-positive individuals (n = 405) was accomplished. The serum profile of COVID-19 participants was mined from laboratory records. Severity-associated alteration in the serum profile was evaluated using Pearson correlation, regression, VCramer, Bayesian posterior VCramer, and bias factor using R-base-RStudio-version-3.3.0 with a significant cut-off of $p < 0.05$.

Results: Significantly different mean ± standard deviation (SD) (highly versus moderately severe) of C-reactive protein (CRP), ferritin, neutrophil-lymphocyte ratio (NLR), D-dimer, platelets, prothrombin time (PT), partial prothrombin time (PTT), troponin 1, lactate dehydrogenase (LDH), aspartate-aminotransferase (AST), alanine aminotransferase (ALT), and AST/ALT ratio was observed ($p < 0.001$). Highly severe COVID-19 associated with CRP, ferritin, NLR, in D-dimer, PT, PTT, troponin 1, AST/ALT ratio, AST and ALT (adjusted odds ratio (AOR): 1.346, 1.05, 1.46, 1.33, 1.42, 1.23, 4.07, 3.9, 1.24, 1.45, $p < 0.001$). CRP with ferritin ($r = 0.743$), NLR ($r = 0.77$), white blood cells (WBC) ($r = 0.8$), troponin1 with LDH ($r = 0.757$), and D-dimer with platelets ($r = -0.81$) were highly correlated. X^2pearson ($p < 0.001$), V_{Cramer} (0.71), Bayesian-V_{Cramer} (0.7), and bias-factor (−125) for troponin 1 indicate the strong association of troponin 1 level and with COVID-19 severity. X^2pearson ($p < 0.001$), V_{Cramer} (1), Bayesian-V_{Cramer} (0.98), and bias-factor (−266.3) for NLR exhibited a very strong association of pathologic conditions with the high severity of the disease.

Conclusion: These biomarkers of inflammation (CRP, Ferritin, NLR), coagulation disorders (D-dimer, PT, and PTT) cardiac abnormality (troponin 1), and liver injury (AST/ALT) could be crucial in low-medical resource settings as potential prognosticator/predictors of the COVID-19 severity and clinical outcomes. Moreover, the outcome of this study could be leveraged for the early prediction of disease severity during SARS-CoV or Middle East Respiratory Coronavirus (MERS-CoV) infection.

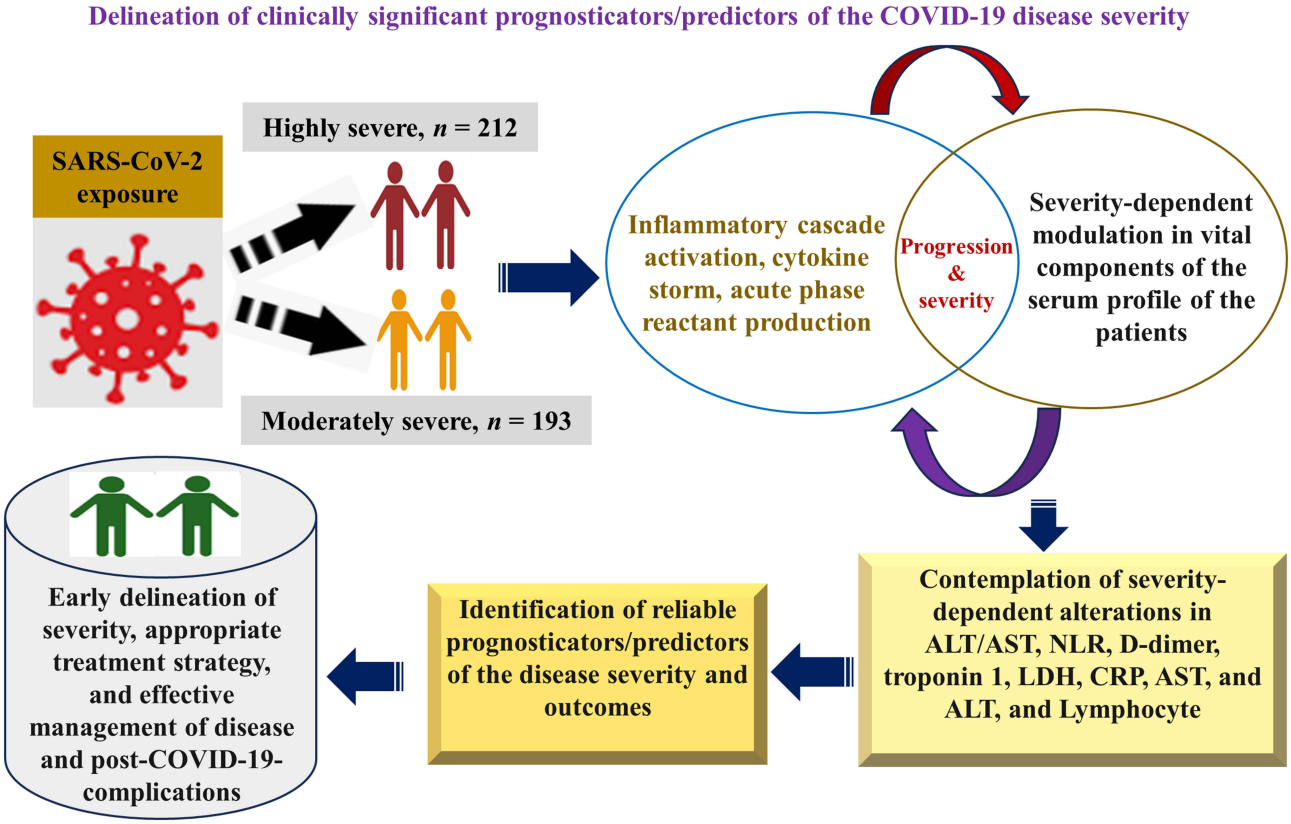
Keywords: AST/ALT, NLR, PLR, regression, association, severity, COVID-19, biomarkers, D-dimer, CRP, ferritin, troponin 1, LDH, PT, PTT

Introduction

The resurgence of COVID-19 caused by variants and/or sub-variants of SARS-CoV-2, a pathogenic virus,[1] remains a noteworthy health concern across the globe despite the comprehensive vaccine drive.[2,3] COVID-19 dynamics and continuing burden are largely dependent on waning immune status and emerging SARS-CoV-2 variants/sub-variants.[3–5] Reports indicate that COVID-19 could eventuate in the endemic state instead of eliminated.[3,6] Furthermore, the trend of decrease in daily reported cases and the origin of a high number of the viral variants/sub-variants[7,8] even after four years of the pandemic suggest that COVID-19 could take longer to be endemic and SARS-CoV-2 regional circulation will continue.[3,6] Variation in COVID-19 outbreak in different regions has been reported[9] probably due to the dependence of the incidence of the disease on climatic conditions.[10] Additionally, COVID-19 causes substantial pulmonary pathology but also extrapulmonary clinical manifestations: arrhythmia, hepatocellular damage, coagulopathy, and coronary syndrome.[11] Moreover, in the last decades, apart from the SARS-CoV-2 outbreak in China in 2019, two other major outbreaks of the respiratory syndrome caused by SARS-CoV from China in

