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Cluster Analysis of Biochemical Markers as Predictor of COVID-19 Severity

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Abstract:

Numerous blood biomarkers are altered in COVID-19 patients; however, no early biochemical markers are currently being used in clinical practice to predict COVID-19 severity. COVID-19, the most recent pandemic, is caused by the SRS-CoV-2 coronavirus. The study was aimed to identify patient groups with a high and low risk of developing COVID-19 using a cluster analysis of several biomarkers. 137 women with confirmed SARS CoV-2 RNA testing were collected and analyzed for biochemical profiles. Two-dimensional automated hierarchy clustering of all biomarkers was applied, and patients were sorted into classes. Biochemistry marker variations (Ferritin, lactate dehydrogenase LDH, D-dimer, and C- reactive protein CRP) have split COVID-19 patients into two groups (severe cases and non-severe cases groups). Ferritin, lactate dehydrogenase LDH, D-dimer and CRP were markedly increased in COVID-19 patients in the first group (severe cases). Our findings imply that early measured levels of (Ferritin, lactate dehydrogenase LDH, D-dimer, and C- reactive protein CRP) are linked to a decreased probability of COVID-19 severity. Elevated levels of this biomarker may predict COVID severity development.

Keywords: COVID-19, Cluster Analysis, C- reactive protein CRP, D-dimer, Ferritin, Lactate dehydrogenase LDH.

Introduction:

COVID-19, the most recent pandemic, is caused by the SRS-CoV-2 (sARS-CoV-2) coronavirus¹. COVID-19 has been declared a pandemic by the World Health Organization (WHO) in March 2020.² Since then, 112,931,685 COVID-19 cases have been documented, with 502,906 deaths confirmed in 216 countries and regions as of February 2021³. SARS-Covid-2 shares clinical characteristics with SARS-Covid-1 and MERS-Cov.⁴ Although a large number of Covid-19 patients have a moderate influenza-like disease or are asymptomatic, a tiny percentage of patients suffer severe pneumonia, multi-organ failure, acute respiratory distress syndrome, or even death⁵. It's still a mystery why some people become seriously ill and others don't. Risk categorization has been suggested using comorbidities and laboratory indicators^{6,7}. Hyperinflammation is associated with

hyperferritinemia, high serum procalcitonin (PCT), D-dimer, and C-reactive protein (CRP) in critically ill patients, according to accumulating evidence. These data show that a cytokine storm may play a crucial role in covid-19 pathogenesis. Laboratory biomarkers that predict the severity of COVID-19 are crucial in the case of a pandemic because resource allocation must be carefully planned, especially in the case of respiratory support readiness.⁸ In this study, we look at the relationship between serum ferritin, LDH, C-reactive protein, and D-dimer, as well as the severity of COVID19.

Material and Methods:

Sample collection

The studies have been approved by the Committee of the University of Baghdad, College of Science for Women, and have been conducted according to

the ethical standard as outlined in the 1964 Helsinki Declaration and its subsequent corrections or comparable ethical standards, also accordance to the ministry of Iraqi health protocols. Each participant in the study gave their informed consent. 137 patients infected with the SARS CoV -2 RNA testing were collected and analyzed for clinical, biochemical, radiological and hematological profiles. Patients were divided into 2 groups (severe cases and non-severe cases).

Sample analysis

Five ml of blood was obtained from each patient by venipuncture using a 5 ml syringe. 2 mL was dispensed into a tube containing ethylenediaminetetraacetic acid (EDTA) and applied to the hematology autoanalyzer. The obtained serum was used to determine the concentration of ferritin, D-Dimer, LDH, and CRP. Serum ferritin and LDH were measured using kits provided by (Hitachi, Germany & Japan), (Biolabo, France) respectively, and the kits provided by (Genrui, China) were used to determine D-Dimer and CRP.

Results

The current study includes 137 individuals with SARS CoV2 infection, as shown in fig1, the

patients split into two clusters depending on the severity of the illness. The percent of COVID-19 infection was severe cases 50.9% and non-severe cases 49.1%.

