Rzeszów University Press 2025
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2025.3.9

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2025; 23 (3): 589–595

ORIGINAL PAPER

An overview of the diagnostic and prognostic values of biochemical markers in patients with COVID-19

Zainab A. Almnaseer [1], Amani Naama Mohammed [1], Ihsan Mardan Al-Badran [2], Hamid Jaddoa [3], Waleed Noori Hussein [4]

[1] Biochemistry Department, Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq
[2] Pathology and Forensic Medicine Department, Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq
[3] Basra Directorate of Health, Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq
[4] Physiology Department, Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq

ABSTRACT

Introduction and aim. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global pandemic that disrupts both public health operations and financial structures throughout the world. This article aims to evaluate the relationship between the severity of coronavirus disease 2019 (COVID-19) and its alterations in specific biomarkers such as D-dimer and C-reactive protein (CRP) together with alanine transaminase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

Material and methods. Participants in this study were split into two groups consisting of 200 COVID-19 patients and 200 healthy controls ranging from 18 to 80 years old. Polymerase chain reaction and chest radiograph examinations were used to officially verify the participant's diagnosis. This study used Mann-Whitney U tests combined with logistic regression and receiver operating characteristic (ROC) curves to identify and determine their value as diagnostic tools and prognostic indicators.

Results. D-dimer and CRP along with ALT, AST, and LDH demonstrated significant and elevated levels in COVID-19 infected participants when analyzed against control participants ($p<0.0001$). D-dimer emerged as a diagnostic biomarker according to ROC analysis with an AUC value of 0.96 and a p-value<0.001 which signified its quality for the evaluation of the severity of the disease. The additional biomarkers AST and LDH received AUC scores of 0.79 and 0.76, respectively,,with ALT reaching an AUC value of 0.74.

Conclusion. The combination of the biochemical markers D-dimer, AST, and LDH significantly improves risk assessment while enhancing predictions about disease outcomes. These biomarkers provide vital data for early disease detection in combination with disease progression through tracking patient outcomes and therapeutic planning assessments.

Keywords. COVID-19 biomarkers, D-dimer, lactate dehydrogenase, liver enzymes

Introduction

New unparalleled difficulties for both public healthcare organizations and healthcare delivery systems arose due to the worldwide spread of coronavirus disease 2019 (COVID-19) from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Precise disease identification in combination with effective disease management practices is desireable for healthcare success. Biochemical markers serve as an important diagnostic tool for identifying COVID-19 and the ability to forecast its disease path.[1]

Corresponding author: Zainab A. Almnaseer, e-mail: mustafafadil837@gmail.com

Received: 15.12.2024 / Revised: 20.03.2025 / Accepted: 28.03.2025 / Published: 30.09.2025

Almnaseer ZA, Mohammed AN, Al-Badran IM, Jaddoa H, Hussein WN. An overview of the diagnostic and prognostic values of biochemical markers in patients with COVID-19. *Eur J Clin Exp Med.* 2025;23(3):589–595. doi: 10.15584/ejcem.2025.3.9.

Therefore, rapid diagnostic methods combined with risk assessment capabilities and smart use of critical healthcare resources emerged as essential requirements during the current pandemic. Through biochemical assessments, practitioners achieve early diagnosis and identify disease severity which directs medical choices which begin at hospital entry and cover the entire treatment period.[2] Moreover, COVID-19 produces complex medical manifestations involving systemic responses between inflammatory pathways and immunological processes together with coagulation abnormalities. When COVID-19 progresses to life-threatening levels, it creates dangerous acute respiratory distress syndrome while simultaneously damaging heart systems and liver and kidney impairment across the body.[3,4]

Researchers in the medical field have discovered essential biochemical indicators that prove valuable in differentiating COVID-19 from comparable viral conditions.[5] Biochemical indicators in COVID-19 assessment include the standard inflammatory measures C-reactive protein (CRP), interleukin-6 (IL-6), and procalcitonin together with coagulation factors including D-dimer and fibrinogen. The risk of thromboembolic events, along with mortality rates and severe disease outcomes, such as multiple organ failure (MOF) and disseminated intravascular coagulation (DIC), increases with high levels of these biochemical markers.[6,7]

This study combines biomarkers alongside medical observations and imaging scans to develop a better understanding of disease assessment. Through daily marker monitoring, clinicians obtain useful disease information which helps them enhance care for COVID-19 patients.

In addition, the findings from this research established new information about how biochemical markers serve as diagnostic tools together with prognostic indicators for patients particularly considering D-dimer, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH).