Received: 7 March 2024
Accepted: 22 April 2024
Published: 29 April 2024

Graphical Abstract



2002[12] and MERS-CoV from Saudi Arabia in 2012[13] were reported, however, reports suggest that the SARS-CoV-2 is less pathogenic and highly transmissible compared to SARS-CoV and MERS.[14] Because of the high transmissibility, it posed an unprecedented life threat to the global population.[15] Since coronaviruses exhibit similar clinical manifestations such as severe acute respiratory distress,[16] therefore, it could be anticipated that the perturbation in serum proteome brought about by SARS-CoV -2 infection could also help understand the pathophysiology of SARS-CoV and MERS-CoV infections. It is noteworthy that for the implementation of precise diagnostic, prognostic, and therapeutic strategies to curb and contain the regional outbreak, assessment of the association of disease severity with COVID-19-dependent alterations in components of serum profile (vital disease predictors): hematological profile,[17] inflammatory profile,[18] coagulopathy parameter,[18] cardiac profile, and liver function test (LFT) is paramount.[19] Along with the indispensable clinical evaluation, insight into the level of laboratory biomarkers as predictors of disease severity offers objective information that potentially impacts various components of the patient's care and control of the outbreaks of the SARS-CoV-2 and other major related human coronaviruses such as SARS-CoV, and MERS-CoV, especially in suburban regions with low medical resources. The objective evaluation of biomarkers serves as the indicator of pathological, physiological, and pharmacological responses to treatment strategies.[20] SARS-CoV-2 infection triggers an inflammatory cascade and produces acute-phase reactants.[21] A hyperactive immune system leads to dysregulated and uncontrolled release of cytokines (cytokine storm)[22] with raised inflammatory and other biomarkers such as coagulopathy markers that worsen the COVID-19 disease outcome.[23–25] Furthermore, information about C-reactive proteins (CRP) and ferritin (inflammatory biomarkers), coagulopathy marker (D-dimer), cardiac biomarkers (troponin and lactate dehydrogenase (LDH)), and hepatocellular markers: alanine aminotransferase (ALT) enzyme, aspartate aminotransferase (AST) enzyme and AST/ALT ratio unravel comprehensive insight into tissue injury, inflammation and immunological modulation caused during COVID-19 disease progression, therefore, evaluation of such circulating serological markers could be potentially beneficial to understand the disease

severity.[19,26] Raised levels of the above-mentioned potential biomarkers have been delineated to exhibit an association with the worst disease prognosis and highly severe status of the disease that leads to the necessity of lengthy hospitalization and intensive care unit (ICU).[27,28] A few studies delineated the COVID-19-severity-associated perturbance in these crucial markers, such as raised troponin,[29] raised procalcitonin (PCT),[30] lactate dehydrogenase (LDH),[31] enhanced serum ferritin,[32] higher erythrocyte sedimentation rate (ESR),[33] complete blood count (CBC)[34] increased C-reactive protein (CRP),[35] liver enzyme[36] and raised interleukin-6 (IL-6).[37] More interestingly, getting initial insight into these biomarkers is critical for understanding the pathophysiology of the COVID-19 and thus helps assess severity, framing hospitalization criteria, the need for ICU admission, deciding timely and precise therapeutic intervention, and predicting disease outcomes.[38] Potential clinical outcomes of COVID-19 disease are the consequence of the highly complex pathophysiological interactions of the virulence factors of the SARS-CoV-2 virus with the various host-immune factors. Since the host factors are highly variable among the individuals of the population.[39] Moreover, reports suggest that different strains/sub-strains of SARS-CoV-2 were involved in different geographical regions, which implies the significance of the study in population to gain insight into regional context. Though some studies on a few markers have been accomplished, however, to my knowledge, information on COVID-19-associated alterations in most of the components of serum profile and their association with severity in this population is lacking. Due to the diagnostic, prognostic, and therapeutic suitability of these serological markers, the present study aimed to determine COVID-19 severity-dependent modulation in the level of these serological biomarkers in this region.

Materials and Methods

Research Design, Population, and Study Area

A retrospective hospital-based research design was implemented to achieve the aim of this study. The analytical procedures were applied to the data retrieved after an inquisitive review and examination of hospital records at Al-Qunfudah Hospital, Saudi Arabia for various laboratory parameters: WBC, platelets, neutrophil, lymphocyte, PT, PTT, D-dimer, troponin, LDH, AST, CRP, ALT, and ferritin, and severity status (Moderately severe and highly severe), and demographic characteristics (age and gender) of the COVID-19 patients from the period of Jan 2020 to Dec 2020. Relevant data of the COVID-19-positive individuals who satisfied the laid down eligibility criteria for this study were acquired from the hospital record. The analytical approaches for execution of the study is illustrated in Figure 1.

Key Parameters of Serum Profile and Their Operational Definition

NLRs of 1–2, >3-5 or <0.7, >5, and 2–3 were considered normal, pathologic, marked pathologic, and gray zone, respectively.[40] The reference value of PLR (36.63–149.13 in males and 43.36–172.68 in females) was considered.[41] Troponin 1 less than 0.04 ng/mL, between 0.04 to 0.3 ng/mL and greater than 0.3nng/mL were defined as normal, low risk, and risk level, respectively.[42] Ferritin (24–336 µg/L in males) and (24–307 µg/L in females) were defined to be normal values while the participants with ferritin levels higher than the latter were hyperferritinemic. D-dimer >500 ng/mL was defined as a high D-dimer level. LDH (135–225 U/L in males) and LDH (>135–214 U/L in females) were reference ranges. LDH < 135 U/L was low LDH and above 225 in males, and 214 in females were characterized as high LDH. AST< 39 U/L and ALT < 52 U/L) were identified as set as reference range.[43] AST/ALT lesser than 1 and AST/ALT greater than 1 were defined as normal and high-risk groups, respectively. The severity status (exposure status) was deduced from the information in the hospital record: highly severe based on the need for a ventilator, ICU admission, CT score, and moderately severe patients based on their admission to the COVID-19 ward with normal oxygen saturation but symptomatic.

Ethical Approval Declaration for the Study

Ethical approval for data collection and study was obtained from the Ethics board in Al-Qunfudah Hospital, Qunfudah, Saudi Arabia, with approval number (Ref: 605–44-0057116) on 06/10/2022. Written informed consent was not required for the collection of the data because the secondary data generated through routine laboratory tests were collected for this retrospective study by explaining the purpose of the collection of data ethical committee through a written study proposal. The data confidentiality and protection policies were well implemented during collection and the execution of this study.

