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Levels Of D-Dimer, Ferritin, Neutrophil To Lymphocyte Ratio, And Monocyte To Lymphocyte Ratio For Hospitalized COVID-19 Patients In Baghdad-Iraq: A Case-Control Study

Shahad Sabah Khalid [1], Zahraa Mohammed Ali [1], Laith G. Shareef [2], Mohanad Faris Raheem[1]

[1] Department of laboratory sciences, College of Pharmacy, University of Baghdad, Baghdad, 10011, Iraq
[2] Department of Pharmacy, Al-Rasheed University College, Baghdad, 10011, Iraq
Corresponding author:
Laith G. Shareef: laithalkunani@yahoo.com

Abstract

Background: A novel form of coronavirus, "severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was described in Wuhan in 2019. The pandemic has become one of humanity's most significant health challenges, quickly increasing infected people. As a result, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) in the United States have approved interim clinical guidelines for the management of patients with confirmed coronavirus disease (COVID-19). However, there is limited evidence of the clinical manifestations for the prognosis of COVID-19.

Methods: During the study period, a total of 90 subjects were enrolled, 45 of which were healthy as controls and 45 of which were hospitalized patients given a diagnosis of COVID-19 by real-time reverse transcription-polymer chain reaction (RT-PCR). A total of five ml of venous blood specimen was reserved for every individual to start measuring D-dimer, Ferritin, neutrophil to lymphocyte ratio, and monocyte to lymphocyte ratio in their blood to determine if there is a correlation between these markers' levels and COVID-19 infection by using SPSS version 23.

Results: This study revealed a remarkable increase in the measured values of D-dimer, Ferritin, and neutrophil to lymphocyte ratio (NLR), while a decrease in monocyte to lymphocyte ratio (MLR) in patients' blood compared to the control group, with p-values < 0.001 for each of these markers.

Conclusions: D-dimer, ferritin, and NLR all increase, while MLR decrease in COVID-19 disease; in this study, D-dimer and NLR were shown to be excellent diagnostic biomarkers, ferritin very good, while MLR suitable diagnostic biomarkers for severe COVID-19 infection

Keywords: COVID-19, SARS-CoV-2, D-dimer, Ferritin, Neutrophil to lymphocyte ratio, Monocyte to lymphocyte ratio.

Introduction

Chinese physicians discovered a person with pneumonia on December 29, 2019, and informed the World Health Organization (WHO) on December 31, 2019. On January 26, 2020, the infection was recognized as a coronavirus [1-3]. The SARS-CoV-2 virus and the disease were later named COVID-19 by the WHO, the third RNA virus in the coronavirus family [4, 5]. Leukopenia, lymphocytopenia, high CRP, high D-dimer, prolonged PT, and high fibrinogen levels have been observed in the early stages of COVID-19 illness [6, 7]. Several investigations have shown that individuals with significant COVID-19 have a poor prognosis for coagulopathy [8-10]. Following the establishment of a clot, the fibrin mesh is degraded by the fibrinolytic system. The D-dimer, consisting of two D segments of fibrin, is produced when the plasmin enzyme is activated. This suggests that degraded fibrin is present in the circulation. D-dimer is indicative of the activation of the coagulation and fibrinolysis systems [11]. Due to the transformation of 2 to 3 percent of fibrinogen into fibrin and subsequent lysis, modest levels of D-dimer might be found in the plasma of healthy individuals. Plasma D-dimer levels are elevated in all physiologic and pathologic conditions associated with increased fibrin formation and subsequent lysis by plasmin [12], including deep vein thrombosis, malignancy, sepsis, acute aortic syndrome, acute coronary syndrome, recent surgery, trauma, and pregnancy [13]. Elevated D-dimer values are one of the sensitive changes in coagulation parameters in COVID-19 and are thought to be more problematic for developing thrombosis [14]. Ferritin is an iron-binding protein that serves to support iron soluble and non-toxic. It is present in most tissues in humans as cytosolic protein in cells; it is also found in blood and body fluids, particularly serum and plasma [15]. It is assumed that the high ferritin level protects organisms by reducing iron bioavailability to pathogens and reducing the damage caused by free radicals in the presence of ferrous iron (Fe II) [16]. Elevated ferritin levels in the blood are a hallmark of hemophagocytic lymphohistiocytosis, a common complication of viral infection, and are associated with a poor prognosis in patients with COVID-19 disease; individuals with impaired lung lesions are more likely to have elevated ferritin levels [17-19]. Ferritin plays an essential role in immunological dysregulation, most notably in hyperferritinemia, by rapid immune suppressive and pro-inflammatory activity, which may be followed by a cytokine storm [20]. Ferritin might function in inflammation after COVID-19 infection. Macrophages produce active ferritin, and cytokines may generate hyperferritinemia, which can promote the production of a range of pro-inflammatory and anti-inflammatory cytokines, including interleukin-1 (IL-1) (IL-2, IL-10) [16, 21]. Inflammatory indicators such as the neutrophil to lymphocyte ratio (NLR) and the monocyte to lymphocyte ratio (MLR) have been used to predict the severity of COVID-19 using routine tests (MLR). NLR and MLR are biomarkers that show systemic inflammation and have been linked to an increased risk of death in people with cardiovascular disease [22]. This study aimed to measure D-dimer, Ferritin, NLR, and MLR levels in the blood of severe COVID-19 infected patients and compare their levels with the healthy controls group participating in this study to find if there is a relationship between these markers and COVID-19 severe infection.