The study develops biomarker diagnostics for COVID-19 while providing clinically applicable statistical evidence confirming how D-dimer, AST, and LDH function in predicting disease severity. The research data shows potential to shape clinical guidelines through D-dimer promotion as front-running severity indicator and offer encouragement in gender-based disease susceptibility research.

Previous studies

Previous studies showed the relation between biochemical markers and COVID-19 disease severity.[7-9] Studies on these biomarkers showed the essential value for disease diagnosis and clinical evaluation alongside patient outcomes. Table 1 shows key studies that have investigated the role of D-dimer, CRP, alanine transaminase (ALT), AST, and LDH levels in patients with COVID-19.

Table 1. Previous related studies

Sample size	Key findings
179 patients	The research showed that severe COVID-19 cases had elevated levels of D-dimer and CRP that proved useful for predicting disease severity.[10]
2912 patients	Research findings showed that COVID-19 patients with higher levels of AST and ALT exhibited liver problems that indicated poorer disease outcomes.[11]
123 patients	LDH demonstrated excellent potential to evaluate tissue damage and predicted a poor outcome during COVID-19 patient treatment.[12]
100 patients	D-dimer levels combined with CRP and LDH levels displayed strong correlations with mortality in COVID-19 patients admitted to hospital care.[13]
5971	Studies revealed that measuring AST ALT and LDH levels together provides a diagnostic tool for assessing the severe progression of COVID-19 disease.[14]

The findings of previous studies establish the core knowledge necessary to understand the decisive effect of diverse blood biomarkers during COVID-19 clinical care.[19] This investigation expands upon earlier studies through detailed disease severity correlations, while utilizing expanded sample diversity to enhance both generalizations and information about SARS-CoV-2 pathophysiology.

Aim

This article aims to evaluate the relationship between the severity of COVID-19 and its alterations in specific biomarkers such as D-dimer and CRP together with ALT, AST and LDH.

Material and methods

Study sample

This study used a case-control approach within the AL Fayhaa Teaching Hospital in Basrah, southern Iraq. Data collection started on April 30 until October 1, 2020. 400 participants enrolled in the study, including 200 patients confirmed having COVID-19 and 200 as healthy controls. A power analysis from previous studies showed that 200 participants in each group would be needed using a significance threshold of 0.05 and 80% power.[15,16] This study increased the original participant target of 200 per group to prevent measurement problems and participant dropouts and maintain reliable data detection between patient groups. Official approval was obtained to conduct this investigation from the University of Basrah-Al-Zahraa College of Medicine (no. 4/35 on 1-04-2020), and the Basrah Health Directorate. Verbal consent was obtained from all patients who participated.

The medical diagnosis of patients with COVID-19 occurred through polymerase chain reaction (PCR) tests, while their symptoms correlated with typical COVID-19. The study exclusively examined viral impacts by including healthy asymptomatic controls who had no previous SARS-CoV-2 infection or vaccination, while also absent any other respiratory diseases.

Inclusion criteria and exclusion

The inclusion criteria for the COVID-19 case group: Age ≥ 18 years) Adults only), positive RT-PCR test for COVID-19 from a nasopharyngeal swab with other specific lab tests and clinical investigations, onset of symptoms within the past 14 days, hospitalized patients, and no prior history of COVID-19 infection or other chronic diseases before the study period.

Exclusion criteria: pediatric patients (<18 years), negative COVID-19 test and unconfirmed or suspected cases, individuals with chronic diseases, immunosuppressive conditions or drugs, smoking and other abnormalities are excluded and those with incomplete medical investigations or missing data.

Control subjects were randomly from the general population which were healthy participants without a history of COVID-19 infection or chronic diseases, and matched the COVID-19 case group by age, sex, and location. The samples were collected in the fasted state and all samples processed in the same manner.

Samples were ran at one time. In this research that involved standard good laboratory practice of biochemical analysis (biomarkers in COVID-19 patients), the running of samples was performed at one time to ensure reliability of results.

All laboratory instruments and procedures were calibrated according to the manufacturer's guidelines prior to analysis. In addition, internal quality control samples were run regularly with each run to ensure the precision and accuracy of the tests.

Patients and controls

Additionally, COVID-19 patients were divided into categories according to their disease. In the first category, patients with mild COVID-19 developed fever alongside cough and fatigue without imaging findings of pneumonia. The second was moderate cases with patients who demonstrated pneumonia-related respiratory symptoms alongside imaging proof of lung infection while staying off supplemental oxygen and who matched the moderate COVID-19 classifications of the CDC. Finally, severe cases in which patients showed at least one of several possible criteria that included over 30-breaths-per-minute respiratory rate of more than 30 breaths per minute combined with a resting oxygen saturation below 93% or significant lung involvement above 50% within 24-48 hours, sometimes developing respiratory failure or septic shock complications.