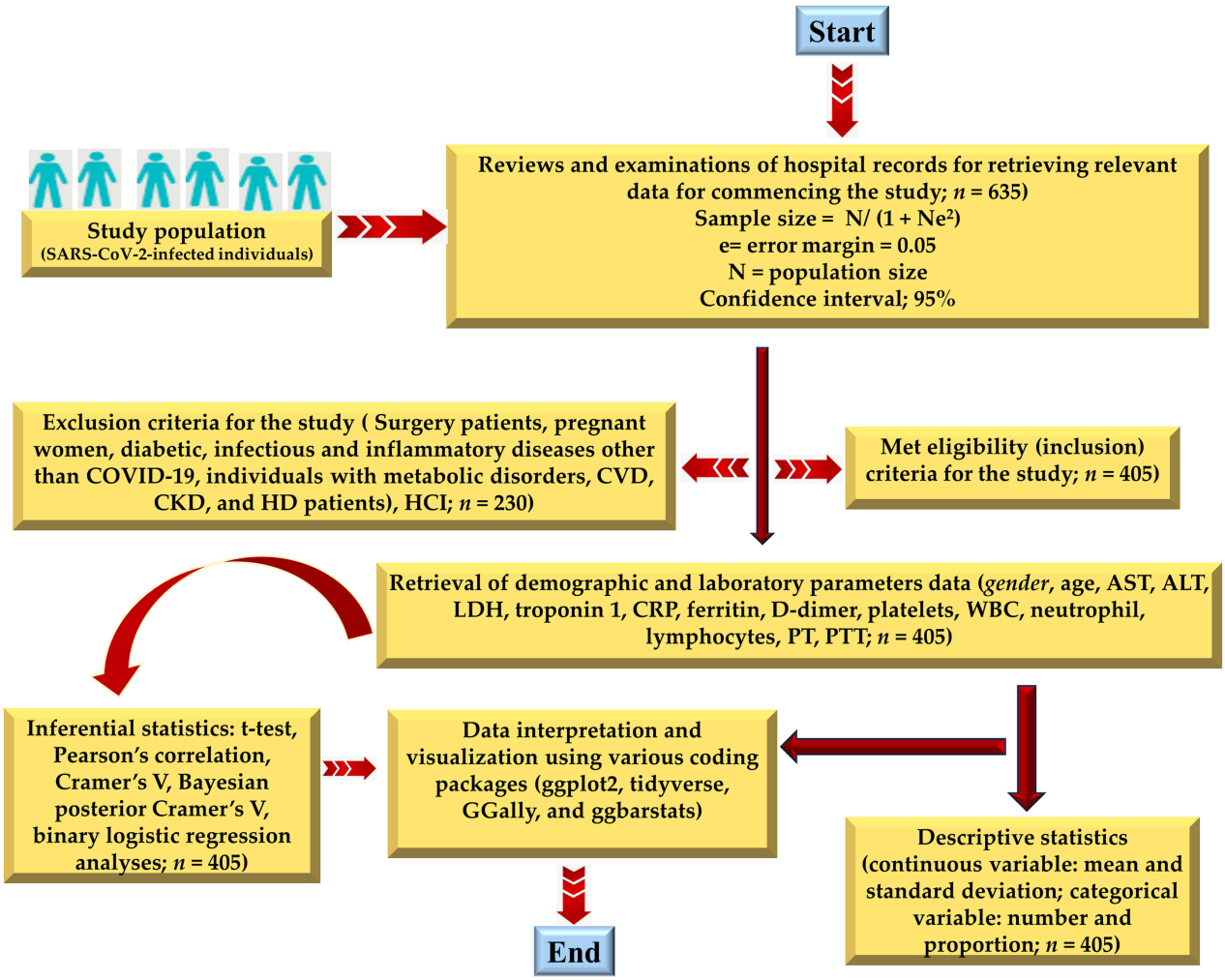


Figure 1 Illustrative explanation of the methodological approach for analysis, interpretation, and visualization of the findings.

Computation of Sample Size

An equation: sample-size (n) equals $N/(1 + Ne^2)$; N expresses population size, and "e" represents the error margin used to compute the sample size. A 95% confidence level (95% CI) and e = 0.05 were taken into consideration.[44] Approximately a sample size of n = 397 was evaluated to be suitable for the study. Nonetheless, a sample size of n = 405 participants was suitable for inclusion in this research based on eligible criteria.

Eligibility Criteria (Inclusion and Exclusion Criteria)

SARS-CoV-2-infected male and female individuals of all ages were encompassed in this research. Individuals infected with microbes other than SARS-CoV-2 were excluded from the study. Pregnant, and participants with hematological disorders, chronic kidney disease, metabolic diseases, inflammatory diseases, hepatocellular injuries (HCI), chronic kidney disease (CKD), hemodialysis (HD), cardiovascular disease (CVD), and diabetes were excluded from this study. Participants with surgery were also excluded to minimize the confounding effects.

Nature of the Data and Methods to Generate at the Primary Level

For evaluating the exposure and infectiousness of participants, sterile nasopharyngeal (NP) swabs were collected aseptically from the COVID-19 suspected individuals for further diagnostic processing at a specifically allocated collection section with special precautionary arrangements at Al-Qunfudah Hospital. SARS-CoV-2 exposure assessment

was confirmed by processing the clinical sample by reverse-transcription (RT)-real-time polymerase-chain-reaction (RT-real-time PCR) using ORF 1b (open-reading frame)/N gene PCR diagnostic kit (KOGENEBIOTECH) following manufacturer's instructions.[45] Fasting blood specimens were collected to measure patients' laboratory parameters. CBC was assessed by Beckman Coulter DxH 900 (USA), ALT, AST, troponin, and CRP by Vitros System Integrated XT 7600, ferritin by DxI 800 analyzer (Beckman Coulter, USA), and D-dimer by Stago automated analyzer. Data on age, gender (males and females), and COVID-19 severity status (Highly severe and moderately severe) were retrieved by conducting multiple reviews and analyzing the hospital records of the participants.

Data Quality-Assurance

The data collected was plugged into MS Office/excel sheet and several reviews were executed to eliminate errors and discrepancies while collecting data before the commencement of further processing of the data for statistical analysis. Participants with incomplete information were not included in this study to ensure the completeness and quality of the data.

Analytical Techniques, Resources, Analysis, and Interpretation

Descriptive followed by inferential statistical techniques were applied for analyzing the continuous and categorical variables using various packages of R-base/R-Studio-4.3.1. The shape of the continuous variables was delineated by descriptive statistics (mean, median, interquartile range, and standard deviation) to understand the shape of the data. Following descriptive statistics, inferential statistics (*t*-test) was used to compare the mean of the two groups using a statistical significance level of $p < 0.05$, while categorical variables were explained by frequencies as well as proportions. Proportional effect size was determined by VCramer, bias factor, VCramer Bayesian posterior, and Chi-square. $V_{Cramer\ and}$ Bayesian-V_{Cramer} test for effect size estimation was interpreted as <0.4 (week/no association), 0.2–0.4 (moderate association), 0.4–0.6 (relatively strong association), 0.6–0.8 (strong association), and 0.8–1.0 (very strong association). The cut-off for interpreting the bias factor was used as - 3.4 to −2.3 (strong evidence for an alternative hypothesis) and < - 4.61 is decisive evidence for a strong association between variables or alternative hypothesis).

COVID-19 severity-associated alteration in the components of the serum profile was ascertained by Pearson correlation (negative/positive value of correlation coefficient gives negative/positive correlation between variables, while the magnitude of the coefficient ranges from 0.0 to 1.0 indicates the strength of the association), and regression (logistic) analyses. P-value below 0.05 was the significant cut-off level. Data illustration and depiction were accomplished by using R-package ggplot2, GGally, and corrgram.

Results

Characteristics of the Serum Profile Components in COVID-19 Patients

The participants' serum profile characteristics in terms of statistical parameters (mean ± SD and p-value) are tabulated in Table 1. The mean ± SD of inflammatory biomarkers (CRP, ferritin, and NLR) by gender (males vs female/p-value) was 51.5 ± 41.4 vs 73.0 ± 43.1/p < 0.001, 700.4 ± 155.6 vs 775.0 ± 180.0/p-value <0.001, and 6.0 ± 5.1 versus 7.5 ± 3.9/p-value = 0.001 while that by severity status (highly severe vs moderately severe/p-value) was 101.7 ± 23 vs 21.7 ± 7.1/p < 0.00, 876.9 ± 123.9 vs 594.6 ± 67.4/p < 0.001, 10.5 ± 3.2 vs 2.8 ± 0.5/p < 0.001 respectively as summarized in Table 1.

The mean ± SD for coagulation disorder biomarkers (platelets, PT, PTT, and D-dimer) by gender (males vs female/p-value) was 468.1 ± 160.1 vs 547.8 ± 171.7/p < 0.001, 226.3 ± 74.8 vs 199.4 ± 61.8/p < 0.001, 13.7 ± 1.6 vs 14.9 ± 4.8/p < 0.001, and 32.4 ± 6.5 vs 34.7 ± 7.0/p < 0.001. At the same time, that by the severity status (highly severe vs moderately severe/p-value) was 663.4 ± 82.1 vs 347.7 ± 42.6/p < 0.001, 153.4 ± 31.2 vs 274.6 ± 35.2/p < 0.001, 15.2 ± 3.8 vs 13.5 ± 3.5/p < 0.001, 36.3 ± 6.4 vs 30.9 ± 6.2/p < 0.001, respectively (Table 1).