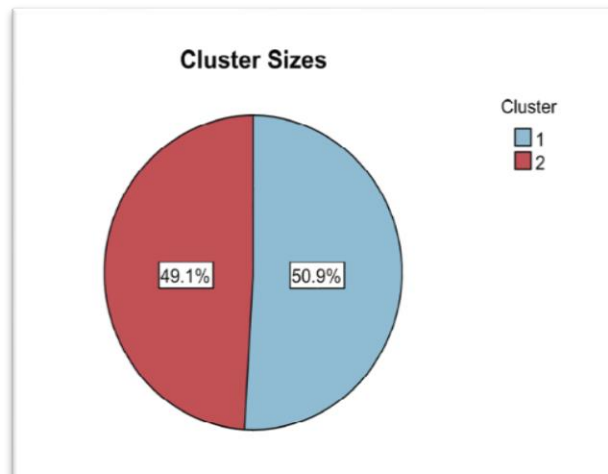


Figure 1. Distribution of patients into two clusters depending on the severity of the illness.

The studied parameters were analyzed and described in Table 1.

Table 1. Descriptive Statistics of Duration of disease in the day, D-Dimer, S. Ferritin, C. R. Protein, and LDH.

Factors	Number	Minimum	Maximum	Mean ± SE
Duration	137	3	13	6.8 ±0.15
D- Dimer	137	0.1	22	3.0±0.4
S. Ferritin	137	3.8	3193	743±50
C.R. Protein	137	0.1	421	40±6
LDH	137	177	963	451±58

The descriptive statistics appeared that the maximum duration of COVID-19 patients was 13 days, after this time the symptom declined, which led to the delivery of the patients from the hospital, the mean the duration of the disease was 6.8. According to the duration data the rest of the parameter levels were taken at the peak of each one. The result showed a high level of D-Dimer with a mean 3.0, a high level of S.Ferritin with a mean 743, a high level of C.R. Protein 40, and also a high level of LDH with a mean 451, as compared to the reference range of the lab.

The studied parameters show clear evidence for their involvement in the estimation of COVID-19 patients, so to improve the involvement of these variables the Predictor of Importance of variables was studied using two- step cluster analysis table 2. The two-step cluster analysis organized the studied parameters into two clusters. Cluster 1 with 50.9 %

of the patients was organized as severe cases, whereas cluster 2 with 49.1 % of the patients was organized as non-sever cases. Input Predictor Importance was listed, the first important variable was S. Ferritin, the second one was LDH, the third one was C.R. Protein, and the last one was D-Dimer. Even though the gradation on the importance of variables, still all of the variables appeared it's associated with the disease.

All Ferritin, LDH, CRP and D-dimer, are linked to the severity of the covid19 infection. The mean of these parameters is significantly increased in patients with severe coronavirus infection and reached 1, 287.6, 509.8, 69.4, and 64.68 for ferritin, (LDH), CRP and D-Dimer respectively, while in non-severe patients, the levels of ferritin, Lactate Dehydrogenase(LDH), D-Dimer and CRP is reached 282.6, 334.8, 18.3, and 0.46 respectively.

Table 2. Predictor of Importance of variables

Cluster	1	2
Size	50.9%	49.1%
Inputs	Severity	Severity
	100% severe cases	100% non-severe cases
↑↑↑↑	S. Ferritin 1,287.6	S. Ferritin 282.6
↑↑↑	LDH 509.8	LDH 334.8
↑↑	C.R. Protein 69.4	C.R. Protein 18.3
↑	D- Dimer 64.68	D- Dimer 0.46
↑↑↑↑	Input(predictor)Importance	
↑↑↑↑		
↑↑		
↑		

S. Ferritin, LDH, C.R. Protein, and D-dimer are indicated by the frequency as shown in Figs 2, 3, 4, 5 respectively. The results of S. ferritin showed the high frequency of the data presented in cluster 2.

Also, LDH with higher frequency appeared in cluster 2, whereas C.R. Protein showed higher frequency in both clusters, finally, D-Dimer also showed high frequency in the two clusters.