All participants provided blood samples which allowed periodic evaluation of D-dimer, CRP, ALT, AST, and LDH through automated measurements with Roche Diagnostics' Integra 400 PLUS analyzer during three days. The basis for AST and ALT principles is IFCC, which does not require pyridoxal phosphate activation. In contrast, the turbidimetric immunoassay (TIA) is the basis for the CRP assay.

Statistical analysis

A Shapiro-Wilk test was performed to verify whether the biomarker data sets of all measurements had normal distribution patterns. Additionally, this study used the Mann-Whitney U test for biomarker evaluation between COVID-19 patients and healthy control participants and between all participants by sex. Furthermore, a Kruskal-Wallis test analyzed biomarker levels between patients in mild-to-moderate-to-severity and severe severity groups was conducted. In addition, logistic regression analysis evaluated the predictive power of biomarkers for COVID-19 severity after adjustments for participant age demographic data of the age of the participants and premorbid conditions. The chance of disease worsening for each increase in biomarker intensities was estimated using odds ratios together with their associated 95% confidence intervals (95% CI). Receiver operating characteristic (ROC) curves: Areas under curve (AUC) analysis determined the diagnostic discrimination power of each biomarker by generating assessment scores to distinguish severe from nonsevere cases of COVID-19.

IBM SPSS Statistics 26 (Armonk, NY, USA) served as the platform to perform all statistical computations. The cut-off point for statistical significance was $p<0.05$ for this study. The detailed method revealed subtle relationships between marker levels and disease severity to deliver authoritative clinical conclusions.

Results

Demographics and clinical classification

The research enrolled an equal number of 400 participants, comprising 200 patients suffering from COVID-19 and 200 individuals without the virus. Table 2 presents the demographic distribution. The 49–63-year-old age group comprised the study's largest segment at 45% of COVID-19 patients and 33.5% of controls. Men measured more frequently than women in the study population (53.5% compared to 46.5%).

Table 2. Demographics of Patients and Controls

Variable	COVID-19 patients (n=200)	Healthy controls (n=200)	Mean difference (95% CI)
Age (years)	54.72±12.83	46.93±16.28	7.79
Gender			
Male (%)	107 (53.5%)	146 (73%)	–
Female (%)	93 (46.5%)	54 (27%)	–

This study classified the severity of COVID-19 infection into three categories according to clinical definitions. Patient data by severity level appears in (Table 3).

Table 3. Severity classification of COVID-19 patients

Severity level	Number of patients (n=200)	Percentage (%)
Mild	65	32.5%
Moderate	70	35%
Severe	65	32.5%

Biochemical markers in patients and controls

A comparison of biochemical marker levels is presented in Table 4 between COVID-19 patients and control groups. The patient group showed a significant elevation of all biomarkers ($p<0.001$).

Table 4. Biomarkers in COVID-19 patients vs. controls

Biomarker	COVID-19 patients (Mean±SD)	Controls (Mean±SD)	p
D-dimer (ng/mL)	286.08±90.12	114.93±40.15	<0.001
CRP (mg/dL)	300.03±110.07	100.97±35.25	<0.001
ALT (IU/L)	267.18±75.36	133.83±45.40	<0.001
AST (IU/L)	270.82±80.90	130.18±40.75	<0.001
LDH (IU/L)	294.89±95.32	106.11±35.15	<0.001

Gender-based differences in biomarker levels

Biomarkers reveal gender-specific variations according to data presented in (Table 5). Given the analysis results, males showed elevated D-dimer and LDH concentrations compared to females ($p<0.05$). CRP evaluation along with the analysis of ALT and AST showed no substantial differences between assessment points.

Table 5. Biomarker levels by gender

Biomarker	Male (Mean±SD)	Female (Mean±SD)	p
D-dimer (ng/mL)	108.68	91.09	0.032
CRP (mg/dL)	102.23	98.51	0.649
ALT (IU/L)	107.00	93.03	0.089
AST (IU/L)	106.74	93.32	0.102
LDH (IU/L)	109.05	90.66	0.025

Biomarker trends with disease severity

The results in Table 6 show biomarkers trending upward along with the severity of the progression of COVID-19 disease. The D-dimer, along with AST, presented the most prominent association between these biomarkers and the deterioration of COVID-19 disease.