The mean ± SD of cardiac (troponin 1 and LDH) and biomarkers hepatic function (AST enzyme, ALT enzyme, and AST/ALT ratio) by gender (males vs female/p-value) and by severity status (highly severe vs moderately severe/p-value) was computed to be (0.1 ± 0.1 vs 0.1 ± 0.1/p < 0.001 and 407.4 ± 142.7 vs 465.1 ± 142.9/p < 0.001) and (95.5 ± 52.7 vs 120.5 ± 56.1/p < 0.001, 98.5 ± 47.7 vs 126.1 ± 52.0/p-value <0.001 and 1.0 ± 0.3 versus 1.0 ± 0.3/p-value >0.9), and (0.2 ± 0.1 versus 0.04 ± 0.06/p-value <0.001 and 528.4 ± 138.9 vs 342.5 ± 71.8/p-value <0.001) and (155.8 ± 35.7 versus 58.8 ± 15.6/p < 0.001,

Table 1 Characteristics of the Patients by Gender and COVID-19 Severity Status (n = 405)

Characteristic	Table 1	Table 2			Table 3		
	Overall N = 405[a]	Female, N = 177[a]	Male, N = 228[a]	p-value[b]	Highly severe, N = 212[a]	Moderately severe, N = 193[a]	p-value[b]
CRP (mg/L)	63.6 ± 43.6	51.5 ± 41.4	73.0 ± 43.1	<0.001	101.7 ± 23.0	21.7 ± 7.1	<0.001
D-dimer(ng/mL)	513.0 ± 171.1	468.1 ± 160.1	547.8 ± 171.7	<0.001	663.4 ± 82.1	347.7 ± 42.6	<0.001
WBC (103/µL)	12.3 ± 4.3	11.3 ± 4.2	13.1 ± 4.2	<0.001	16.0 ± 2.2	8.3 ± 1.4	<0.001
Neutrophil (%)	78.7 ± 10.7	76.1 ± 10.5	80.7 ± 10.5	<0.001	87.4 ± 7.1	69.1 ± 3.5	<0.001
ANC (103/µL)	10.0 ± 4.5	9.0 ± 4.4	10.8 ± 4.4	<0.001	13.9 ± 2.4	5.8 ± 1.1	<0.001
Lymphocyte (%)	16.6 ± 9.1	18.8 ± 9.0	14.9 ± 8.8	<0.001	8.6 ± 1.5	25.4 ± 4.8	<0.001
ALC (103/µL)	1.8 ± 0.9	1.8 ± 0.6	1.7 ± 1.1	0.2	1.4 ± 1.0	2.1 ± 0.5	<0.001
Platelets (103/µL)	211.2 ± 69.0	226.3 ± 74.8	199.4 ± 61.8	<0.001	153.4 ± 31.2	274.6 ± 35.2	<0.001
Troponin I (ng/mL)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	<0.001	0.2 ± 0.1	0.04± 0.06	<0.001
Ferritin (µg/L)	742.4 ± 173.6	700.4 ± 155.6	775.0 ± 180.0	<0.001	876.9 ± 123.9	594.6 ± 67.4	<0.001
NLR	6.9 ± 4.5	6.0 ± 5.1	7.5 ± 3.9	0.001	10.5 ± 3.2	2.8 ± 0.5	<0.001
PLR	126.8 ± 42.0	130.3 ± 49.0	124.1 ± 35.6	0.2	117.8 ± 45.8	136.7 ± 35.0	<0.001
LDH (U/L)	439.8 ± 145.5	407.4 ± 142.7	465.1 ± 142.9	<0.001	528.4 ± 138.9	342.5 ± 71.8	<0.001
Age (in years)	46.3 ± 23.8	49.8 ± 24.3	43.6 ± 23.0	0.009	51.6 ± 25.1	40.5 ± 20.8	<0.001
AST (U/L)	109.6 ± 56.0	95.5 ± 52.7	120.5 ± 56.1	<0.001	155.8 ± 35.7	58.8 ± 15.6	<0.001
ALT (U/L)	114.0 ± 52.0	98.5 ± 47.7	126.1 ± 52.0	<0.001	158.4 ± 30.6	65.4 ± 10.0	<0.001
AST/ALT	1.0 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	>0.9	1.0 ± 0.3	0.9 ± 0.3	<0.001
PT (sec)	14.4 ± 3.8	13.7 ± 1.6	14.9 ± 4.8	<0.001	15.2 ± 3.8	13.5 ± 3.5	<0.001
PTT (sec)	33.7 ± 6.9	32.4 ± 6.5	34.7 ± 7.0	<0.001	36.3 ± 6.4	30.9 ± 6.2	<0.001

Notes: [a]Mean ± SD, [b]Welch Two Sample *t*-test.

Abbreviations: CRP = C-reactive protein, ALC = absolute lymphocyte count, ANC = absolute neutrophil count, NLR = neutrophil/lymphocyte ratio, PLR = platelets/lymphocyte ratio, LDH = lactate dehydrogenase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, PT = prothrombin time, PTT = partial prothrombin time, and WBC = white blood cell.

158.4 ± 30.6 versus 65.4 ± 10.0/p-value <0.001, and 1.0 ± 0.3 versus 0.9 ± 0.3/p-value <0.001), respectively, as described in Table 1. For the overall (n = 405) participants, by gender, and by severity status, the mean ± SD of various components of the serum profile are summarized in Table 1.

The Magnitude of Effect Sizes and Proportionality Analysis

Based on troponin 1 concentration, a difference (significant) in the proportion of low-risk troponin level and high-risk troponin level participants was observed in the highly severe group (N/%; 180/85% and N/%; 32/15%; p-value <0.001), a difference (statistically significant) in proportion was also noticed between normal troponin level and low-risk troponin level patients in moderately severe participants (N/%; 124/64% and N/%; 69/36%; p < 0.001). The overall effect sizes calculated by X^2pearson, Bayesian V_{Cramer}, and BF tests were statistically significant. (X^2pearson = 205; $p < 0.001$, V_{Cramer} = 0.71: 95% CI; 0.62, 1.00, Bayesian V_{Cramer} = 0.7; HDI: 0.64, 0.75, $BF_{(01)} = -125$) as illustrated in Figure 2a.

Moreover, a difference (statistically significant) in the proportion of normal/low-risk and high-risk patients based on the AST/ALT ratio was observed in the moderately severe group (N/%; 124/64% and N/%; 69/36%; p < 0.001), on the contrary, the difference in proportions of normal/low-risk and high-risk was not a significant highly severe group (N/%; 116/55% and N/%; 96/45%; p = 0.17). However, the overall effect sizes calculated by X^2pearson, Bayesian V_{Cramer}, and BF tests were not significant. (X^2pearson = 0.38; $p = 0.5$, V_{Cramer} = 0.08: 95% CI; 0.00, 1.00, Bayesian V_{Cramer} = 0.08; HDI: 0.00, 0.18, $BF_{(01)}$ = 0.18) as illustrated in Figure 2b.

Furthermore, NLR proportionality analysis indicates the marked-pathological conditions for all the highly severe patients conversely, a difference (significant) in the proportions of normal, pathologic, and grey zone participants in moderately severe participants was observed (N/%; 0/5%, N/%; 77/40%, N/%; 107/55%; p < 0.001). The overall effect sizes determined by X^2pearson, Bayesian V_{Cramer}, and BF tests were statistically significant. (X^2pearson = 405; $p < 0.001$, V_{Cramer} = 1: 95% CI; 0.91, 1.00, Bayesian V_{Cramer} = 0.98; HDI: 0.96, 0.91, $BF_{(01)} = -266$) as illustrated in Figure 2c.