Methods

Study design

This is an observational Case-Control Study including adult Iraqi hospitalized patients

diagnosed with COVID-19 to determine whether there is a relationship between D-dimer, Ferritin, neutrophil to lymphocyte ratio, and monocyte to lymphocyte ratios in their blood and COVID-19 infection.

Sample size

The sample size was calculated using G*Power version 3.1.9.7 software. The smallest total sample size was 70 patients with 90% power at a 95% confidence interval, a two-tailed alpha of 0.05, and an effect size of 0.80. (f). The research involved 90 individuals, 45 in the diseased group and 45 in the healthy controls.

Eligibility criteria:

Adult (30-60) years old, patients with positive COVID-19 PCR test, willing to participate, both sexes were eligible, and have no chronic disease.

This research involved. Ninety individuals, 45 of whom were healthy controls, and 45 hospitalized individuals with COVID-19 illness had a positive result of nucleic acid amplification testing of nasal swabs for COVID-19 by real-time reverse transcription-polymerase chain reaction (RT-PCR). This multicenter trial was held in Dar-Alslam and Alshefaa hospitals in Baghdad from September 2021 to January 2022. All COVID-19 patients were severe cases who had the same treatment plan and wore a non-rebreather mask. Blood samples were collected from participants under the same circumstances, and participants were questioned by the researcher, who collected data such as their name, age, gender, and illness history, as well as medication.

Exclusion criteria:

People with chronic illness, an autoimmune condition, pregnant and lactating women, individuals with a history of substance abuse, cancer, and individuals on long-term corticosteroid or immunological medication or who have previously been infected with COVID-19.

Study procedure:

Each participant provided 3 ml of venous blood samples; one milliliter (ml) of this specimen was immediately collected in a sodium citrate tube for D-Dimer testing. And one milliliter (ml) of these specimens was placed in an Ethylene diamine tetra acetic acid (EDTA) tube to be used for the analysis of complete blood count (CBC); the reminder specimens were collected in a gel tube and then centrifuged for 10-15 minutes at 4400 round per minute (rpm) to get the serum to analyze. Ferritin, Table 1 summarizes the chemical components, equipment, and kits utilized in this work.

Bias

During the process of selecting the study sample, selection bias may occur. This is particularly true in retrospective cohort studies when exposures and results already have occurred before participants are recruited for the research. However, since the outcome is unknown at the time of enrollment, sampling error is less probable in this Prospective study. The ideal study population is well-defined, conveniently accessible, trustworthy, and has a high likelihood of producing the intended outcome.

Table1: Summary of the Chemical Materials, Equipment, and Kits Used

Chemicals	Provider

D-Dimer	Roche (Germany)
Ferritin	Roche (Germany)
Centrifuge	Eppendorf (Germany)
Gel Tube	Biozek – Holland
Sodium citrate tube	BIOZEK – Holland
EDTA tube	BIOZEK – Holland
CD-Ruby analyzer	Cobas (Germany)

Ethical consideration

The research was carried out with the agreement of Iraq's Ministry of Health's Human Research Ethics Committee. Before commencing the investigation, each participant signed an informed consent form.