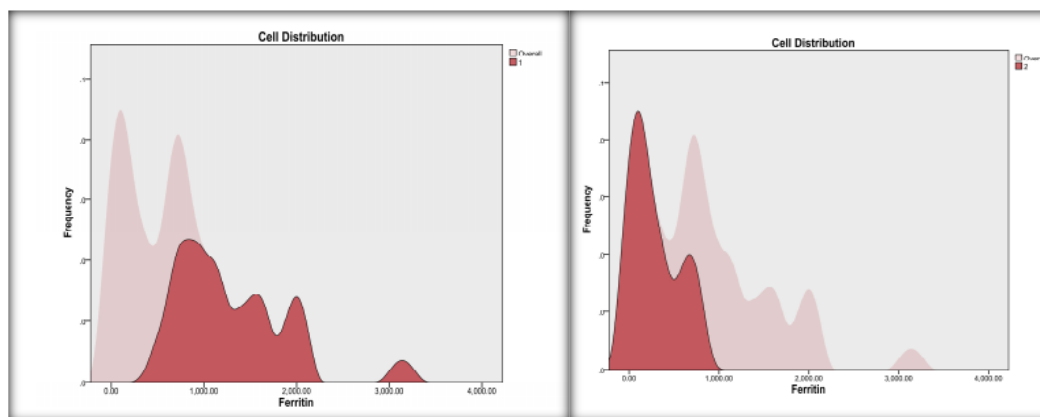


Figure 2. S. Ferritin frequency in the two clusters

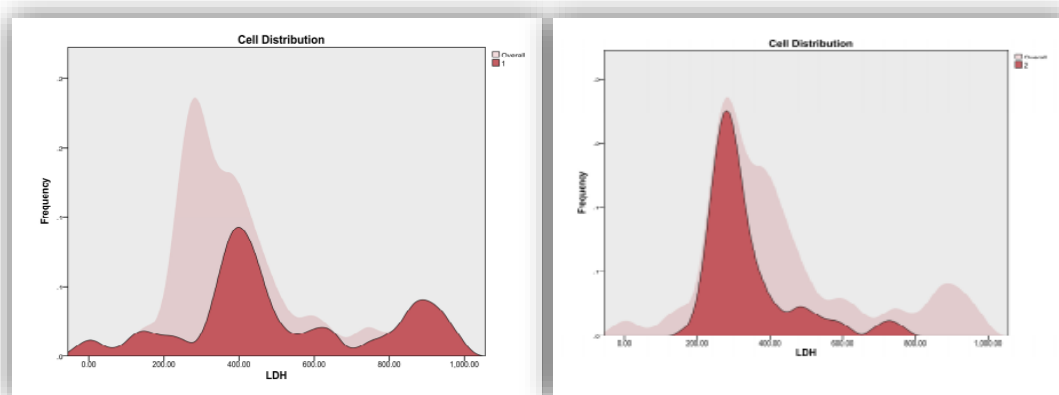


Figure 3. LDH frequency in the two clusters

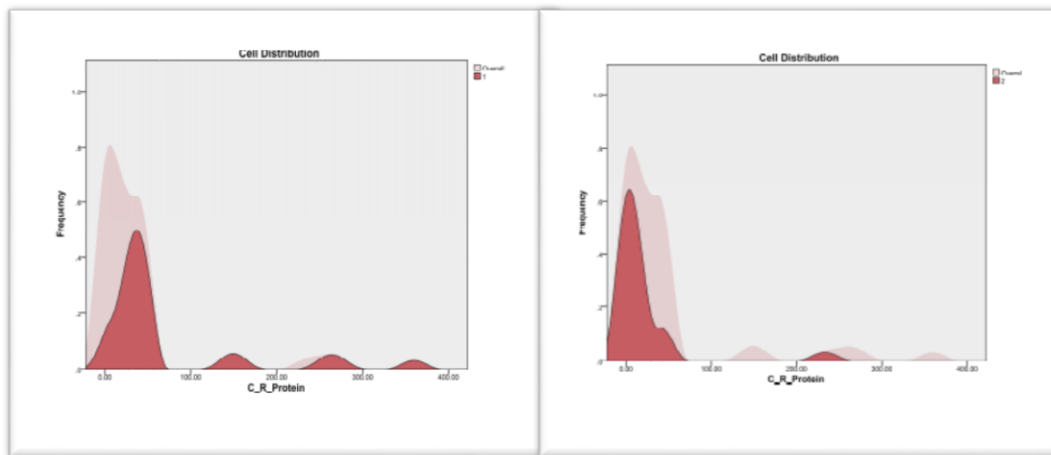


Figure 4. C. R. Protein frequency in the two clusters

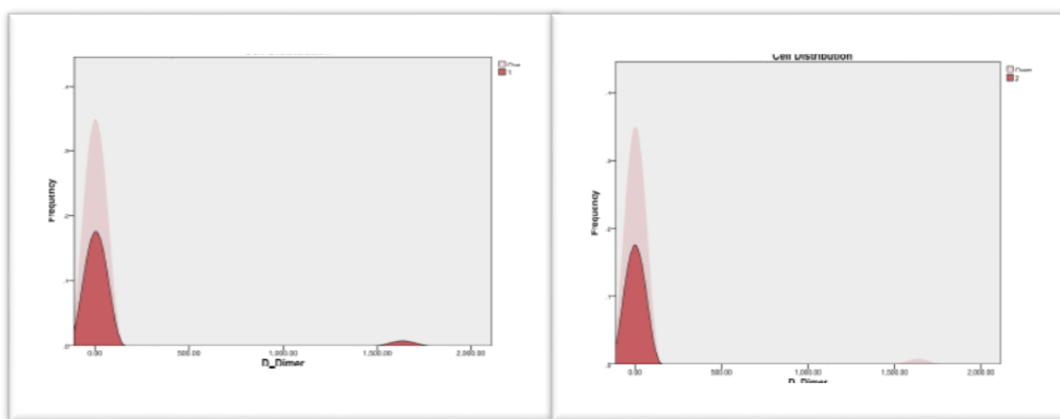


Figure 5. D-Dimer frequency in the two clusters

Discussion:

The clinical course of the COVID-19 pandemic is considered to be highly variable. Although most patients had only mild symptoms, a significant percentage of patients had severe symptoms due to virus-induced hyperinflammation up to respiratory failure requiring mechanical ventilation^{9,10} since, the development of the clinical condition of these patients is difficult to predict, early identification of prognostic indicators is an essential basis for regulating treatment plans and quickly identifying the severity of the patient's condition.¹¹ Through a cluster analysis of 137 patients, we were able to identify two unique COVID-19 groupings in this study. The severity of Cluster 1 was the highest (severe cases 50.9%), and significant increase in serum Ferritin, LDH, D-dimer and CRP when compared with cluster 2 (nonsevere cases 49.1%). This result was in line with earlier reports^{12,13,14,15}. Serum ferritin is an iron-storing protein that is commonly used to assess iron levels, but it is also a well-known inflammatory marker¹⁶. Inflammation and a variety of disorders can cause large increases in serum ferritin levels¹⁷. Although the processes underlying

the link between high ferritin and the severity in COVID-19 patients are unknown, there are various possibilities: 1) cytokines like interleukin-1 (IL1), interleukin-6 (IL6), and tumor necrosis factor- α (TNF α) can increase ferritin production. As a result, we hypothesized that the release of proinflammatory (cytokines) generated by SARS-CoV-2, which are reported to be high in COVID-19, would stimulate ferritin production earlier in the inflammation process. 2) Inflammation-induced cellular damage can lead to intracellular ferritin leakage, which raises serum ferritin levels.¹⁸ Several studies have reported an increase in LDH levels to the severity of COVID-19.^{14,19,20} LDH is a glycolytic enzyme found in the cytoplasm of practically all tissues. Its rise is usually indicative of tissue injury. An elevated LDH level was a common observation in MERS-CoV patients, and it was also one of the most significantly related biomarkers with ARDS death.²¹ According to our findings, in the early stages of severe COVID-19 individuals, elevated LDH revealed possible subclinical tissue harm. Although the virus attaches to the angiotensin-converting enzyme 2 (ACE2) receptor in the lungs²², the lungs are one of the first organs