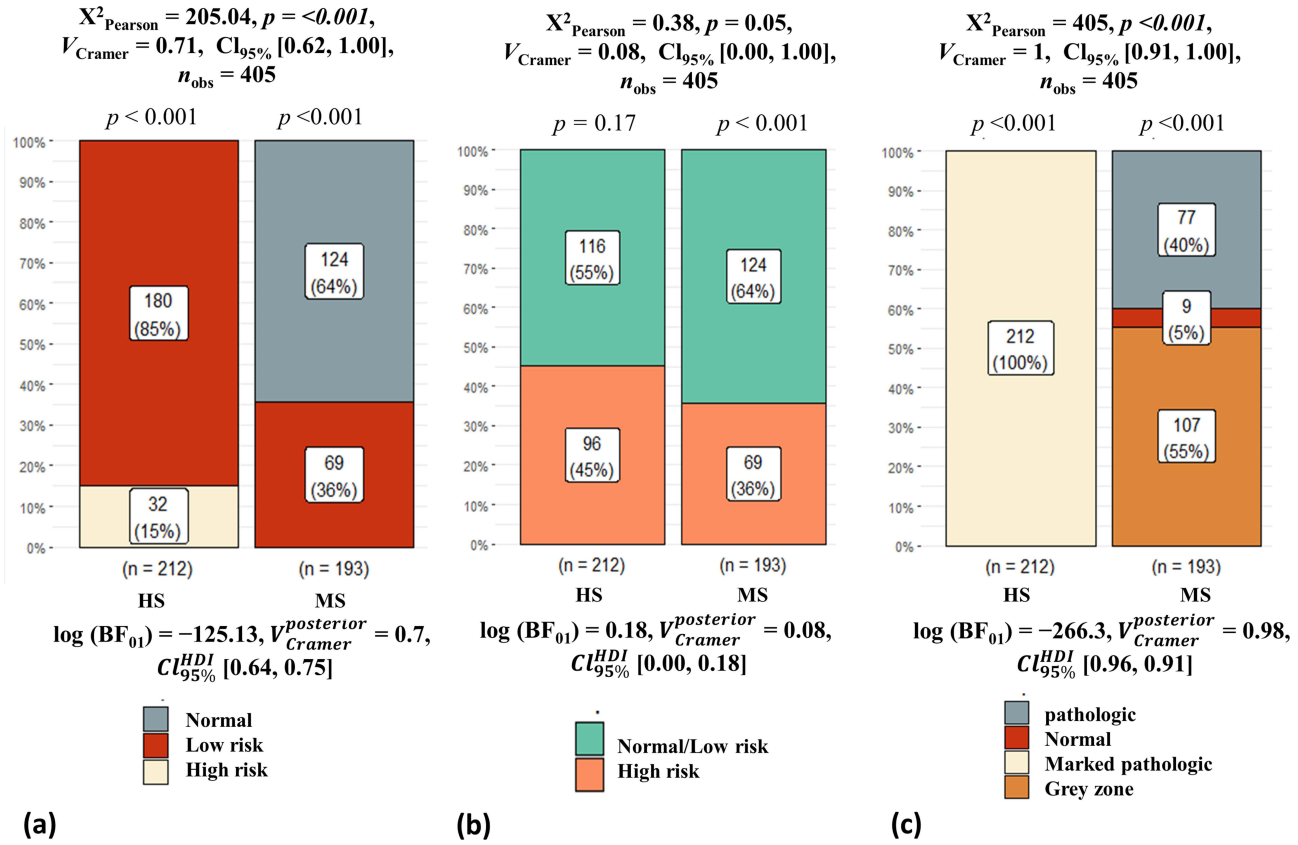


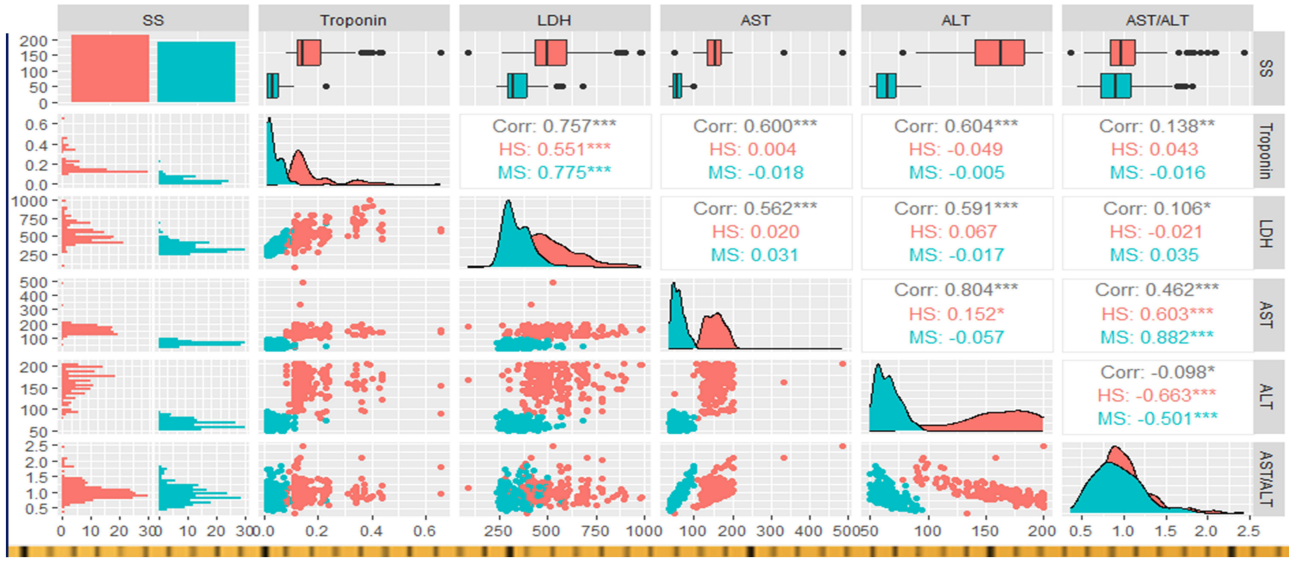
Figure 2 Depictive description of proportionality distribution by severity status. (**a**) represents the troponin I by severity status, (**b**) represents the AST/ALT ratio by severity status, and (**c**) depicts the neutrophil/lymphocyte ratio (NLR) status by severity scenario.
Abbreviations: HS, highly severe; MS, moderately severe; HDI, highest-density interval; BF, bias factor; CI, confidence interval.

The Magnitude of Correlations Between Serum Profile Components by Severity Status

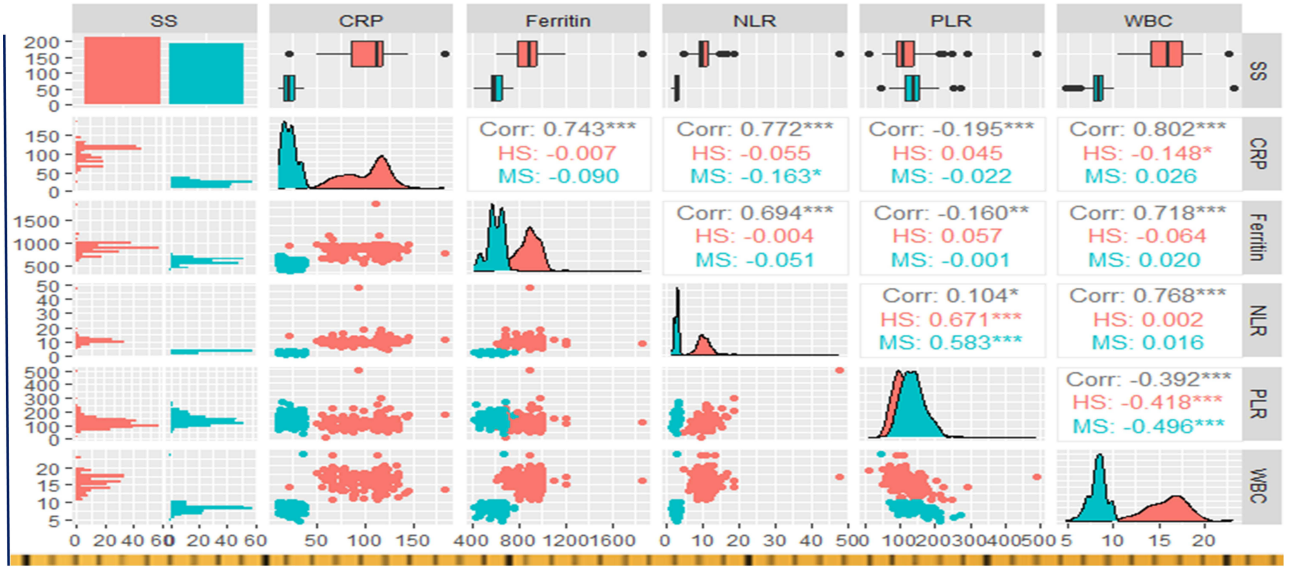
The overall correlation between troponin 1 with LDH (r/coef = 0.757), AST (r/coef = 0.6), ALT (r/coef = 0.743), AST/ALT ratio (r/coef = 0.138) was statistically significant (p-value under 0.01) that is depicted in Figure 3a. The overall correlation between CRP with ferritin (r/coef = 0.743), NLR (r/coef = 0.77), PLR (r/coef = −0.195), and WBC (r/coef = 0.8) was found to be significant (p-value under 0.01) that is depicted in Figure 3b. The overall correlation between D-dimer with platelets (r/coef = −0.81), PT (r/coef = 0.37), and PTT (r/coef = 0.23) was statistically significant (p-value below 0.01) which is depicted in Figure 4. Moreover, group-specific correlations between parameters of cardiac, liver function, inflammatory markers, and coagulation-disorder biomarkers with the level of significance are also illustrated in Figures 3a and b and 4 respectively.