Statistical analysis:

SPSS version 23 software for Windows was used for statistical analysis. The median, interquartile range, and mean rank T-test- Mann Whitney were employed to compare patients and control groups with non-normally distributed data. The receiver operating characteristic curve (ROC) was also used to calculate the area under the curve, optimum cut-off value specificity, and sensitivity; the extreme value for AUC is one [23].

Results:

The current research found a considerable increase in the measured value of D-dimer in patients compared to controls, with the median (IQR) of D-dimer for the COVID-19 patients group being 1.630 (3.33) g/ml and the healthy control group being 0.150 (0.24) g/ml. In addition, the analysis also found a significantly higher ferritin level p-value < 0.05 in the COVID-19 patients' group than in the healthy control group, with patients having a median (IQR) of 917 (1020.5) g/L and healthy controls having a median (IQR) of 115 (109.0) g/L. Also, it showed a more significant level of NLR and MLR in the COVID-19 patients' group than in the controls p-value for both < 0.05; the median (IQR) for NLR and MLR in the patients' group was 10.705 (15.505) and 4.800 (5.539) respectively, while for healthy controls group was 2.000 (1.404) and 10.20 (9.750) respectively as shown in Table 2, Figure1, Figure 2, and Figure 3.

Table 2: Comparison of D-Dimer, Ferritin, NLR, and MLR Levels in Studied Groups

Parameter	**Group**	**median**	**IQR**	**Mean rank**	**P-value**
D-dimer (µg/ml)	patients	1.6300	3.33	64.74	*<0.001
	control	0.1500	0.24	26.26	
	patients	917	1020.5	62.96	

Ferritin (µg/L)	control	115	109.0	28.04	*<0.001
NLR	patients	10.705	15.505	64.38	*<0.001
	control	2.000	1.404	267.62	
LMR	patients	4.800	5.539	33.53	*<0.001
	control	10.20	9.750	57.47	

Where: *P< 0.05 statistically significant; P-value was for T-Mann Whitney test; IQR, interquartile range.

Figure 1: D-dimer levels in studied groups.

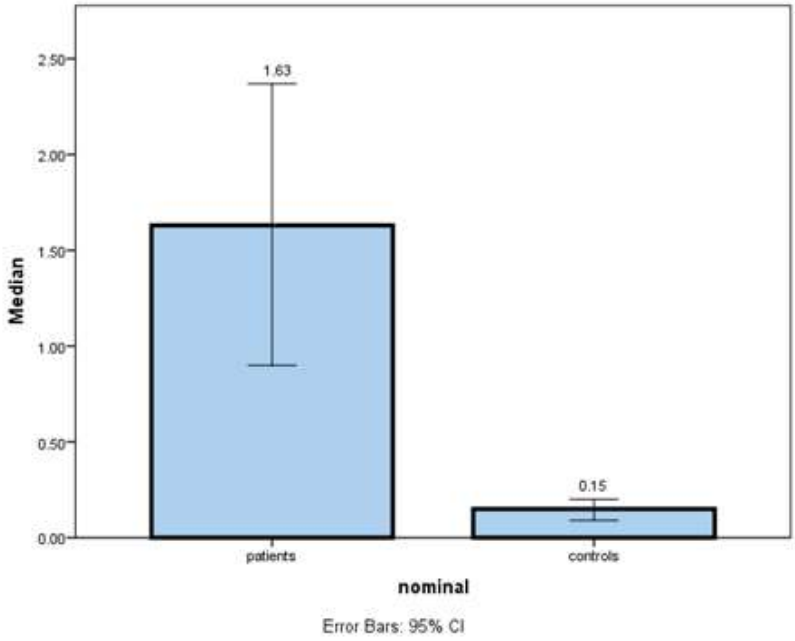


Figure 2: ferritin levels in studied groups.