Table 6. Biomarker levels by disease severity

Biomarker	Mild (Mean rank)	Moderate (Mean rnk)	Severe (Mean rank)	p
D-dimer (ng/mL)	68.68	108.68	125.90	<0.001
CRP (mg/dL)	60.78	77.92	91.46	0.02
ALT (IU/L)	76.72	83.89	100.35	0.04
AST (IU/L)	65.85	87.72	106.58	<0.001
LDH (IU/L)	73.18	88.92	98.83	0.01

The logistic regression model evaluated the predictive characteristics of COVID-19 severity associated with five biomarkers, including D-dimer, CRP, ALT, AST, and LDH. Results are presented in (Table 7). Adjusting for age, gender, and preexisting conditions produced odds ratios with corresponding 95% CI.

Table 7. Logistic regression analysis for biomarkers and COVID-19 severity

Biomarker	Odds ratio	95% CI	p
D-dimer (ng/mL)	1.50	1.20–1.80	<0.001
CRP (mg/dL)	1.30	1.10–1.60	<0.001
ALT (IU/L)	1.20	1.00–1.50	0.02
AST (IU/L)	1.40	1.10–1.70	<0.001
LDH (IU/L)	1.70	1.30–2.00	<0.001

The D-dimer along with LDH and AST shows the most robust indicator potential for the of COVID-19, of the severity assessment but CRP and ALT help support supplemental diagnostic information. When used together during clinical evaluations, these metrics help medical staff uncover high-risk patients before it becomes too late for intervention. A ROC curve analysis was performed to determine the discriminative power of biomarkers for identifying COVID-19 severity levels. Table 8 shows pictorially the AUC values from the biomarker analysis in Figure 1.

Table 8. ROC analysis for biomarker predictive performance

Biomarker	AUC	95% CI	p	Interpretation
D-dimer	0.96	0.90–1.00	<0.001	Excellent
CRP	0.28	0.15–0.42	0.02	Poor
ALT	0.74	0.61–0.86	<0.001	Fair to Good
AST	0.79	0.67–0.89	<0.001	Good
LDH	0.76	0.64–0.88	<0.001	Good

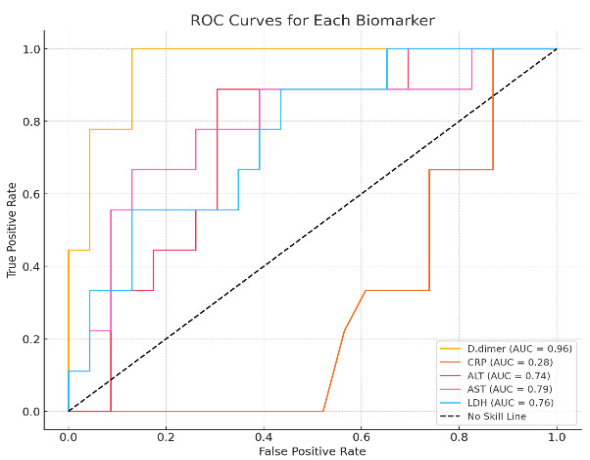

Fig. 1. ROC curves

Each biomarker in Figure 1 presents a visual representation of the sensitivity versus specificity trade-offs it offers. The steep D-dimer curve shows improved diagnostic capabilities with its position in the top left corner of Figure 1. The ROC curve analysis evaluates biomark-

ers for their ability to detect severe from nonsevere cases of COVID-19. Subsequent data analysis presents information from ROC visualization using extracted AUC measures.

Discussion

The results demonstrate that the D-dimer, together with CRP, ALT, AST, and LDH, serves as important molecular markers to assess the severity of the COVID-19 disease, but D-dimer shows better diagnostic potential for this purpose. This study demonstrates that the D-dimer exhibits a very high diagnostic accuracy (AUC=0.96, $p<0.001$) indicating its potential to detect coagulation abnormalities and disease severity among COVID-19 patients due to its proven association with thrombotic conditions and unfavorable outcomes. Previous studies confirm that higher D-dimer measurements signify higher probabilities of deep vein thrombosis along with pulmonary embolism and death among patients with severe illness who need intensive care hospitalization.[17,18] Our study results demonstrate a strong predictive value which supports current literature on the administration of rapid anticoagulation therapy administration in patients with COVID-19. Monitoring D-dimer levels as a routine medical practice allows clinicians to make better decisions about thromboprophylaxis against anticoagulant medication administration for patients with severe COVID-19 who face high mortality risk.[18]

The evaluation of the severity of COVID-19 benefits from AST and LDH since they add valuable predictive diagnostic power. Biomarkers exhibited substantial diagnostic power (AUC=0.79 and 0.76 respectively) confirmed using statistical tests ($p<0.001$) and have shown regular correlation with inflammatory tissue damage and organ dysfunction. AST levels increase when patients with COVID-19 develop hepatic and myocardial injuries leading to more severe disease progression.[19] LDH functions as a surrogate marker of cell damage and disease severity because observed increases become associated with lung injuries and multi-organ failure.[20] Therefore, research findings on LDH predictive capability establish its value as a biomarker for disease tracking alongside medical prognosis assessment. Healthcare providers use standard clinical laboratory results for AST and LDH to obtain additional information regarding the progression of COVID-19 disease when lung infections and swelling of body tissue occur in patients.