The Magnitude of Association Between Serum Profile Components by Severity Status

In addition to that, the magnitude of COVID-19 severity-associated modulation in the major components of the serum profile is summarized in Table 2. COVID-19 severity-associated modulation in the inflammatory biomarkers (CRP, ferritin, and NLR) was measured, and the findings are summarized in Table 2. An increase in CRP, ferritin, and NLR by one unit explains 1.346 (adjusted odds ratio (adOR)/1.346: CI/1.15, 1.57: p-value below 0.001), 1.05 (adOR/1.05: CI; 1.032, 1.067: p < 0.001), and 1.46 (adOR/1.46: CI; 1.15, 1.67: p higher than 0.05) times higher odds of suffering from infection with high severity compared to the moderate severity of the COVID-19 infection. Moreover, the odds of showing marked pathological and pathological conditions as compared to normal were 1.18 and 1.12 times higher in the



(a)



(b)

Figure 3 An illustrative description of correlations between components of serum profile by COVID-19 severity status. (**a**) depicts the correlation between cardiac and liver function biomarkers by COVID-19 severity status, and (**b**) represents the correlation between inflammatory biomarkers by severity status. asterisk number represents the level of statistical significance (* = weak correlation, ** = moderate correlation, and *** = strong correlation), MS = moderately sever, SS = severity status. The top and right view of the correlogram illustrate the continuous and categorical variables by severity status in the form of boxplots and bar diagrams respectively.

highly-severe group compared to the moderately-severe group (adOR/1.18: CI/1.04, 2.18: p-value lesser than 0.001) and (adOR/1.12: CI: 1.01, 1.20/p-value <0.05).

Furthermore, the odds of being highly severe against moderately severe were 1.33, 1.42, and 1.23 times higher with a unit increase in D-dimer (adOR/1.33: CI/1.16, 1.58. p > 0.05), PT (adOR/1.42: CI/1.22, 1.65: p-value <0.001), and PTT (adOR/1.23: CI; 1.156, 1.29: p < 0.001) level. The odds of being highly severe were 0.81 times lower than moderately severe (adOR/0.81: CI/0.732, 0.897: p-value below 0.001) as mentioned in Table 2. The odds of highly severe compared to moderately severe were 4.07 (adOR/4.07: CI:1.52, 11.3: p-value under 0.001) times greater a unit increase in troponin 1 concentration (Table 2). Additionally, the odds of high-risk (HR) troponin 1 status compared to normal troponin status were 1.28 (adjusted odds ratio (adOR/1.28: CI: 1.10, 1.31: p value <0.001) times higher in the highly severe patients

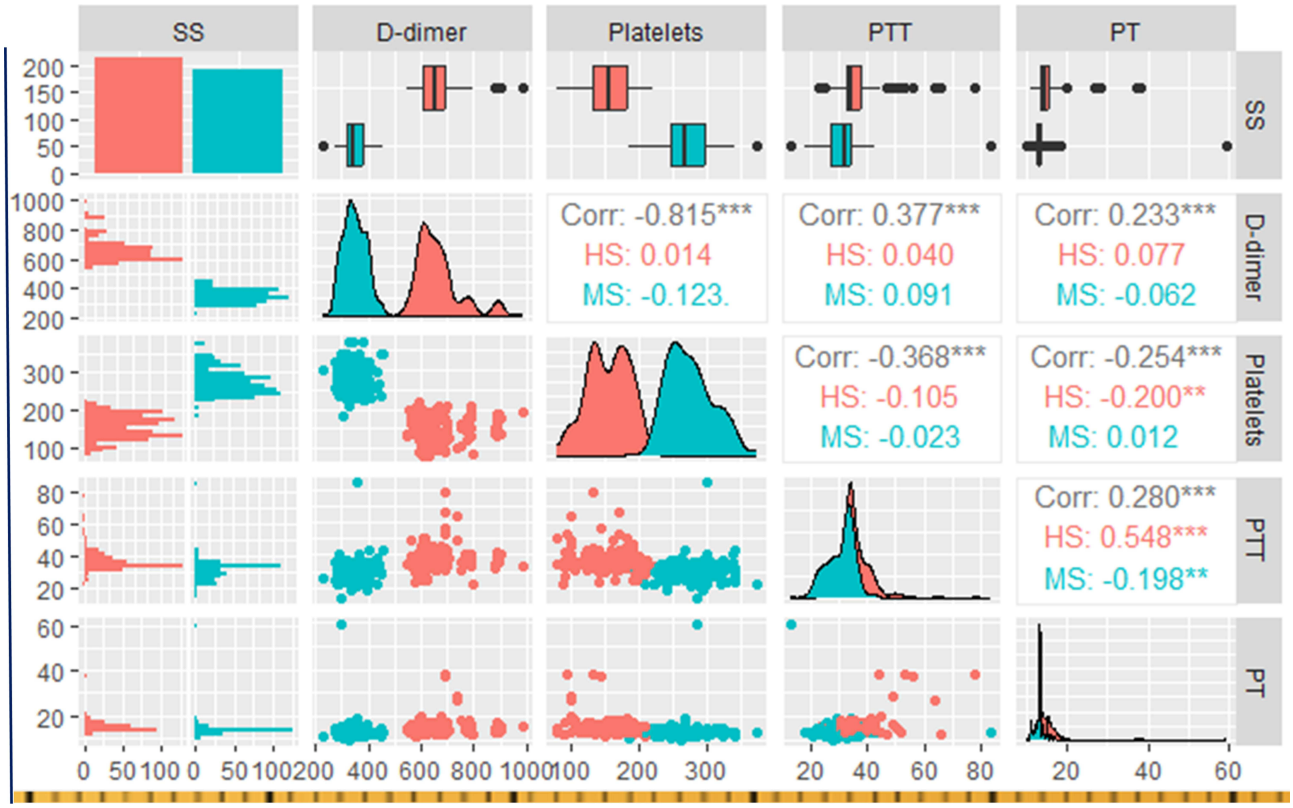


Figure 4 Depiction of correlation between components of serum profile (inflammatory and coagulation disorder biomarkers) by COVID-19 severity. asterisk number represents the level of statistical significance (* = weak correlation, ** = moderate correlation, and *** = strong correlation), MS = moderately severe, SS = severity status. The top and right view of the correlogram illustrate the continuous and categorical variables by severity status in the form of boxplots and bar diagrams respectively.

compared to moderately-sever participants; however, the higher odds (8.45) of low risk (LR) troponin status compared to normal troponin 1 level was measured in highly-severe patients compared to moderately-severe individuals (adOR/8.45: CI: 6.78, 11.31: p-value below 0.05) as tabulated in Table 2.