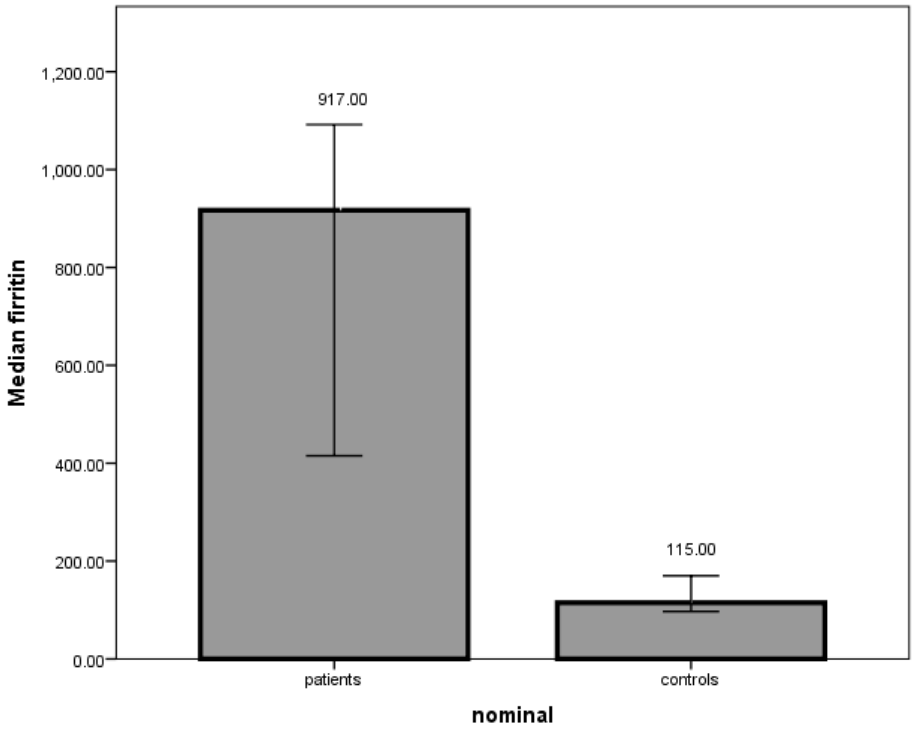
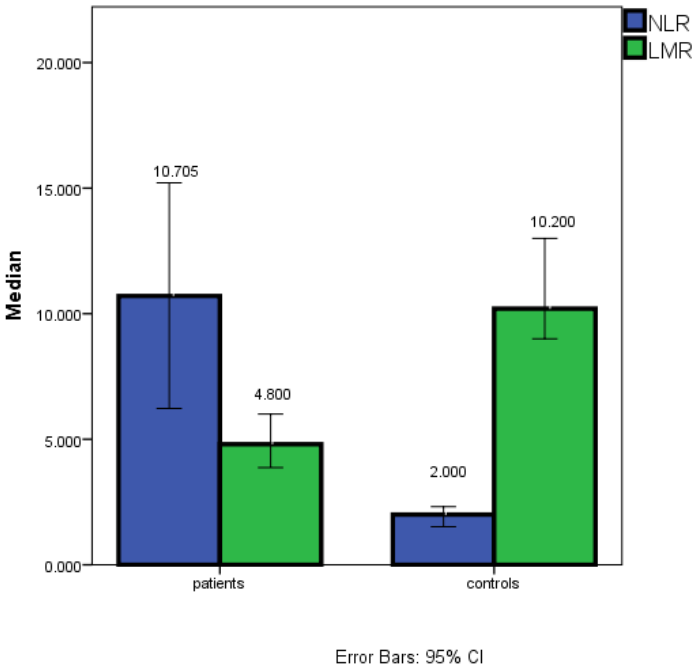


Figure 3: NLR and LMR Levels in Studied Groups



The optimal cut-off value for D-dimer was ≥ 0.2350 µg/ml with sensitivity and specificity of 91.1% and 73.3%, respectively, with an AUC of 0.928, as shown in Table 3 and Figure 4. The optimal cut-off value for Ferritin was ≥ 212.00 (µg/L) with sensitivity and specificity of 82.2% and 95.6%, respectively; the AUC for Ferritin was 0.888, as shown in Table 3 and Figure 5. The optimal cut-off value of NLR was ≥ 2.650, with sensitivity and specificity of 95.6% and 73.3 %, respectively, with an AUC of 0.920. The optimal cut-off value for MLR was ≤7.8263 with sensitivity and specificity of 73.3% and 73.3%, respectively, and UAC was 0.766, as shown in Table 3, Figure 4, Figure 5, Figure 6, and Figure 7.