to be damaged. But as the disease progresses, various cytokine abnormalities as well as multiple organ failure can be identified in severe patients indicating organ damage by the immune system's over activation. The LDH isoenzymes test can also assist in the identification of damaged tissues or organs²¹. COVID-19 is thought to produce direct liver damage as a result of viral hepatitis as well as immunological reactions involving intrahepatic cytotoxic T cells and Kupffer cells²³. Han et al²⁴ concluded that there is either a substantial association between LDH and lung damage as well as the severity of the disease, or that the destruction of the heart muscle and liver caused by COVID-19 is owing to direct damage of the virus to the target organ rather than hypoxia generated by lung injury. It was also observed that increasing lactate generation by LDH leads to a rise in immune suppressive cells and a decline in cytolytic cells, both of which are substantially connected with disease severity²³. D-dimer is fibrin breaks down product, a small fraction of protein detected in blood after fibrinolysis of blood clots. In clinical practice, determining circulation D-dimer is a sensitive assay for diagnosing thrombotic conditions, including pulmonary embolism²⁵. As a result, D-dimer increases in COVID-19 individuals may be useful in identifying those who have severe disease, lung problems, and a high risk of venous thromboembolism. This would be beneficial with risk stratification as well as early therapeutic measures that may reduce morbidity and mortality associated with COVID-19. A new meta-analysis showed that patients with severe COVID-19 have higher D-dimer levels than milder forms²⁶. COVID-19, as a result of the cytokine storm, causes severe thromboembolic complications in severe patients. In autopsies of small pulmonary arteries, scientists identified thrombi, endothelial damage, and fibrin, and determined that the most dangerous coagulation disorder was disseminated intravascular coagulation (DIC). However, it is unclear whether this is a direct consequence of the SARS-CoV2 virus or a result of the coagulation system being activated in reaction to disease severity. High D-dimer levels, according to the researchers, could indicate disease severity, pulmonary problems, and thromboembolic events before they happen. With early identification and treatment, they hoped to lessen the disease's morbidity and death²⁷. The liver produces large amounts of CRP, an acute-phase protein in response to infections. This protein is an extremely sensitive marker for tissue injury and infection. CRP levels are linked to inflammation, increase activation of the complement system and enhanced phagocytosis. CRP, in other words, binds to germs and stimulates

phagocytosis to remove them.²⁸ The current study found that severe patients had considerably higher CRP levels than patients with non-severe conditions, demonstrating that CRP may be a diagnostic of disease severity and progression in COVID-19 patients. According to Liu et al., severe COVID-19 individuals had considerably higher CRP than non-severe patients.¹⁵ Many researchers^{28,29} found a link between the levels of CRP and COVID-19 severity and indicated that it might be used to predict disease severity as an independent risk factor.

Conclusion:

Global healthcare costs are rising due to the COVID-19 pandemic's quick spread. Strong evidence must be gathered in order to accurately diagnose the patient's condition and foresee consequences due to the changeable nature of diseases and complications. Our data suggest that early evaluated levels of (s. Ferritin, LDH, D-dimer, and CRP) may be linked to a reduced likelihood of acquiring COVID-19 severity. Higher levels of (Ferritin, LDH, D-dimer and CRP) may predict COVID severity development.

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration's approval
- Ethical approval: This research was approved by the Committee of the University of Baghdad, College of Science for Women, Department of Chemistry with session 14, number 387 on 19/6/2022, it is performed in accordance with the ethical standard as laid down in 1964 declaration of Helsinki statement and its later corrections or comparable ethical standards.
- Informed consent was obtained from all individual participants included in the study.

Authors' contributions statement:

E. M. T., M. M. T., and S. K. M., conceived of the present idea, E. M. T., and S. K. M. have carried out the experiment. E. M. T., M. M. T., S. K. M., and M. N. A. have contributed to the final version of the manuscript

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التحليل العنقودي للواسمات البايوكيميائية كمتنبى لشدة كوفيد 19

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الخلاصة:

العديد من المؤشرات الحيوية في الدم تتغير لدى مرضى COVID-19 ؛ ومع ذلك ، لا يتم حاليًا استخدام علامات بيوكيميائية مبكرة للتعقب بشدة COVID-19. أراد المؤلفون تحديد مجموعات المرضى ذات الخطورة العالية والمنخفضة للإصابة بـ COVID-19 باستخدام التحليل العنقودي للعديد من المؤشرات الحيوية. تم جمع 137 امرأة مع اختبار SARS CoV-2 RNA المؤكدة وتحليلها. تم تطبيق التجميع الهرمي الآلي ثنائي الأبعاد لجميع المؤشرات الحيوية ، وتم تصنيف المرضى إلى فئتين. تم زيادة الفيبريتين ، اللاكتات ديهيدروجيناز LDH ، D-dimer و CRP بشكل ملحوظ في مرضى COVID-19 في المجموعة الأولى (الحالات الشديدة). تشير النتائج التي توصلنا إليها إلى أن المستويات التي تم قياسها في وقت مبكر من Ferritin و LDH lactate dehydrogenase و D-dimer ، و C- CRP التفاعلي بروتين مرتبطة بانخفاض احتمال الإصابة بفيروس COVID-19. قد تتنبأ المستويات المرتفعة من هذا المرقم الحيوي بتطور شدة ال COVID19.

الكلمات المفتاحية: كوفيد 19، تحليل عنقودي، البروتين C التفاعلي، دي دايمر، فيبريتين، لاكتيت دي هايدروجيناز.