Furthermore, higher odds (1.46) of High-risk (HR) AST/ALT ratio compared to normal/low-risk AST/ALT were measured in highly severe patients compared to moderately severe individuals (adOR/1.46: CI; 0.79, 2.88: p-value below

Table 2 Tabulation of the Magnitude of Association (Regression Analyses) of COVID-19 Infection with Modulations in Components of Serum Profile (n = 405)

Characteristic	Multivariate binary logistic regression			Univariate binary logistic regression		
	AOR[a]	95% CI[a]	p-value	COR[b]	95% CI[a]	p-value
CRP (mg/L)	1.346	1.15, 1.57	<0.001	1.322	1.16, 1.51	<0.001
D-dimer(ng/mL)	1.33	1.16, 1.58	>0.05	1.38	0.98, 1.59	>0.05
WBC (103/μL)	4.85	3.1, 7.54	<0.001	4.93	3.21, 7.55	<0.001
Neutrophil (%)	1.56	1.42, 1.72	<0.001	1.56	1.52, 1.72	<0.001
ANC (103/μL)	3.97	2.76, 5.712	<0.001	4.05	2.83, 5.80	<0.001
Lymphocyte (%)	0.146	0.014, 1.54	>0.05	0.14	0.01, 1.45	<0.001
ALC (103/μL)	0.057	0.29, 0.11	<0.001	0.047	0.025, 0.091	<0.001
Platelets (103/μL)	0.81	0.732, 0.897	<0.001	0.821	0.758, 0.88	<0.001
Troponin 1 (ng/mL)	4.07	1.52, 11.3	<0.001	1.84	2.24, 1.51	<0.001
Ferritin (μg/L)	1.05	1.032, 1.067	<0.001	1.051	1.03, 1.07	<0.001

(*Continued*)

Table 2 (Continued).

Characteristic	Multivariate binary logistic regression			Univariate binary logistic regression		
	AOR[a]	95% CI[a]	p-value	COR[b]	95% CI[a]	p-value
NLR	1.46	1.15, 1.67	>0.05	2.71	1.53, 3.04	>0.05
PLR	0.98	0.983, 0.985	<0.001	0.99	0.98, 0.99	<0.001
LDH (U/L)	1.022	1.018, 1.027	<0.001	1.02	1.01, 1.025	<0.001
AST (U/L)	1.24	1.13, 1.36	<0.001	1.19	1.12, 1.27	<0.001
ALT (U/L)	1.45	1.18, 1.76	<0.001	1.46	1.2, 1.7	<0.001
AST/ALT	3.9	1.76, 8.66	=0.001	3.45	1.67, 7.13	=0.001
PT (sec)	1.42	1.22, 1.65	<0.001	1.56	1.34, 1.80	<0.001
PTT (sec)	1.23	1.156, 1.29	<0.001	1.22	1.15, 1.28	<0.001
Troponin status (HR)	1.28	1.10, 1.31	<0.001	1.34	0.73, 2.01	<0.001
Troponin status (LR)	8.45	6.78, 11.31	<0.05	8.88	6.67, 11.99	<0.05
NLR status (Grey zone)	1.00	0.88, 1.18	<0.05	1.01	0.7, 1.19	<0.05
NLR status (Pathologic)	1.12	1.01, 1.20	<0.05	1.09	1.014, 1.21	<0.05
NLR status (Marked pathologic)	1.18	1.04, 2.18	<0.001	1.19	1.04, 2.18	<0.001
AST/ALT ratio (HR)	1.46	0.79, 2.88	<0.001	1.487	0.98, 2.69	<0.001

Notes: [a]AOR = Adjusted Odds Ratio, [b]COR, = Crude Odds Ratio.
Abbreviations: CI, Confidence Interval; AOR was obtained after adjustment for gender and age. The reference category for dependent variables was set as a moderately severe group. The dummy variable for troponin status, AST/ALT ratio, and NLR status were normal, normal/low risk, and normal respectively.

0.001). With an increase in the AST/ALT ratio by unit 1, the odds of being highly severe compared to a moderately severe increase by 3.9 (adOR/3.9: CI; 1.76, 8.66: p-value = 0.001). With a unit increase in AST and ALT enzyme levels, the odds of being highly severe as compared to moderately severe increases by 1.24 (adOR/1.24: CI: 1.13, 1.36: p value below 0.001) and 1.45 (adOR/1.45: CI: 1.18, 1.76: p value below 0.001), respectively (Table 2).

Discussion

Pulmonary and extra-pulmonary (coagulation disorder, hepatological dysfunction, cardiac function related-risk) impact of COVID-19 is paramount.[11] Biomarkers are objectively measurable characteristics that are assessed as indicators of normal and abnormal physiological processes and the pharmacological responses to therapy.[46] The role of various classes of potential biomarkers (antecedent, screening, diagnostic, staging, and prognostic biomarkers)[46] is crucial for the development of medical therapeutic interventions.[47] The current study explains COVID-19 severity-associated alteration in the major components (biomarkers) of the serum profile. A set of biomarkers of the serum profile evaluated in this study explains the specific normal or abnormal pathophysiological process such as evaluation of AST, ALT, AST/ALT ratio gives insight into the hepatological implications of SARS-CoV-2 infection.[48] Level of the n = 18 laboratory parameters by disease-severity status, magnitude of overall and severity group-specific correlations between different parameters, and strength of COVID-19-associated alterations in these parameters have been determined. Significantly high concentrations of CRP and ferritin and high NLR were observed in highly severe patients ($p < 0.001$) Table 1. In addition, the NLR suggests that all highly severe patients were in marked-pathologic (NLR >5) condition, nevertheless, in the moderately severe group, patients were in pathologic (NLR >3-5 or <0.7) and grey zone (NLR = 2–3) state compared to a normal state (p-value < 0.001) that is represented in Figure 2c which corroborates reports that is indicative of the COVID-19 severity-associated neutrophilia, high-NLR, high WBC counts.[49,50] The present study result showed the association of CRP, ferritin, and NLR with highly severe conditions (adOR /1.346: CI; 1.15, 1.57: p-value below 0.001), (adOR /1.05: CI; 1.032, 1.067: p-value below 0.001), and (adOR) /1.46:CI/1.15, 1.67: p-value greater than 0.05) as compared to moderately severe state of the COVID-19 infection. Turgunova et al also reported the prognostic value of NLR along with other potential biomarkers termed soluble Trigger receptor-expressed on myeloid cells-1 (sTREM-1) in predicting the COVID-19-associated-mortality.[51] Significant elevation in CRP and ferritin concentration was associated