Table 3: Receiver Operating Characteristic Curve for Measuring the Area Under the Curve of D-Dimer, Ferritin, NLR, and MLR Levels in Studding groups

Variable	AUC	95%CI Of AUC	P-Value	Optimal cut-Off	Sensitivity	Specificity
D-dimer (µg/ml)	0.928	0.877-0.979	*<0.001	≥0.2350	0.911	0.733
Ferritin (µg/L)	0.888	0.810 –0.965	*<0.001	≥212.00	0.822	0.956
NLR	0.920	0.866 – 0.973	*<0.001	≥2.650	0.956	0.733
LMR	0.766	0.667 – 0.865	*<0.001	≤7.8263	0.733	0.733

Figure 4: Receivers Operating Characteristic Curve for Measuring the Area Under Curve of D-Dimer for COVID-19 Infection.

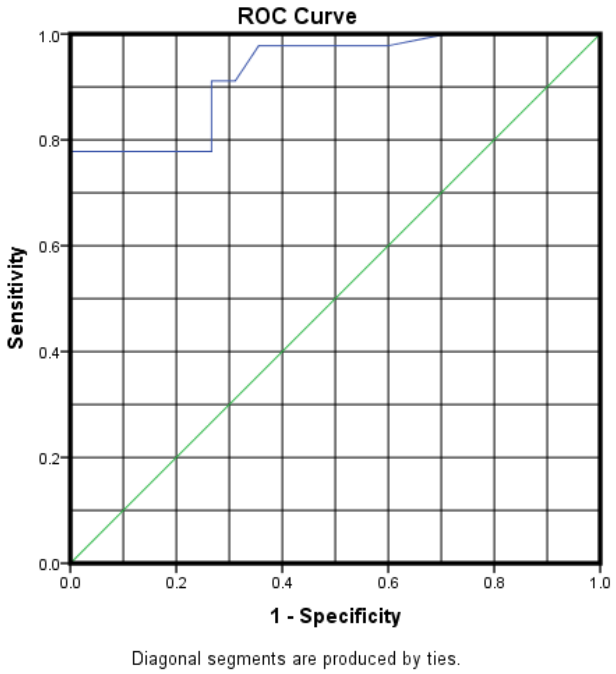


Figure 5: Receivers Operating Characteristic Curve for Measuring the Area Under the Curve of Ferritin for COVID-19 Infection.

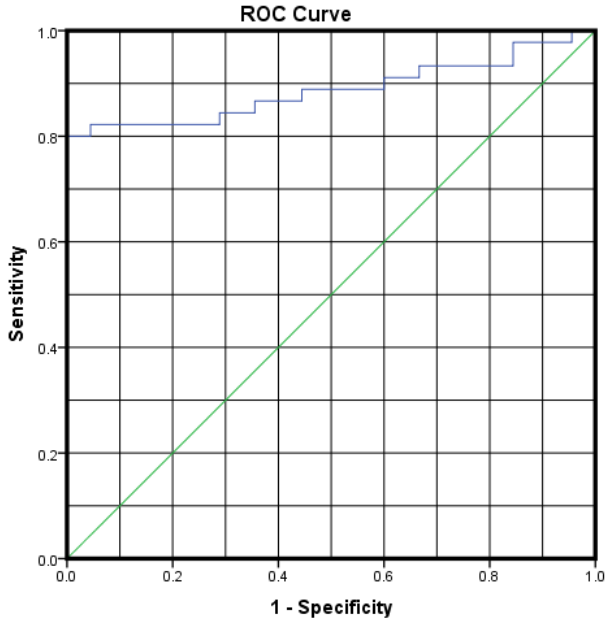


Figure 6: Receiver Operating Characteristic Curve for Measuring the Area Under the Curve of NLR for Covid-19 Infection