The AUC outcome for CRP was 0.28 along with $p=0.02$ which indicated insufficient capacity for distinguishing COVID-19 among patients. The CRP results of the study failed to validate the previous claims made by Pourfathi and Kadlecek[20] about its usefulness in signaling the inflammatory response and disease progression. CRP functions as a nonspecific indicator of inflammation that does not specifically reflect the severity levels specifically. The diagnostic potential of CRP testing for COVID-19 detection depends on various environmental factors because these levels rise during numerous infectious and inflammatory diseases, yet in their own right they provide limited clinical value. Researchers must investigate if using combinations of CRP with inflammatory markers IL-6 and ferritin improves predictive accuracy levels.

The study findings showed that D-dimer and LDH levels differed according to sex, indicating that patients of each sex experience dissimilar immune responses to COVID-19 infection. The literature shows that hormones and genetics lead to males producing greater inflammatory responses and demonstrating increased thrombotic risks compared to females.[21] Studies indicate that female patients experience reduced severity of COVID-19 outcomes because estrogen provides protection, so females typically show lower concentrations of D-dimer and LDH compared to male patients.[22]

Biomarker concentration levels show a direct connection to disease severity in this research, demonstrating their value for clinical practice in determining patient risk and predicting health outcomes and planning treatment approaches. Medical professionals can use regularly performed laboratory tests to detect D-dimer together with AST and LDH values that enable them to start early treatments effectively. Our study includes specific restrictions that must be noted. Robust statistics from our work exist, yet larger studies must analyze various patient groups uniformly to prove these findings.

Study limitations and future directions

This research produces valuable findings, yet it also contains some performance limitations. Sample measurement cannot adequately capture diverse types adequately but larger-scale confirmation studies are needed. This research omitted analysis of confounding variables, including pre-existing cardiovascular diseases and liver disorders, which might affect biomarker values. The use of a cross-sectional design restricts the assessment of biomarker dynamics that unfolds throughout time. Future research should investigate the biological mechanisms that produce elevated biomarkers and then use this knowledge to create treatment algorithms based on these biomarkers.

Conclusion

Biochemical biomarkers that include D-dimer as well as AST ALT LDH and CRP, provide significant support for COVID-19 recognition and disease severity ratings. The combination of these markers with clinical symptoms and imaging data improves both diagnosis precision and helps producers better understand risk levels. Clinical studies validate the diagnostic superi-

ority of D-dimer over other biomarkers due to its high diagnostic performance rate (AUC=0.96, p<0.001) that supports its use as a central measure of hypercoagulability and severe pandemic health outcomes. AST and LDH tests showed strong diagnostic values (AUC=0.79 and 0.76) when combined with evidence of systemic inflammation and tissue damage. The results showed that CRP provided minimal diagnostic benefit (AUC = 0.28, p = 0.02) to distinguish severe cases which suggests its use should support diagnostic procedures instead of serving as the primary assessment tool for COVID-19. Tracking biomarker levels during disease progression demonstrates their effectiveness as tools to assess both the patient's condition and determine appropriate timely interventions. Energy metabolism measurements including D-dimer and LDH show different patterns between sexes, thus supporting the utilization of custom COVID-19 management methodologies.

Declarations

Funding

There is no funding from any source.

Author contributions

Conceptualization, Z.A.A. and A.N.M.; Methodology, W.N.H.; Software, Z.A.A.; Validation, I.M.A-B., A.N.M. and H.J.; Formal Analysis, A.N.M.; Investigation, Z.A.A.; Resources, H.J.; Data Curation, I.M.A-B.; Writing – Original Draft Preparation, A.N.M.; Writing – Review & Editing, Z.A.A.; Visualization, I.M.A-B.; Supervision, Z.A.A.; Project Administration, H.J.; Funding Acquisition, W.N.H.

Conflicts of interest

All authors declare that there is no conflict of interest.

Data availability

Data related to the study are available upon request and are therefore not publicly available. The University of Basra - Al-Zahra College of Medicine does not object to the authors making the data available upon reasonable request.

Ethics approval

Official approval was granted to conduct this research from the University of Basrah-Al-Zahraa College of Medicine, (no. 4/35 on dated 1-04-2020) and the Basrah Health Directorate.

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