significantly with COVID-19 severity.[50] Enhanced CRP levels and marked CRP concentration were reported in highly severe participants as compared to mild/non-severe individuals.[35,52–56] A higher proportion of increased CRP concentration in highly severe compared to non-sever patients has been reported too.[57] Neutrophilia and high NLR in highly severe patients suggest the stress on neutrophil reserves contained in the bone marrow during a severe state of COVID-19 infection, and the evaluation of the biomarkers provides information on the severity and prognosis of the disease.[58] Serum ferritin (high) in highly severe, autopsy patients and ICU patients compared to moderately/non-severe individuals has been reported in other studies suggesting the usefulness of ferritin biomarker for COVID-19 severity.[27,59,60] Enhance mortality rate associated with the high ferritin concentration (ferritin greater than 1000 units).[61] Moreover, the present study also showed a strong positive overall correlation of CRP with ferritin (r/coef = 0.743; p-value below 0.01), NLR (r/coef = 0.77; p-value below 0.01), and WBC (r/coef = 0.8; p-value below 0.01) which shows that these inflammatory markers could be useful in early-stage prediction the disease severity. The current study's observations and those of other studies describe the correlation (positive) of disease severity with CRP levels.[62,63] CRP and ferritin could be considered potential prognosticators/predictors for COVID-19 severity. Fukui et al also observed the CRP as a potent prognosticator of COVID −19 corroborating with our finding while evaluating other biomarkers such as sialylated carbohydrate antigen KL-6 (KL-6), procalcitonin (PCT), and presepsin (PSP).[64] Furthermore, a difference (p-value under 0.001) in coagulation disorder markers (platelets, PT, D-dimer, and PTT) both by severity status was observed.[49] In this study, higher D-dimer, PT, PTT, and thrombocytopenia were found to be associated with COVID-19 severity (adOR/1.33: CI; 1.16, 1.58. p-value greater than 0.05), (adOR/1.42: CI/1.22, 1.65: p-value under 0.001), (adOR/1.23: CI; 1.156, 1.29: p-value under 0.001) level, and (adOR/0.81: CI; 0.732, 0.897: p-value <0.001) as mentioned in Table 2. Increased D-dimer, thrombocytopenia, prolonged PT, and PTT were found in highly severe compared to moderately severe participants, which is consistent with various other studies.[50,58,65–68] D-dimer, thrombocyte count, PT, and PTT could provide worthwhile information on COVID-19 severity-related coagulation disorders (pulmonary and venous embolisms) at the initial phase of COVID-19. Moreover, two other prognostic indicators of COVID-19 chitinase-3-like protein 1 (CHI3L1) and insulin-like growth factor-binding protein acid labile subunit (IGFALS) have been reported to be more reliable than the biomarkers (CRP and D-dimer) evaluated in this study based on the receiver operating characteristic (ROC) and area under ROC curve (AUC) analysis.[69] Which is suggestive of the application of CHI3L1, IGFALS in conjunction with CRP, and D-dimer biomarkers could be used as potential predictors COVID-19 severity.

The observations of this study also unraveled the raised troponin 1, LDH, AST, ALT, and AST/ALT ratios measured in highly severe compared to moderately severe participants and the level of these parameters by severity status (highly vs moderately severe) were significant (p-value below 0.001) which is in line with observations of other studies.[64,70–73] Moreover, the higher odds of highly severe compared to moderately severe were observed (adOR/4.07: CI: 1.52, 11.3: p-value under 0.001), the higher odds of high-risk (HR) troponin 1 level status compared to normal troponin status (adOR/1.28: CI; 1.10, 1.31: p-value <0.001), and the higher odds (8.45) of low risk (LR) troponin status compared to normal troponin 1 level was measured in highly-severe patients compared to moderately-severe individuals (adOR/8.45: CI; 6.78, 11.31: p-value <0.05) were also observed in this study. Additionally, a difference (significant, p below 0.001) in the proportion of troponin 1 between highly severe and moderately severe groups along with strongly positive correlations of the troponin 1 with LDH (r/coef = 0.757; p-value under 0.001) suggests the extra-pulmonary cardiac impact of the severity of the disease, which may lead to the heart muscle damage in a severe state of the disease along with inflammatory cytokine storm-mediated myocardial necrosis.[74] Troponin 1 could be characterized as a potential biomarker for early prediction of cardiac implications of the COVID-19 severe disease.

Current study observations included the significant difference in the values of hepatic function test parameters (AST enzyme, ALT enzyme, AST/ALT) of highly severe and moderately severe groups (p-value under 0.001). The elevated level of these markers was found to be allied with the high COVID-19 severity, which was consistent with the reports of another study.[72] Also, a difference (significant, p-value below 0.05) in the proportions of individuals with normal/low-risk levels and high-risk enzyme levels based on the AST/ALT was observed. Elevated AST/ALT ratio, a potential marker for hepatocellular injury and mortality risk, has been reported.[73] Additionally, another observation was that with an increase in the AST/ALT by unit 1, the odds of having a highly severe condition compared to a moderately severe condition increased by 3.9, and with one unit enhancement in AST enzyme and ALT enzyme levels the odds of being

highly severe as compared to moderately severe increased by 1.24 and 1.45 times. Higher level of liver enzymes are associated with hepatic injury in hospitalized SARS-CoV-2-infected patients.[71] The association of Liver injury and increased liver enzymes with highly severe COVID-19 cases was reported.[75] The observations of this research suggest that attention should be given to liver function tests during the clinical management of COVID-19 especially when it is in a severe state. In addition to the predictors/prognosticators evaluated in this research, other biomarkers such as neurofilament light chain (sNfL) and glial fibrillar acidic protein (sGFAp) explaining the neurological implication were reported to be the potential predictor of COVID-19 in-hospital mortality.[76] Papadopoulou et al highlighted the significance of evaluating molecular biomarkers or transcriptomic signature (differentially expressed genes) in conjunction with the serological biomarkers, such as proinflammatory markers (IL-6), NLR and WBC counts and reported the enriched pathways of inflammatory response, and monocyte and neutrophil chemotaxis.[77]

Furthermore, the emergence of new variants/subvariants of SARS-CoV-2 with high transmissibility may cause future COVID-19 resurgence nationally or regionally, in that scenario the result of this study could used for effective clinical management of the regional outbreak especially with poor healthcare facilities. Furthermore, SARS-CoV-2 shares genomic sequence similarity with SARS-CoV and MERS-CoV by up to 80%.[78] Given the similar clinical manifestation,[79] and pathophysiology of the disease caused by these human coronaviruses (HCo-Vs),[80] the diagnostic/prognostic values of evaluated biomarkers could be potentially applied to clinically manage the future outbreak of MERS-CoV and SARS-CoV beyond the SARS-CoV-2 resurgence in the region. Additionally, the findings of the study may also contribute to the prognostic and therapeutic requirements for other related less virulent HCo-Vs such as HCoV 229E and HCoV-OC43[80,81] in a scenario when they turn highly virulent and transmissible to cause a future outbreak or resurge in the region.

Conclusion

Evaluating biomarkers (serological markers and transcriptomic signatures) is critical for understanding disease progression, diagnostic measures, prognostic indicators, and response to therapeutic interventions. In conclusion, the increased levels of CRP, NLR, D-dimer, troponin 1, LDH, AST, ALT, and AST/ALT ratio, leukocytosis, and neutrophilia were assessed to be associated with COVID-19 severity-associated. Moreover, low PLR, absolute lymphocyte count (ALC), thrombocytopenia, prolonged PT, and PTT were associated with COVID-19 severity. The finding of the research suggests that the CRP, ferritin, NLR, PLR, D-dimer, platelets, PT, PTT, AST, ALT, AST/ALT ratio, and troponin 1 biomarkers could be used as potential and promising prognosticators/predictors of COVID-19-disease severity. Clinicians' attention to the implications of SARS-CoV-2 infection and frequent monitoring and assessment of these biomarkers in conjunction with other reported biomarkers (sNfL, sGFAp, CHI3L1, IGFALS, sTREM-1 and transcriptomic signatures such as differentially expressed genes for pathways of inflammation neutrophil and monocyte chemotaxis) are recommended to be focused on laying down the policies and decision-making for managing the infected patients clinically, in a holistic and evidence-based manner.

Data Sharing Statement

Data related to this research can be requested from the corresponding author with appropriate reasons.

Acknowledgment

The author acknowledges the contribution of Al-Baha University for facilitating the dry laboratory and the Qunfudah Hospital, Al-Qunfudah, KSA for providing the secondary data of various laboratory parameters.

Funding

The research did not receive funds.

Disclosure

The author reports no conflicts of interest in this work.

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