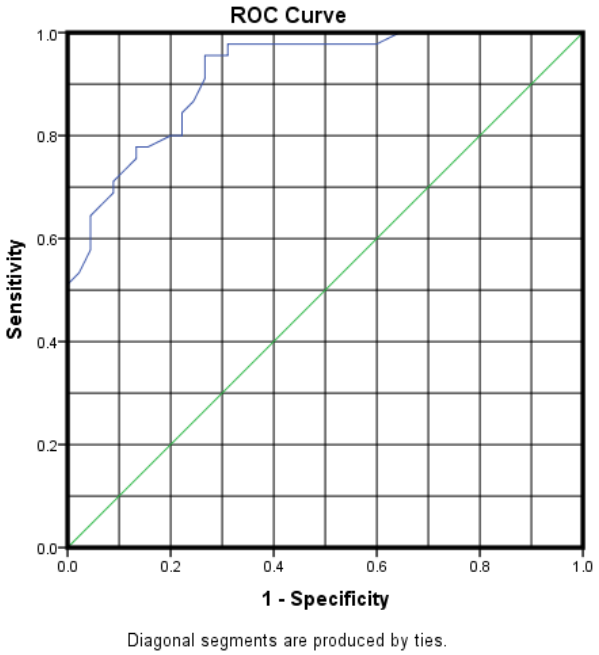
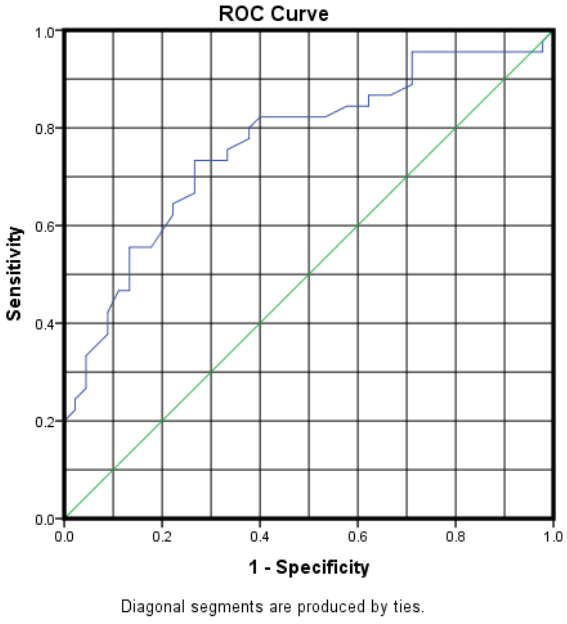


Figure 7: Receiver Operating Characteristic Curve for Measuring the Area Under Curve of MLR for Covid-19 Infection



Discussion:

D-dimer levels are often abnormal in COVID-19 patients. In patients hospitalized with COVID-19, D-dimer levels correspond with disease severity and are an accurate predictive diagnostic for in-hospital mortality [24]. This research aligned with a case-control study conducted by Spiezia L et al., which included 44 COVID-19-infected patients and 22 healthy cases as controls; their results revealed that patients with COVID-19 infection exhibited a substantial increase in D-dimer levels when compared to controls [25]. Thachil J et al. (2020) investigated 1099 known cases of COVID-19 infection, including around 550 health centers in China; their results indicated that D-dimer measurements in non-survivor specimens were significantly higher than in people who have survived, with a D-dimer equal or greater than 0.5 mg/L documented in 260 of 550 (46.4 %) patients evaluated, with only 43 % starting to experience raised D-dimer if the illness was not severe [26]. The ideal cut-off value for D-dimer has been 0.2350 (g/ml), with sensitivity and specificity of 91.1 and 73.3 %, in both, and an AUC of 0.928, going to make D-dimer an excellent predictive indicator for COVID-19 severe infection. Because the cut-off value was 0.2350 (g/ml), the severity of study participants was connected with a D-dimer higher than 0.2350 (g/ml). Blood hypercoagulability may emerge from the very active immunological response associated with SARS-CoV-2 infection and systemic inflammation associated with cytokine storms, as seen by higher D-dimer concentrations in COVID-19 patients. Hypoxia-reoxygenation, oxidative stress, and acid-base balance loss may be combined with pressure (such as acute respiratory distress syndrome, sepsis, or shock) or toxicity with chemical agents such as medicines, leading to a very high D-dimer level rise [27]. The fundamental function of ferritin is iron-binding and storage; its concentration corresponds with immunological and inflammatory responses [28]. When a person contracts a viral infection, an increase in blood ferritin levels correlates to the release of iron in the reticuloendothelial system, a decrease in ferritin transport capacity in the liver and spleen, and an increase in intracellular ferritin synthesis and release [29]. This research found a significantly higher ferritin level (p-value < 0.05) in the COVID-19 patients' group than in the healthy control group, with the median (IQR) of Ferritin for patients being 917 (1020.5) g/L and the healthy control group having 115 (109.0) g/L. Many researchers believe hyperferritinemia syndrome is a significant modulator of COVID-19 infection [30, 31]. Ahmed S. et al. (2021) observed that non-survivor hospitalized patients had ferritin levels of about 1400 ng/mL, approximately 3 to 4 times higher than that reported in survivors when comparing ferritin levels in their research on admitted COVID-19 patients [32]. The optimal cut-off value of Ferritin was 212 μg/L, with sensitivity and specificity of 82.2% and 95.6%, respectively, indicating that the severity of patients in this study was associated with ferritin levels greater than 212 (μg/L). The area under the curve was 0.888 which mean it was an excellent predictive marker for COVID-19 infection severity. This study disagrees with Zhou B et al. (2020) in a retrospective analysis of 942 adult COVID-19 patients hospitalized in March 2020 at a prominent New York City health center that found the weak performance of ferritin levels for the prediction of mortality [33]. Since NLR is related to systemic inflammation and disease activity, it has predictive validity in conditions including cardiovascular disease, autoimmune disease, cancer, and other infectious illnesses. In COVID-19 patients, NLR is also utilized as a variable in a risk score to anticipate the onset of a critical condition. Some studies have shown that it may assist in discriminating between severe diseases and predicting mortality [34]. In a trial of 245 hospitalized COVID-19 patients, Liu Y et

al. at Wuhan University's Zhongnan Hospital revealed that the neutrophil-to-lymphocyte ratio was an independent highly significant predictor of mortality. Every unit greater neutrophil-to-lymphocyte ratio increased mortality by 8% [35]. The ideal cut-off value for MLR was 7.8263, with 73.3 % sensitivity and specificity, and a UAC of 0.766. Keskin A et al. (2022) recommend that the AUC for MLR should be 0.918, and the optimal cut-off value should be 0.275, with 86.4 % sensitivity and 85.3 % specificity [36].

Conclusions

With an AUC of excellent diagnostic value (0.928), D-dimer tests revealed a substantial rise in plasma of patients with severe COVID-19 infection. Therefore, in the treatment of COVID-19 infection, D-dimer control will be the primary strategy. Furthermore, due to its statistically significant outcome gained and availability in laboratories, ferritin concentration is a helpful marker in COVID-19 disease that can be evaluated in conjunction with clinical investigations and other laboratory analyses while planning the cases-centered treatment plans. In addition, ferritin concentration is a hopeful diagnostic of severe COVID-19 infection. Also essential inflammatory indicators found by regular analysis include NLR and MLR.

Competing interests:
No competing interests were disclosed.

Grant information:

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References

1. Naser NH, Alibeg AAAJIJoPS. Exacerbation of COVID-19 in Hypertensive Patients? A review? 2021;30(2):23-30.
2. Alqubbanchi FB, Al-Hamadani FYJIJoPS. A Pharmacoeconomics Study for Anticoagulants used for Hospitalized COVID-19 Patients in Al-Najaf Al-Ashraf city–Iraq (Conference Paper). 2021;30(Suppl.):48-59.
3. Abd HA, Kasim AA, Shareef LG. Serum levels of α1-antitrypsin, interleukin-1β and interleukin-6 in Iraqi COVID-19 patients: A cross-sectional study. F1000Research. 2022;11(921):921.
4. Shareef LG, Abdulwahab SMJEJPMR. Trends in covid-19 therapeutic modalities: A narrative literature. 2020;7:757-67.
5. Sabah Khalid S, Mohamed Ali Z, Shareef LG. Levels of cardiac troponin-T and LDL-C to HDL-C ratio of hospitalized COVID-19 patients: A case-control study. F1000Research. 2022;11:860.
6. Bonyan FA, Shareef LG, Al-waily A, Abdulrazaq AA, Al-Rubayee WAJMS. COVID-19 clinical characteristics and outcomes in 60 hospitalized Iraqi patients-Case series. 2020:2251-8.
7. Khalid SS, Ali ZM, Raheem MF. Serum Levels of Homocysteine, Troponin-I, and High Sensitive C-Reactive Protein in Iraqi COVID-19 Patients. J Contemp Med Sci| Vol. 2022;8(3):189-93.
8. Shareef LGJGB, Sciences P. COVID-19 vaccine coverage and the necessity of its urgent development towards Omicron the new SARS CoV-2 B. 1.1. 529 variant. 2021;17(3):058-60.
9. Shareef LG, Al-Hussainy AF, Hameed SMJF. COVID-19 vaccination hesitancy among Iraqi general population between beliefs and barriers: An observational study. 2022;11.
10. Chen R, Sang L, Jiang M, Yang Z, Jia N, Fu W, et al. Longitudinal hematologic and immunologic variations associated with the progression of COVID-19 patients in China. 2020;146(1):89-100.

11. Imberti DJI, medicine e. D-dimer testing: advantages and limitations in emergency medicine for managing acute venous thromboembolism. 2007;2(1):70-1.
12. Squizzato A, Ageno WJIEM. What is the next step in D-dimer research? Education of physicians. 2006;1:165.
13. Schutte T, Thijs A, Smulders YJNJM. Never ignore extremely elevated D-dimer levels: they are specific for serious illness. 2016;74(10):443-8.
14. Righini M, Perrier A, De Moerloose P, Bounameaux HJJot, haemostasis. D-dimer for venous thromboembolism diagnosis: 20 years later. 2008;6(7):1059-71.
15. Torti FM, Torti SVJB. Regulation of ferritin genes and protein. 2002;99(10):3505-16.
16. Kernan KF, Carcillo JAJIi. Hyperferritinemia and inflammation. 2017;29(9):401-9.
17. Fu S, Fu X-y, Song Y, Li M, Pan P-h, Tang T, et al. Virologic and clinical characteristics for prognosis of severe COVID-19: a retrospective observational study in Wuhan, China. 2020.
18. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, et al. Across Speciality (2020).395(10229):1033.
19. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. 2020;395(10229):1054-62.
20. Abbaspour N, Hurrell R, Kelishadi RJJorimstojoIUoMS. Review on iron and its importance for human health. 2014;19(2):164.
21. Rosário C, Zandman-Goddard G, Meyron-Holtz EG, D'Cruz DP, Shoenfeld YJBm. The hyperferritinemic syndrome: macrophage activation syndrome, Still's disease, septic shock and catastrophic antiphospholipid syndrome. 2013;11(1):1-11.
22. Citu C, Gorun F, Motoc A, Sas I, Gorun OM, Burlea B, et al. The predictive role of NLR, d-NLR, MLR, and SIRI in COVID-19 mortality. 2022;12(1):122.
23. Hajian-Tilaki KJCjoim. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. 2013;4(2):627.
24. Tang N, Li D, Wang X, Sun ZJJot, haemostasis. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. 2020;18(4):844-7.
25. Spiezia L, Boscolo A, Poletto F, Cerruti L, Tiberio I, Campello E, et al. COVID-19-related severe hypercoagulability in patients admitted to intensive care unit for acute respiratory failure. 2020;120(06):998-1000.
26. Barrett CD, Moore HB, Yaffe MB, Moore EEJJTH. ISTH interim guidance on recognition and management of coagulopathy in COVID-19: a comment. 2020;18(8):2060-3.
27. Abou-Ismail MY, Diamond A, Kapoor S, Arafah Y, Nayak LJTr. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. 2020;194:101-15.
28. Lalueza A, Ayuso B, Arrieta E, Trujillo H, Folgueira D, Cueto C, et al. Elevation of serum ferritin levels for predicting a poor outcome in hospitalized patients with influenza infection. 2020;26(11):1557. e9-. e15.
29. Senjo H, Higuchi T, Okada S, Takahashi OJH. Hyperferritinemia: causes and significance in a general hospital. 2018;23(10):817-22.
30. Colafrancesco S, Alessandri C, Conti F, Priori RJAr. COVID-19 gone bad: A new character in the spectrum of the

hyperferritinemic syndrome? 2020;19(7):102573.
31. Perricone C, Bartoloni E, Bursi R, Cafaro G, Guidelli GM, Shoenfeld Y, et al. COVID-19 as part of the hyperferritinemic syndromes: the role of iron depletion therapy. 2020;68(4):213-24.
32. Ahmed S, Ahmed ZA, Siddiqui I, Rashid NH, Mansoor M, Jafri LJAom, et al. Evaluation of serum ferritin for prediction of severity and mortality in COVID-19-A cross sectional study. 2021;63:102163.
33. Zhou B, She J, Wang Y, Ma X. Utility of ferritin, procalcitonin, and C-reactive protein in severe patients with 2019 novel coronavirus disease. 2020.
34. Yang A-P, Liu J-p, Tao W-q, Li H-mJIi. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. 2020;84:106504.
35. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. 2020;323(11):1061-9.
36. Keskin A, U Ustun G, Aci R, Duran UJBiM. Homocysteine as a marker for predicting disease severity in patients with COVID-19. 2022;16(7):559-68.